Published in Journals: Microplastics, Sustainability, Toxics and Water

Topic Reprint

Microplastics Pollution

Edited by
Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

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Topic Editors

Grigorios L. Kyriakopoulos
School of Electrical and Computer Engineering
National Technical University of Athens
Athens
Greece

Vassilis J. Inglezakis
Department of Chemical and Process Engineering
University of Strathclyde
Glasgow
UK

Antonis A. Zorpas
School of Pure and Applied Science
Open University of Cyprus
Nicosia
Cyprus

María Rocío Rodríguez Barroso
Department of Environmental Technologies
University of Cádiz
Cádiz
Spain

Editorial Office
MDPI AG
Grosspeteranlage 5
4052 Basel, Switzerland

This is a reprint of the Topic, published open access by the journals *Microplastics* (ISSN 2673-8929), *Sustainability* (ISSN 2071-1050), *Toxics* (ISSN 2305-6304) and *Water* (ISSN 2073-4441), freely accessible at: https://www.mdpi.com/topics/0CLXJ5R9AJ.

For citation purposes, cite each article independently as indicated on the article page online and as indicated below:

Lastname, A.A.; Lastname, B.B. Article Title. *Journal Name* **Year**, *Volume Number*, Page Range.

ISBN 978-3-7258-3753-3 (Hbk)
ISBN 978-3-7258-3754-0 (PDF)
https://doi.org/10.3390/books978-3-7258-3754-0

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About the Editors

Grigorios L. Kyriakopoulos

Dr. Grigorios L. Kyriakopoulos (2 PhDs) holds the following university qualifications: Chemical Engineering (MEng, 2 MScs, PhD, post-doc), Environmental Science (BSc, MSc, PhD), Business Management (BA, MA), Hellenic Culture (BA, MA). He works as a Teaching and Research Associate and Senior Researcher at the School of Electrical and Computer Engineering, National Technical University of Athens (NTUA). He has been credited with more than 150 publications (books, contributions to books, papers in journals, international conferences). His current Google Scholar h-index is 45, with more than 5600 citations. The main research areas of his specialization are as follows: engineering, environment, renewable energy sources, low-carbon economy, waste and water management, business administration, education, urban and regional development, development economics, circular economy, and behavioral ecology. He has been recognized since 2020 and across the last four consecutive years among the world's top 1% researchers in the list of the most influential researchers in the world in the main section of "Enabling and Strategic Technologies", two subsections of "Energy", and "Business and Management", based on bibliometric data from the Stanford University (USA) and Elsevier Report.

Vassilis J. Inglezakis

Dr. Inglezakis joined Strathclyde University, Department of Chemical & Process Engineering in 2020. At present, he is the chair of the Laboratory Operations Committee and acted as Director of Research from 2021 to 2023. His area of research is within the broader area of environmental chemical engineering, and he has published two books with Elsevier and more than 145 journal papers (Scopus data; h-index = 47, $>$ 6900 citations). He has been PI/Co-PI in 16 research projects, with a total budget of $>$£3M, and participated in 44 other research and consultancy projects. His experience as consultant engineer is rich and recognized at an international level, including two appointments as Pre-Accession Advisor, coordinating candidate countries' alignment with EU environmental legislation (2004-2007). He has been a project proposal evaluator for the European Commission, EPSRC (UK), UEFISCDI (Romania), RSF (Russia), NCST (Kazakhstan), and HFRI (Greece). At the Chemical Engineering Department of Nazarbayev University, Kazakhstan (2013-2020), he founded the Environmental Science & Technology Group and the Environment and Recourse Efficiency Cluster, and he served as Scientific Supervisor of the Analytical Chemistry Core Facility and as Chair of the Biosafety & Chemical Safety Committee of the university. He led the development of 19 teaching and research labs, and in 2016, he received an award from the Ministry of Education of Kazakhstan for his distinguished contribution to the country's education system. He also led the development of the Unit of Environmental Science and Technology Research Lab, Chemical Engineering Department, National Technical University of Athens (2002-2003).

Antonis A. Zorpas

Dr. Antonis Zorpas is a chemical engineer actively engaged with several universities and research institutes across Europe. He is a Professor at the Open University of Cyprus, Director of the Lab of Chemical Engineering & Engineering Sustainability, Director of the Sustainable Environmental Engineering Postgraduate Master of Science (MSc) and a member of the OUC Senate. He has been credited with more than 650 publications (books, contributions to books, papers in journals, international conferences). His current Google h-index is 59, with more than 11000 citations.

He has been recognized since 2019 among the world's top 1% researchers in the list of the most influential researchers in the world in the section of Environmental Science and among <1% in the section of Chemical Engineering, based on bibliometric data from the Stanford University (USA) and Elsevier Report. Hence, the database from Google Scholar GPS places him 5th globally in the field of waste management and among the 0.5% of the most influential scientists on the planet in the field of chemical engineering. His research background is in strategic planning development within the framework of circular economy and bioeconomy, waste management, life cycle assessment, end-of waste criteria, material flow analysis, food waste, fashion waste, microplastics, etc. He has participated in more than 50 EU research projects in the last 15 years. For more than 20 years, he has been acting as consulting engineer on behalf of several industrial activities and governmental authorities. In 2019, he received an honorary distinction for his long-standing contribution to the environmental issues of Cyprus from the President of the Republic of Cyprus. He has collaborated with the European Commission since 2008 (e.g., as Strategic Advisor for EURASHE 2016-2029, as Technical Expert on behalf of European Defense Agency since 2020, etc.).

María Rocío Rodríguez Barroso

Dr. Rodríguez Barroso holds a degree in Marine Sciences and a Ph.D. in Chemical Engineering, and he is currently a full professor at the University of Cadiz, Faculty of Marine and Environmental Sciences (Cadiz). Her teaching activities have focused on environmental technologies and environmental management tools. Since 2016, her research interests have focused on the training and development of projects related to the circular economy, including coordinating expert courses on the subject, research on the recovery of organic and radioelectronic waste, and research on microplastics in the urban water cycle (drinking water and wastewater).

Preface

Plastics are amazing products with infinite applications, for example, in food packaging, the aviation and automotive industry, pharmaceuticals, medicine, telecommunication, the apparel industry, etc. Plastic particles less than 5 mm in diameter are considered microplastics, derived from commercial products such as cosmetics, microfibers in textiles, and the degradation of larger plastic waste in the environment. This reprint summarizes the current knowledge on microplastic accumulation, impacts, and monitoring approaches.

Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas, and María Rocío Rodríguez Barroso

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Editorial

The Silent Invaders: Microplastic Accumulation, Impacts, and Monitoring Approaches

Grigorios L. Kyriakopoulos [1,*], Antonis A. Zorpas [2], Vassilis J. Inglezakis [3] and Rocío Rodríguez-Barroso [4]

[1] Photometry Laboratory, Electric Power Division, School of Electrical and Computer Engineering, National Technical University of Athens, 9 Heroon Polytechniou Street, 15780 Athens, Greece
[2] Laboratory of Chemical Engineering and Engineering Sustainability, Faculty of Pure and Applied Sciences, Open University of Cyprus, Giannou Kranidioti 89, Latsia, 2231 Nicosia, Cyprus; antonis.zorpas@ouc.ac.cy or antoniszorpas@yahoo.com
[3] Department of Chemical & Process Engineering, University of Strathclyde, Glasgow G1 1XJ, UK; vasileios.inglezakis@strath.ac.uk
[4] Department of Environmental Technologies, Faculty of Marine and Environmental Sciences, University of Cádiz, 11510 Cádiz, Spain; rocio.rodriguez@uca.es
* Correspondence: gregkyr@chemeng.ntua.gr

1. Introduction

Environmental pollution caused by microplastics (MPs) has evolved into a global concern; however, the knowledge about MP accumulation in the environment, potential impacts, and monitoring approaches is limited. MPs, defined as plastic particles smaller than 5 mm, have emerged as pervasive pollutants infiltrating ecosystems, food chains, and even human bodies according to several reports. MPs are now recognized as a major environmental challenge due to their microscopic size, with far-reaching consequences. This editorial summarizes the current knowledge on MP accumulation, impacts, and monitoring approaches.

MPs are plastic particles less than 5 mm in diameter coming from commercial products such as cosmetics and microfibers in textiles and the degradation of larger plastic waste in the environment. They have been recognized as a major environmental and human health hazard. This is because MPs take a long time to decompose, they are widespread, and they find their way into the food chain through consumption by marine animals. Also, they will continue to be released into the environment since plastic usage is widespread. Plastics are amazing products with infinite applications, such as food packaging, the aviation and automotive industry, pharmaceuticals, medicine, telecommunication, apparel industry, etc. Nafea et al. [1] pointed out that global plastic production has doubled since 2000, reaching 460 million tonnes in 2019, and at the same time, plastic waste generation reached 353 million tonnes. Regrettably, only 9% of all plastics produced are recycled, with 68% ending up in landfills or being incinerated and 22% being mismanaged and entering the natural environment [2]. Chlorinated plastics and MPs can release harmful chemicals into the soil, which can then seep into groundwater or other water sources. Landfill areas are constantly piled high with many different types of plastics, and there are many microorganisms which speed up their biodegradation, releasing methane.

Due to the highly complex nature of plastic pollution, encompassing a wide range of polymers, various products and packaging, diverse societal uses, and numerous pathways into different environmental compartments [3], monitoring approaches are equally challenging. Each sector of plastic usage in society must implement customized solutions to address the specific polymers, products, and packaging it utilizes, as well as their potential release into the environmental media.

Received: 5 February 2025
Revised: 4 March 2025
Accepted: 6 March 2025
Published: 7 March 2025

Citation: Kyriakopoulos, G.L.; Zorpas, A.A.; Inglezakis, V.J.; Rodríguez-Barroso, R. The Silent Invaders: Microplastic Accumulation, Impacts, and Monitoring Approaches. *Toxics* **2025**, *13*, 192. https://doi.org/10.3390/toxics13030192

At the same time, plastic pollution is a defining characteristic of modern society, and its ubiquity has been noted by scientists from every continent. Researchers have found plastic particles in the deep sea [4], rainwater [5], on top of mount Everest [6], and even in human placenta [7], blood [8], drinking water [9], plankton [10], coral reef [11], and sea mammals and birds [12]. Despite the increased attention given to plastic pollution in the last decade, evidence suggests that the problem is worsening [13].

To combat plastic pollution, countries have begun regulating the production, consumption, and distribution of plastics. The European Commission (EC) is actively implementing initiatives from the European Green Deal and the newly introduced Circular Economy Action Plan. Several directives, including the Packaging and Packaging Waste Directive and the Waste Framework Directive, and the ban on single-use plastics, are being enforced to tackle this problem. The issue of plastic pollution, and therefore MPs, is not strictly economic or environmental pollution, but it can be said to reflect environmental justice [13]. Innovative strategies are essential, requiring a comprehensive understanding of plastic pollution, especially its root causes and the role of global sustainability agendas in addressing the issue. A strategic approach to mitigate pollution (including plastic pollution) was proposed by Zorpas [14]. In general, plastic pollution can be reduced by adopting several R strategies [15]. These methods reflect circular economy principles, SDGs, and the new circular economy framework standard ISO 59000 series [15].

There are several technologies for the plastic clean-up of seas, lakes, rivers, oceans, and shores, but they are insufficient in addressing the vast extent of microplastic pollution [1]. Moreover, to the best of the authors' knowledge, there are no technologies for the removal of MPs from agricultural lands or forests. Hence, effective plastic waste management, policies, and legislation play a crucial role in reducing environmental plastic and microplastic pollution. Regulations can encourage research, innovation, and adoption of sustainable practices leading to a more circular resource-efficient approach to plastic and microplastic management [16]. For example, the European policy "Strategy for Plastics in a Circular Economy" aims to establish a new circular economy for plastics across the continent, promoting the reuse, repair, and recycling of plastics and plastic products while striving to reduce the introduction of microplastic products. In general, proper waste management practice can mitigate the negative impacts of plastic pollution on the environment.

2. Research Topic Overview

The Research Topic titled "Microplastics Pollution" contains 26 articles that have been published by the following five participating MDPI journals: *International Journal of Environmental Research and Public Health*, *Microplastics*, *Sustainability*, *Toxics*, and *Water*. These articles are presented below:

"Enhancing Pb Adsorption on Crushed Microplastics: Insights into the Environmental Remediation", by Sen Li, Lu Cao, Qiyuan Liu, Shuting Sui, Jiayin Bian, Xizeng Zhao, Yun Gao. This study investigates the pollution characteristics and environmental risks of crushed microplastics (MPs) generated during plastic recycling, emphasizing their adsorption capacity for heavy metals, particularly lead (Pb), by employing a variety of advanced analytical techniques, such as that of SEM-EDS, revealing that crushed MPs exhibit significantly higher adsorption capacity than primary MPs. Nevertheless, the presence of adsorbed Pb slightly reduced recovery performance, emphasizing the need to optimize recovery conditions for maximum efficiency. Therefore, the study demonstrated the dual threat posed by crushed MPs: their capacity to both adsorb and concentrate harmful substances, and thus, the increase in ecological toxicity and challenge to their recovery.

"Evaluation of Microplastic Toxicity in Drinking Water Using Different Test Systems", by Natalya S. Salikova, Anna V. Lovinskaya, Saule Zh. Kolumbayeva, Ainash U. Bekte-

missova, Saltanat E. Urazbayeva, María-Elena Rodrigo-Clavero, Javier Rodrigo-Ilarri. This study investigated the toxicological and genotoxic effects of various microplastic types (polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE)) on plant and animal models. The findings of this study underscored the need to mitigate risks associated with microplastic pollution, particularly in drinking water sources. Future research works could closely approach the long-term health impacts of microplastic exposure, including carcinogenic potential coupling with the exploration of the synergistic effects of other pollutants.

"Microplastic Transport and Accumulation in Rural Waterbodies: Insights from a Small Catchment in East China", by Tom Lotz, Wenjun Chen, Shoubao Su. This study investigates the distribution and sources of MP in drainage ditches influenced by pond connectivity, land use, and soil properties. Interestingly, site-specific MP sources have been determined, with fluororubber (FR) linked to road runoff and polyethylene terephthalate (PET) being suitable for agricultural practices. Correlations between MP shape and soil properties proved that more compact and filled shapes were more commonly associated with coarser soils. In this context, PE particle size was negatively correlated with organic matter. The most general applicability issues of this study refer to the need for targeted strategies in order to reduce MP pollution in rural landscapes, including reduction in plastic use, ditch maintenance, and improved road runoff management.

"Sustainable Wastewater Treatment Strategies in Effective Abatement of Emerging Pollutants", by Hafiz Waqas Ahmad, Hafiza Aiman Bibi, Murugesan Chandrasekaran, Sajjad Ahmad, Grigorios L. Kyriakopoulos. This study underscores how the fundamental existence of any living organism requires the availability of pure and safe water. In this context, this study is a review analysis on MPs, considering the ever-increasing population that has led to extensive industrialization and urbanization, which have subsequently escalated micropollutants and water contamination, as well as the MPs' environmental impact on various life forms. Environmentally friendly, cost-effective, and sustainable strategies are envisaged for efficient environmental protection. The review study also proposed a novel strategy that combines nanomaterials to improve micropollutant degradation with bioremediation techniques, particularly the creative application of phytoremediation technologies like floating wetlands. The critical domains of water pollution abatement included floating wetland treatments, facilitating micropollutant elimination, landscape management, ecosystem conservation, and aesthetic enhancement in diverse environments. The novelty of this research focus includes the incorporation of nanomaterials in the bioremediation of toxic micropollutants, augmenting novel and innovative strategies.

"Retention, Degradation, and Runoff of Plastic-Coated Fertilizer Capsules in Paddy Fields in Fukushima and Miyagi Prefectures, Japan: Consistency of Capsule Degradation Behavior and Variations in Carbon Weight and Stable Carbon Isotope Abundance", by Shigeki Harada, Itsuki Yajima, Keitaro Fukushima, Youji Nitta. In this study, paddy field runoff containing plastic capsules that are used to coat fertilizers has been receiving increased attention. However, the behavior of these capsules, especially their degradation behavior, has not been extensively investigated, which was the research focus of this study. The behaviors of the capsules in both types of runoff were monitored in 2022 and 2023 at four paddy fields in Fukushima and Miyagi prefectures in northern Japan. Weight loss of the plastic capsules could be attributed to a combination of capsule degradation and the release of urea inside the capsules, which was explained by carbon isotopic analyses. The following types of degraded capsules were identified: shrunken, broken, and spherical, making it statistically significant to signify the differences among the weights of each type.

"Sorption-Based Removal Techniques for Microplastic Contamination of Tap Water", by Natalya S. Salikova, Almagul R. Kerimkulova, Javier Rodrigo-Ilarri, Kulyash K. Alimova,

María-Elena Rodrigo-Clavero, Gulzhanat A. Kapbassova. This study investigates the presence of MPs in tap drinking water and evaluates the efficacy of various sorbents for their removal in the context of Kazakhstan's water treatment system. Water samples were taken in the cities of Kokshetau and Krasny Yar (Akmola Region). The research objective of this study was the sorption efficiency of different sorbents, indicating especially high retention rates (ranging from 82.7 to 97.8%) for microplastic particles of different shapes and synthesis. Indeed, sorbents synthesized by carbon sorption material (CSM) demonstrated the highest efficiency in both microplastic retention and improvement in water quality parameters, making it a promising technology especially suitable for water treatment facilities and household filters.

"Spatial and Temporal Distribution Characteristics and Potential Sources of Microplastic Pollution in China's Freshwater Environments" by Hualong He, Sulin Cai, Siyuan Chen, Qiang Li, Pengwei Wan, Rumeng Ye, Xiaoyi Zeng, Bei Yao, Yanli Ji, Tingting Cao, Yunchao Luo, Han Jiang, Run Liu, Qi Chen, You Fang, Lu Pang, Yunru Chen, Weihua He, Yueting Pan, Gaozhong Pu. This study investigated the characteristics of microplastic pollution in the freshwater environments of 21 major cities across China. Through indoor and outdoor experimental analysis, their spatial and temporal distribution characteristics were identified. Authors observed that the abundance of MPs in China's freshwater environments was generally increased from west to east and from south to north, while other factors of positive correlation were that of (a) areas of intense human activity, including agricultural, transport, and urban land, (b) seasonal changes peaking in summer, followed by spring and autumn, and (c) rainfall variations.

"Microplastics in Groundwater: Pathways, Occurrence, and Monitoring Challenges", by Elvira Colmenarejo Calero, Manca Kovač Viršek, Nina Mali. In this study, MPs are considered a fast-emerging pollutant, whose presence in the water cycle and interaction with ecological processes pose a significant environmental threat to surface and groundwater, which represent the primary sources of drinking water. Monitoring MPs in groundwater (GW) is determined primarily by the contamination pathways of MPs from surface water, seawater, and soil into the GW. Such an analysis identified the challenges associated with their monitoring in GW, among which (challenges) are that of applying standardized techniques for MP sampling and detection, by better understanding the specific hydrogeological and hydrogeographic conditions in the process of sample collection using sampling devices with comparable specifications and comparable laboratory techniques for MPs' identification.

"Monitoring of Microplastics in Water and Sediment Samples of Lakes and Rivers of the Akmola Region (Kazakhstan)", by Natalya S. Salikova, Javier Rodrigo-Ilarri, Lyudmila A. Makeyeva, María-Elena Rodrigo-Clavero, Zhulduz O. Tleuova, Anar D. Makhmutova. This study provides a detailed description of the findings and methodology related to the monitoring of MPs in three lakes and one river of the Akmola Region in Kazakhstan. The research variables were the concentration of microplastic particles and the connection between microplastic content and turbidity, particularly notable during the spring season. Sediment analysis revealed a decrease in microplastic concentrations from the coastal zones. In addition, the spatial and temporal distribution of MPs necessitated the ongoing monitoring and management strategies to address emerging environmental concerns.

"Comparison of Methodologies for Microplastic Isolation through Multicriteria Analysis (AHP)", by Valentina Phinikettou, Iliana Papamichael, Irene Voukkali, Antonis A. Zorpas. This study developed an inexpensive, rapid method with user-friendly and environmentally sustainable outcomes, considering the limited knowledge that exists about MPs in soils due to the absence of standardized extraction methods. In better understanding the mitigation of MPs in soils, a comprehensive multicriteria analysis proved that saturated

sodium chloride solution emerged as the optimal scenario for MP extraction in terms of demonstrated economic feasibility, safety, and reliability, followed closely by the canola oil scenario.

"Interaction between Microplastics and Pathogens in Subsurface System: What We Know So Far", by Hongyu Zhao, Xiaotao Hong, Juanfen Chai, Bo Wan, Kaichao Zhao, Cuihong Han, Wenjing Zhang, Huan Huan. This study has focused on MPs that are abundant in soil and the subsurface environment. Authors stated that MPs can co-transport with pathogens or act as vectors for pathogens, potentially causing severe ecological harm. The interaction of MPs with pathogens is environmentally important, especially when focusing on interaction mechanisms and environmental factors and their co-transport. Such environmental factors affecting their interaction include particle size, specific surface area, shape and functional groups of MPs, the zeta potential and auxiliary metabolic genes of pathogens, and hydrophobicity.

"Application and Efficacy of Management Interventions for the Control of Microplastics in Freshwater Bodies: A Systematic Review", by Suveshnee Munien, Puspa L. Adhikari, Kimberly Reycraft, Traci J. Mays, Trishan Naidoo, MacKenzie Pruitt, Jacqueline Arena, Sershen. This study is a review analysis that systematically represents one of the first attempts to trace the evolution of research and compare the efficacy of the full suite of management interventions developed to control (prevent or remove) MPs in freshwater bodies, both man-made and natural. Among the key findings of this review paper are that physical types (particularly membrane filtration) were most common. It is notable that wastewater/sludge, stormwater, and in situ water/sediment categories exhibited removal efficacies of more than 90% under laboratory conditions. It is also questionable whether scalability and suitability could be achieved across different settings. Moreover, downstream interventions lack sustainability without effective upstream interventions, and when in situ methods are technically achievable, they may not be feasible in resource-limited settings.

"Release of Microplastics from Urban Wastewater Treatment Plants to Aquatic Ecosystems in Acapulco, Mexico", by Enrique J. Flores-Munguía, José Luis Rosas-Acevedo, Aurelio Ramírez-Hernández, Alejandro Aparicio-Saguilan, Rosa M. Brito-Carmona, Juan Violante-González. This study denotes that contamination of aquatic ecosystems by MPs is mainly due to the release of high levels of microplastic particles from treated effluents by wastewater treatment plants (WWTPs). The authors also point out the lack of policies and regulations establishing criteria for the control and elimination of hazard pollutants from aquatic ecosystems, such as that of Acapulco, Mexico, which is the research area of their study. The MPs' average daily emissions to the receiving bodies of the three selected WWTPs ranged from 9.5×10^6 to 4.70×10^8 particles, while the annual emissions ranged from 3.05×10^9 to 1.72×10^{11} particles. The generalized prospects are relevant to the urgency of implementing regulatory policies to avoid the continuous emission of MPs in the studied area and its WWTPs.

"Microplastic in the Snow on Sledding Hills in Green Areas of Krakow", by Jarosław Lasota, Wojciech Piaszczyk, Sylwester Tabor, Ewa Błońska. This study aims to determine the amount of microplastic in the snow on sledding hills in green areas of Krakow, considering the intensive use of these sledding hills in winter. After the snow melts, microplastics are transferred to the soil surface, which can lead to changes in the properties of the soil. Subsequently, due to their strong hydrophobicity, they will play an important role in the transport of toxic compounds, e.g., polycyclic aromatic hydrocarbons (PAHs). The research objective is to limit the use of plastic sleds and replace them with wooden sleds, which will not be a source of pollution for urban green spaces used by residents regardless of the season.

"Influence of Microplastics on Morphological Manifestations of Experimental Acute Colitis", by Natalia Zolotova, Dzhuliia Dzhalilova, Ivan Tsvetkov, Olga Makarova. This study demonstrates that microplastic pollution poses a threat to human health, and it is highly possible that the increase in the incidence of inflammatory bowel disease is associated with exposure to MPs. For this reason, authors investigated the effect of the consumption of polystyrene microparticles with a diameter of 5 μm, focusing on the acute colitis body episodes. Exposure of healthy mice to MPs resulted in an increase in endocrine cell numbers, an increase in the content of highly sulfated mucins in goblet cells, an increase in the number of cells in the lamina propria, and a decrease in the volume fraction of macrophages. The extent to which microplastic consumption caused more severe acute colitis can be characterized by a greater prevalence of ulcers and inflammation and a decrease in the content of neutral mucins in goblet cells.

"Environmental Assessment of Microplastic Pollution Induced by Solid Waste Landfills in the Akmola Region (North Kazakhstan)", by Natalya S. Salikova, Javier Rodrigo-Ilarri, María-Elena Rodrigo-Clavero, Saltanat E. Urazbayeva, Aniza Zh. Askarova, Kuandyk M. Magzhanov. This study presents the outcomes derived from an environmental assessment of microplastic pollution resulting from solid waste landfills in the Akmola Region, situated in North Kazakhstan. This research was conducted on MPs within this specific region. The utilized methodologies were focused on the "soft" removal of organic substances through the use of oxidants which do not damage plastics and were tested using a water-bath therapeutic treatment. Furthermore, an analysis of soil samples taken from the landfills unveiled the ultimate retention of microplastic particles, attributed to leachate and rainwater runoff.

"Spatial–Temporal Distribution and Ecological Risk Assessment of Microplastics in the Shiwuli River", by Lei Hong, Xiangwu Meng, Teng Bao, Bin Liu, Qun Wang, Jie Jin, Ke Wu. This study aims to investigate the distribution of MPs within the Shiwuli River in Hefei, a Chinese inland city. Water and sediment samples were collected during both flood season (May to September) and non-flood season (October to April) at 10 representative sites to assess the potential risk posed by MP pollution in the Shiwuli River to the quality of drinking water sources in Chaohu Lake in Hefei. The analytical part of this study contained an analysis of the main variables that determine the level of MP existence. Such variables include tributaries that are also close to residential and industrial development, agricultural areas, wetland ecological regions, and flood season or non-flood season. The research findings provide valuable insights into management, pollution control, and integrated management strategies pertaining to MPs in urban inland rivers (with a research focus on Hefei).

"A Review of the Current State of Microplastic Pollution in South Asian Countries", by Lee Tin Sin, Vineshaa Balakrishnan, Soo-Tueen Bee, Soo-Ling Bee. This study is a review analysis that discusses the development of microplastic pollution based on a selection of South Asian countries consisting of Bangladesh, Iran, Philippines, Thailand, India, Indonesia, and Vietnam. The presence of MPs in food items, mainly tea bags, sugar, shrimp paste, and salt packets, has been reported. Microplastic contamination includes the ingestion of MPs by aquatic creatures in water environments. The impacts on terrestrial environments relate to MPs sinking into the soil, leading to the alteration of the physicochemical parameters of soil. Moreover, the impact on the atmospheric environment included the settling of MPs on the external bodies of animals and humans.

"Assessing the Occurrence and Distribution of Microplastics in Surface Freshwater and Wastewaters of Latvia and Lithuania", by Reza Pashaei, Viktorija Sabaliauskaitė, Sergej Suzdalev, Arūnas Balčiūnas, Ieva Putna-Nimane, Robert M. Rees, Reda Dzingelevičienė. This study focuses on microplastic concentrations in surface water and wastewater collected

from the cities of Daugavpils and Liepaja in Latvia, as well as Klaipeda and Siauliai in Lithuania, which were measured in July and December 2021. In this study, a variety of advanced analytical techniques contained the use of optical microscopy, and polymer composition can be characterized using micro-Raman spectroscopy. Surface water and wastewater samples showed that municipal and hospital wastewater from catchment areas were the main reasons for the contamination of MPs in the surface water and wastewater of Latvia and Lithuania. Reduction in pollution loads can be realistic by implementing measures such as raising awareness, installing more high-tech wastewater treatment plants, and reducing plastic use.

"Social Cognitive Theory and Reciprocal Relationship: A Guide to Single-Use Plastic Education for Policymakers, Business Leaders and Consumers", by Sarah Fischbach, Brielle Yauney. This study concentrates on the social cognitive theory framework for sustainable consumption. The authors employed a US-based survey in which the reciprocal relationship among three factors—personal (green consumer values), environmental (bans and rebate/reward programs), and behavioral (consumer decision-making related to single-use plastic waste)—was examined. The key findings of this study are that those states with bans or rebate/reward programs tend to have higher green consumer values, and consumers in those states report less use of single-use plastic waste. Education level is a determining factor that impacts green consumer values and plastic waste usage. A resource guide was developed for decision makers to implement programs in five areas, including Business Resources, Public Policy Resources, Non-Profit Resources, Education Resources, and Personal Resources.

"High-Efficiency Microplastic Sampling Device Improved Using CFD Analysis", by Seonghyeon Ju, Jongchan Yi, Junho Lee, Jiyoon Kim, Chaehwi Lim, Jihoon Lee, Kyungtae Kim, Yeojoon Yoon. In response to the fact that MPs are considered harmful to the human body, studies on their samplings, pre-treatments, and environmental media analyses, such as water, are continuously being conducted. However, there is an imperative need to develop a standard sampling and pre-treatment method, particularly because MPs of a few micrometers in size are easily affected by external contamination. For the evaluation of the developed device, microplastic reference materials were produced and used, and computational fluid dynamics (CFD) analysis was performed. This device has been applied to the relatively large previously studied microplastics (100 μm), but it is also suited to MPs of approximately 20 μm that are vulnerable to contamination. A recovery rate of 94.2% was obtained using this device, and the particles were separated by filtration through a three-stage cassette. Accuracy and reproducibility of results for microplastic contamination in the environment are needed. This method is able to consistently obtain and manage MP data, which are often difficult to compare using various existing methods.

"Occurrence Characterization and Contamination Risk Evaluation of Microplastics in Hefei's Urban Wastewater Treatment Plant", by Xiangwu Meng, Teng Bao, Lei Hong, Ke Wu. This study denotes that MPs are one of the primary nodes in their flow through the environment, making it critical to examine and assess sewage treatment, occurrence, and removal of MPs in a waste treatment plant (WWTP). The examined variables contain the shape, size, and composition of MPs at various stages of the WWTP process in the south of the city of Hefei, China, considering both the dry and the rainy weather conditions, as well as the removal effectiveness of MPs in a three-stage process. The methodology and findings of this study are case specific to the examined area. In particular, pollution indices of MPs in row water and tail water were 2.40 and 2.46, respectively, which were heavily contaminated, and 1.0 and 1.2, which were moderately polluted. MPs in dewatered sludge had severely polluted indexes of 3.5 and 3.4, respectively. MP efflux or build-up in sludge during and after the WWTP process presents an ecological contamination concern.

"Experimental Investigation of Water-Retaining and Unsaturated Infiltration Characteristics of Loess Soils Imbued with Microplastics", by Jiahui Gu, Liang Chen, Yu Wan, Yaozong Teng, Shufa Yan, Liang Hu. This study explores the effect of MPs on agricultural soil permeability by simulating the rainfall irrigation process. For this reason, a one-dimensional vertical soil column rainfall infiltration test device was used to study the unsaturated infiltration characteristics of loss soil imbued with MPs under rainfall conditions. MPs represent negative effects on rainfall infiltration and soil water retention, so it is recommended to dispose of them.

"Assessment of Microplastics in Green Mussel (Perna viridis) and Surrounding Environments around Sri Racha Bay, Thailand", by Jitraporn Phaksopa, Roochira Sukhsangchan, Rangsiwut Keawsang, Kittipod Tanapivattanakul, Bojara Asvakittimakul, Thon Thamrongnawasawat, Suchai Worachananant. This study analyses the characteristics of MPs in the seawater, sediments, and green mussels (Perna viridis) around Sri Racha Bay, Thailand. In the field of food management and practices this study signified that the excessive riverine freshwater discharge is transported terrestrial plastic debris into the estuarine system, thus, higher microplastic contamination in surface seawater and sediment was evidenced. The presence of colorants in organisms revealed an anthropogenic origin through the use of a wide array of applications.

"Adsorption Behavior of Nonylphenol on Polystyrene Microplastics and Their Cytotoxicity in Human $CaCO_2$ Cells", by Fangfang Ding, Qianqian Zhao, Luchen Wang, Juan Ma, Lingmin Song, Danfei Huang. This study points out that -as two environmental pollutants of great concern, polystyrene microplastics (PS-MPs) and nonylphenol (NP) often coexist in the environment and cause combined pollution- authors carried out batch adsorption experiments by varying parameters such as pH, the particle sizes of the PS-MPs, the initial concentration of NP, and metal ion content. The NP adsorption process of the PS-MPs was optimally matching to pseudo-second-order kinetic model and Langmuir isotherm model, while the intraparticle diffusion and Bangham models were also involved in determining the NP adsorption process.

"Investigating the Epigenetic Effects of Polystyrene Nanoplastic Exposure in Bluegill (*Lepomis macrochirus*) Epithelial Cells Using Methylation Sensitive-AFLPs", by Sheridan M Wilkinson, Justine M Whitaker, Alexis M Janosik. This study has focused on MPs, defining them as remnants of macroplastics that have broken down to fragments smaller than 5 mm, and nanoplastics, broken down even further to sizes < 1 μm, are pervasive in aquatic ecosystems. The accumulation of plastic in the organ gut can result in various repercussions, including cellular contamination and genomic modifications such as DNA methylation. The study delves into such a largely uncharted territory, investigating the accumulation of methylation due to nanoplastic exposure within the genome of cultured bluegill BF-2 cells (*Lepomis macrochirus*) using methylation-sensitive AFLPs. It is also noteworthy that higher 21 dosages and exposure times to nanoplastics do not result in increased methylation levels in congruence with the dosage and exposure time, but rather only the presence of nanoplastics is enough to cause DNA methylation changes.

Based on the aforementioned framework of the 26 articles, and considering the perspective of opening up a scientific discussion, the top five most frequently reported terms and phrases among the keywords of the total of these 26 articles of this Research Topic "Microplastics Pollution", in descending order (in parentheses is the total times of appearance), are as follows: "microplastics" (MPs) (19) > "sediment samples" (11) > "contamination" = "degradation" = "wastewater treatment plants" (5). Based on this overview of keywords, it is noteworthy that the main fields of MP investigation in the relevant literature are that of agricultural soils (agriculture) followed by concerns in wastewater treatment technologies and, at the same time, weak research focus on topics of food and health interest.

3. Conclusions

Addressing existing data gaps and prioritizing research in challenging areas are crucial for developing methods and processes that can mitigate the impact of microplastics (MPs) on natural environments. MPs are commonly disposed of and transferred through water channels and trophic transfers, posing risks to human and animal tissues, as well as cultivated soils and natural water sources. Therefore, it is essential to explore ways to either eliminate MPs or render them safe for further consumption and accumulation.

One of the most promising yet challenging areas of future research involves the physical adherence of polymeric-based MPs to organic debris with similar fabrication and texture. This process may stimulate the development of attractive forces and molecular bonds, allowing MPs and organic matter to aggregate under natural outdoor conditions without requiring additional energy or human intervention. By leveraging these natural bonding mechanisms, the agglomerated materials could undergo physical degradation in the environment under two key conditions:

(a) The agglomerating matter should be soluble in aqueous media, including seawater, river water, underground water, runoff, rainfall, or through evapotranspiration cycles.
(b) The agglomerating matter should be dispersed in solution at relatively low concentrations to ensure interaction with natural components and promote degradation in living organisms and environmental systems.

These conditions aim to regulate MP degradation within safe thresholds, such as the lethal concentration 50% (LC50) for living organisms and the carrying capacity of environmental contexts. Ensuring these safe disposal mechanisms would not only address MPs but also other polymeric-fabricated and organic matter that coexist in natural environments.

This future research proposal is conceptualized based on published experimental studies that examine the adsorption of organic-based pesticide molecules onto polymeric absorbents at low concentrations. The adsorption process presents an advantageous, low-energy-consuming mechanism for promoting the adherence of pesticide molecules without excessive energy input. For a more refined fabrication design, researchers should consider the physicochemical properties of hydrophobicity and hydrophilicity, as well as other morphological parameters influencing agglomeration, including pH behavior in aqueous solutions and fluctuations in ionic strength [17,18].

This research approach has the potential to enhance the protection of soil and water sources by removing MPs through interactions with chemically affiliated substances, eliminating the need for human intervention or additional energy consumption. Furthermore, it may facilitate the biomagnification of MPs and associated chemicals from soil and aquifers through trophic transfers [19–21]. Rather than focusing solely on MP recovery—which could complicate treatment due to safety concerns in mechanical collection and large-scale environmental storage—this research prioritizes natural compatibility and degradation via bio-modular synergistic processes.

This approach aligns with the study by Jiahui Gu et al. (List of Contributions (24)), which highlights the negative effects of MPs on rainfall infiltration and soil water retention, further supporting the recommendation for their safe disposal in natural environments.

Author Contributions: Conceptualization, G.L.K.; methodology, G.L.K. and A.A.Z.; software, V.J.I. and R.R.-B.; validation, G.L.K., A.A.Z., V.J.I. and R.R.-B.; formal analysis, G.L.K. and A.A.Z.; investigation, G.L.K. and A.A.Z.; resources, A.A.Z. and G.L.K.; data curation, R.R.-B., V.J.I. and G.L.K.; writing—original draft preparation, G.L.K.; writing—review and editing, A.A.Z. and V.J.I.; visualization, G.L.K.; supervision, A.A.Z., V.J.I. and R.R.-B.; project administration, G.L.K.; funding acquisition, no needed. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflicts of interest.

List of Contributions

1. Wilkinson, S.M.; Whitaker, J.M.; Janosik, A.M. Investigating the Epigenetic Effects of Polystyrene Nanoplastic Exposure in Bluegill (*Lepomis macrochirus*) Epithelial Cells Using Methylation-Sensitive AFLPs. *Microplastics* **2025**, *4*, 10. https://doi.org/10.3390/microplastics4010010.
2. Li, S.; Cao, L.; Liu, Q.; Sui, S.; Bian, J.; Zhao, X.; Gao, Y. Enhancing Pb Adsorption on Crushed Microplastics: Insights into the Environmental Remediation. *Water* **2024**, *16*, 3541. https://doi.org/10.3390/w16233541.
3. Salikova, N.S.; Lovinskaya, A.V.; Kolumbayeva, S.Z.; Bektemissova, A.U.; Urazbayeva, S.E.; Rodrigo-Clavero, M.-E.; Rodrigo-Ilarri, J. Evaluation of Microplastic Toxicity in Drinking Water Using Different Test Systems. *Water* **2024**, *16*, 3250. https://doi.org/10.3390/w16223250.
4. Lotz, T.; Chen, W.; Su, S. Microplastic Transport and Accumulation in Rural Waterbodies: Insights from a Small Catchment in East China. *Toxics* **2024**, *12*, 761. https://doi.org/10.3390/toxics12100761.
5. Ahmad, H.W.; Bibi, H.A.; Chandrasekaran, M.; Ahmad, S.; Kyriakopoulos, G.L. Sustainable Wastewater Treatment Strategies in Effective Abatement of Emerging Pollutants. *Water* **2024**, *16*, 2893. https://doi.org/10.3390/w16202893.
6. Harada, S.; Yajima, I.; Fukushima, K.; Nitta, Y. Retention, Degradation, and Runoff of Plastic-Coated Fertilizer Capsules in Paddy Fields in Fukushima and Miyagi Prefectures, Japan: Consistency of Capsule Degradation Behavior and Variations in Carbon Weight and Stable Carbon Isotope Abundance. *Water* **2024**, *16*, 1782. https://doi.org/10.3390/w16131782.
7. Salikova, N.S.; Kerimkulova, A.R.; Rodrigo-Ilarri, J.; Alimova, K.K.; Rodrigo-Clavero, M.-E.; Kapbassova, G.A. Sorption-Based Removal Techniques for Microplastic Contamination of Tap Water. *Water* **2024**, *16*, 1363. https://doi.org/10.3390/w16101363.
8. He, H.; Cai, S.; Chen, S.; Li, Q.; Wan, P.; Ye, R.; Zeng, X.; Yao, B.; Ji, Y.; Cao, T.; et al. Spatial and Temporal Distribution Characteristics and Potential Sources of Microplastic Pollution in China's Freshwater Environments. *Water* **2024**, *16*, 1270. https://doi.org/10.3390/w16091270.
9. Colmenarejo Calero, E.; Kovač Viršek, M.; Mali, N. Microplastics in Groundwater: Pathways, Occurrence, and Monitoring Challenges. *Water* **2024**, *16*, 1228. https://doi.org/10.3390/w16091228.
10. Salikova, N.S.; Rodrigo-Ilarri, J.; Makeyeva, L.A.; Rodrigo-Clavero, M.-E.; Tleuova, Z.O.; Makhmutova, A.D. Monitoring of Microplastics in Water and Sediment Samples of Lakes and Rivers of the Akmola Region (Kazakhstan). *Water* **2024**, *16*, 1051. https://doi.org/10.3390/w16071051.
11. Phinikettou, V.; Papamichael, I.; Voukkali, I.; Zorpas, A.A. Comparison of Methodologies for Microplastic Isolation through Multicriteria Analysis (AHP). *Microplastics* **2024**, *3*, 184–204. https://doi.org/10.3390/microplastics3010011.
12. Zhao, H.; Hong, X.; Chai, J.; Wan, B.; Zhao, K.; Han, C.; Zhang, W.; Huan, H. Interaction between Microplastics and Pathogens in Subsurface System: What We Know So Far. *Water* **2024**, *16*, 499. https://doi.org/10.3390/w16030499.
13. Munien, S.; Adhikari, P.L.; Reycraft, K.; Mays, T.J.; Naidoo, T.; Pruitt, M.; Arena, J.; Sershen. Application and Efficacy of Management Interventions for the Control of Microplastics in Freshwater Bodies: A Systematic Review. *Water* **2024**, *16*, 176. https://doi.org/10.3390/w16010176.
14. Flores-Munguía, E.J.; Rosas-Acevedo, J.L.; Ramírez-Hernández, A.; Aparicio-Saguilan, A.; Brito-Carmona, R.M.; Violante-González, J. Release of Microplastics from Urban Wastewater Treatment Plants to Aquatic Ecosystems in Acapulco, Mexico. *Water* **2023**, *15*, 3643. https://doi.org/10.3390/w15203643.
15. Lasota, J.; Piaszczyk, W.; Tabor, S.; Błońska, E. Microplastic in the Snow on Sledding Hills in Green Areas of Krakow. *Sustainability* **2023**, *15*, 12995. https://doi.org/10.3390/su151712995.
16. Zolotova, N.; Dzhalilova, D.; Tsvetkov, I.; Makarova, O. Influence of Microplastics on Morphological Manifestations of Experimental Acute Colitis. *Toxics* **2023**, *11*, 730. https://doi.org/10.3390/toxics11090730.

17. Salikova, N.S.; Rodrigo-Ilarri, J.; Rodrigo-Clavero, M.-E.; Urazbayeva, S.E.; Askarova, A.Z.; Magzhanov, K.M. Environmental Assessment of Microplastic Pollution Induced by Solid Waste Landfills in the Akmola Region (North Kazakhstan). *Water* **2023**, *15*, 2889. https://doi.org/10.3390/w15162889.
18. Hong, L.; Meng, X.; Bao, T.; Liu, B.; Wang, Q.; Jin, J.; Wu, K. Spatial–Temporal Distribution and Ecological Risk Assessment of Microplastics in the Shiwuli River. *Water* **2023**, *15*, 2330. https://doi.org/10.3390/w15132330.
19. Sin, L.T.; Balakrishnan, V.; Bee, S.-T.; Bee, S.-L. A Review of the Current State of Microplastic Pollution in South Asian Countries. *Sustainability* **2023**, *15*, 6813. https://doi.org/10.3390/su15086813.
20. Pashaei, R.; Sabaliauskaitė, V.; Suzdalev, S.; Balčiūnas, A.; Putna-Nimane, I.; Rees, R.M.; Dzingelevičienė, R. Assessing the Occurrence and Distribution of Microplastics in Surface Freshwater and Wastewaters of Latvia and Lithuania. *Toxics* **2023**, *11*, 292. https://doi.org/10.3390/toxics11040292.
21. Fischbach, S.; Yauney, B. Social Cognitive Theory and Reciprocal Relationship: A Guide to Single-Use Plastic Education for Policymakers, Business Leaders and Consumers. *Sustainability* **2023**, *15*, 3946. https://doi.org/10.3390/su15053946.
22. Ju, S.; Yi, J.; Lee, J.; Kim, J.; Lim, C.; Lee, J.; Kim, K.; Yoon, Y. High-Efficiency Microplastic Sampling Device Improved Using CFD Analysis. *Sustainability* **2023**, *15*, 3907. https://doi.org/10.3390/su15053907.
23. Meng, X.; Bao, T.; Hong, L.; Wu, K. Occurrence Characterization and Contamination Risk Evaluation of Microplastics in Hefei's Urban Wastewater Treatment Plant. *Water* **2023**, *15*, 686. https://doi.org/10.3390/w15040686.
24. Gu, J.; Chen, L.; Wan, Y.; Teng, Y.; Yan, S.; Hu, L. Experimental Investigation of Water-Retaining and Unsaturated Infiltration Characteristics of Loess Soils Imbued with Microplastics. *Sustainability* **2023**, *15*, 62. https://doi.org/10.3390/su15010062.
25. Phaksopa, J.; Sukhsangchan, R.; Keawsang, R.; Tanapivattanakul, K.; Asvakittimakul, B.; Thamrongnawasawat, T.; Worachananant, S. Assessment of Microplastics in Green Mussel (*Perna viridis*) and Surrounding Environments around Sri Racha Bay, Thailand. *Sustainability* **2023**, *15*, 9. https://doi.org/10.3390/su15010009.
26. Ding, F.; Zhao, Q.; Wang, L.; Ma, J.; Song, L.; Huang, D. Adsorption Behavior of Nonylphenol on Polystyrene Microplastics and Their Cytotoxicity in Human Caco-2 Cells. *Water* **2022**, *14*, 3288. https://doi.org/10.3390/w14203288.

References

1. Nafea, H.T.; Chan, S.K.F.; Xu, H.; Wang, C.; Xia, H.; He, J. Status of management and mitigation of microplastic pollution. *Crit. Rev. Environ. Sci. Technol.* **2024**, *54*, 1734–1756. [CrossRef]
2. OECD Global Plastics Outlook Database. *Plastic Pollution is Growing Relentlessly as Waste Management and Recycling Fall Short*; OECD: Washington, DC, USA, 2023. Available online: https://www.oecd.org/environment/plastic-pollution-is-growing-relentlessly-as-waste-management-and-recycling-fall-short.htm (accessed on 20 January 2025).
3. Erdle, M.L.; Eriksen, M. Monitor compartments, mitigate sectors: A framework to deconstruct the complexity of plastic pollution. *Mar. Pollut. Bull.* **2023**, *193*, 115198. [CrossRef] [PubMed]
4. Peng, X.; Chen, M.; Chen, S.; Dasgupta, S.; Xu, H.; Ta, K.; Bai, S. Microplastics contaminate the deepest part of the world's ocean. *Geochem. Perspect. Lett.* **2018**, *9*, 1–5. [CrossRef]
5. Brahney, J.; Hallerud, M.; Heim, E.; Hahnenberger, M.; Sukumaran, S. Plastic rain in protected areas of the United States. *Science* **2020**, *368*, 1257–1260. [CrossRef] [PubMed]
6. Napper, I.E.; Davies, B.F.; Clifford, H.; Elvin, S.; Koldewey, H.J.; Mayewski, P.A.; Thompson, R.C. Reaching new heights in plastic pollution—Preliminary findings of microplastics on Mount Everest. *One Earth* **2020**, *3*, 621–630. [CrossRef]
7. Ragusa, A.; Svelato, A.; Santacroce, C.; Catalano, P.; Notarstefano, V.; Carnevali, O.; Giorgini, E. Plasticenta: First evidence of microplastics in human placenta. *Environ. Int.* **2021**, *146*, 106274. [CrossRef] [PubMed]
8. Leslie, H.A.; van Velzen, M.J.; Brandsma, S.H.; Vethaak, A.D.; Garcia-Vallejo, J.J.; Lamoree, M.H. Discovery and quantification of plastic particle pollution in human blood. *Environ. Int.* **2022**, *163*, 107199. [CrossRef] [PubMed]
9. Marsden, P.; Koelmans, A.A.; Bourdon-Lacombe, J.; Gouin, T.; D'Anglada, L.; Cunliffe, D.; De France, J. *Microplastics in Drinking Water*; World Health Organization: Geneva, Switzerland, 2019.

10. Amin, R.M.; Sohaimi, E.S.; Anuar, S.T.; Bachok, Z. Microplastic ingestion by zooplankton in Terengganu coastal waters, southern South China Sea. *Mar. Pollut. Bull.* **2020**, *150*, 110616. [CrossRef] [PubMed]
11. Zientek, A.; Schager, M.; Nagy, M.; Wanek, W.; Heinz, P.; Ali, S.S.; Lintner, M. Effect of microplastic particles in coral reef foraminifera. *Sci. Rep.* **2024**, *14*, 12423. [CrossRef] [PubMed]
12. Wilcox, C.; Van Sebille, E.; Hardesty, B.D. Threat of plastic pollution to seabirds is global, pervasive, and increasing. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 11899–11904. [CrossRef] [PubMed]
13. Stoett, P.; Scrich, M.V.; Elliff, I.C.; Andrade, M.M.; Grilli, N.d.M.; Turra, A. Global plastic pollution, sustainable development, and plastic justice. *World Dev.* **2024**, *184*, 106756. [CrossRef]
14. Zorpas, A.A. Strategy Development in the Framework of Waste Management. *Sci. Total Environ.* **2020**, *716*, 137088. [CrossRef] [PubMed]
15. Zorpas, A.A. The hidden concept and the beauty of multiple "R" in the framework of waste strategies development reflecting to circular economy principles. *Sci. Total Environ.* **2024**, *952*, 175508. [CrossRef] [PubMed]
16. Usman, S.; Abdull Razis, A.F.; Shaari, K.; Azmai, M.N.A.; Saad, M.Z.; Mat Isa, N.; Nazarudin, M.F. The burden of microplastics pollution and contending policies and regulations. *Int. J. Environ. Res. Public Health* **2022**, *19*, 6773. [CrossRef] [PubMed]
17. Kyriakopoulos, G.; Doulia, D. Morphology of polymeric resins in adsorption of organic pesticides. *Fresenius Environ. Bull.* **2007**, *16*, 731–734.
18. Kyriakopoulos, G.; Doulia, D.; Hourdakis, A. Effect of ionic strength and pH on the adsorption of selected herbicides on Amberlite. *Int. J. Environ. Anal. Chem.* **2006**, *86*, 207–214. [CrossRef]
19. Lang, T.; Jelić, F.; Wechselberger, C. From Cradle to Grave: Microplastics—A Dangerous Legacy for Future Generations. *Environments* **2024**, *11*, 263. [CrossRef]
20. Rednikin, A.R.; Frank, Y.A.; Rozhin, A.O.; Vorobiev, D.S.; Fakhrullin, R.F. Airborne Microplastics: Challenges, Prospects, and Experimental Approaches. *Atmosphere* **2024**, *15*, 1380. [CrossRef]
21. Zeng, W.; He, S.; Zhao, Y.; Jiang, M.; Wang, W.; Yang, L.; Du, W.; Zhuang, W. Microplastics Exposure Aggravates Synovitis and Pyroptosis in SLE by Activating NF-κB and NRF2/KEAP1 Signaling. *Toxics* **2024**, *12*, 840. [CrossRef] [PubMed]

water

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Article

Enhancing Pb Adsorption on Crushed Microplastics: Insights into the Environmental Remediation

Sen Li [1], Lu Cao [1,*], Qiyuan Liu [2], Shuting Sui [1], Jiayin Bian [2], Xizeng Zhao [1,3] and Yun Gao [1]

[1] School of Marine Engineering Equipment, Zhejiang Ocean University, Zhoushan 316022, China; w1157491613@163.com (S.L.); 13406747750@163.com (S.S.); xizengzhao@zju.edu.cn (X.Z.); zjgaoyun@163.com (Y.G.)
[2] Oceanic Environmental Monitoring and Forecasting Center of Zhoushan City, Zhoushan 316022, China; 13867219761@163.com (Q.L.); 19818079522@163.com (J.B.)
[3] Ocean College, Zhejiang University, Zhoushan 316021, China
* Correspondence: lucaogh@zjou.edu.cn

Abstract: This study investigates the pollution characteristics and environmental risks of crushed microplastics (MPs) generated during plastic recycling, emphasizing their adsorption capacity for heavy metals, particularly lead (Pb). SEM-EDS analysis revealed that crushed MPs exhibit significantly higher adsorption capacity than primary MPs due to their larger surface area and more available adsorption sites, including oxygen-containing functional groups. The adsorption behavior of MPs was influenced by key factors such as MP size, MP quantity, pH, salinity, and biofilm formation. Smaller MPs demonstrated higher adsorption efficiency, while elevated pH enhanced Pb adsorption. Conversely, increased salinity reduced adsorption due to competition for adsorption sites. Increasing MP concentrations improved Pb removal efficiency, but higher MP quantities led to a decrease in maximum adsorption capacity, demonstrating a trade-off between removal efficiency and adsorption capacity. Isothermal adsorption experiments revealed that Pb adsorption on MPs follows a multi-layer mechanism, best characterized by the Freundlich model. The adsorption capacity increased nonlinearly with Pb concentration and stabilized as surface sites became saturated. The formation of biofilms on MPs further enhanced their adsorption capacity by providing additional functional groups and facilitating multi-layer adsorption, increasing ecological risks. Adsorption kinetics were best described by pseudo-second-order and intra-particle diffusion models, indicating chemical adsorption and boundary layer diffusion as dominant mechanisms. Magnetic Fe_3O_4 nanoparticles demonstrated a high recovery efficiency of 99.3% for MPs, highlighting their potential for environmental remediation. However, the presence of adsorbed Pb slightly reduced recovery performance, emphasizing the need to optimize recovery conditions for maximum efficiency. These findings underscore the dual threat posed by crushed MPs: their capacity to adsorb and concentrate harmful substances, increasing ecological toxicity, and the challenges associated with their recovery. This research provides critical insights into mitigating MP pollution and developing effective recovery strategies under realistic environmental conditions.

Keywords: crushed microplastics; Pb; adsorption capacity; ecological threat; environmental remediation

Citation: Li, S.; Cao, L.; Liu, Q.; Sui, S.; Bian, J.; Zhao, X.; Gao, Y. Enhancing Pb Adsorption on Crushed Microplastics: Insights into the Environmental Remediation. *Water* **2024**, *16*, 3541. https://doi.org/10.3390/w16233541

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

Received: 29 October 2024
Revised: 2 December 2024
Accepted: 6 December 2024
Published: 9 December 2024

1. Introduction

Microplastics (MPs), plastic fragments smaller than 5 mm in diameter [1–3], have been reported to be widely distributed across diverse ecosystems, including oceans, land, freshwater, and even polar regions [4–7]. MPs are normally generated by the breaking down of plastics under various environmental factors, such as ultraviolet radiation, thermal degradation, biodegradation, and oxidative weathering. However, plastic recycling processes are an overlooked way of MP generation but have been reported recently, and research has shown that the plastic recycling industry and wastewater treatment plants could be major sources of MP pollution entering the environment [8–10]. In the recycling

industry, MPs are mainly generated during the mechanical crushing of plastic waste, and they are subsequently released into the environment through washing processes [10]. Due to their smaller sizes, larger surface areas, and higher stability, MPs are easily prone to absorb persistent organic pollutants (POPs) and heavy metals, which exacerbates their ecological toxicity and presents significant challenges to environmental health [11–14]. Studies have detected trace metals such as cadmium (Cd), chromium (Cr), copper (Cu), and lead (Pb) in plastic particles collected from beaches on the island of Vis, Croatia [15]. Additionally, harmful metals, including Pb, have been detected in MP samples collected from the Musi River [16]. So, MPs have the potential to passivate heavy metals in water. Compared to other MPs in soils or oceans, crushed MPs are easier to collect. However, the adsorption capacity of crushed MPs remains unclear and requires further research into its various influencing factors, such as MP type, MP size, the number of MPs, pH, salinity, and the presence of biofilms on MPs.

Furthermore, due to their large surface areas and strong hydrophobicity, MPs are highly susceptible to microbial colonization in aquatic environments, leading to the formation of biofilms on their surfaces. These biofilms alter the physical and chemical properties of the MPs, significantly affecting their ability to adsorb heavy metals. As the biofilm matures, the concentration of heavy metals on the MPs increases [17,18]. This is because the presence of biofilms enhances electrostatic interactions, coordination, complexation, and surface precipitation between MPs and heavy metals [18]. During the adsorption process, MPs may form complex pollutants that pose more severe threats to aquatic ecosystems and are more likely to be ingested by aquatic organisms, introducing new ecological risks. Therefore, investigating the effects of biofilm attachment on MPs' adsorption of heavy metals is of great environmental significance.

To address MP pollution, researchers have explored various physical, chemical, and biological methods. In recent years, magnetic nanomaterials, especially Fe_3O_4 nanoparticles, have emerged as a promising environmental remediation technology due to their excellent adsorption performance and magnetic recovery capabilities [19]. Studies have shown that magnetic Fe_3O_4 nanoparticles can effectively adsorb various types of MPs, such as polyethylene (PE), polypropylene (PP), and polystyrene (PS), and can be rapidly separated from water by applying a magnetic field [20]. By optimizing the density and contact time of Fe_3O_4 nanoparticles, experiments have achieved over 80% removal efficiency, with high recovery rates observed in environmental waters such as rivers and seawater [19]. This magnetic separation-based MP recovery technology, while effective, faces challenges in dealing with the diverse types, shapes, and sizes of MPs in the environment, which can lead to variations in removal efficiency. Therefore, improving the production process of magnetic nanomaterials and enhancing recovery efficiency, particularly under real-world environmental conditions, remain pressing issues.

In this study, we systematically investigate the adsorption capacity of crushed MPs for Pb under various environmental conditions, including MP type, MP size, the number of MPs, pH, salinity, and the presence of biofilms on MPs. By analyzing both kinetic and isothermal adsorption models, this work provides a comprehensive understanding of the adsorption mechanisms and highlights the factors that exacerbate the toxicity of MPs and their environmental impact. Additionally, this study addresses the effects of biofilm formation, a naturally occurring process in aquatic environments, on the adsorption properties of MPs. Biofilm attachment significantly alters the physical and chemical characteristics of MPs, enhancing their adsorption of heavy metals and thereby increasing ecological risks. Furthermore, this study explores the application of magnetic Fe_3O_4 nanoparticles specifically for the recovery of crushed MPs after adsorption. The recovery efficiency was evaluated based on the amount of Fe_3O_4 nanoparticles added, the adsorption capacity of MPs, and their interaction during the recovery process. This method demonstrates its practicality by providing insights into optimizing Fe_3O_4 nanoparticle usage and improving recovery efficiency under controlled experimental conditions. These findings highlight

the potential of Fe_3O_4 nanoparticles as a practical solution for addressing MP pollution, contributing to the development of sustainable remediation strategies.

2. Methods and Materials

2.1. Materials and Chemicals

The PVC MP particles used in the experiments included primary MP particles and crushed MP particles. The primary PVC particles were purchased from Guangdong Guangyuan Plastic Raw Material Co., Ltd. (Guangzhou, China) and the crushed PVC particles were derived from PVC-U pipes produced by Ningbo Yongcai Plastic Co., Ltd. (Ningbo, China); they were crushed into MPs with a diameter smaller than 5 mm, followed by sieving using screens with diameters of 1 mm, 0.5 mm, and 0.2 mm. Before the experiments, the MP particles underwent a pretreatment process, including washing with 95% ethanol, 2% nitric acid, and deionized water (three times for each solution), followed by air drying in a well-ventilated area. Reagents such as lead chloride ($PbCl_2$), nitric acid (HNO_3), ethanol (CH_3CH_2OH), hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium chloride (NaCl), iron trichloride hexahydrate ($FeCl_3{\cdot}6H_2O$), sodium acetate (CH_3COONa), L-Lysine ($C_6H_{14}N_2O_2$), and dopamine hydrochloride ($C_8H_{12}ClNO_2$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Characterization

In this study, the morphological changes of MPs were observed both before and after the experiment, utilizing a scanning electron microscope (SEM, Sigma500, Zeiss, Oberkochen, Germany). Furthermore, the element composition of the MPs after the adsorption process was analyzed through energy-dispersive X-ray spectroscopy (EDS, Oxford Instruments, Abingdon, Britain).

2.3. Adsorption Experiments

Before conducting the adsorption experiments, conducting preliminary experiments using MPs (PVC, PP, PET, PE) obtained from a recycling process in a certain company under the same experimental conditions; MP size of 0.5 mm, initial Pb concentration of 30 mg/L, and MPs concentration of 1 g/L. The aim was to determine the type of MPs to be used in the subsequent experiments, and detailed results are provided in Table S1. The research findings indicated that PVC exhibited the best adsorption efficiency for Pb, and thus, PVC was predominantly used in the subsequent experiments.

The research goal is to explore the possibility of MPs carrying a significant amount of heavy metal Pb in highly polluted environments. Considering the detected Pb concentrations in North Africa (14.19 ± 7.5 mg/L) and in the Ganges river of Rishikesh–Allahabad (ranging from 2.4 to 26.9 mg/L) [21,22], the Pb solution concentrations were set at 30 mg/L for the majority of the experiments.

To investigate the influence of various factors on the adsorption performance of Pb onto crushed PVC MPs, multiple experimental conditions were designed, including MP type, MP size, pH, salinity, the number of MPs, and the presence of biofilms on MPs. The specific experimental procedures are described as follows. A predetermined amount of MPs was weighed and placed into 300 mL glass conical flasks containing 250 mL of Pb solution. The flasks were then placed on a shaker operating at 120 rpm and kept at room temperature. Sampling intervals were set at 4, 8, 16, 24, 48, 96, 144, 192, 240, 288, and 336 h. At each sampling point, 10 mL of the solution was extracted using a pipette, filtered through a 0.45 μm membrane to separate the MPs from the solution, and subsequently analyzed to determine the Pb concentration in the filtrate. A UV–visible spectrophotometer (UV2365, Unico, Shanghai, China) was used for Pb concentration analysis. The adsorption capacity of MPs was calculated based on the change in Pb concentration over time. To ensure reproducibility, three parallel samples were prepared for each experimental condition. The average of the three parallel results was used as the experimental data. Prior to use, all glass conical flasks were soaked in 2% HNO_3 for two hours and rinsed thoroughly with

Milli-Q water to eliminate potential contamination. Detailed experimental setups for each factor are summarized in Table 1. For each factor, the experimental procedures followed the same general protocol as described above.

Table 1. Table of experimental conditions and settings.

Experimental Influencing Factors	MP Type	MP Size	Initial Pb Concentration	The Number of MPs	pH	Salinity
MP type	Primary PVC MPs and crushed PVC MPs	0.5 mm	30 mg/L	1 g/L	7	0
MP size	Crushed PVC MPs	1, 0.5 and 0.2 mm	30 mg/L	1 g/L	7	0
Number of MPs	Crushed PVC MPs	0.2 mm	30 mg/L	0.1, 0.5, 1, 1.5 and 2 g/L	7	0
pH	Crushed PVC MPs	0.2 mm	30 mg/L	0.3 g/L	4, 5, 6, 7 and 8	0
Salinity	Crushed PVC MPs	0.2 mm	30 mg/L	0.3 g/L	7	0, 1% and 3.5%
Presence of biofilms on MPs	Biofilm-coated and non-coated MPs	1, 0.5 and 0.2 mm	30 mg/L	1 g/L	7	0

To further understand the adsorption behavior of Pb in crushed PVC MPs, adsorption kinetics and isothermal adsorption experiments were conducted. For the adsorption kinetics experiments, the initial Pb concentration was set as the variable, with concentrations of 10, 30, and 50 mg/L. A constant MP concentration of 1 g/L and a particle size of 0.2 mm were used. The experimental setup and sampling intervals followed the same protocol as described above. The adsorption kinetics were analyzed by fitting the experimental data to pseudo-first-order and pseudo-second-order kinetic models to determine the adsorption rate and underlying mechanisms. For the isothermal adsorption experiments, MPs with a particle size of 0.2 mm were used at concentrations of 0.1, 0.2, and 0.3 g/L, while the initial Pb concentrations ranged from 30 to 55 mg/L (30, 35, 40, 45, 50, and 55 mg/L). The experiments were carried out under the same shaking conditions (120 rpm) and room temperature. The samples were allowed to reach equilibrium after 192 h, after which the equilibrium Pb concentrations in the solutions were measured. The adsorption isotherms were evaluated using Langmuir and Freundlich models to determine the adsorption capacity, adsorption mechanisms, and the nature of the adsorption process. All adsorption models and parameters are listed in Table S2.

This experiment utilized a UV–Vis spectrophotometer (UV2365, Unico, China) to monitor the changes in the concentration of Pb solution before and after the experiment. The method to detect Pb was the improved xylene orange spectrophotometric method, described as follows: (1) Two drops of 0.1% thymol blue solution were added into a 5 mL sample. The solution was then neutralized to a slightly red color with hydrochloric acid and further neutralized to yellow with ammonium hydroxide. (2) Six drops of 1% ascorbic acid, 1 mL of 0.1% potassium ferrocyanide, and 3 mL of acetic acid-sodium acetate buffer solution (pH 5.1) were added to the solution, which was then diluted to 22 mL with water and thoroughly mixed. (3) An amount of 1 mL of 0.5% ammonium fluoride solution and 1 mL of 0.2% xylene orange solution were added to the solution. The resulting solution was further diluted to 25 mL and shaken for 10 min. (4) A portion of the solution was transferred to a 3 cm colorimetric dish (or a 2 cm colorimetric dish for high concentrations) with water as the reference liquid, and the absorbance was measured at a wavelength of 580 nm after subtracting the blank absorbance obtained from the control sample.

2.4. Approaches to Microplastic Recovery

To investigate the effectiveness of magnetic Fe_3O_4 nanoparticles in removing MPs, the following steps were conducted for the synthesis of magnetic Fe_3O_4: The synthesis of magnetic Fe_3O_4 nanoparticles: 3.40 g of $FeCl_3 \cdot 6H_2O$ and 6 g of CH_3COONa were added to 100 mL of ethylene glycol. The mixture was stirred at 50 Hz for 90 min and then placed in

an oven and heated to 190 °C for 10 h. The product was washed with ethanol five times and dried in an oven at 65 °C for 3 h to obtain magnetic Fe_3O_4 nanoparticles [23].

Dopamine coating of magnetic particles: 100 mg of Fe_3O_4 and 2 mg of L-lysine were added to 20 mL of a water/ethanol solution (1:1) and the mixture was sonicated for thorough dispersion. The mixture was stirred at 100 Hz for 4 h, during which 5 mL of dopamine hydrochloride solution was added. After stirring, the mixture was dried at 90 °C in an oven for 3 h, followed by carbonization in a tube furnace at 700 °C for 3 h.

To ensure consistency with previous studies, the concentration of MPs was maintained at 1 g/L using PVC particles with an average diameter of 0.2 mm. In the initial adsorption experiments, PVC particles were placed in 40 mL of water, with three parallel samples prepared for each group. After 3 days, magnetic Fe_3O_4 nanoparticles were added to the solution containing the MPs for adsorption experiments. Upon the addition of the magnetic Fe_3O_4 nanoparticles, the mixture was stirred at 100 Hz for 10 min to allow the Fe_3O_4 to adsorb onto the MPs. The magnetized MPs were then removed using a magnet, separated, and washed with pure water in a beaker. After filtration and drying, the removal efficiency was calculated. The removal efficiency of MPs under different conditions was compared to identify the factors influencing MP adsorption.

3. Results and Discussion

3.1. Adsorption Capacity Between Primary MPs and Crushed MPs

Scanning electron microscopy (SEM) images reveal distinct differences in the surface morphology of MPs. As depicted in Figure 1a, the surface of primary MPs exhibits a smooth and flat characteristic with only subtle depressions. In contrast, Figure 1b illustrates the rough and multi-layered surface of crushed MPs, featuring prominent protrusions and depressions. This morphology may provide additional adsorption sites for heavy metals, leading to a significantly enhanced adsorption capacity of MPs for heavy metals [24,25]. Figure 1c highlights the presence of columnar structures absorbed on the surface of MPs, consistent with the Pb ions confirmed in the EDS spectrum in Figure 1c. EDS scans of MPs used in the heavy metal adsorption experiment clearly show a significant accumulation of Pb ions on the surface in Figure 1b,c. Analysis of the EDS spectrum indicates the presence of elements such as Ca (18.04%) on the surface of crushed MPs, potentially influencing the adsorption capacity of MPs for Pb. This aligns with previous research findings [26].

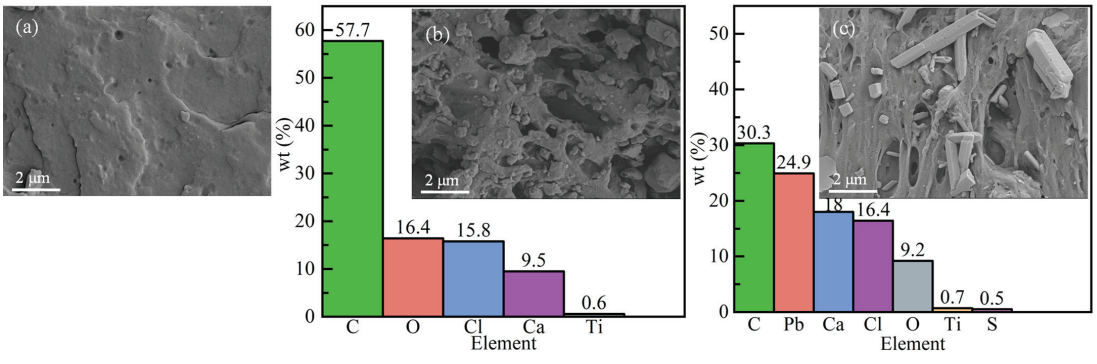

Figure 1. (**a**) SEM image of primary MPs (magnification: 10.00KX); (**b**) SEM image and corresponding elemental composition (EDS) bar chart of crushed MPs; (magnification: 10.00K X) (**c**) SEM image and corresponding elemental composition (EDS) bar chart of Pb-adsorbed MPs (magnification: 10.00K X).

Figure 2 shows the comparison of the adsorption capacities between primary and crushed MPs. It is worth noting that the adsorption performance of primary MPs is relatively poor, with little change in Pb concentration, resulting in an adsorption capacity of 0.169 mg/g. In contrast, for crushed MPs, a significant trend is observed. Over the next 96 h, the Pb concentration reached an adsorption equilibrium state. As previously

reported, primary MPs indeed exhibit limitations in the adsorption of heavy metals, with a slow adsorption process and low adsorption capacity [27,28]. The significant disparity in adsorption capacities among MPs of different morphologies suggests that untreated primary MPs have a relatively lower adsorption capacity for heavy metals compared to other types of MPs. This also implies that crushed MPs have a stronger adsorption capacity for heavy metal substances in the environment, posing greater environmental toxicity. The adsorption capacity of crushed PVC in the final experiment was 28.272 mg/g. The crushing treatment significantly enhanced the MPs' adsorption capacity for pollutants, thereby increasing environmental risk.

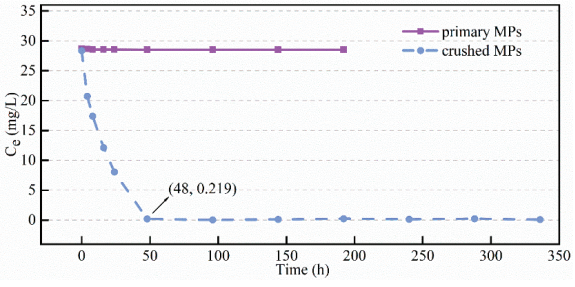

Figure 2. The adsorption of Pb on primary MPs and crushed MPs with time.

3.1.1. Effect of MP Size on Adsorption

The impact of MP size on Pb adsorption was evaluated, as illustrated in Figure 3. In the process of Pb adsorption in crushed MPs, the equilibrium was reached at approximately 48 h for MPs with diameters of 0.2 mm and 0.5 mm, whereas MPs with a diameter of 1 mm required up to 192 h to achieve adsorption equilibrium. This delay in reaching adsorption equilibrium for larger MPs is attributed to the saturation of adsorption sites as the adsorption progresses, limiting the interaction between MPs and Pb ions [29]. The adsorption capacity of MPs for heavy metals is influenced by their specific surface area, which is related to different particle sizes [30,31]. As evidenced by Figure 3, it is apparent that MPs with diameters of 0.2 mm and 0.5 mm reach adsorption equilibrium faster than those with a diameter of 1 mm, exhibiting higher adsorption capacities. This phenomenon is attributed to the increased complexity of surface morphology as the MP size decreases, resulting in smaller MPs having larger surface areas and more unoccupied adsorption sites [32,33]. This finding is consistent with previous research results. For instance, Wang et al. [31] compared three different-sized polyethylene MPs and found that smaller-sized MPs exhibited the highest adsorption capacity for Cd. Similarly, Gao et al. [34] compared four different-sized polypropylene MP particles and observed a decrease in the adsorption capacity of MPs for Pb, copper, and cadmium with increasing particle size. This experimental study confirms the high sensitivity of crushed MPs to Pb ions and their characteristic of rapidly reaching adsorption saturation, thereby increasing their toxicity in a shorter duration.

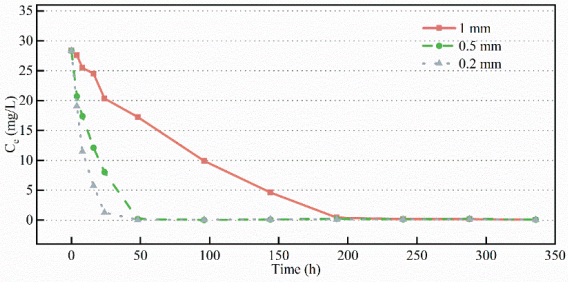

Figure 3. Adsorption curves of crushed MPs with different particle sizes in Pb solution. Experimental conditions: [adsorbents] = 1 g/L; [Pb] = 30 mg/L.

3.1.2. Adsorption Kinetics

From Figure 4a, it can be observed that under the same number of MPs, the higher the Pb concentration in the solution, the smaller the adsorption rate and the larger the adsorption capacity. The adsorption process of MPs for Pb exhibits distinct stages. The initial stage occurs from 0 to 24 h, demonstrating rapid adsorption, reaching 80.39% to 97.45% of the maximum adsorption capacity within the first 24 h. Subsequently, a slow adsorption stage occurs from 24 to 48 h, during which 93% to 99.81% of the maximum adsorption capacity is attained. After 48 h, the adsorption rate gradually approaches zero, indicating the achievement of adsorption equilibrium. The maximum adsorption capacity of MPs in this experiment is calculated to be 49.3 mg/g. When the initial concentration of Pb is 10 mg/L, the Pb concentration in the solution decreases to 0. However, for initial concentrations of 30 mg/L and 50 mg/L, the concentrations decrease to 0.1045 mg/L and 0.8943 mg/L, respectively. These results confirm the effective removal capacity of crushed PVC for Pb ions from the solution. In a study by Liu et al. (2022) on Pb adsorption isotherms, a significant positive correlation was observed between the increased concentration of Pb and the adsorption capacity of MPs [26]. Therefore, varying the quantity of crushed PVC MPs at different Pb concentrations may reduce the Pb concentration in the solution to 0. Crushed MPs demonstrate significant potential for removing heavy metal pollutants from water environments. The strong adsorption capacity can be utilized for environmental applications, and over time, the adsorption of heavy metal pollutants by MPs increases, enhancing their toxicity and causing irreversible harm to organisms [13].

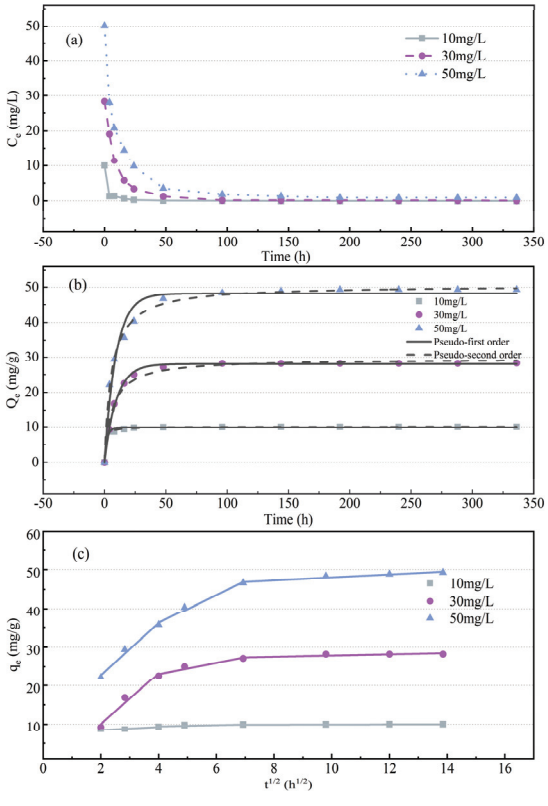

Figure 4. Adsorption characteristics of crushed MPs in Pb solutions: adsorption curves (**a**), adsorption kinetics (**b**), and intra-particle diffusion model (**c**). Experimental conditions: [adsorbents] = 1 g/L; [MP size] = 0.2 mm.

To gain a deeper understanding of the adsorption process of Pb, we explored pseudo-first-order and pseudo-second-order kinetic models. The fitting results of the experimental data are shown in Figure 4b, and the corresponding adsorption parameters are listed in Table S3. The adsorption rate of MPs with initially high concentrations is significantly lower than those at low concentrations, and the adsorption capacity is larger [35]. This may be attributed to the increase in Pb concentration, leading to an elevated collision probability between particles [36]. By comparing the fitting results of the two kinetic models in Table S3, it can be inferred that the pseudo-second-order kinetic model with correlation coefficients (R^2 values ranging from 0.986 to 0.997) outperforms the pseudo-first-order kinetic model (values from 0.970 to 0.997). This suggests that chemical adsorption may occur during the adsorption process, consistent with previous research. The adsorption process of crushed PVC for Pb is evidently complex and likely involves chemical adsorption mechanisms [26,37].

Further investigation of the mass transfer process of Pb adsorption was conducted using the intra-particle diffusion model, as shown in Figure 4c, and the corresponding adsorption parameters are listed in Table S4. The fitted line of the model does not pass through the origin, indicating that internal diffusion is not the sole limiting step but is also influenced by the surface water film on MPs [38]. The adsorption process is divided into three stages, with the diffusion rates continually decreasing ($k_{t1} > k_{t2} > k_{t3}$). This could be attributed to the decreasing Pb solution concentration and the reduction in adsorption sites over time [29]. Firstly, the first stage involves surface diffusion, where adsorption sites are unoccupied, leading to a fast adsorption rate. In the second stage, internal pore diffusion occurs where metal ions slowly diffuse into the particles. As adsorption sites decrease, the adsorption rate begins to decline. The third stage involves micropore diffusion, reaching adsorption equilibrium, and the adsorption rate returns to zero [35,39]. In this process, the *C* value continually increases, indicating a growing boundary layer effect.

3.1.3. Adsorption Isotherms

To further investigate the adsorption behavior of Pb on MPs, an isothermal adsorption experiment was conducted to complement the kinetic studies. Building on the findings that the adsorption of Pb on MPs involves chemical adsorption and is influenced by initial concentrations, the isothermal adsorption experiment aimed to explore the adsorption capacity and its relationship with Pb concentrations under equilibrium conditions.

In this experiment, 0.2 mm MPs at concentrations of 0.1, 0.2, and 0.3 g/L were placed in Pb solutions with initial concentrations ranging from 30 to 55 mg/L (30, 35, 40, 45, 50, and 55 mg/L). The adsorption process was monitored over 192 h to ensure equilibrium, and the maximum adsorption capacity was determined. The results revealed that the maximum adsorption capacity of MPs increased with higher Pb concentrations, exhibiting a nonlinear growth pattern. At lower Pb concentrations (30–40 mg/L), the adsorption rate was highest, suggesting that abundant adsorption sites on the surface of MPs facilitated efficient adsorption. However, as the Pb concentration increased, the adsorption sites on the surface of MPs gradually became saturated, limiting further adsorption [26].

Figure 5 and Table S5 present the corresponding results and parameters. As shown in Figure 5, the maximum adsorption capacity increased nonlinearly with Pb concentration, eventually stabilizing as the surface adsorption sites became saturated at an MP concentration of 0.3 g/L. The data in Table S5 further indicate that the Freundlich model provides a better fit for the adsorption behavior of Pb on MPs, with correlation coefficients (R^2 values ranging from 0.9604 to 0.9910) surpassing those of the Langmuir model (R^2 values ranging from 0.9412 to 0.9936). This suggests that the adsorption mechanism involves multi-layer adsorption, as characterized by the Freundlich model. The value of 1/n being less than 0.500 indicates an uneven distribution of adsorption sites on the surface of MPs, which was further confirmed through SEM. The SEM images revealed a heterogeneous surface morphology of MPs, consistent with the observation of non-uniform surface multi-layer adsorption [40]. These results align with the kinetic findings and highlight the complex and heterogeneous nature of adsorption sites on MPs during the Pb adsorption process.

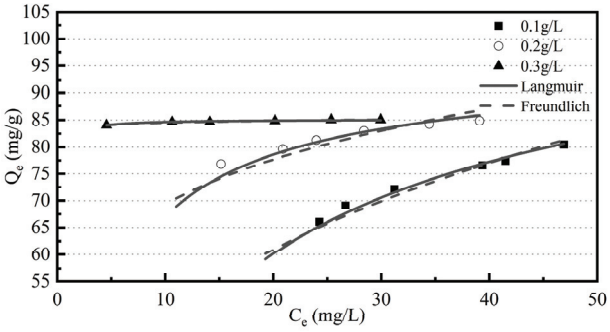

Figure 5. Adsorption isotherm of heavy metal Pb solution on MPs. Experimental conditions: [adsorbent] = (0.1 g/L, 0.2 g/L, 0.3 g/L); [MP size] = 0.2 mm.

3.1.4. Effect of pH on Adsorption

The pH of the water body highly influences the surface charge of the adsorbent and the metal ions. Figure 6a depicts the dependence of Pb adsorption on MPs within the pH range of 4.0 to 8.0 under experimental conditions. The experiments were conducted with a Pb solution concentration of 30 mg/L at room temperature, and samples were analyzed after 192 h to ensure equilibrium. In the deionized water system, as the solution pH increases, the adsorption of Pb on MPs also increases. Initially, there is the competitive adsorption of H^+ ions, and as the pH reaches or exceeds 7, Pb ions in the solution mainly exist in the forms of Pb^{2+}, $Pb(OH)^+$, and $Pb(OH)_2$, with the precipitation observed in the solution when adjusting the pH to 8.0, indicating the occurrence of precipitation. This phenomenon is attributed to precipitation, enhanced electrostatic forces, and the reduced competitive effect of H^+ ions in the solution. Conversely, as the solution pH increases, the adsorption capacity for anionic pollutants gradually decreases [41]. The highest adsorption at pH 8.0 was observed, consistent with similar findings reported by Liu et al. [26].

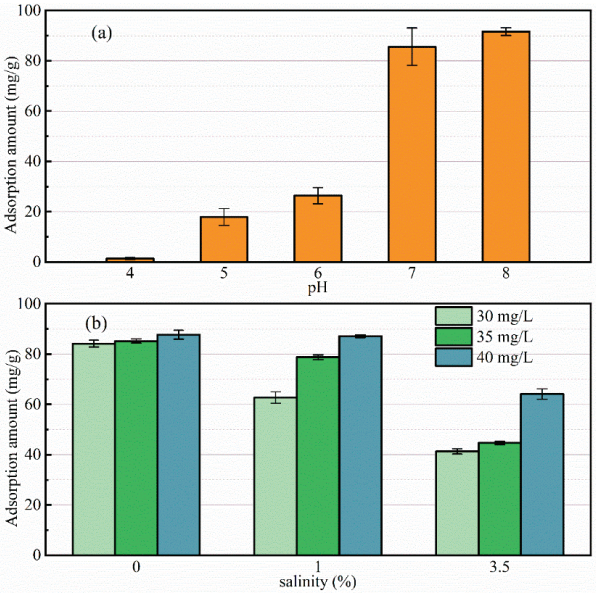

Figure 6. Effect of the pH (**a**) and salinity (**b**) on the adsorption of Pb on crushed PVC. Experimental conditions: [adsorbents] = 0.3 g/L; [MP size] = 0.2 mm.

3.1.5. Effect of Salinity on Adsorption

Using NaCl to simulate seawater, we investigated the impact of salinity on the adsorption capacity of MPs for Pb. Given that the average salinity of the world's oceans is 3.5%, with the Baltic Sea having the lowest at 1%, we selected 1% and 3.5% as contrasting experimental conditions. As shown in Figure 6b, salinity significantly affects the ability of MPs to adsorb Pb, with a decrease in Pb adsorption as salinity increases. This aligns with our previous research findings [26,42]. Due to the negative charge present in MPs, Na ions compete with Pb ions for adsorption. With the increasing concentration of Na ions, limited adsorption sites on MPs lead to a reduction in the specific surface area for Pb ion accumulation, hindering the adsorption of Pb by MPs. Therefore, the inhibitory effect of seawater is limited, and crushed MPs with higher adsorption capacities still pose considerable ecological toxicity [13].

3.1.6. Effect of MPs Quantity on Adsorption

To evaluate the effect of MP concentration on Pb adsorption, varying amounts of 0.2 mm-sized MPs (0.1 g/L, 0.5 g/L, 1 g/L, 1.5 g/L, and 2 g/L) were added to a 30 mg/L Pb solution. The results demonstrate a clear trend of increased Pb adsorption capacity with increasing MP concentrations (Figure 7). Notably, when the MP concentration was 0.1 g/L, the Pb concentration only decreased by 29.7%. However, as the MP concentration increased to 0.5 g/L, the Pb concentration dropped significantly by 97.1%, indicating a substantial reduction in Pb in the solution. Further increasing the MP concentration to 1.5 g/L and 2 g/L resulted in the complete removal of Pb from the solution, with the Pb concentration dropping to 0 mg/L. Across all experimental groups, a rapid initial adsorption process was observed during the first 48 h. At the highest MP concentration of 2 g/L, Pb removal efficiency reached 99% within this period. Additionally, for MP concentrations exceeding 1 g/L, the adsorption process approached equilibrium within 48 h. Interestingly, despite the increased Pb removal efficiency with higher MP concentrations, the maximum adsorption capacity of MPs decreased as the MP concentration increased. Specifically, the maximum adsorption capacity decreased from 86.2 mg/g at 0.1 g/L to 15 mg/g at 2 g/L.

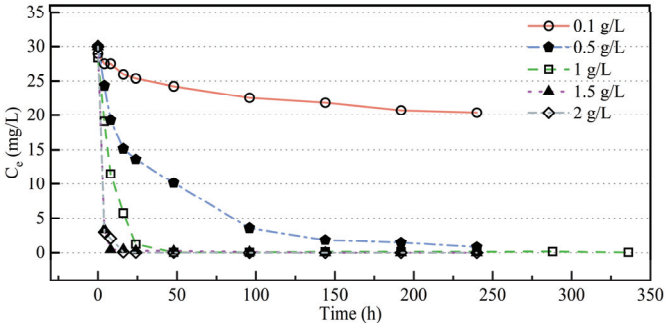

Figure 7. Adsorption curves of crushed MPs with different concentrations in Pb solution. Experimental conditions: [MP size] = 0.2 mm; [Pb] = 30 mg/L.

3.2. Effect of Surface Biofilm on Pb Adsorption by MPs

To assess the impact of biofilms on Pb adsorption, crushed MP samples of varying sizes (0.2, 0.5, and 1 mm) were exposed to seawater for two weeks, allowing for natural biofilm formation (Figure 8). Following biofilm development, both biofilm-coated and non-coated MPs were subjected to adsorption experiments using a Pb solution with a concentration of 30 mg/L. The experimental conditions included an adsorbent concentration of 1 g/L, and the adsorption was monitored for 48 h to track the equilibrium point.

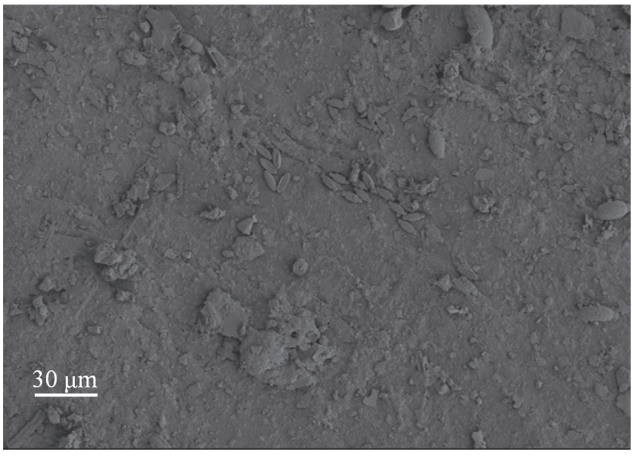

Figure 8. Biofilm formation on the surface of MPs (magnification: 350X).

As shown in Figure 9, biofilm-coated MPs exhibited a significantly higher Pb adsorption capacity compared to non-coated MPs, quickly reaching the adsorption equilibrium within 48 h. The extracellular polymeric substances (EPS) within the biofilm matrix are believed to play a crucial role in enhancing Pb adsorption by providing additional binding sites [17]. Overall, biofilm-coated MPs demonstrated a markedly higher adsorption capacity for Pb ions compared to their non-coated counterparts. This result aligns with previous studies, suggesting that biofilm presence enhances pollutant adsorption by increasing the available surface area and providing additional functional groups for metal binding. The EPS in the biofilm, which include polysaccharides, proteins, and lipids, play a crucial role in facilitating this enhanced adsorption [18].

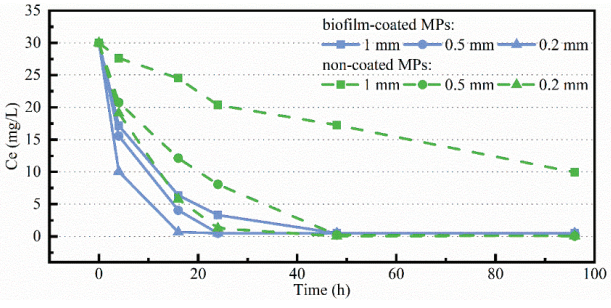

Figure 9. Effect of biofilm-coated MPs and non-coated MPs on Pb adsorption in crushed PVC. Experimental conditions: [adsorbent] = 1 g/L; [Pb] = 30 mg/L.

The results highlight the influence of biofilm formation in enhancing the adsorption of heavy metals, such as Pb, onto MPs. The biofilm matrix acts as a secondary adsorbent, contributing to the overall adsorption capacity of the MPs and accelerating the adsorption speed of MPs for Pb. This finding suggests that in natural environments, where biofilms are likely to form on MPs over time, the ecological risks posed by heavy metal-laden MPs could be greater than previously anticipated. On the contrary, this characteristic of biofilm-coated MPs can have a high potential to control Pb pollution by passivating it.

3.3. MP Recovery

Magnetic nanoparticles, particularly Fe_3O_4, have shown significant potential for the recovery of MPs. However, it remains unclear whether this method is equally effective for MPs with Pb on their surface. In this study, Fe_3O_4 nanoparticles were introduced

into a system containing Pb-contaminated wastewater and crushed MPs to evaluate their recovery efficiency.

3.3.1. The Effect of Different Masses of Fe_3O_4 on Microplastic Adsorption

To assess the feasibility of the recovery process, initial experiments were conducted using deionized water and crushed MPs. A total of 40 mg of crushed MPs was added to 40 mL of deionized water, and three parallel samples were prepared. These samples were shaken at 120 rpm in a large-capacity shaker for 3 days to ensure sufficient interaction. Following this, varying amounts of Fe_3O_4 nanoparticles (20 mg, 30 mg, 40 mg, 50 mg, and 60 mg) were added to the samples for adsorption experiments, with the mixtures stirred at 100 Hz for 10 min. The MPs were then recovered by applying a magnetic field, which separated them from the solution.

As shown in Figure 10, the recovery efficiency of MPs increased with the mass of Fe_3O_4 nanoparticles, ranging from 85.2% at 20 mg to 99.3% at 60 mg. These results clearly demonstrate that increasing the amount of Fe_3O_4 nanoparticles significantly enhances recovery efficiency. The large surface area, surface modifications, and magnetic properties of Fe_3O_4 facilitate efficient interaction with MPs, promoting their effective recovery. Moreover, the magnetic properties of Fe_3O_4 enable easy and rapid separation of adsorbed MPs using a magnet, further demonstrating the practicality of this approach in real-world applications. Compared to other recovery methods reported in the literature, Fe_3O_4 nanoparticles provide highly competitive recovery efficiency. For example, studies on Fe_3O_4-based MP removal have shown removal rates exceeding 80% under optimal conditions [19,20]. The combination of high adsorption efficiency and the simplicity of magnetic separation underscores Fe_3O_4 nanoparticles as an effective and scalable solution for MP recovery.

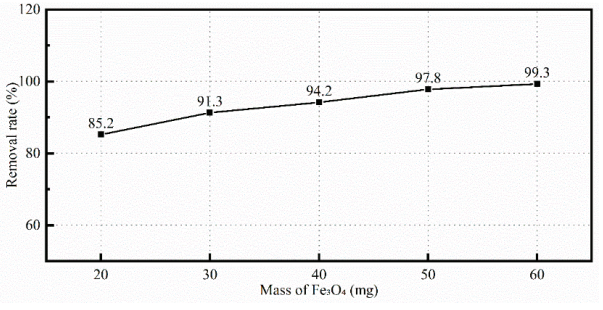

Figure 10. The effect of Fe_3O_4 mass on the recovery efficiency of MPs.

The increase in recovery efficiency with a higher mass of Fe_3O_4 can be attributed to the nanoparticles' large surface area and superparamagnetic properties, which provide more adsorption sites and enhance their contact with MPs. However, as seen in Figure 10, when the mass of Fe_3O_4 is lower (e.g., 20 mg), the adsorption capacity is limited, resulting in a significant decrease in the removal rate. This suggests that, in practical applications, the amount of Fe_3O_4 should be optimized based on the concentration of MPs to maximize recovery efficiency. Compared to membrane technologies, which also demonstrate high removal efficiency, such as membrane bioreactors combined with activated sludge achieving up to 99.5% removal [43], Fe_3O_4-based recovery offers a simpler, more cost-effective alternative.

3.3.2. The Effect of Different Adsorption Capacities of MPs on Fe_3O_4 Adsorption

In the next stage of the experiments, Fe_3O_4 nanoparticles were tested in Pb-contaminated wastewater to evaluate whether the adsorption of Pb by MPs would impact the efficiency of Fe_3O_4 in recovering these MPs. In this setup, 40 mg of crushed MPs was added to 40 mL of Pb-contaminated solutions at three different concentrations of Pb (10, 30, and 50 mg/L), and UV–visible spectrophotometry confirmed that the Pb concentrations decreased

significantly, nearing zero. The adsorption capacities of the MPs were calculated as 10, 30, and 50 mg/g, respectively.

Subsequently, 60 mg of Fe_3O_4 nanoparticles were introduced into each sample to assess the recovery efficiency of Fe_3O_4 under varying adsorption capacities of MPs. Figure 11 shows the relationship between the removal rate of MPs and their adsorption capacity. At low adsorption capacities (0 mg/g), the recovery rate of MPs remains high at 99.3%. However, as the adsorption capacity of MPs increases, a gradual decline in the recovery rate is observed, with the rate decreasing to 92.5% when the adsorption capacity reaches 50 mg/g. This decline in efficiency suggests that as more Pb ions are adsorbed in the MPs, they begin to compete for the active adsorption sites on Fe_3O_4 nanoparticles, reducing the ability of Fe_3O_4 to effectively adsorb the MPs. Additionally, the surface properties of Pb-adsorbed MPs may change, weakening their interaction with Fe_3O_4 nanoparticles and contributing to the reduced recovery rate.

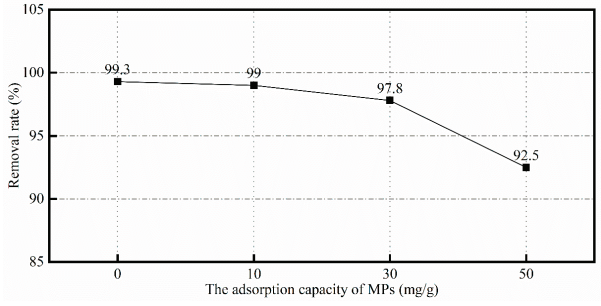

Figure 11. The effect of adsorption capacity of MPs on their recovery efficiency by Fe_3O_4 nanoparticles in Pb-contaminated wastewater.

These findings indicate that the adsorption of Pb onto MPs negatively affects the interaction between Fe_3O_4 nanoparticles and MPs. This points to the need for optimization in real-world applications, where MPs may carry significant loads of heavy metals. Adjustments to Fe_3O_4 dosage and other recovery parameters may help counteract this decline in efficiency and maintain high removal rates. For example, pre-screening water to capture larger MPs before Fe_3O_4 adsorption could enhance the overall recovery efficiency by reducing the burden on Fe_3O_4 in heavy metal-laden environments [44].

Overall, Fe_3O_4 nanoparticles remain a promising solution for scalable and cost-effective MP removal. The combination of high recovery efficiency (over 85%) and the ease of magnetic separation makes Fe_3O_4 an attractive alternative to more complex and expensive technologies like membrane filtration. However, further research into the interaction between heavy metal adsorption and MP recovery is essential to optimize this approach across varying environmental conditions and contamination levels.

4. Conclusions

This study investigated the pollution characteristics of crushed PVC MPs, providing insights into their environmental and health risks when carrying harmful substances. SEM-EDS analysis revealed that crushed MPs exhibit a significantly larger adsorption capacity compared to primary MPs, attributed to their increased surface area and more adsorption sites, including oxygen-containing functional groups. This study confirmed the critical influence of MP size, MP quantity, pH, salinity, and biofilm formation on their adsorption capacity. Smaller MPs demonstrated higher adsorption due to their larger surface area, while elevated pH enhanced adsorption efficiency. Conversely, increased salinity reduced adsorption, likely due to competition between ions for adsorption sites. Increasing MP concentrations improved Pb removal efficiency but higher MP quantities led to a decrease in maximum adsorption capacity, highlighting a trade-off between removal efficiency and adsorption capacity. The formation of biofilms on MPs further increased

their adsorption capacity, highlighting the potential for biofilm-coated MPs to act as vectors for transporting harmful substances through aquatic ecosystems, exacerbating ecological risks. Adsorption kinetics were best described by the pseudo-second-order and intra-particle diffusion models, indicating chemical adsorption and boundary layer diffusion as dominant processes. Isothermal adsorption followed a multi-layer mechanism, best characterized by the Freundlich model, with SEM confirming heterogeneous surface morphology. Additionally, Fe_3O_4 nanoparticles demonstrated a high recovery efficiency of 99.3% for MPs, emphasizing their potential for environmental remediation, but they experienced reduced performance when Pb was adsorbed. These findings provide critical evidence for understanding how crushed MPs interact with environmental pollutants and contribute to ecological risks. The results underscore the importance of addressing the pollution risks associated with crushed MPs in aquatic environments, offering a theoretical foundation for evaluating and mitigating their impact.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16233541/s1, Table S1 The adsorption results parameters for heavy metal Pb by MPs of different materials; Table S2 Adsorption kinetic and isotherm models used in this study; Table S3 adsorption kinetics fitting parameters of heavy metal Pb on MPs; Table S4 Adsorption kinetics parameters on crushed MPs in different background solutions; Table S5 Adsorption isotherms parameters on crushed MPs in different background solutions.

Author Contributions: S.L.: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed data; Wrote the paper. L.C.: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents materials, analysis tools or data; Wrote the paper. Q.L.: Contributed reagent materials and analysis tools. S.S.: Performed the experiments. J.B.: Contributed reagent materials and analysis tools. X.Z.: Supervision and project administration. Y.G.: Conceived and designed the experiments. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the project of Zhoushan Science and Technology Bureau (grant no. 2020C11246).

Data Availability Statement: The datasets generated and analyzed in this current study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interests.

References

1. Fei, J.; Xie, H.; Zhao, Y.; Zhou, X.; Sun, H.; Wang, N.; Wang, J.; Yin, X. Transport of degradable/nondegradable and aged microplastics in porous media: Effects of physicochemical factors. *Sci. Total Environ.* **2022**, *851*, 158099. [CrossRef] [PubMed]
2. Ouyang, W.; Zhang, Y.; Wang, L.; Barcelo, D.; Wang, Y.; Lin, C. Seasonal relevance of agricultural diffuse pollutant with microplastic in the bay. *J. Hazard. Mater.* **2020**, *396*, 122602. [CrossRef] [PubMed]
3. Thompson, R.C.; Olsen, Y.; Mitchell, R.P.; Davis, A.; Rowland, S.J.; John, A.W.; McGonigle, D.; Russell, A.E. Lost at sea: Where is all the plastic? *Science* **2004**, *304*, 838. [CrossRef] [PubMed]
4. Fragao, J.; Bessa, F.; Otero, V.; Barbosa, A.; Sobral, P.; Waluda, C.M.; Guimaro, H.R.; Xavier, J.C. Microplastics and other anthropogenic particles in Antarctica: Using penguins as biological samplers. *Sci. Total Environ.* **2021**, *788*, 147698. [CrossRef] [PubMed]
5. Liu, B.; Zhao, S.; Qiu, T.; Cui, Q.; Yang, Y.; Li, L.; Chen, J.; Huang, M.; Zhan, A.; Fang, L. Interaction of microplastics with heavy metals in soil: Mechanisms, influencing factors and biological effects. *Sci. Total Environ.* **2024**, *918*, 170281. [CrossRef]
6. Liu, Q.; Wu, H.; Chen, J.; Guo, B.; Zhao, X.; Lin, H.; Li, W.; Zhao, X.; Lv, S.; Huang, C. Adsorption mechanism of trace heavy metals on microplastics and simulating their effect on microalgae in river. *Environ. Res.* **2022**, *214 Pt 1*, 113777. [CrossRef]
7. Liu, Y.; Zhang, K.; Xu, S.; Yan, M.; Tao, D.; Chen, L.; Wei, Y.; Wu, C.; Liu, G.; Lam, P.K. Heavy metals in the "plastisphere" of marine microplastics: Adsorption mechanisms and composite risk. *Gondwana Res.* **2022**, *108*, 171–180. [CrossRef]
8. Sun, Y.; Cao, L.; Wang, Y.; Chen, W.; Li, Y.; Zhao, X. Sources and distribution of microplastics in the east China sea under a three-dimensional numerical modelling. *Environ. Pollut.* **2022**, *311*, 119910. [CrossRef]
9. Brown, E.; MacDonald, A.; Allen, S.; Allen, D. The potential for a plastic recycling facility to release microplastic pollution and possible filtration remediation effectiveness. *J. Hazard. Mater. Adv.* **2023**, *10*, 100309. [CrossRef]
10. Stapleton, M.J.; Ansari, A.J.; Ahmed, A.; Hai, F.I. Evaluating the generation of microplastics from an unlikely source: The unintentional consequence of the current plastic recycling process. *Sci. Total Environ.* **2023**, *902*, 9. [CrossRef]

11. Green, D.S.; Boots, B.; Sigwart, J.; Jiang, S.; Rocha, C. Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (*Arenicola marina*) and sediment nutrient cycling. *Environ. Pollut.* **2016**, *208*, 426–434. [CrossRef] [PubMed]
12. Guo, X.; Wang, J. The chemical behaviors of microplastics in marine environment: A review. *Mar. Pollut. Bull.* **2019**, *142*, 1–14. [CrossRef] [PubMed]
13. Lv, M.; Zhang, T.; Ya, H.; Xing, Y.; Wang, X.; Jiang, B. Effects of heavy metals on the adsorption of ciprofloxacin on polyethylene microplastics: Mechanism and toxicity evaluation. *Chemosphere* **2023**, *315*, 137745. [CrossRef] [PubMed]
14. Zhang, H.; Pap, S.; Taggart, M.A.; Boyd, K.G.; James, N.A.; Gibb, S.W. A review of the potential utilisation of plastic waste as adsorbent for removal of hazardous priority contaminants from aqueous environments. *Environ. Pollut.* **2020**, *258*, 113698. [CrossRef]
15. Maršić-Lučić, J.; Lušić, J.; Tutman, P.; Varezić, D.B.; Šiljić, J.; Pribudić, J. Levels of trace metals on microplastic particles in beach sediments of the island of Vis, Adriatic Sea, Croatia. *Mar. Pollut. Bull.* **2018**, *137*, 231–236. [CrossRef]
16. Purwiyanto, A.I.S.; Suteja, Y.; Ningrum, P.S.; Putri, W.A.E.; Agustriani, F.; Cordova, M.R.; Koropitan, A.F. Concentration and adsorption of Pb and Cu in microplastics: Case study in aquatic environment. *Mar. Pollut. Bull.* **2020**, *158*, 111380. [CrossRef]
17. Liu, S.T.; Shi, J.F.; Wang, J.; Dai, Y.X.; Li, H.Y.; Li, J.Y.; Liu, X.H.; Chen, X.C.; Wang, Z.Y.; Zhang, P.P. Interactions Between Microplastics and Heavy Metals in Aquatic Environments: A Review. *Front. Microbiol.* **2021**, *12*, 652520. [CrossRef]
18. Pan, H.; Zhao, X.; Zhou, X.; Yan, H.; Han, X.; Wu, M.; Chen, F. Research progress on the role of biofilm in heavy metals adsorption-desorption characteristics of microplastics: A review. *Environ. Pollut.* **2023**, *336*, 122448. [CrossRef]
19. Shi, X.; Zhang, X.; Gao, W.; Zhang, Y.; He, D. Removal of microplastics from water by magnetic nano-Fe3O4. *Sci. Total Environ.* **2022**, *802*, 149838. [CrossRef]
20. Li, W.; Liu, S.; Huang, K.; Qin, S.; Liang, B.; Wang, J. Preparation of magnetic Janus microparticles for the rapid removal of microplastics from water. *Sci. Total Environ.* **2023**, *903*, 166627. [CrossRef]
21. Abdallah, M.A.M. Trace metal behavior in Mediterranean-climate coastal bay: El-Mex Bay, Egypt and its coastal environment. *Glob. J. Environ. Res.* **2008**, *2*, 23–29.
22. Paul, D. Research on heavy metal pollution of river Ganga: A review. *Ann. Agrar. Sci.* **2017**, *15*, 278–286. [CrossRef]
23. Yang, F.; Hu, X.; Gao, S.; Liu, X.; Zhou, S.; Kong, Y. Enabling synchronous activation of inner-core and mesoporous outer-shell of monodispersed Fe_3O_4@SiO_2 by in-situ implanted MnO to synergistically deliver enhanced catalytic activity. *J. Alloys Compd.* **2020**, *842*, 155817. [CrossRef]
24. Gao, L.; Fu, D.; Zhao, J.; Wu, W.; Wang, Z.; Su, Y.; Peng, L. Microplastics aged in various environmental media exhibited strong sorption to heavy metals in seawater. *Mar. Pollut. Bull.* **2021**, *169*, 112480. [CrossRef]
25. Lang, M.; Yu, X.; Liu, J.; Xia, T.; Wang, T.; Jia, H.; Guo, X. Fenton aging significantly affects the heavy metal adsorption capacity of polystyrene microplastics. *Sci. Total Environ.* **2020**, *722*, 137762. [CrossRef]
26. Liu, S.; Huang, J.; Zhang, W.; Shi, L.; Yi, K.; Zhang, C.; Pang, H.; Li, J.; Li, S. Investigation of the adsorption behavior of Pb(II) onto natural-aged microplastics as affected by salt ions. *J. Hazard. Mater.* **2022**, *431*, 128643. [CrossRef]
27. Li, X.; Mei, Q.; Chen, L.; Zhang, H.; Dong, B.; Dai, X.; He, C.; Zhou, J. Enhancement in adsorption potential of microplastics in sewage sludge for metal pollutants after the wastewater treatment process. *Water Res.* **2019**, *157*, 228–237. [CrossRef]
28. Li, Y.; Zhang, Y.; Su, F.; Wang, Y.; Peng, L.; Liu, D. Adsorption behaviour of microplastics on the heavy metal Cr(VI) before and after ageing. *Chemosphere* **2022**, *302*, 134865. [CrossRef]
29. Ma, J.; Zhao, J.; Zhu, Z.; Li, L.; Yu, F. Effect of microplastic size on the adsorption behavior and mechanism of triclosan on polyvinyl chloride. *Environ. Pollut.* **2019**, *254*, 113104. [CrossRef]
30. Brennecke, D.; Duarte, B.; Paiva, F.; Caçador, I.; Canning-Clode, J. Microplastics as vector for heavy metal contamination from the marine environment. *Estuar. Coast. Shelf Sci.* **2016**, *178*, 189–195. [CrossRef]
31. Wang, F.; Yang, W.; Cheng, P.; Zhang, S.; Zhang, S.; Jiao, W.; Sun, Y. Adsorption characteristics of cadmium onto microplastics from aqueous solutions. *Chemosphere* **2019**, *235*, 1073–1080. [CrossRef] [PubMed]
32. Han, X.; Wang, S.; Yu, X.; Vogt, R.D.; Feng, J.; Zhai, L.; Ma, W.; Zhu, L.; Lu, X. Kinetics and Size Effects on Adsorption of Cu(II), Cr(III), and Pb(II) Onto Polyethylene, Polypropylene, and Polyethylene Terephthalate Microplastic Particles. *Front. Mar. Sci.* **2021**, *8*, 785146. [CrossRef]
33. Zhang, L.; Li, Y.; Wang, W.; Zhang, W.; Zuo, Q.; Abdelkader, A.; Xi, K.; Heynderickx, P.M.; Kim, K.-H. The potential of microplastics as adsorbents of sodium dodecyl benzene sulfonate and chromium in an aqueous environment. *Environ. Res.* **2021**, *197*, 111057. [CrossRef] [PubMed]
34. Gao, F.; Li, J.; Sun, C.; Zhang, L.; Jiang, F.; Cao, W.; Zheng, L. Study on the capability and characteristics of heavy metals enriched on microplastics in marine environment. *Mar. Pollut. Bull.* **2019**, *144*, 61–67. [CrossRef]
35. Shen, M.; Song, B.; Zeng, G.; Zhang, Y.; Teng, F.; Zhou, C. Surfactant changes lead adsorption behaviors and mechanisms on microplastics. *Chem. Eng. J.* **2021**, *405*, 126989. [CrossRef]
36. Fotopoulou, K.N.; Karapanagioti, H.K. Surface properties of beached plastic pellets. *Mar. Environ. Res.* **2012**, *81*, 70–77. [CrossRef]
37. Yin, Y.; Guo, X.; Peng, D. Iron and manganese oxides modified maize straw to remove tylosin from aqueous solutions. *Chemosphere* **2018**, *205*, 156–165. [CrossRef]
38. Hu, B.; Li, Y.; Jiang, L.; Chen, X.; Wang, L.; An, S.; Zhang, F. Influence of microplastics occurrence on the adsorption of 17beta-estradiol in soil. *J. Hazard. Mater.* **2020**, *400*, 123325. [CrossRef]

39. Dong, Y.; Gao, M.; Song, Z.; Qiu, W. As(III) adsorption onto different-sized polystyrene microplastic particles and its mechanism. *Chemosphere* **2020**, *239*, 124792. [CrossRef]
40. Sun, H.; Shi, X.; Mao, J.; Zhu, D. Tetracycline sorption to coal and soil humic acids: An examination of humic structural heterogeneity. *Environ. Toxicol. Chem.* **2010**, *29*, 1934–1942. [CrossRef]
41. Gao, X.; Hassan, I.; Peng, Y.; Huo, S.; Ling, L. Behaviors and influencing factors of heavy metals adsorption onto microplastics: A review. *J. Clean. Prod.* **2021**, *319*, 128777. [CrossRef]
42. Wang, L.; Guo, C.; Qian, Q.; Lang, D.; Wu, R.; Abliz, S.; Wang, W.; Wang, J. Adsorption behavior of UV aged microplastics on the heavy metals Pb(II) and Cu(II) in aqueous solutions. *Chemosphere* **2023**, *313*, 137439. [CrossRef] [PubMed]
43. Lv, X.; Dong, Q.; Zuo, Z.; Liu, Y.; Huang, X.; Wu, W.-M. Microplastics in a municipal wastewater treatment plant: Fate, dynamic distribution, removal efficiencies, and control strategies. *J. Clean. Prod.* **2019**, *225*, 579–586. [CrossRef]
44. Staplevan, M.J.; Ansari, A.J.; Ahmed, A.; Hai, F.I. Effect of embedding a sieving phase into the current plastic recycling process to capture microplastics. *J. Water Process Eng.* **2024**, *66*, 106075. [CrossRef]

water

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Article

Evaluation of Microplastic Toxicity in Drinking Water Using Different Test Systems

Natalya S. Salikova [1], Anna V. Lovinskaya [2], Saule Zh. Kolumbayeva [2], Ainash U. Bektemissova [3], Saltanat E. Urazbayeva [1], María-Elena Rodrigo-Clavero [4] and Javier Rodrigo-Ilarri [4,*]

[1] Department of Ecology, Life Safety and Environmental Protection, Abay Myrzakhmetov Kokshetau University, Kokshetau 020000, Kazakhstan; natsal66@mail.ru (N.S.S.); lily06_95@mail.ru (S.E.U.)
[2] Department of Molecular Biology and Genetics, Al-Farabi Kazakh National University, Almaty 050000, Kazakhstan; anna.lovinskaya@kaznu.edu.kz (A.V.L.); s_kolumb@mail.ru (S.Z.K.)
[3] Department of Chemistry and Technology of Organic Substances, Natural Compounds and Polymers, Al-Farabi Kazakh National University, Almaty 050000, Kazakhstan; abektemisova@mail.ru
[4] Instituto de Ingeniería del Agua y del Medio Ambiente (IIAMA), Universitat Politècnica de València, 46022 Valencia, Spain; marodcla@upv.es
* Correspondence: jrodrigo@upv.es

Abstract: Microplastic pollution poses a significant threat to environmental and human health. This study investigated the toxicological and genotoxic effects of various microplastic types (polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE)) on plant and animal models. Aqueous extracts of microplastics in different size fractions (0.175 mm, 0.3 mm, 1 mm, 2 mm, and 3 mm) were evaluated for their impact on barley seed germination and cell division. Results indicated that smaller microplastic fractions exhibited higher toxicity, particularly for PP and PE. Significant reductions in germination rates and root growth were observed, along with increased chromosomal aberrations in barley cells. Furthermore, the migration of formaldehyde, a known toxicant, from microplastics exceeded permissible limits. These findings highlight the potential risks associated with microplastic pollution, particularly in drinking water sources. Future research should focus on the long-term health impacts of microplastic exposure, including carcinogenic potential, and explore the synergistic effects with other pollutants. Stricter regulations on microplastic pollution and advancements in water treatment technologies are urgently needed to mitigate these risks.

Keywords: microplastic; phytotoxicity; mutagenicity; toxicant migration; acute toxicity

Citation: Salikova, N.S.; Lovinskaya, A.V.; Kolumbayeva, S.Z.; Bektemissova, A.U.; Urazbayeva, S.E.; Rodrigo-Clavero, M.-E.; Rodrigo-Ilarri, J. Evaluation of Microplastic Toxicity in Drinking Water Using Different Test Systems. *Water* **2024**, *16*, 3250. https://doi.org/10.3390/w16223250

Academic Editors: Grigorios L. Kyriakopoulos, Antonis A. Zorpas, María Rocío Rodríguez Barroso and Vassilis J. Inglezakis

Received: 5 October 2024
Revised: 5 November 2024
Accepted: 9 November 2024
Published: 12 November 2024

1. Introduction

The widespread use of polymeric materials has introduced a novel source of environmental pollution. Although polymeric materials are inherently inert, they can release unreacted and unincorporated toxic oligomers and monomers into the environment, thereby imparting toxic properties [1,2]. Additives used to enhance the properties of polymer products (such as plasticizers, modifiers, solvents, and other raw material components) are also toxic and, in most cases, chemically unrelated to the plastic polymer. In such instances, non-covalently bound chemical components may migrate from the polymer product into the surrounding media [3,4]. While the migration of chemicals from plastics typically occurs in small amounts, it can persist over extended periods [5]. The potential hazard posed by microplastics to living organisms is exacerbated by their high sorption capacity for environmental toxicants, attributed to their high surface area-to-volume ratio [6]. Consequently, the risk of contamination of the soil, air, and water by toxic migration products from polymer matrices may be significant.

The situation is exacerbated by the widespread distribution of degraded plastic particles (microplastics) in various environmental compartments, including surface waters, soils, and atmospheric air [7–9]. A key source of microplastic introduction into the natural

environment is landfills, where waste plastics, primarily under the influence of abiotic factors, degrade into smaller fractions and subsequently migrate into the environment [10].

The literature indicates that microplastics can exert physical, chemical, and biological effects on living organisms and their habitats. Previous studies demonstrated that microplastics can influence plants by altering the physical structure of the soil, thereby affecting the availability of water and nutrients [11]. Several researchers have reported that microplastics are capable of adsorbing and transporting toxic substances, such as heavy metals and organic pollutants, thereby amplifying their toxic effects on plants. Additionally, microplastics may contain or release substances that disrupt physiological processes in plants [12–16].

Published studies highlight the dangers of microplastics to aquatic organisms and humans [17,18]. One of the primary mechanisms of exposure to microplastics is their ingestion through the gastrointestinal tract of living organisms via contaminated food and water, ultimately making their way up the food chain into the human body. Microplastics have been detected in various foods, including non-alcoholic beverages, fish, vegetables, fruits, packaged foods, and honey [19–21], as well as in drinking water (both bottled and tap water) [22,23]. Additionally, scientists have provided approximate estimates of microplastic intake in humans, based on their presence in different food and water sources [24].

The indirect negative impacts of microplastics are associated with their ability to transport sorbed chemicals. Microplastics can contain a wide range of chemical additives, such as bisphenol A (BPA), phthalates, and polybrominated diphenyl ethers, which are used in plastic synthesis to enhance plasticity [25,26]. These additives can disrupt the endocrine system and exert toxic effects on the human body when they migrate from the polymer matrix [27]. Due to their small size, microplastics can easily be ingested by aquatic organisms, enter the bloodstream, and accumulate in the gastrointestinal tract and other organs, thereby transporting chemicals and inducing oxidative stress [28–30].

The available data on microplastics in food and drinking water are difficult to generalize due to the absence of standardized sampling and analysis protocols, as well as inconsistencies in the qualitative and quantitative expression of results. The assessment of microplastic toxicity is even more challenging; studies in this field are not only limited but also vary in terms of plastic types, particle size, exposure doses, and test models. Nevertheless, the outcomes of these studies consistently demonstrate various manifestations of microplastic toxicity. For example, ref. [31] investigated the effects of single-dose oral exposure to polyethylene terephthalate (PET) microplastics in adult male Wistar rats, revealing tissue and organ damage and dysfunction. Similarly, hepatotoxicity was observed following oral and nasal exposure to nano/microplastics of polylactic acid in experiments on mice [24].

These findings underscore the urgent need for further research into the toxicity of microplastics, particularly those in contact with food and water. Such research will support the development of effective strategies for managing the production and use of plastic products in everyday life, thereby mitigating the exposure pathways of toxic plastics to the human body.

Although the number of experiments studying the toxicity of microplastics is constantly growing, there are still insufficient data to assess the toxic effects on human health, including the lack of epidemiological studies. Most studies conducted on animals and human cells indicate the presence of oxidative stress, failure of the antioxidant defense system against toxicity caused by microplastics, and induction of reproductive and developmental toxicity [32,33]. In separate experiments, genomic instability was observed at low concentrations of PE microplastics, as revealed in an experiment on human blood lymphocytes [34], as well as a negative impact of PS particles on immature cardiomyocytes (in an experiment on newborn rats) [35], functional disorder of respiratory tract organelles (on polyester particles) [36], and the disruption of the function of forebrain, liver, and intestinal organelles, established on polystyrene particles [37]. Moreover, the toxic effects, such as decreased cell viability, depended on the dose of exposure [38].

Although microplastics are too large to penetrate the skin, they can cause skin irritancy when in contact with creams, soil, children's toys, etc. [39,40].

Additives in plastics are thought to be toxic, carcinogenic, and mutagenic, but due to the impossibility of conducting human studies, the exact effects on the body have not yet been studied [32].

A review of 133 articles [33] describes the presence of adverse effects of nano- and microplastics on human health, but 78.9% of the studies are devoted to the areas of PS and 12% of the studies describe conflicting results. The presence of studies indicating the absence of a toxic effect (under certain experimental conditions) [41] supports our proposal for the need for additional toxicological studies under harmonized/standardized experimental conditions. It is therefore hoped that this study, which covers four types of microplastics of different sizes, the toxicity of which was studied simultaneously using different methods, expands the existing knowledge about the potential toxicity of microplastics.

Determining the mechanisms of the toxic effects of microplastics on living organisms is an important task, but at the same time is not fully understood. At present, it has been established that microplastics can cause genetic damage through mechanical damage to cells, chemical pollution, and interference with cellular processes [42]. Microplastics can block the pores of seeds, including due to the presence of plasticizers [43], and thus prevent water absorption [44]. In turn, a decrease in the swelling process leads to a decrease in the rate of germination [45]. Microplastics can penetrate the root system of plants, disrupting their normal functions [46]. The toxic effect of microplastics may also be linked to their ability to cause DNA breaks in cell cultures [47,48] and influence dominant pathways leading to the modulation of inflammation and cell proliferation [38]. The ability of microplastics to carry toxic chemicals such as heavy metals and organic pollutants on their surface exacerbates their toxic impact [49,50].

Our previous studies on microplastic monitoring have confirmed its presence in all surveyed environmental samples, including natural waters and sediments, in the Akmola region of Kazakhstan [51,52]. The existing water supply system in Kokshetau, as in Kazakhstan as a whole, is based on the predominant use of surface water as drinking water (96.1%), while 94.7% of the population is provided with a centralized water supply [52]. The critical condition of water supply systems (built in the 1960s–1970s) and outdated water treatment technologies (coarse filtration, filtration through sand filters, coagulation, and flotation, settling, and disinfection) do not ensure effective water purification from insoluble microimpurities [53]. In addition, the failure to comply with water treatment regimes in the city of Kokshetau, the absence of coagulation and flotation workshops, and the small number of sand filters and their low productivity lead to the presence of microplastics in samples of tap water in the city of Kokshetau with a size of more than 300 μm in a concentration of (2.0×10^{-2}–6.0×10^{-2}) particles/dm^3 [52]. The weak regional environmental policies regarding plastic waste management lead to the widespread presence of plastic waste in unauthorized landfills on the coast of water bodies, which ultimately leads to the entry of microplastics into natural and drinking waters [54]. Assuming a systemic intake of microplastics into the body of the population of Kokshetau with drinking water, the toxic properties of individual types of microplastics, mostly found in tap water in Kokshetau, were studied [52]. Investigating the toxicity of the types of microplastics found in the tap water of Kokshetau city, Akmola region (Kazakhstan) will facilitate predictions of the toxic risks associated with drinking water.

Focusing on the assessment of toxicity not from the polymers themselves, but from their constituent components that migrate, our study evaluated the toxicity of aqueous extracts from polymer particles fragmented to microplastic size. Similarly, the Office of Food Additive Safety (OFAS) of the U.S. FDA's Center for Food Safety and Applied Nutrition assesses food-contact substances by examining the toxicity of migrating toxicants rather than the polymers themselves [55]. In the context of polymers, the OFAS investigates oligomers and low molecular weight compounds capable of migrating into the contact medium, as they are considered more biologically relevant. We propose that studying the

toxicity of polymer material extracts serves as a reliable indicator of a plastic's potential to impart toxic properties to various liquids. A negative result in such studies may indicate the harmlessness of the polymeric material under the specific experimental conditions (such as exposure temperature, microplastic type, and particle size).

The investigation of microplastic aqueous extract toxicity brings us closer to understanding the safety of exposure to drinking water in contact with plastics, such as when using plastic water pipes or plastic water storage tanks.

Recognizing the insufficiency and ambiguity of assessing the toxic properties of synthetic materials through a single method, along with ethical considerations such as avoiding human experiments and reducing animal testing, the aim of this study was to comprehensively assess the toxicity of aqueous extracts from microplastics (PP, PE, PET, and PS) with different degrees of dispersibility. This assessment was conducted using plant and animal test organisms, frozen bull semen, and through an analysis of the migration of organic substances into the aquatic environment.

The use of plant test objects (phytotoxicity assessment) provides a method to evaluate toxicity without resorting to animal or human testing, and it also helps to assess the potential impact of microplastic extracts on phytocenoses. Additionally, some studies suggest that animal tests may not always exhibit high sensitivity to certain toxicants [56]. Since seed germination has a significant impact on plant yield, it is one of the most common indicators for assessing the phytotoxicity of environmental pollutants [57,58].

Studies on the genotoxicity of microplastics are very limited, yet this assessment is crucial, particularly in relation to drinking water that comes into contact with plastic products. Toxic substances released into the environment disrupt natural cellular processes, leading to structural modifications in DNA and chromosomal abnormalities [12,59–61]. We consider the detection of chromosomal damage in plant test objects to be an effective method for studying the genotoxic activity of microplastics [62,63].

Animal studies (using rabbits in this study) were deemed necessary to investigate the epicutaneous effects of water extracts from the studied microplastics. It is hypothesized that toxic substances migrating from microplastics into water may have an irritant effect on human skin during activities such as bathing. The use of rabbit skin in studies of skin irritancy in rabbits has long been known, as they show good correlations between the results obtained in rabbits and the results obtained in humans, including for non-irritating or highly irritating substances [64,65]. Furthermore, the skin of rats and hamsters is not sensitive enough to be useful in studies of the skin-irritating properties of toxicants [65]. The in vivo rabbit skin irritation test is currently the primary method for testing skin irritation and is the reference method against which non-animal alternatives are compared [66].

One effective method for assessing the acute toxicity of chemical substances is the evaluation of changes in sperm motility using bovine semen. Many researchers consider this method to be more sensitive than tests for hemolytic or toxic effects of plastic products [67]. The method is simple to perform, can be used for large-scale studies, and has high reproducibility. These advantages make it suitable for use in the Kazakhstan system for certifying plastic products, as well as for other Eurasian Economic Union (EurAsEU) countries [68–70]. Although studies on the toxicity of substances often involve cattle semen, they are typically conducted on metal particles (e.g., magnetite [71], gold [72], or iron [73]). Research on the toxic effects of microplastics on semen parameters and reproductive function is scarce. However, one of the few studies on polystyrene microparticles demonstrated decreased sperm functionality and increased oxidative stress in embryos [73]. Our study will contribute to the investigation of fertility and male infertility, which has gained relevance given the widespread accumulation of microplastics in the environment [74,75].

The migration behavior of microplastic components is also poorly studied, likely due to a lack of awareness of the potential public health risks posed by toxicants migrating from polymeric materials. Phenol and formaldehyde are two common chemicals known to migrate into contact media from polymer products [76]. Formaldehyde can cause degenerative changes in parenchymatous organs and skin sensitization, and has significant effects

on the central nervous system. It also inactivates several enzymes in organs and tissues, inhibits nucleic acid synthesis, disrupts vitamin C metabolism, and exhibits mutagenic properties. Formaldehyde is particularly hazardous due to its ability to be rapidly and completely absorbed through any route of entry into the body [77].

The comprehensive nature of this study will provide a more thorough evaluation of the potential hazards posed by aqueous extracts from microplastics of various structures and sizes to living organisms. The findings can be used to assess the potential risks to humans and biota in contact with water containing microplastics.

2. Materials and Methods

2.1. Objects of the Study

All toxicological studies on microplastics were conducted using four types of plastics: polypropylene (PP, from water pipes), polyethylene (PE, from water pipes), polyethylene terephthalate (PET, from plastic bottles), and polystyrene (PS, from packaging containers). These types of plastics were selected based on their prevalence in natural and drinking waters, as established in our earlier studies [51,52]. The structures of the selected polymers were determined by infrared (IR) spectroscopy using an IR-Prestige 21 spectrometer (Shimadzu Corporation, Kyoto, Japan) within the wavelength range of 4000–400 cm^{-1}. No special sample preparation was required, and the analysis was conducted using a DuraSampl IR II single-reflection total internal reflection (ATR) attachment (prism material: diamond on ZnSe substrate) (Smiths Detection, Danbury, CT, USA).

The IR spectra were analyzed using the Polymer2, Polymer, T-Polymer, and T-Organic library databases, as well as by interpreting absorption bands corresponding to the stretching and bending vibrations of functional groups characteristic of specific polymers. Polypropylene (PP) was identified by the presence of absorption bands corresponding to the stretching and bending vibrations of CH, CH_2, and CH_3 groups at 2950, 2918, 2836, 1456, and 1376 cm^{-1}. Similarly, the IR spectrum of polyethylene (PE) showed absorption bands at 2916, 2846, 1468, and 717 cm^{-1}, corresponding to the stretching and bending vibrations of the CH_2 group [52,78]. The structures of polyethylene terephthalate (PET) and polystyrene (PS) were identified by comparing their Fourier-transform infrared (FTIR) spectra with those in polymer library databases.

All types of plastics were manually shredded and further processed through various laboratory mills to simulate the fragmentation of microplastics as they naturally degrade in the environment. In order to standardize all studies throughout the project, we studied the fractions of microplastics obtained by us with the available set of sieves (0.175 mm; 0.3 mm; 1.0 mm; 2.0 mm; and 3.0 mm). Thus, in the toxicological study, we covered the range of sizes of microplastics found by us in surface waters and in tap water (100–500 μm on the largest side [51,52]), which correlates with other published data, where the detected microplastic particles were in the range of more than 0.7 mm, less than 0.3 mm, and in the range of 0.3–0.7 mm [79]. Given that the entire surface area of a plastic container or a significant surface area of a plastic water pipe may be in contact with water, in this study we also included microplastics larger than 1 mm (1 mm and 3 mm), taking into account that the maximum microplastic size is generally considered to be particles smaller than 5 mm [80].

Thus, the shredded plastic was then sieved into fractions of 3 mm, 2 mm, 1 mm, 0.3 mm, and 0.175 mm using a series of sieves. All experiments were carried out on the following 20 different variants of microplastic:

- Aqueous extracts from plastic bottle (PET) with particle sizes of 0.175 mm; 0.3 mm; 1.0 mm; 2.0 mm; and 3.0 mm;
- Aqueous extracts from plastic container (PC) with particle sizes of 0.175 mm; 0.3 mm; 1.0 mm; 2.0 mm; and 3.0 mm;
- Water extracts from water pipe (PP) with particle sizes of 0.175 mm; 0.3 mm; 1.0 mm; 2.0 mm; and 3.0 mm;

- Water extracts from water pipe (PE) with particle sizes of 0.175 mm; 0.3 mm; 1.0 mm; 2.0 mm; and 3.0 mm.

In order to simulate the contact of drinking water with the surface of plastic containers and to ensure the prevention of external contamination, the process of microplastic extraction was carried out in closed containers. After the specified extraction time, the microplastic was separated from the liquid fraction by filtration through No. 42 Whatman filters, which ensured the removal of any particles larger than 2.5 μm. The resulting extracts were then used to assess their toxic properties. The toxicity of aqueous extracts of microplastics was evaluated in a toxicity study:

- Phytotoxicity;
- Genotoxic properties on a plant test subject;
- Local cutaneous irritant action at single applications to the back skin of experimental animals;
- Phenol and formaldehyde migration;
- Acute toxicity using frozen bovine semen.

The test subject in the experiment to assess phytotoxicity and mutagenic activity was common barley (*Hordeum vulgare* L.), often used by scientists as a model species for basic and applied research on agricultural crops. The diploid genome and haploid set of a small number (7) of chromosomes makes barley suitable for genotoxic studies [81,82]. In addition, barley is the most common type of agricultural crop grown in the Akmola region and in Kazakhstan. Given the fact that microplastic pollution has the greatest impact on agrocenoses [83], this study on the toxicity of microplastics on barley is informative for such agrocenoses.

The positive control was methyl methanesulfonate (MMS), a classic mutagen [81,82], and distilled water (dH_2O) was the negative.

Sexually mature laboratory animals, specifically rabbits weighing between 3500–3800 g, were selected as test subjects for studying the epicutaneous effects of aqueous extracts of microplastics. The selection of animals and the formation of homogeneous experimental and control groups were carried out with consideration of similar body weight (with a maximum weight difference of no more than 10% within each group), as well as the absence of differences in behavior and general health condition.

For the acute toxicity experiment, bovine semen frozen in liquid nitrogen vapor was used as a test subject. The frozen bovine semen pellets were obtained from artificial insemination stations and stored in Dewar vessels filled with liquid nitrogen.

2.2. Research Methods

To assess the phytotoxicity and genotoxicity of aqueous extracts of microplastics, all types of shredded plastic were divided into fractions and immersed in distilled water at a concentration of 1 g of microplastic per 100 cm^3 of water. The mixtures were then incubated for 1 month at thermostatic room temperature (22–25 °C). For experiments evaluating skin irritant effects, the migration of phenol and formaldehyde, and acute toxicity using frozen bovine semen, the preparation of aqueous extracts of microplastics was conducted according to the relevant standards described in Sections 2.2.1–2.2.5

In order to standardize the experimental conditions, aqueous extracts were obtained using distilled water with a pH of 5.4–6.6 (without further changing the acid–base balance), at illumination levels regulated by standards for laboratory and thermostatic rooms (400 lux) and for the vivarium (325 lux) [84], and the following thermostatting temperature conditions:

- Assessment of phytotoxicity and frozen bovine semen: obtaining extracts—22–25 °C, seed germination—23–24 °C;
- Assessment of skin irritant properties: preparation of aqueous extracts—(18–24 °C);
- Acute toxicity studies on bovine semen—preparation of aqueous extracts at 40 °C;

- study of phenol and formaldehyde migration—preparation of extracts at a temperature of −22–25 °C.

2.2.1. Method for Analyzing Phytotoxic Properties

To conduct research on the model test subject barley (*Hordeum vulgare* L.), seeds with a germination efficiency of at least 80% were selected at the initial stage. The scheme for determining phytotoxicity is shown in Figure 1a.

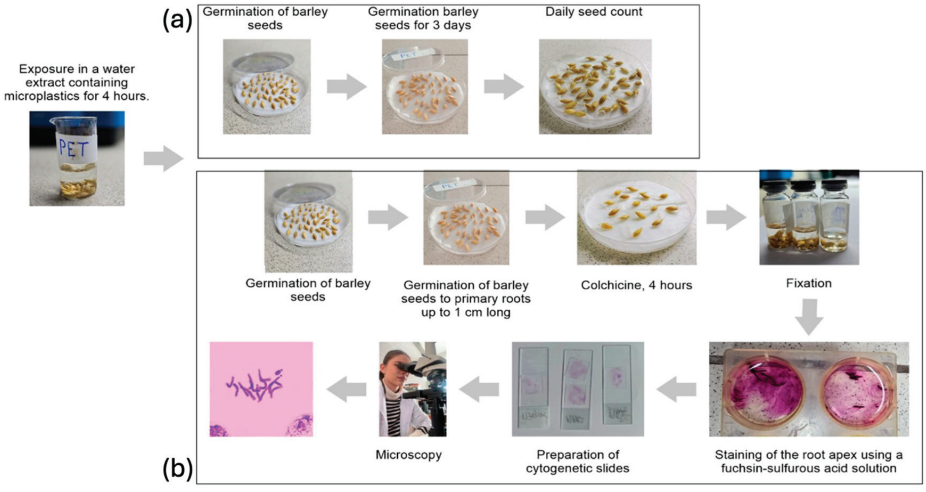

Figure 1. Schematic diagram of (**a**) analysis of phytotoxicity and (**b**) mutagenic activity of microplastic extracts.

The cleaned seeds were soaked in water for 12 h to enhance germination. Following this, the already swollen seeds (50 per treatment) were immersed in aqueous extracts of microplastics for 4 h. After soaking, the seeds were planted in Petri dishes and placed in a thermostat at 23–24 °C for root germination over a period of 3 days. The number of germinated seeds was recorded every 24 h. Seed germination was determined by the presence of a visible root emerging from the split seed coat [44].

The phytotoxic activity of the various microplastic treatments was assessed based on the germination rate, seedling vigor, and overall germination rate of common barley seeds (*Hordeum vulgare* L.) as per the methodology outlined in [85]. The percentage of seed germination (B) was calculated using Equation (1).

$$B = \frac{a}{b} \times 100\% \quad (1)$$

where a is the number of germinated seeds and b is the total number of seeds.

The germination rate (C) is the sum of the average number of seeds germinating daily, calculated using Equation (2).

$$C = a + \frac{b}{2} + \frac{c}{3} \quad (2)$$

where a is the number of seeds germinated during the first day, b is the number of seeds germinated on the second day, and c is the number of seeds germinated on the third day.

Germination unity refers to the average percentage of seeds per day of germination, calculated by Equation (3):

$$D = \frac{P}{A} \quad (3)$$

where D is the germination unity, P the number of germinated seeds for the first day, and A the number of germinated seeds for the fourth day.

2.2.2. Method of Mutagenic Activity Analysis

The general scheme of the analysis of mutagenic activity is presented in Figure 1b.

Methods of Preparation of Cytogenetic Preparations from Cells of the Root Meristem of Common Barley (*Hordeum vulgare* L.)

Cytogenetic preparations from the cells of the root meristem of common barley were prepared and stained with fuchsin–sulfuric acid according to the methodology outlined in [85]. To arrest cell division at the metaphase stage, seeds germinated overnight in the thermostat were transferred to a 0.01% colchicine solution for 4 h. The seeds were then fixed using a freshly prepared solution of glacial acetic acid and 96% ethyl alcohol in a 1:1 ratio.

Initially, the fixed material was thoroughly washed in three portions of distilled water, with each wash lasting 4 min. Following the water washes, cold hydrolysis was performed using a pre-cooled solution of concentrated hydrochloric acid diluted with water at a 1:1 ratio for 50 min at 4–5 °C. After hydrolysis, the samples were washed to remove hydrochloric acid and incubated in a fuchsinic acid solution for a minimum of 12 h in the dark.

The enzymatic maceration method was employed to decompose the intercellular matrix and remove the cell walls. During this stage, the brightly colored root tips containing the zone of actively dividing meristematic cells were carefully excised with a scalpel and placed in an aqueous solution of cytase for 30 min at 30 °C. After washing the enzyme off with distilled water, 2–3 drops of 45% acetic acid were added to the apical parts of the roots and allowed to incubate for 1–2 min. The root tips were then transferred to a microscope slide using a pipette and covered with a coverslip to create a monolayer of cells.

Microscopy was the final step, in which the cytogenetic preparations were analyzed under an MX 100T microscope (MicroOptix, Wiener Neudorf, Austria) at a magnification of 1000×.

Metaphase Method of Accounting for Chromosomal Aberrations

The genotoxic activity of microplastic aqueous extracts was assessed by counting metaphase chromosomal aberrations. For each experimental variant, more than 400 metaphase cells from barley root meristems were examined. In analyzing the structural changes of chromosomes, both the overall frequency of chromosomal aberrations and the frequency of chromosomal and chromatid-type abnormalities were considered. The frequency of aberrations resulting from natural mutation processes in plant cells served as the control. For each experimental variant, the percentage of cells with altered chromosomes was calculated relative to the total number of metaphases studied, as well as the percentage of total chromosomal changes classified as chromosome rearrangements.

Experiments assessing phytotoxicity and genotoxicity were conducted in triplicate. For all cases, mean values and their associated standard errors were computed using the Microsoft Excel data analysis package. The significance of differences between mean values was evaluated using Student's *t*-test for independent samples, with a significance threshold set at 0.05 ($p < 0.05$).

2.2.3. Method for Assessing the Dermal Irritant Effect

This study was conducted at the National Centre of Expertise in the Akmola region, which is accredited to perform toxicological assessments of plastic products on test subjects. Each experimental group consisted of 10 animals, adhering to the state standards that require a minimum of 6 animals per group [86]. Applications were made on the skin of rabbits, covering at least 5% of the total skin surface, which corresponded to an area of 7×8 cm^2.

A day prior to the experiment, the fur of the animals was carefully clipped using an electric trimmer on symmetrical areas of the back, leaving 1–2 cm of fur between the clipped sections. The right side of the back was designated for the application of the test substance, while the left side served as the control. To prevent the animals from licking the applied substance and to minimize inhalation, they were housed individually in specialized enclosures during the exposure period.

Gauze pads soaked in a water extract of microplastics were applied to the skin of the rabbits. The preparation of these extracts involved adding 1 g of the specific type and fraction of microplastic to 100 cm^3 of distilled water at room temperature (18–24 °C, extraction duration—4 days under thermostatic conditions, exposure duration—4 h). Clinical manifestations of intoxication and the condition of the skin were monitored 1 h and 24 h after application and subsequent washing of the substance. The presence of a skin-intoxication effect was evaluated based on indicators of skin functional disorders, including erythema, edema, fissures, ulcers, and changes in the temperature and neutralizing capacity of the skin. The experiment was conducted in triplicate.

2.2.4. Method of Acute Toxicity Study Based on Frozen Bovine Semen

This study was conducted at TEKS LLP, which is accredited to perform toxicological assessments of polymeric materials in accordance with the Methodological Guidelines [87]. The method employed involves examining changes in sperm motility in response to chemical compounds present in the extracts derived from the samples under investigation. The motility index is determined by measuring changes in light intensity as spermatozoa move through an optical probe.

The preparation of aqueous extracts was done as follows. For testing, 30 g of each microplastic fraction (PS, PET, PP, and PE) were prepared. Using a quartering method, 1 g suspensions microplastic were extracted from each fraction and placed in heat-resistant flasks with a capacity of 250 cm^3. A total of 100 cm^3 of boiling distilled water was added, and the flasks were incubated in a thermostat at a temperature of (40 ± 2) °C for 24 h. To establish a toxicity index, the experimental solutions were compared against a control (model) medium. Glucose-citrate medium (glucose—4 g, sodium citrate—1 g, and distilled water—100 cm^3) was selected as the control solution, which also served as the diluent for thawing frozen semen.

The experimental solution consisted of an aqueous extract of microplastics adjusted to isotonicity using dry reagents of glucose and sodium citrate (glucose—4 g, sodium citrate—1 g, and test solution—100 cm^3). Control and experimental solutions of 0.4 cm^3 each were placed in test tubes with lapped plugs and incubated in a water bath at (40 ± 1.5) °C. Both the control and experimental solutions were prepared one hour before the experiment. Subsequently, 0.1 cm^3 of semen stock solution was added to each test tube of the control and experimental series. Each working sample was then transferred into a cuvette, sealed, placed in a hood test stand, and tested immediately for 10–300 s [87].

The test results were evaluated by comparing the sperm motility of bovine semen from the tested samples to that of the control sample, which was designated as 100% (toxicity index). The experiment was repeated until the standard deviation of three parallel tests did not exceed 1%.

2.2.5. Method for Analyzing Phenol and Formaldehyde Migration Values

This study was conducted in accordance with [88]. This normative document establishes the values of PAM (mg/dm^3)—the permissible amount of migration of a chemical substance—as a limiting indicator. Sanitary–hygienic requirements are set for formaldehyde migration in polymeric particles such as PS, PET, PP, and PE. While these polymers are not typically evaluated for phenol migration due to their chemical structures, we opted to include phenol migration assessment in this experiment to evaluate the quality of the tested products.

The experiment was carried out using distilled water under the following conditions: temperature—22–25 °C and exposure time extraction duration—3 h under thermostatic conditions [88]. To ensure that standardized conditions [88] were met (the ratio of the microplastic particle area to the water surface area should be no less than 1 cm^2:2 cm^2) and that the condition of uniform microplastic concentration in the extracted solutions was met in all our experiments, 1 g of microplastic was taken and 100 cm^3 of distilled water at a temperature of 22–25 °C was added to obtain the extracts.

The concentration of formaldehyde was determined using a photocolorimetric method with a Jenway 6320D spectrophotometer (Jenway Ltd., Gransmore Green, Great Britain), with a measurement accuracy of ±0.01 mg/dm^3, in accordance with [89]. The phenol concentration was assessed via gas chromatography using a "Chromos GC-1000" gas chromatograph equipped with a flame ionization detector (Chromos Ltd., Dzerzhinsk, Russia), also with a measurement accuracy of ±0.01 mg/dm^3 [86].

The concentrations of phenol and formaldehyde in the aqueous extracts were then compared against the sanitary norms for the permissible migration quantities (PMQs) of these substances.

3. Results and Discussion

3.1. Assessment of Phytotoxic Properties of Microplastics

When barley seeds were soaked in the aqueous extracts of all studied fractions of microplastics, a deterioration in the physiological parameters of the barley was observed compared to the negative control (Table 1). Additionally, after 2–3 days, rotting formations were noted in the majority of seeds treated with the aqueous extracts of all types of the studied microplastics.

Table 1. Percentage of germination and speed, friendliness of seed germination when soaking seeds with water extract of polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE).

Experiment Variant		Germination, %	Germination Rate, Conventional Units	Germination Density, %
Negative control, dHO_2		86.67 ± 1.76	39.39 ± 0.81	28.89 ± 0.59
Positive control, MMC (5 mg/dm^3)		66.67 ± 1.76 **	31.39 ± 1.19 *	22.22 ± 0.72 **
Polystyrene (PS)	0.175 mm	69.33 ± 3.53 *	30.83 ± 1.30 *	23.11 ± 1.18 *
	0.3 mm	71.3300 ± 3.53	32.83 ± 1.78	23.78 ± 1.18
	1.0 mm	72.00 ± 4.16	29.61 ± 2.14 *	24.00 ± 1.39
	2.0 mm	74.00 ± 3.46	31.89 ± 2.50	24.67 ± 1.15
	3.0 mm	75.33 ± 4.37	33.39 ± 2.31	25.11 ± 1.46
Polyethylene terephthalate (PET)	0.175 mm	73.33 ± 1.76 *	33.72 ± 0.86 *	24.44 ± 0.59 *
	0.3 mm	76.67 ± 3.33	33.72 ± 1.33	25.56 ± 1.11
	1.0 mm	77.33 ± 5.46	34.56 ± 1.53	25.78 ± 1.82
	2.0 mm	78,00 ± 4.62	36.72 ± 0.70	26.00 ± 1.54
	3.0 mm	78.67 ± 2.40	35.61 ± 0.87	26.22 ± 0.80
Polypropylene (PP)	0.175 mm	72.67 ± 2.40 *	33.17 ± 1.42 *	24.22 ± 0.80 *
	0.3 mm	74.67 ± 2.40 *	32.67 ± 0.75 **	24.89 ± 0.80 *
	1.0 mm	76.00 ± 4.00	35.89 ± 2.12	25.33 ± 1.33
	2.0 mm	77.33 ± 2.91	35.22 ± 1.27	25.78 ± 0.97
	3.0 mm	79.33 ± 4.06	34.06 ± 0.48 **	26.44 ± 1.35
Polyethylene (PE)	0.175 mm	69.33 ± 2.91 *	28.83 ± 2.18 *	23.11 ± 0.97 *
	0.3 mm	70.00 ± 3.06 *	31.17 ± 1.04 *	23.33 ± 1.02 *
	1.0 mm	71.33 ± 3.71	30.89 ± 1.65 *	23.78 ± 1.24
	2.0 mm	72.67 ± 3.53	32.28 ± 1.26 *	24.22 ± 1.18
	3.0 mm	75.33 ± 2.91	33.22 ± 0.96 *	25.11 ± 0.97

Note: * $p < 0.05$, ** $p < 0.01$—compared to negative control.

Specifically, when seeds were exposed to polystyrene (PS) microplastic of 0.175 mm size, there was a statistically significant decrease ($p < 0.05$) in seed germination, which was reduced by 1.25 times, along with a 1.28 times decrease in germination rate and a 1.25 times decrease in germination uniformity compared to the negative control. Notably, these decreases in physiological parameters were comparable to those observed in the positive control.

In seeds exposed to PS microplastic particles of sizes 0.3 mm, 1.0 mm, 2.0 mm, and 3.0 mm, an increase in phytotoxic activity was observed; however, these changes were not statistically significant compared to the negative control. An exception was noted with the germination rate of barley seeds influenced by the 1.0 mm fraction of PS microplastic, which showed a statistically significant decrease of 1.33 times ($p < 0.05$).

When seeds were exposed to PET microplastic of 0.175 mm size, a statistically significant decrease in seed germination was observed, reduced by 1.18 times ($p < 0.05$), along with a 1.17 times decrease in germination rate and a 1.18 times decrease in germination uniformity compared to the negative control. This decrease in phytotoxicity indicators was comparable to the positive control.

For seeds exposed to PET microplastic sizes of 0.3 mm, 1.0 mm, 2.0 mm, and 3.0 mm, a decrease in physiological indicators was also noted; however, these changes were not statistically significant compared to the negative control.

In the case of water extracts from PP microplastic fractions of 0.175 mm and 0.3 mm, statistically significant decreases in seed germination were observed, reduced by 1.19 times ($p < 0.05$) and 1.16 times ($p < 0.05$), respectively. Germination rates also decreased significantly by 1.19 times ($p < 0.05$) and 1.21 times ($p < 0.01$) for these fractions, respectively, as well as germination uniformity decreasing by 1.19 times ($p < 0.05$) and 1.16 times ($p < 0.05$). Notably, when seeds were exposed to PP microplastic of 3.0 mm size, a statistically significant decrease in the germination rate of 1.16 times ($p < 0.01$) compared to the negative control was observed. This decline in physiological parameters and the increase in phytotoxic activity of the PP microplastic was at a level similar to that of the positive control. Deterioration in the physiological indicators of barley seeds exposed to PP microplastic fractions of 1.0 mm and 2.0 mm was also observed, although these changes were not statistically significant compared to the negative control.

When seeds were exposed to microplastic PE fractions of 0.175 mm and 0.3 mm, statistically significant decreases in seed germination were observed, reduced by 1.25 times ($p < 0.05$) and 1.24 times ($p < 0.05$), respectively. Similar reductions were noted in the germination rates, which decreased by 1.25 times for the 0.175 mm fraction and 1.24 times for the 0.3 mm fraction compared to the negative control.

Additionally, significant decreases in germination rates were observed for all PE microplastic fractions compared to the negative control: 0.175 mm—1.37 times, 0.3 mm—1.26 times, 1.0 mm—1.28 times, 2.0 mm—1.22 times, and 3.0 mm—1.19 times. The deterioration of the barley's physiological parameters in these cases was similar to that observed in the positive control.

Statistical analysis did not reveal significant differences between the various types of microplastics tested. Thus, the data indicate that all four types of microplastics (PS, PET, PP, and PE) exhibited statistically significant phytotoxic properties, particularly for the smallest fraction studied (0.175 mm). For the 0.3 mm fraction, significant reductions in all studied physiological parameters of barley were found for PP and PE; for the 1.0 mm fraction, significant effects were noted for PS; for the 2.0 mm fraction, significant effects were observed for PE; and for the 3.0 mm fraction, significant effects were noted for both PP and PE.

Phytotoxic activity manifested as growth-inhibiting effects on germinating barley seeds, along with the presence of rot formations within the seeds.

A few studies on the phytotoxicity of microplastics have indicated negative effects on seed growth due to toxicants leached from plastics [43]. These studies also suggest that the effects of plastics can vary based on concentration, polymer type, and plant species [90,91].

Furthermore, nanosized plastic particles may exert physical blocking effects, clogging pores and preventing water entry, thereby inhibiting seed growth [44].

3.2. Assessment of Chromosomal Abnormalities

When *H. vulgare* seeds were exposed to aqueous extracts of all types of the studied microplastics, an increase in the frequency of chromosomal abnormalities in apical meristem cells was observed compared to the baseline levels of spontaneous mutations. Table 2 presents the data on the frequency of chromosomal aberrations in the meristems of common barley germinal roots that were soaked in aqueous extracts of microplastics from polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE).

Table 2. Frequency of chromosomal abnormalities in common barley root meristem cells germinated on aqueous extracts of polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE) microplastics.

Experiment Variant		Total Cells	Frequency of Aberrant Cells (M ± m,%)	Number of Chromosomal Aberrations per 100 Metaphase Cells		
				Total Aberrations	Chromosomal Type	Chromatid Type
Negative control, dHO_2		450	1.11 ± 0.49	1.11 ± 0.49	0.44 ± 0.31	0.67 ± 0.38
Positive control, MMC (5 mg/dm)^3		471	5.31 ± 1.03 ***	5.94 ± 1.09 ***	2.55 ± 0.73 **	3.40 ± 0.83 **
Polystyrene (PS)	0.175 mm	480	3.75 ± 0.87 **	4.58 ± 0.95 **	2.08 ± 0.65 *	2.50 ± 0.71 *
	0.3 mm	511	3.52 ± 0.82 *	3.91 ± 0.86 **	1.37 ± 0.51	2.54 ± 0.70 *
	1.0 mm	512	3.32 ± 0.79 *	3.91 ± 0.86 **	1.56 ± 0.55	2.34 ± 0.67 *
	2.0 mm	458	2.84 ± 0.78	3.28 ± 0.83 *	1.09 ± 0.49	2.18 ± 0.68
	3.0 mm	446	2.69 ± 0.77	2.91 ± 0.80	0.90 ± 0.45	2.02 ± 0.67
Polyethylene Terephthalate (PET)	0.175 mm	462	4.55 ± 0.97 **	5.41 ± 1.05 ***	2.16 ± 0.68 *	3.25 ± 0.82 **
	0.3 mm	482	3.94 ± 0.89 **	4.15 ± 0.91 **	1.87 ± 0.62 *	2.28 ± 0.68 *
	1.0 mm	425	4.24 ± 0.98 **	4.71 ± 1.03 **	1.18 ± 0.52	3.53 ± 0.90 **
	2.0 mm	436	3.67 ± 0.90 *	3.90 ± 0.93 **	1.61 ± 0.60	2.29 ± 0.72 *
	3.0 mm	440	3.64 ± 0.89 *	3.86 ± 0.92 **	1.59 ± 0.60	2.27 ± 0.71 *
Polypropylene (PP)	0.175 mm	500	3.20 ± 0.79 *	3.40 ± 0.81 *	1.60 ± 0.56	1.80 ± 0.59
	0.3 mm	490	3.06 ± 0.78 *	3.06 ± 0.78 *	1.43 ± 0.54	1.63 ± 0.57
	1.0 mm	442	2.94 ± 0.80	2.94 ± 0.80	1.36 ± 0.55	1.58 ± 0.59
	2.0 mm	456	2.85 ± 0.78	2.85 ± 0.78	1.32 ± 0.53	1.54 ± 0.58
	3.0 mm	495	2.63 ± 0.72	2.63 ± 0.72	1.21 ± 0.49	1.41 ± 0.53
Polyethylene (PE)	0.175 mm	495	3.23 ± 0.79 *	3.64 ± 0.84 *	1.82 ± 0.60 *	1.82 ± 0.60
	0.3 mm	500	3.20 ± 0.79 *	3.40 ± 0.81 *	1.60 ± 0.56	1.80 ± 0.59
	1.0 mm	520	2.88 ± 0.73 *	2.88 ± 0.73 *	1.15 ± 0.47	1.73 ± 0.57
	2.0 mm	465	2.80 ± 0.76	2.80 ± 0.76	1.08 ± 0.48	1.72 ± 0.60
	3.0 mm	458	2.40 ± 0.72	2.40 ± 0.72	0.87 ± 0.43	1.53 ± 0.57

Note: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to negative control.

The frequency of aberrant cells exposed to PS microplastic fractions of 0.175 mm, 0.3 mm, and 1.0 mm significantly increased compared to the negative control, by 3.38-fold ($p < 0.01$), 3.17-fold ($p < 0.05$), and 2.99-fold ($p < 0.05$), respectively. For PS microplastic fractions of 2.0 mm and 3.0 mm, the frequency of aberrant cells also increased compared to the negative control, by 2.56-fold and 2.42-fold, respectively; however, these increases were not statistically significant.

When assessing the number of chromosomal aberrations per 100 metaphases in the apical meristem of barley seeds exposed to aqueous extracts of microplastics from PS fractions, statistically significant increases were observed compared to the negative control. Specifically, exposure to the aqueous extract of the 0.175 mm PS fraction resulted in a

4.13-fold increase ($p < 0.01$) in chromosomal aberrations. The number of chromosomal and chromatid aberrations increased 4.73-fold ($p < 0.05$) and 3.73-fold ($p < 0.05$), respectively.

For the 0.3 mm PS fraction, the number of chromosomal structural rearrangements per 100 metaphases increased by 3.52-fold ($p < 0.01$) compared to the negative control, while chromosomal and chromatid aberrations increased 3.11-fold and 3.79-fold ($p < 0.05$), respectively. Similarly, exposure to the aqueous extract of the 1.0 mm PS fraction led to a 3.52-fold increase in chromosomal aberrations ($p < 0.01$), with chromosomal and chromatid aberrations increasing 3.54-fold and 3.49-fold ($p < 0.05$), respectively.

For the 2.0 mm PS fraction, a statistically significant increase in chromosomal aberrations was observed, with a 2.95-fold increase ($p < 0.05$) compared to the negative control. The number of chromosomal and chromatid aberrations increased by 2.48-fold and 3.01-fold, respectively. Exposure to the aqueous extract of the 3.0 mm PS fraction also resulted in a significant increase in chromosomal aberrations, with a 2.62-fold increase compared to the negative control and a 2.05-fold and 3.01-fold increase in chromosomal and chromatid aberrations, respectively.

When barley seeds were exposed to aqueous extracts of PET microplastic of the 0.175 mm fraction, a statistically significant increase in the frequency of aberrant cells was observed, with a 4.10-fold increase ($p < 0.01$) compared to the negative control. The number of chromosomal structural rearrangements per 100 metaphases also significantly increased by 4.87-fold ($p < 0.001$). Additionally, the number of chromosome-type aberrations increased 4.91-fold ($p < 0.05$) and chromatid-type aberrations increased 4.85-fold ($p < 0.01$).

For the 0.3 mm PET fraction, the frequency of aberrant cells significantly increased by 3.55-fold ($p < 0.01$) compared to the negative control, while the number of chromosomal rearrangements per 100 metaphases increased by 3.74-fold ($p < 0.01$). The number of chromosomal type aberrations increased 4.25-fold ($p < 0.05$) and the number of chromatid type aberrations increased 3.40-fold ($p < 0.05$).

Exposure to aqueous extracts of the 1.0 mm PET fraction resulted in a statistically significant increase in the frequency of cells with chromosomal aberrations, with a 3.82-fold increase ($p < 0.01$), and a 4.24-fold increase ($p < 0.01$) in the number of chromosomal aberrations per 100 metaphases compared to the negative control. This fraction also exhibited a significant increase in chromosomal type rearrangements (2.68-fold) and chromatid type rearrangements (5.27-fold, $p < 0.01$).

The 2.0 mm PET fraction caused a statistically significant increase in the frequency of cells with chromosomal aberrations, by 3.31-fold ($p < 0.05$), and in the number of chromosomal rearrangements per 100 cells, by 3.51-fold ($p < 0.01$), compared to the negative control. The number of chromosome-type aberrations increased 3.66-fold and chromatid-type aberrations increased 3.42-fold ($p < 0.05$).

Finally, exposure to aqueous extracts of the 3.0 mm PET fraction resulted in a statistically significant increase in the frequency of cells with chromosomal structural abnormalities, with a 3.28-fold increase ($p < 0.05$) compared to the negative control. The number of chromosomal aberrations per 100 metaphases increased by 3.48-fold ($p < 0.01$), while chromosome-type aberrations increased 3.61-fold and chromatid-type aberrations increased 3.39-fold ($p < 0.05$).

When barley seeds were exposed to aqueous extracts of PP microplastic, the frequency of aberrant cells significantly increased compared to the negative control. For the 0.175 mm and 0.3 mm fractions, the frequency of aberrant cells increased by 2.88-fold ($p < 0.05$) and 2.76-fold ($p < 0.05$), respectively. In contrast, the frequencies of aberrant cells for the 1.0 mm, 2.0 mm, and 3.0 mm fractions increased by 2.65-fold, 2.57-fold, and 2.37-fold compared to the negative control, but these increases were not statistically significant.

When evaluating the number of chromosomal aberrations per 100 metaphases in the apical meristem of barley seeds, significant increases were noted for the 0.175 mm and 0.3 mm fractions compared to the negative control. Specifically, exposure to the aqueous extracts of the 0.175 mm PP fraction resulted in a 3.06-fold increase ($p < 0.05$)

in the number of chromosomal aberrations. Additionally, chromosomal and chromatid aberrations increased by 3.64-fold and 2.69-fold, respectively.

For the 0.3 mm PP fraction, the increase in the number of structural rearrangements of chromosomes per 100 metaphases was 2.76-fold ($p < 0.05$) compared to the negative control, with chromosomal and chromatid aberrations increasing by 3.25-fold and 2.43-fold, respectively.

When barley seeds were exposed to aqueous extracts of the 1.0 mm PP fraction, there was a 2.65-fold increase in the number of chromosomal aberrations per 100 metaphases compared to the negative control. This exposure also led to increases of 3.09-fold and 2.36-fold in chromosomal and chromatid aberrations, respectively.

For the 2.0 mm PP fraction, the number of chromosomal aberrations per 100 metaphases increased by 2.57-fold compared to the negative control, with chromosomal and chromatid aberrations rising by 3.00-fold and 2.30-fold, respectively. Finally, exposure to the aqueous extract of the 3.0 mm PP fraction resulted in a 2.37-fold increase in chromosomal aberrations per 100 metaphases compared to the negative control, with chromosomal and chromatid aberrations increasing by 2.75-fold and 2.10-fold, respectively.

When barley seeds were exposed to aqueous extracts from PE microplastic of the 0.175 mm fraction, a statistically significant increase in the frequency of aberrant cells was observed, rising 2.91 times ($p < 0.05$) compared to the negative control. The number of chromosomal structural rearrangements per 100 metaphases also increased by 3.28 times ($p < 0.05$). Additionally, chromosomal type aberrations increased by 4.14 times ($p < 0.05$), while chromatid type aberrations rose by 2.72 times.

When exposed to aqueous extracts from the 0.3 mm PE microplastic fraction, the frequency of aberrant cells increased significantly by 2.88 times ($p < 0.05$) compared to the negative control and the number of chromosomal rearrangements per 100 metaphases increased by 3.06 times ($p < 0.05$). The number of chromosomal type aberrations increased by 3.64 times, while chromatid type aberrations rose by 2.69 times.

For the 1.0 mm PE microplastic fraction, a statistically significant increase in the level of cells with chromosomal aberrations was noted, increasing by 2.59 times ($p < 0.05$). The number of chromosomal aberrations per 100 metaphases also rose by 2.59 times ($p < 0.05$). Furthermore, chromosomal type rearrangements increased by 2.61-fold and chromatid type rearrangements increased by 2.58-fold.

The 2.0 mm PE microplastic fraction caused an increase in the frequency of cells with chromosomal aberrations by 2.52 times and the number of chromosomal rearrangements per 100 cells also increased by 2.52 times compared to distilled water. However, while the number of chromosomal type aberrations increased by 2.45-fold and the number of chromatid-type aberrations increased by 2.57-fold, these increases were not statistically significant.

A similar trend was observed with the 3.0 mm PE microplastic fraction. The frequency of cells with chromosomal structural abnormalities increased by 2.16 times compared to the negative control, as did the number of chromosomal aberrations per 100 metaphases. The number of chromosomal aberrations rose by 1.97 times and chromatid aberrations increased by 2.28 times; however, these increases were not statistically significant. In the studied cells of the barley apical meristem under the influence of various microplastics, structural disorders of chromosomes were noted at the metaphase stage, irrespective of the type (PS, PET, PP, and PE) or size of the microplastic (0.175 mm, 0.3 mm, 1 mm, 2 mm, and 3 mm). Observed abnormalities included single and paired deletions, centric rings, point fragments, and instances of polyploidy. Anaphase stage abnormalities included chromosome lagging and chromosome bridges (see Figure 2).

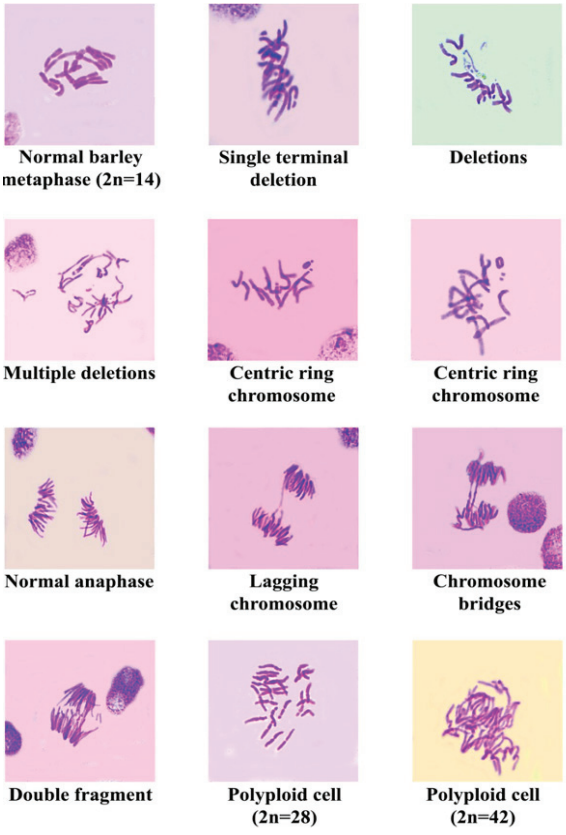

Figure 2. Chromosomal and genomic abnormalities in root germ meristem cells of *Hordeum vulgare* L. induced by PS, PET, PP, and PE of fractions 0.175 mm, 0.3 mm, 1.0 mm, 2.0 mm, and 3.0 mm.

The results of the study on the genotoxicity of aqueous extracts from microplastics demonstrated a statistically significant increase in both the frequency of aberrant cells and the number of chromosomal aberrations in the apical meristem cells of barley. All types of microplastics studied—PS, PET, PP, and PE—exhibited mutagenic effects, with these effects being most pronounced for smaller fractions. The average increases in the frequency of aberrant cells and the number of chromosomal aberrations per 100 metaphases were as follows: for the 0.175 mm fraction, 3.32 and 3.84 times; for the 0.3 mm fraction, 3.09 and 3.27 times; and for the 1 mm fraction, 3.06 and 3.23 times, respectively. For the 2 mm and 3 mm fractions, statistically significant genotoxicity was observed only for PET.

The spectrum of chromosomal aberrations was broad and independent of the type and size of microplastic. It included deletions, multiple breaks, centric rings, polyploids, chromosomal bridges, and lagging chromosomes during anaphase. The order of microplastic types based on decreasing genotoxicity was as follows: PET > PS > PE > PP.

This increase in genotoxicity with smaller particle sizes may be attributed to the enhanced dispersibility of microplastics, which leads to an increased surface area for extractable substances to migrate into aqueous solutions (solvents, monomers, etc.). These extracted chemicals can impart toxicity to the aqueous extracts of microplastics and exhibit phytotoxic effects and mutagenic activity in the presence of inert particles [13,14]. Similar unexplained genotoxic effects, which intensified with decreasing particle size, were also reported in experiments involving polystyrene [92].

In contrast, the study of the dermal irritant properties of aqueous extracts from all investigated types of microplastics (PS, PET, PP, and PE) across all fractional sizes revealed no significant changes to the skin compared to the control group. This suggests that a single application of aqueous extracts obtained at room temperature for a short duration (1 day) does not reveal toxic properties of the microplastics. However, further research is necessary, involving repeated exposure to microplastic extracts over extended extraction times (up to 30 days), varying the temperature of distilled water used for the extraction, and simulating conditions that mimic the use of plastics in a domestic setting.

3.3. Results of Acute Toxicity Studies on Frozen Bull Semen

The results of the experiments are presented in Table 3.

Table 3. Toxicity index of aqueous fractions of microplastics established in the experiment on frozen bull semen.

Polymer	Fraction, mm	Toxicity Index, %	Deviation of Sperm Motility of Bull Semen from the Norm, %
Polystyrene (PS)	0.175	94.2	5.8
	0.3	94.0	6.0
	1.0	92.4	7.6
	2.0	93.0	7.0
	3.0	93.8	6.2
The average deviation of the PS is 6.52%			
Polyethylene terephthalate (PET)	0.175	94.2	5.2
	0.3	93.1	6.9
	1.0	96.7	3.3
	2.0	99.4	0.6
	3.0	90.9	9.1
The average deviation on PET is 5.02%			
Polypropylene (PP)	0.175	98.7	0.3
	0.3	99.8	0.2
	1.0	105.6	5.6
	2.0	109.9	9.9
	3.0	107.2	7.2
The average deviation on PP is 4.64%			
Polyethylene (PE)	0.175	97.9	2.1
	0.3	103.7	3.7
	1.0	96.8	3.2
	2.0	101.3	1.3
	3.0	99.5	0.5
The average deviation in PE is 2.16%			

As a result of the experiment, no significant effect of microplastic fractional size within the range of 0.175 to 3 mm on the motility of bovine spermatozoa was observed. However, an increase in toxicity was noted with greater complexity in the polymer structure. The smallest deviation in sperm motility from the control was recorded for polyethylene (PE), which has a simple structure characterized by a straight chain of methylene groups (CH_2). In contrast, polypropylene (PP) has a more complex structure due to the presence of a methyl group in the side chain, resulting in a 2.16% reduction in sperm motility.

Polyethylene terephthalate (PET) features a benzene ring (C_6H_4) in its main chain, which not only enhances the rigidity of the PET macromolecule but also elevates its glass transition temperature and melting point. This structural complexity appears to influence its toxic properties. According to the experimental results, the reduction in sperm motility for PET microplastic particles was 2.32% compared to PE and 8.19% compared to PP.

In contrast, polystyrene (PS) has a benzene ring located in its side chain, which contributes to its brittleness and likely impacts its toxic properties adversely. In our experiment, the change in sperm motility for PS was 1.3% lower than for PET and 3.02% lower than for PE.

It is hypothesized that the experimental conditions—including treatment with boiled water and daily thermostatting at elevated temperatures (40 ± 1.5 °C)—may facilitate the formation of toxic organic compounds such as bisphenol A (BPA) [93,94]. BPA is a known toxicant with detrimental effects on the reproductive system [95,96]. This concern is particularly relevant for polymers with chemical structures that include benzene rings. However, these assumptions warrant further investigation [97].

3.4. Results of Phenol and Formaldehyde Migration Analyses

The results of the experiment are presented in Table 4.

Table 4. Migration of phenol and formaldehyde into aqueous extracts from microplastic fractions of polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE) microplastics.

Polymer PAM	Fraction (mm)	Phenol (mg/dm^3)/PAM Share Not More than 0.05	Formaldehyde (mg/dm^3)/PAM Share Not More than 0.1
Polystyrene (PS)	0.175	<0.01/0.02	0.30/3.0
	0.3	<0.01/0.02	0.40/4.0
	1.0	<0.01/0.02	0.31/3.1
	2.0	<0.01/0.02	0.11/1.1
	3.0	<0.01/0.02	0.12/1.2
Polyethylene terephthalate (PET)	0.175	<0.01/0.02	1.04/10.4
	0.3	<0.01/0.02	0.82/8.2
	1.0	<0.01/0.02	0.50/5.0
	2.0	<0.01/0.02	0.61/6.1
	3.0	<0.01/0.02	0.40/4.0
Polypropylene (PP)	0.175	<0.01/0.02	1.04/10.4
	0.3	<0.01/0.02	1.21/12.1
	1.0	<0.01/0.02	0.30/3.0
	2.0	<0.01/0.02	0.35/3.5
	3.0	<0.01/0.02	0.26/2.6
Polyethylene (PE)	0.175	<0.01/0.02	0.41/4.1
	0.3	<0.01/0.02	0.40/4.0
	1.0	<0.01/0.02	0.34/3.4
	2.0	<0.01/0.02	0.30/3.0
	3.0	<0.01/0.02	0.32/3.2

As shown in Table 4, the migration of phenol into the aqueous extract is observed at levels five times lower than the permissible value for dichloromethane (PAM). In contrast, the concentration of formaldehyde in the water extracts exceeds the PAM limit by factors ranging from 1.1 to 12.1, depending on the type and fraction of microplastic. The highest levels of formaldehyde migration are detected in polypropylene (PP), which we selected as a representative of water pipe material (averaging 6.32 PAM for all fractions), and in polyethylene terephthalate (PET), chosen as a sample of disposable plastic bottles (averaging 6.74 PAM across all fractions).

Notably, the migration of formaldehyde increases as the size of the microplastic fraction decreases. Therefore, it can be concluded that using water pipes made from polyethylene (PE) and PP, as well as disposable tableware crafted from PET and polystyrene (PS), even at room temperature and after a short exposure period (3 h), may result in

formaldehyde migration that surpasses the permissible sanitary standards of the Eurasian Economic Union (EurAsEU).

Furthermore, the use of water pipes made from PP and PE with hot water, and disposable containers made from PET and PS for hot products, may lead to significantly higher levels of formaldehyde migration. This warrants additional research in both aqueous and gaseous environments.

Thus, a comparative analysis of published sources (see Table 5) showed that a number of studies of the toxicity of micro- and nanoplastics on plant test systems (seed germination, growth, and development of plant test objects) were carried out in soil, which, unlike in our experiment and some other studies on plant seeds, allows for climatic factors, soil quality, and microbial community to influence the results obtained [98].

Table 5. Comparative analysis of published sources.

Type of Microplastic (MP or NP)	Concentration	Origin of MP	Object and Studied Indicators	Research Result	Source
PP-MP PE-MP PS-MP	1 g/100 dm^3 of water (extractant, for seeds)	Hand-crushed to resemble natural form	Barley *Hordeum vulgare* L.: seed growth indicators, genotoxicity in apical meristem cells Acute toxicity to bull semen Formaldehyde migration Phenol migration Skin irritant effect on the dermis of rabbits	decreased germination rates, increased frequency of aberrant cells and number of chromosomal aberrations presence of influence on sperm motility exceeding permissible levels of migration no toxic effects detected no toxic effects detected	This study
PS-MP PS-NP	10^{-3}–10^{-7} particles/sm^3 extractant (water)	spherical (standardized production)	Watercress *Lepidium sativum:* seed germination, root and shoot growth, chlorophyll content, seed growth indicators	significant effect on seed germination and root growth on the first day, the adverse effect increased with the transition from NP to MP	[99]
PET-MP PVC-MP	0.5% (by soil weight)	spherical (standardized production)	Tomato *Solanum lycopersicum* L.: growth and number of fruits	Negative effects on growth and physiology, fruit set; increased anti-nutritional properties	[100]
LDPE-MP	0.4% (by soil weight) in a climate-controlled chamber	Hand-crushed	Wheat Triticum aestivum: Wheat growth exposed to low-density polyethylene (LDPE) and biodegradable starch-PET plastics	Negative impact on aboveground and underground parts of the plant during vegetative and reproductive growth	[101]
MP: PET, PA, PP, PE, PU, PS, PC, PES	0.4% (by soil weight)	Hand-chopped	Wild carrot *Daucus carota:* seed germination	Deterioration of seed germination rates depending on MPs shape	[102]
PE-MP	0.25, 0.50, 0.75 and 1.0% of water (extractant, for seeds)	Fragments (industrial production)	Blackgram (*Vigna mungo* L.) and tomato *Solanum lycopersicum* L.): growth and physiological parameters	effect on seed germination, root length and shoots, depending on the dose, type and duration of exposure	[91]
PS (MP, NP)	2 µm–80 nm in 0, 10, 50, 100 and 500 mg/L of water (extractant, for plants in the soil)	Fragments (industrial production)	Ornamental plants: *Trifolium repens*, *Orychophragmus violaceus* and Impatiens balsamina: seed germination tendency, germination rate and various physiological and biochemical parameters	inhibitory effect on seed germination processes	[103]
PMMA (MP, NP)	0, 0.01, 0.1, 1, 5 y 10 g/L (for plants, on soils)	Fragments (industrial production)	Rape *Brassia campestris* L.: Single and combined effects	Suppression of growth index GI, biomass growth biomass, root length and shoot length	[104]

Table 5. *Cont.*

Type of Microplastic (MP or NP)	Concentration	Origin of MP	Object and Studied Indicators	Research Result	Source
HDPE Mater-bi®, MB пакеты	pieces of plastic bags of size approximately 1 cm^2, in liquid (water) to solid (plastic) ratios of 100, 10 and 5, corresponding to approximately 4.1×10^{-4}, 4.1×10^{-3} and 8.3×10^{-3} bag/mL respectively (extractant, for seeds and seedlings)	Hand-crushed to resemble natural form	Garden cress *Lepidium sativum* L: seed germination	developmental abnormalities and decreased plant growth	[43]
PES-MP PP-MP	0.4% (by soil weight)	Fragments (industrial production)	*Corn Zea mays*, *Soybean Glycine max*, *Peanut Arachis hypogaea*: growth, physiological and biochemical parameters	negative consequences for plant growth, biomass accumulation and its quality	[105]
PS-NP	200 nm 0.1–1000 mg/L (for plants in the soil)	Fragments (industrial production)	Rice seeds: seed germination, root growth, antioxidant enzyme activity and transcriptome	gene expression, changes in growth rates, root length, accumulation of reactive oxygen species in the roots. No significant effect on seed germination was found. Significant increase in root length and decrease in antioxidant enzyme activity	[45]
PET-MP PET-NP	0.02% (*w*/*w* microplastic/soil)	industrial pellet crushing	Watercress *Lepidium sativu:* seed germination, plant height, fresh biomass production, oxidative stress response, photosynthetic apparatus disruption, aminolevulinic acid and proline production	The percentage of inhibition of seed germination was the only parameter that showed statistically significant changes	[106]
PS-MP	0.6–0.7 μg/day, 6–7 μg/ day, 60–70 μg/da	spherical (standardized production)	Mice	Decreased sperm quality, abnormal testicular spermatogenesis	[107]
PS-NP	50 mg/kg/day orally	spherical (standardized production)	Mice	Decreased fertility, expression of genes associated with apoptosis and inflammation	[108]

Only in the work [101] was it possible to ensure that climatic factors did not influence the growth of wheat seeds, but the influence of soil parameters remained. Various crops have been chosen as plant test objects, such as cress salad [43,99,106], ornamental plants [103] and wild carrot [102]. Of the agricultural crops, the following plant species were studied that are most typical for the study region: barley (our study), tomatoes and fruits [100], wheat [101], blackgram and tomatoes [91], rapeseed [104], corn, soybean, peanut [105], and rice [45]. Most studies have examined the effects of microplastics on growth, biochemical, and productivity characteristics of plants. However, only a few studies have focused on the effects of microplastic toxicity on seed germination, a critical stage in the plant life cycle. All studies confirm the negative impact of plastic particles on seed germination [103], as established in our experiment, depending on the dose, type of plastics, and duration of exposure, increasing the negative impact on seed germination and root growth in the first day when switching from microplastics to nanoplastics [99], which justifies our choice of the size of the studied particles. The importance of studying seed germination when studying the toxicity of micro- and nanoplastics is confirmed by

the study [106]. Among the parameters studied in the study (seed germination, plant height, fresh biomass production, oxidative stress response, photosynthetic apparatus impairment, and aminolevulinic acid and proline production), the percentage of seed germination inhibition was the only parameter that showed statistically significant changes. In the work [104], inhibition of root growth was observed along with suppression of the growth index GI, biomass growth, and shoot length. One of the studies included the results of a genetic test, which determined gene expression as well as changes in physiological parameters—changes in growth rates, root length, and accumulation of active oxygen species in rice roots [45]. However, the authors did not find any significant effect of polystyrene nanoparticles on seed germination.

The effect of microplastic toxicity on sperm was studied in our experiment on frozen bull sperm, but a comparative assessment with other studies could not be carried out due to the lack of similar experiments. Most studies are conducted on animals (with oral administration), which is subject to the influence of other factors (diet, hunched position of the animals, etc.) and does not allow a clear assessment of the contribution of microplastic toxicity to the effects detected. However, such studies have found decreased sperm quality, abnormal testicular spermatogenesis, decreased fertility, and expression of genes associated with apoptosis and inflammation for microplastics [107] and nanoplastics [108].

The presence of contradictory research results, the organization of phytotoxicity and genotoxicity studies on individual types of microplastics (mostly represented by industrial samples of spherical particles, far from the real shape of micro- and nanoplastics in water), a small selection of plastic types (often polystyrene as an object of influence), and toxicity assessment only on single test objects, in contrast to the comprehensive approach in our case, make this study relevant and new.

4. Conclusions

The presence of microplastics (polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), and polyethylene (PE)) in the tap water of Kokshetau city (Akmola region, Kazakhstan) has raised concerns regarding the potential toxic risks associated with microplastics in the region's drinking water and the possibility of skin irritation during bathing. Our study evaluated the toxicity of aqueous extracts of polymer particles fragmented to microplastic sizes rather than the polymers themselves. The investigation encompassed various microplastic fractions (0.175 mm, 0.3 mm, 1 mm, 2 mm, and 3 mm), thus varying the contact surface area of microplastics with water and their extraction efficiency.

Recognizing the inadequacy of assessing the toxic properties of synthetic materials through a singular method, and to mitigate ethical concerns regarding human and extensive animal testing, we conducted a comprehensive evaluation of the toxicity of aqueous extracts of microplastics on both plant and animal test organisms, including frozen bull semen, alongside analyses of organic matter migration into the aquatic environment.

Germination experiments with *Hordeum vulgare* L. seeds revealed phytotoxic effects of water extracts from PS, PET, PP, and PE microplastics, particularly for the 0.175 mm fraction. The 0.3 mm fraction of PP and PE, the 1.0 mm fraction of PS, the 2.0 mm fraction of PE, and the 3.0 mm fractions of PP and PE significantly reduced various physiological indices of barley. Phytotoxicity manifested as inhibited growth and seed rot. No significant differences in phytotoxicity were observed among the different microplastic types.

Genotoxicity studies demonstrated a significant increase in the frequency of aberrant cells and chromosomal aberrations in barley apical meristem cells exposed to aqueous extracts of all microplastic types (PS, PET, PP, and PE), with smaller fractions exhibiting more pronounced effects. The 0.175 mm fraction increased the frequency of aberrant cells and chromosomal aberrations by 3.32 and 3.84 times, respectively, compared to the control. Similar increases were observed for the 0.3 mm and 1 mm fractions. PET microplastics, especially in the 2 mm and 3 mm fractions, also exhibited significant genotoxicity. The spectrum of chromosomal aberrations was broad, encompassing deletions, multiple breaks, centric rings, polyploidy, chromosomal bridges, and lagging chromosomes, and was in-

dependent of microplastic type or size. In terms of decreasing genotoxicity, the order was PET > PS > PE > PP.

No significant skin irritation was observed from aqueous extracts of any microplastic type or size. The assessment of bovine semen motility revealed increased toxicity with increasing polymer complexity, regardless of particle size within the 0.175–3 mm range. The mean motility reduction was 6.52% for PS, 5.02% for PET, 4.64% for PP, and 2.16% for PE, indicating a toxicity order of PS > PET > PP > PE.

While phenol migration from microplastics was within permissible limits in Kazakhstan, formaldehyde migration exceeded limits for all microplastic types and sizes. Formaldehyde migration increased with decreasing particle size, with the highest levels observed for PET, followed by PP, PE, and PS.

These findings suggest that both particle size and polymer structure influence microplastic toxicity. Smaller particles with larger surface areas facilitate the leaching of non-covalently bound chemicals into the aqueous phase. The discrepancy in toxicity rankings across different experiments indicates that factors beyond formaldehyde migration may contribute to the observed toxic effects and warrant further investigation.

Based on these findings, several recommendations emerge. Policymakers should implement stricter regulations on microplastic pollution in water bodies and invest in advanced water treatment technologies. Additionally, regulatory limits for water pipes and plastic containers should be reevaluated to account for the potential release of hazardous compounds like formaldehyde.

This study provides a foundational understanding of the toxicity and mutagenicity of aqueous microplastic extracts. While human exposure was not directly assessed, the results can inform predictions of human health impacts. Future research should quantify the toxic risks associated with chronic microplastic ingestion, identify safe exposure limits, and investigate the potential carcinogenicity of these particles. Moreover, future studies should expand the scope to include a wider range of microplastic types, long-term exposure assessments, and the combined effects of multiple contaminants.

Author Contributions: Conceptualization, N.S.S.; methodology, N.S.S., A.V.L. and S.Z.K.; software, A.V.L., S.Z.K. and A.U.B.; validation, A.V.L., S.Z.K., A.U.B. and S.E.U.; formal analysis, N.S.S. and J.R.-I.; investigation, A.V.L., S.Z.K., S.E.U. and A.U.B.; resources, N.S.S.; data curation, A.V.L., S.Z.K. and A.U.B.; writing—original draft preparation, N.S.S. and J.R.-I.; writing—review and editing, J.R.-I. and M.-E.R.-C.; visualization, J.R.-I. and M.-E.R.-C.; supervision, N.S.S. and J.R.-I.; project administration, N.S.S.; funding acquisition, N.S.S. and J.R.-I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan through the research project entitled "Health Risk Modelling Based on the Identification of Microplastics in Water Systems and the Reasoning About Actions to Manage the Water Resources Quality" (Grant No. AP14869081).

Data Availability Statement: Data is contained within the article.

Acknowledgments: The authors acknowledge Erasmus + CBHE project «Land management, Environment and SoLId-WastE: inside education and business in Central Asia» (LESLIE). Project number: ERASMUS-EDU-2023-CBHE no. 101129032. for its cooperation in the dissemination of this work.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Khare, R.; Khare, S. Polymer and its effect on environment. *J. Indian. Chem. Soc.* **2023**, *100*, 100821. [CrossRef]
2. Wiesinger, H.; Wang, Z.; Hellweg, S. Deep Dive into Plastic Monomers, Additives, and Processing Aids. *Environ. Sci. Technol.* **2021**, *55*, 9339–9351. [CrossRef] [PubMed]
3. Viljoen, S.J.; Brailsford, F.L.; Murphy, D.V.; Hoyle, F.C.; Chadwick, D.R.; Jones, D.L. Leaching of phthalate acid esters from plastic mulch films and their degradation in response to UV irradiation and contrasting soil conditions. *J. Hazard. Mater.* **2023**, *443*, 130256. [CrossRef] [PubMed]

4. Teuten, E.L.; Saquing, J.M.; Knappe, D.R.; Barlaz, M.A.; Jonsson, S.; Björn, A.; Rowland, S.J.; Thompson, R.C.; Galloway, T.S.; Yamashita, R.; et al. Transport and release of chemicals from plastics to the environment and to wildlife. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **2009**, *364*, 2027–2045. [CrossRef] [PubMed]
5. Gilbert, J.; Startin, J.R.; McGuinness, J.D. Compositional analysis of commercial PVC bottles and studies of aspects of specific and overall migration into foods and stimulants. *Food. Addit. Contam.* **1986**, *3*, 133–143. [CrossRef]
6. Bostan, N.; Ilyas, N.; Akhtar, N.; Mehmood, S.; Saman, R.U.; Sayyed, R.Z.; Shatid, A.A.; Alfaifi, M.Y.; Elbehairi, S.E.I.; Pandiaraj, S. Toxicity assessment of microplastic (MPs); a threat to the ecosystem. *Environ. Res.* **2023**, *234*, 116523. [CrossRef]
7. Auta, H.S.; Emenike, C.U.; Fauziah, S.H. Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environ. Int.* **2017**, *102*, 165–176. [CrossRef]
8. Guo, J.J.; Huang, X.P.; Xiang, L.; Wang, Y.Z.; Li, Y.W.; Li, H.; Cai, Q.Y.; Mo, C.H.; Wong, M.H. Source, migration and toxicology of microplastics in soil. *Environ. Int.* **2020**, *137*, 105263. [CrossRef]
9. O'Brien, S.; Rauert, C.; Ribeiro, F.; Okoffo, E.D.; Burrows, S.D.; O'Brien, J.W.; Wang, X.; Wright, S.L.; Thomas, K.V. There's something in the air: A review of sources, prevalence and behaviour of microplastics in the atmosphere. *Sci. Total Environ.* **2023**, *874*, 162193. [CrossRef]
10. Calero, M.; Martín-Lara, M.A.; Godoy, V.; Quesada, L.; Martínez, D.; Peula, F.; Soto, J.M. Characterization of plastic materials present in municipal solid waste: Preliminary study for their mechanical recycling. *Detritus* **2018**, *4*, 104. [CrossRef]
11. de Souza Machado, A.A.; Lau, C.W.; Till, J.; Kloas, W.; Lehmann, A.; Becker, R.; Rillig, M.C. Impacts of Microplastics on the Soil Biophysical Environment. *Environ. Sci. Technol.* **2018**, *52*, 9656–9665. [CrossRef] [PubMed]
12. Yu, Y.; Kumar, M.; Bolan, S.; Padhye, L.P.; Bolan, N.; Li, S.; Wang, L.; Hou, D.; Li, Y. Various additive release from microplastics and their toxicity in aquatic environments. *Environ. Pollut.* **2024**, *343*, 123219. [CrossRef] [PubMed]
13. Wu, P.; Huang, J.; Zheng, Y.; Yang, Y.; Zhang, Y.; He, F.; Chen, H.; Quan, G.; Yan, J.; Li, T.; et al. Environmental occurrences, fate, and impacts of microplastics. *Ecotoxicol. Environ. Saf.* **2019**, *184*, 109612. [CrossRef] [PubMed]
14. Liu, G.; Wang, J.; Wang, M.; Ying, R.; Li, X.; Hu, Z.; Zhang, Y. Disposable plastic materials release microplastics and harmful substances in hot water. *Sci. Total Environ.* **2022**, *818*, 151685. [CrossRef] [PubMed]
15. Galloway, T.S.; Cole, M.; Lewis, C. Interactions of microplastic debris throughout the marine ecosystem. *Nat. Ecol. Evol.* **2017**, *1*, 0116. [CrossRef]
16. Horton, A.A.; Walton, A.; Spurgeon, D.J.; Lahive, E.; Svendsen, C. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total Environ.* **2017**, *586*, 127–141. [CrossRef]
17. Wojnowska-Baryła, I.; Bernat, K.; Zaborowska, M. Plastic Waste Degradation in Landfill Conditions: The Problem with Microplastics, and Their Direct and Indirect Environmental Effects. *Int. J. Environ. Res. Public. Health* **2022**, *19*, 13223. [CrossRef]
18. Caldeira, D.F.; Paulini, F.; Silva, R.C.; de Azevedo, R.B.; Lucci, C.M. In vitro exposure of bull sperm cells to DMSA-coated maghemite nanoparticles does not affect cell functionality or structure. *Int. J. Hyperth.* **2018**, *34*, 415–422. [CrossRef]
19. Crosta, A.; Parolini, M.; De Felice, B. Microplastics Contamination in Nonalcoholic Beverages from the Italian Market. *Int. J. Environ. Res. Public Health* **2023**, *20*, 4122. [CrossRef]
20. Sewwandi, M.; Wijesekara, H.; Rajapaksha, A.U.; Soysa, S.; Vithanage, M. Microplastics and plastics-associated contaminants in food and beverages; Global trends, concentrations, and human exposure. *Environ. Pollut.* **2023**, *317*, 120747. [CrossRef]
21. Makhdoumi, P.; Hossini, H.; Pirsaheb, M. A review of microplastic pollution in commercial fish for human consumption. *Rev. Environ. Health* **2023**, *38*, 97–109. [CrossRef] [PubMed]
22. Mintenig, S.M.; Löder, M.G.J.; Primpke, S.; Gerdts, G. Low numbers of microplastics detected in drinking water from ground water sources. *Sci. Total Environ.* **2019**, *648*, 631–635. [CrossRef] [PubMed]
23. Welle, F.; Franz, R. Microplastic in bottled natural mineral water-literature review and considerations on exposure and risk assessment. *Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess.* **2018**, *35*, 2482–2492. [CrossRef] [PubMed]
24. Senathirajah, K.; Attwood, S.; Bhagwat, G.; Carbery, M.; Wilson, S.; Palanisami, T. Estimation of the mass of microplastics ingested–A pivotal first step towards human health risk assessment. *J. Hazard. Mater.* **2021**, *404*, 124004. [CrossRef] [PubMed]
25. Deng, L.; Liu, Y.X.; Chen, P.Y.; Wang, L.; Deng, N.S. Determination of Trace Bisphenol A in Leachate by Solid Phase Microextraction Coupled with High Performance Liquid Chromatography. *Anal. Lett.* **2006**, *39*, 395–404. [CrossRef]
26. Fromme, H.; Küchler, T.; Otto, T.; Pilz, K.; Müller, J.; Wenzel, A. Occurrence of phthalates and bisphenol A and F in the environment. *Water Res.* **2002**, *36*, 1429–1438. [CrossRef]
27. Lehel, J.; Murphy, S. Microplastics in the Food Chain: Food Safety and Environmental Aspects. *Rev. Environ. Contam. Toxicol.* **2021**, *259*, 1–49. [CrossRef]
28. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. Microplastics as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* **2011**, *62*, 2588–2597. [CrossRef]
29. Zarfl, C.; Fleet, D.; Fries, E.; Galgani, F.; Gerdts, G.; Hanke, G.; Matthies, M. Microplastics in oceans. *Mar. Pollut. Bull.* **2011**, *62*, 1589–1591. [CrossRef]
30. Browne, M.A.; Dissanayake, A.; Galloway, T.S.; Lowe, D.M.; Thompson, R.C. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environ. Sci. Technol.* **2002**, *42*, 5026–5031. [CrossRef]
31. Guševac Stojanović, I.; Drakulić, D.; Todorović, A.; Martinović, J.; Filipović, N.; Stojanović, Z. Acute Toxicity Assessment of Orally Administered Microplastic Particles in Adult Male Wistar Rats. *Toxics* **2024**, *12*, 167. [CrossRef] [PubMed]

32. Blackburn, K.; Green, D. The potential effects of microplastics on human health: What is known and what is unknown. *Ambio* **2021**, *51*, 518–530. [CrossRef] [PubMed]
33. Xu, J.L.; Lin, X.; Wang, J.J.; Gowen, A.A. A review of potential human health impacts of micro- and nanoplastics exposure. *Sci. Total Environ.* **2022**, *851*, 158111. [CrossRef] [PubMed]
34. Çobanoğlu, H.; Belivermiş, M.; Sıkdokur, E.; Kılıç, Ö.; Çayır, A. Genotoxic and cytotoxic effects of polyethylene microplastics on human peripheral blood lymphocytes. *Chemosphere* **2021**, *272*, 129805. [CrossRef] [PubMed]
35. Roshanzadeh, A.; Oyunbaatar, N.E.; Ganjbakhsh, S.E.; Park, S.; Kim, D.S.; Kanade, P.P.; Lee, S.; Lee, D.W.; Kim, E.S. Exposure to nanoplastics impairs collective contractility of neonatal cardiomyocytes under electrical synchronization. *Biomaterials* **2021**, *278*, 121175. [CrossRef] [PubMed]
36. Winkler, A.S.; Cherubini, A.; Rusconi, F.; Santo, N.; Madaschi, L.; Pistoni, C.; Moschetti, G.; Sarnicola, M.L.; Crosti, M.; Rosso, L. Human airway organoids and microplastic fibers: A new exposure model for emerging contaminants. *Environ. Int.* **2022**, *163*, 107200. [CrossRef]
37. Li, Y.; Tao, L.; Wang, Q.; Wang, F.; Li, G.; Song, M. Potential Health Impact of Microplastics: A Review of Environmental Distribution, Human Exposure, and Toxic Effects. *Environ. Health* **2023**, *1*, 249–257. [CrossRef]
38. Wu, S.; Wu, M.; Tian, D.; Qiu, L.; Li, T. Effects of polystyrene microbeads on cytotoxicity and transcriptomic profiles in human Caco-2 cells. *Env. Toxicol.* **2020**, *35*, 495–506. [CrossRef]
39. Wu, P.; Lin, S.; Cao, G.; Wu, J.; Jin, H.; Wang, C.; Wong, M.H.; Yang, Z.; Cai, Z. Absorption, distribution, metabolism, excretion and toxicity of microplastics in the human body and health implications. *J. Hazard. Mater.* **2022**, *437*, 129361. [CrossRef]
40. Prata, J.C. Airborne microplastics: Consequences to human health? *Environ. Pollut.* **2018**, *234*, 115–126. [CrossRef]
41. Bunge, A.; Lugert, V.; McClure, M.; Kammann, U.; Hanel, R.; Scharsack, J.P. Less impact than suspected: Dietary exposure of three-spined sticklebacks to microplastic fibers does not affect their body condition and immune parameters. *Sci. Total Environ.* **2022**, *819*, 153077. [CrossRef] [PubMed]
42. Jemec, A.; Horvat, P.; Kunej, U.; Bele, M.; Kržan, A. Uptake and effects of microplastic textile fibers on freshwater crustacean Daphnia magna. *Environ. Pollut.* **2016**, *219*, 201–209. [CrossRef] [PubMed]
43. Balestri, E.; Menicagli, V.; Ligorini, V.; Fulignati, S.; Galletti, A.M.R.; Lardicci, C. Phytotoxicity assessment of conventional and biodegradable plastic bags using seed germination test. *Ecol. Indic.* **2019**, *102*, 569–580. [CrossRef]
44. Bosker, T.; Bouwman, L.J.; Brun, N.R.; Behrens, P.; Vijver, M.G. Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant Lepidium sativum. *Chemosphere* **2019**, *226*, 774–781. [CrossRef]
45. Zhang, Q.; Zhao, M.; Meng, F.; Xiao, Y.; Dai, W.; Luan, Y. Effect of polystyrene microplastics on rice seed germination and antioxidant enzyme activity. *Toxics* **2021**, *9*, 179. [CrossRef]
46. Jia, L.; Liu, L.; Zhang, Y.; Fu, W.; Liu, X.; Wang, Q.; Tanveer, M.; Huang, L. Microplastic stress in plants: Effects on plant growth and their remediations. *Front. Plant Sci.* **2023**, *14*, 1226484. [CrossRef]
47. Yang, Z.; DeLoid, G.M.; Baw, J.; Zarbl, H.; Demokritou, P. Assessment of Ingested Micro- and Nanoplastic (MNP)-Mediated Genotoxicity in an In Vitro Model of the Small Intestinal Epithelium (SIE). *Nanomaterials* **2024**, *14*, 807. [CrossRef]
48. Roursgaard, M.; Rothmann, M.H.; Schulte, J.; Karadimou, I.; Marinelli, E.; Møller, P. Genotoxicity of Particles From Grinded Plastic Items in Caco-2 and HepG2 Cells. *Front. Public Health* **2022**, *10*, 906430. [CrossRef]
49. Wang, F.; Wong, C.S.; Chen, D.; Lu, X.; Wang, F.; Zeng, E.Y. Interaction of toxic chemicals with microplastics: A critical review. *Water Res.* **2018**, *139*, 208–219. [CrossRef]
50. Revel, M.; Châtel, A.; Mouneyrac, C. Micro(nano)plastics: A threat to human health? *Curr. Opin. Env. Sci. Health* **2018**, *1*, 17–23. [CrossRef]
51. Salikova, N.S.; Rodrigo-Ilarri, J.; Makeyeva, L.A.; Rodrigo-Clavero, M.-E.; Tleuova, Z.O.; Makhmutova, A.D. Monitoring of Microplastics in Water and Sediment Samples of Lakes and Rivers of the Akmola Region (Kazakhstan). *Water* **2024**, *16*, 1051. [CrossRef]
52. Salikova, N.S.; Kerimkulova, A.R.; Rodrigo-Ilarri, J.; Alimova, K.K.; Rodrigo-Clavero, M.E.; Kapbassova, G.A. Sorption-Based Removal Techniques for Microplastic Contamination of Tap Water. *Water* **2024**, *16*, 1363. [CrossRef]
53. Salikova, N.S.; Alimova, K.K. Communal, Domestic and Industrial Water Supply in Kazakhstan: Interim Report on the Project IRN AP14869081 'Modelling of Health Risks Based on Identification of Microplastics in Water Systems and Justification of Actions to Manage the Quality of Water Resources. Kokshetau. 2022. Available online: https://kuam.edu.kz/sites/default/files/2022/salikova_n.s._alimova_k.k._communal_domestic_and_industrial_water_supply_in_kazakhstan._interim_report_on_the_project_irn_ap14869081.pdf (accessed on 4 November 2024).
54. Salikova, N.S.; Rodrigo-Ilarri, J.; Rodrigo-Clavero, M.E.; Urazbayeva, S.E.; Askarova, A.Z.; Magzhanov, K.M. Environmental Assessment of Microplastic Pollution Induced by Solid Waste Landfills in the Akmola Region (North Kazakhstan). *Water* **2023**, *15*, 2889. [CrossRef]
55. Nelson, C.P.; Patton, G.W.; Arvidson, K.; Lee, H.; Twaroski, M.L. Assessing the toxicity of polymeric food-contact substances. *Food Chem. Toxicol.* **2011**, *49*, 1877–1897. [CrossRef]
56. Lytle, J.S.; Lytle, T.F. Use of plants for toxicity assessment of estuarine ecosystems. *Environ. Toxicol. Chem.* **2001**, *20*, 68–83. [CrossRef]
57. Ribé, V.; Nehrenheim, E.; Odlare, M. Assessment of mobility and bioavailability of contaminants in MSW incineration ash with aquatic and terrestrial bioassays. *Waste Manag.* **2014**, *34*, 1871–1876. [CrossRef]

58. Hubai, K.; Kováts, N.; Sainnokhoi, T.-A.; Eck-Varanka, B.; Hoffer, A.; Tóth, Á.; Teke, G. Phytotoxicity of particulate matter from controlled burning of different plastic waste types. *Bull. Environ. Contam. Toxicol.* **2022**, *109*, 852–858. [CrossRef]
59. Sekar, V.; Shaji, S.; Sundaram, B. Microplastic prevalence and human exposure in the bottled drinking water in the west Godavari region of Andhra Pradesh, India. *J. Contam. Hydrol.* **2024**, *264*, 104346. [CrossRef]
60. Mohanan, N.; Montazer, Z.; Sharma, P.K.; Levin, D.B. Microbial and Enzymatic Degradation of Synthetic Plastics. *Front. Microbiol.* **2020**, *11*, 580709. [CrossRef]
61. Jahan, I.; Chowdhury, G.; Rafi, S.; Ashab, A.; Sarker, M.; Chakraborty, A.; Couetard, N.; Kabir, M.A.; Hossain, M.A.; Iqbal, M.M. Assessment of dietary polyvinylchloride, polypropylene and polyethylene terephthalate exposure in Nile tilapia, Oreochromis niloticus: Bioaccumulation, and effects on behaviour, growth, hematology and histology. *Environ. Pollut.* **2024**, *345*, 123548. [CrossRef]
62. Giorgetti, L.; Spanò, C.; Muccifora, S.; Bottega, S.; Barbieri, F.; Bellani, L.; Castiglione, M.R. Exploring the interaction between polystyrene nanoplastics and Allium cepa during germination: Internalization in root cells, induction of toxicity and oxidative stress. *Plant Physiol. Biochem.* **2020**, *149*, 170–177. [CrossRef] [PubMed]
63. Maity, S.; Chatterjee, A.; Guchhait, R.; De, S.; Pramanick, K. Cytogenotoxic potential of a hazardous material, polystyrene microparticles on *Allium cepa* L. *J. Hazard Mater.* **2020**, *385*, 121560. [CrossRef] [PubMed]
64. Phillips, L.; Steinberg, M.; Maibach, H.I.; Akers, W.A. A comparison of rabbit and human skin response to certain irritants. *Toxicol. Appl. Pharmacol.* **1972**, *21*, 369–382. [CrossRef] [PubMed]
65. Marzulli, F.N.; Maibach, H.I. The rabbit as a model for evaluating skin irritants: A comparison of results obtained on animals and man using repeated skin exposures. *Food Cosmet. Toxicol.* **1975**, *13*, 533–540. [CrossRef]
66. Rooney, J.P.; Choksi, N.Y.; Ceger, P.; Daniel, A.B.; Truax, J.; Allen, D.; Kleinstreuer, N. Analysis of variability in the rabbit skin irritation assay. *Regul. Toxicol. Pharmacol.* **2021**, *122*, 104920. [CrossRef]
67. Knasiak-Paluch, D. Toxicity of plastic materials as evaluated by means of bull semen (preliminary report). *Pol. J. Pharmacol. Pharm.* **1980**, *32*, 109–114.
68. Petzoldt, R.; Wolf, H.; Reefschläger, J.; Karge, M.; Berger, G. A rapid quantitative method based on motility of bull sperm cells for in vitro toxicity testing of biomaterials. *Biomaterials* **1985**, *6*, 105–109. [CrossRef]
69. Paluch, D.; Borzemska-Szymonowicz, M.; Olszewska-Błach, Z. In vitro screening studies of the toxicological testing of synthetic biomaterials. Introduction and comparative evaluation of a new method of testing applying breeding bull semen. *Polim. Med.* **1980**, *10*, 193–204.
70. Paluch, D. Comparative studies in vitro of the toxic effect of water extracts from synthetic materials in biological tests on living cells. *Polym. Med.* **1982**, *12*, 79–130.
71. Makhluf, S.B.D.; Qasem, R.; Rubinstein, S.; Gedanken, A.; Breitbart, H. Loading magnetic nanoparticles into sperm cells does not affect their functionality. *Langmuir* **2006**, *22*, 9480–9482. [CrossRef]
72. Zakhidov, S.T.; Marshak, T.L.; Malolina, E.A.; Kulibin, A.Y.; Zelenina, I.; Pavluchenkova, S.M.; Rudoi, V.M.; Dement'eva, O.V.; Skuridin, S.G.; Evdokimov, Y.M. Gold nanoparticles disturb nuclear chromatin decondensation in mouse sperm in vitro. *Biochem. (Mosc.) Suppl. Ser. A Membr. Cell Biol.* **2010**, *4*, 293–296. [CrossRef]
73. Grechi, N.; Ferronato, G.A.; Devkota, S.; Ferraz, M.A.M.M. Microplastics are detected in bull and dog sperm and polystyrene microparticles impair sperm fertilization. *bioRxiv* **2023**. [CrossRef]
74. Zhang, C.; Chen, J.; Ma, S.; Sun, Z.; Wang, Z. Microplastics May Be a Significant Cause of Male Infertility. *Am. J. Men's Health* **2022**, *16*, 15579883221096549. [CrossRef] [PubMed]
75. Hong, Y.; Wu, S.; Wei, G. Adverse effects of microplastics and nanoplastics on the reproductive system: A comprehensive review of fertility and potential harmful interactions. *Sci. Total Environ.* **2023**, *903*, 166258. [CrossRef] [PubMed]
76. Hahladakis, J.N.; Velis, C.A.; Weber, R.; Iacovidou, E.; Purnell, P. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. *J. Hazard. Mater.* **2018**, *344*, 179–199. [CrossRef]
77. Gami, A.A.; Shukor, M.Y.; Khalil, K.A.; Dahalan, F.A.; Khalid, A.; Ahmad, S.A. Phenol and its toxicity. *J. Environ. Microbiol. Toxicol.* **2014**, *2*, 11–23. [CrossRef]
78. Kuptsov, A.; Zhizhin, G. *Fourier-Raman and Fourier-IR Spectra of Polymers*; Fizmatlit: Moscow, Russia, 2001. Available online: https://www.researchgate.net/publication/262416516_Kupcov_AH_Zizin_GN_Fure-KR_i_Fure-IK_spektry_polimerov_m_Fizmatlit_2001_657_s (accessed on 1 May 2024).
79. Fan, J.; Zou, L.; Zhao, G. Microplastic abundance, distribution, and composition in the surface water and sediments of the Yangtze River along Chongqing City, China. *J. Soils Sediments* **2021**, *21*, 1840–1851. [CrossRef]
80. Crawford, C.B.; Quinn, B. *Microplastic Pollutants*, 1st ed.; Elsevier Limited: Amsterdam, The Netherlands, 2016.
81. Boehme, K.; Dietz, Y.; Hewitt, P.; Mueller, S.O. Activation of P53 in HepG2 cells as surrogate to detect mutagens and promutagens in vitro. *Toxicol. Lett.* **2010**, *198*, 272–281. [CrossRef]
82. Doak, S.H.; Brüsehafer, K.; Dudley, E.; Quick, E.; Johnson, G.; Newton, R.P.; Jenkins, G.J. No-observed effect levels are associated with up-regulation of MGMT following MMS exposure. *Mutat. Res./Fundam. Mol. Mech. Mutagen.* **2008**, *648*, 9–14. [CrossRef]
83. Khalid, N.; Aqeel, M.; Noman, A. Microplastics could be a threat to plants in terrestrial systems directly or indirectly. *Environ. Pollut.* **2020**, *267*, 115653. [CrossRef]

84. Minister of Health of the Republic of Kazakhstan. Order of the Acting Minister of Health of the Republic of Kazakhstan Dated March 24, 2005 No. 136. Sanitary and Epidemiological Rules and Norms "Sanitary and Epidemio-logical Requirements for the Content and Working Conditions in Laboratories Per-forming Chemical, Toxicological, and Radiological Research". 2005. Available online: https://adilet.zan.kz/rus/docs/V1700015990 (accessed on 4 November 2024).
85. Kolumbayeva, S.J.; Lovinskaya, A.V.; Kalimagambetov, A.M. *Cytogenetic Methods in Genetic Monitoring*; Kazak University: Almaty, Kazakhstan, 2017.
86. USSR Ministry of Health. Assessment of the Impact of Harmful Chemical Compounds on the Skin and Justification of Maximum Permissible Levels of Skin Contamination.-Methodical Instructions No. 2102. Moscow. 1979. Available online: https://meganorm.ru/Data2/1/4293782/4293782372.htm (accessed on 3 May 2024).
87. *MU 1.1.037-95*; Biotesting of Products from Polymeric and Other Materials. Russian Technical Standard: Moscow, Russia, 1996.
88. Eurasian Economic Commission. Uniform Sanitary-Epidemiological and Hygienic Requirements for Products (Goods) Subject to Sanitary and Epidemiological Supervision (Control) № 299 of 28 May 2010. Section 16: Requirements for Materials and Products Made of Polymeric and Other Materials Intended for Contact with Food Products and Media. 2010. Available online: https://docs.eaeunion.org/docs/en-us/0027349/cuc_28062010_299_doc.pdf (accessed on 4 November 2024).
89. Russia Ministry of Health. Guideline Document RD 52.24.492-2006 p.11 Mass Concentration of Formaldehyde in Waters Methodology of Measurements by Photometric Method with Acetylacetone. Moscow. 2009. Available online: https://meganorm.ru/Data2/1/4293837/4293837383.pdf (accessed on 4 November 2024).
90. Liwarska-Bizukojc, E. Phytotoxicity assessment of biodegradable and non-biodegradable plastics using seed germination and early growth tests. *Chemosphere* **2022**, *289*, 133132. [CrossRef] [PubMed]
91. Sahasa, R.G.K.; Dhevagi, P.; Poornima, R.; Ramya, A.; Moorthy, P.S.; Alagirisamy, B.; Karthikeyan, S. Effect of polyethylene microplastics on seed germination of Blackgram (*Vigna mungo* L.) and Tomato (*Solanum lycopersicum* L.). *Environ. Adv.* **2023**, *11*, 100349. [CrossRef]
92. Jiang, X.; Chen, H.; Liao, Y.; Ye, Z.; Li, M.; Klobučar, G. Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant Vicia faba. *Environ. Pollut.* **2019**, *250*, 831–838. [CrossRef] [PubMed]
93. Fan, Y.Y.; Zheng, J.L.; Ren, J.H.; Luo, J.; Cui, X.Y.; Ma, L.Q. Effects of storage temperature and duration on release of antimony and bisphenol A from polyethylene terephthalate drinking water bottles of China. *Environ. Pollut.* **2014**, *192*, 113–120. [CrossRef] [PubMed]
94. Kazemi, A.; Younesi, H.; Bahramifar, N. Migration of bisphenol A and nonylphenol from mineral water bottles and disposable plastic containers into water at different temperatures. *Iran. J. Health Environ.* **2014**, *6*, 515–522.
95. Ginter-Kramarczyk, D.; Zembrzuska, J.; Kruszelnicka, I.; Zając-Woźnialis, A.; Ciślak, M. Influence of Temperature on the Quantity of Bisphenol A in Bottled Drinking Water. *Int. J. Environ. Res. Public Health* **2022**, *19*, 5710. [CrossRef]
96. Casajuana, N.; Lacorte, S. Presence and release of phthalic esters and other endocrine disrupting compounds in drinking water. *Chromatographia* **2003**, *57*, 649–655. [CrossRef]
97. Daniels, D.; Eberhardt, A.B. Climate change, microplastics, and male infertility. *Curr. Opin. Urol.* **2024**, *34*, 366–370. [CrossRef]
98. Hasan, M.M.; Jho, E.H. Effect of Microplastics on the Germination and Growth of Terrestrial Plants. *J. Korean Soc. Environ. Eng.* **2022**, *44*, 375–382. [CrossRef]
99. De Silva, Y.S.K.; Rajagopalan, U.M.; Kadono, H. Microplastics on the growth of plants and seed germina-tion in aquatic and terrestrial ecosystems. *Glob. J. Environ. Sci. Manag.* **2021**, *7*, 347–368. [CrossRef]
100. Dainelli, M.; Pignattelli, S.; Bazihizina, N.; Falsini, S.; Papini, A.; Baccelli, I.; Mancuso, S.; Coppi, A.; Castellani, M.B.; Colzi, I.; et al. Can microplastics threaten plant productivity and fruit quality? Insights from Micro-Tom and Micro-PET/PVC. *Sci. Total Environ.* **2023**, *895*, 165119. [CrossRef] [PubMed]
101. Qi, Y.; Yang, X.; Pelaez, A.M.; Lwanga, E.H.; Beriot, N.; Gertsen, H.; Garbeva, P.; Geissen, V. Macro- and micro- plastics in soil-plant system: Effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth. *Sci. Total Environ.* **2018**, *645*, 1048–1056. [CrossRef] [PubMed]
102. Lozano, Y.M.; Caesaria, P.U.; Rillig, M.C. Microplastics of different shapes increase seed germination syn-chrony while only films and fibers affect seed germination velocity. *Front. Environ. Sci.* **2022**, *10*, 1017349. [CrossRef]
103. Guo, M.; Zhao, F.; Tian, L.; Ni, K.; Lu, Y.; Borah, P. Effects of polystyrene microplastics on the seed germination of herbaceous ornamental plants. *Sci. Total Environ.* **2022**, *809*, 151100. [CrossRef] [PubMed]
104. Dong, R.; Liu, R.; Xu, Y.; Liu, W.; Wang, L.; Liang, X.; Huang, Q.; Sun, Y. Single and joint toxicity of polymethyl methacrylate microplastics and As (V) on rapeseed (*Bras-sia campestris* L.). *Chemosphere* **2022**, *291*, 133066. [CrossRef]
105. Zhou, W.; Wang, Q.; Wei, Z.; Jiang, J.; Deng, J. Effects of microplastic type on growth and physiology of soil crops: Implications for farmland yield and food quality. *Environ. Pollut.* **2023**, *326*, 121512. [CrossRef]
106. Pignattelli, S.; Broccoli, A.; Piccardo, M.; Terlizzi, A.; Renzi, M. Effects of polyethylene terephthalate (PET) microplastics and acid rain on physiology and growth of Lepidium sativum. *Environ. Pollut.* **2021**, *282*, 116997. [CrossRef]

107. Hou, B.; Wang, F.; Liu, T.; Wang, Z. Reproductive toxicity of polystyrene microplastics: In vivo experimental study on testicular toxicity in mice. *J. Hazard. Mater.* **2021**, *405*, 124028. [CrossRef]
108. Xu, W.; Yuan, Y.; Tian, Y.; Cheng, C.; Chen, Y.; Zeng, L.; Yuan, Y.; Li, D.; Zheng, L.; Luo, T. Oral exposure to polystyrene nanoplastics reduced male fertility and even caused male infertility by inducing testicular and sperm toxicities in mice. *J. Hazard. Mater.* **2023**, *454*, 131470. [CrossRef]

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Microplastic Transport and Accumulation in Rural Waterbodies: Insights from a Small Catchment in East China

Tom Lotz [1,2,*], Wenjun Chen [3,4] and Shoubao Su [1,2,5]

[1] School of Computer Engineering, Jinling Institute of Technology, Hongjing Avenue 99, Nanjing 211169, China; showbo@jit.edu.cn
[2] Jiangsu Key Laboratory of Data Science & Smart Software, Jinling Institute of Technology, Hongjing Avenue 99, Nanjing 211169, China
[3] School of Software Engineering, Jinling Institute of Technology, Hongjing Avenue 99, Nanjing 211169, China; chenwenjun@jit.edu.cn
[4] Key Laboratory of Watershed Geographic Science, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China
[5] School of Computer, Jiangsu University of Science and Technology, Changhui Road 666, Zhenjiang 212003, China
* Correspondence: tomlotz@jit.edu.cn or tom.lotz@outlook.com

Abstract: Microplastic (MP) pollution in agricultural ecosystems is an emerging environmental concern, with limited knowledge of its transport and accumulation in rural waterbodies. This study investigates the distribution and sources of MP in drainage ditches influenced by pond connectivity, land use, and soil properties within a small catchment in Nanjing, East China. Sediment was collected from ditches in 18 sites across forest, agricultural, horticultural, and urban areas. Using laser-directed infrared spectroscopy (LDIR), 922 MP particles were identified. Six materials were dominant: fluororubber (FR), polyethylene terephthalate (PET), polyurethane (PU), acrylonitrile (ACR), chlorinated polyethylene (CPE), and polyethylene (PE). MP concentrations varied by land use and pond connectivity, with ditches above ponds exhibiting higher counts (1700 particles/kg) than those below (1050 particles/kg), indicating that ponds act as MP sinks. The analysis revealed site-specific MP sources, with FR linked to road runoff and PET associated with agricultural practices. Correlations between MP shape and soil properties showed that more compact and filled shapes were more commonly associated with coarser soils. PE particle size was negatively correlated with organic matter. This study highlights the need for targeted strategies to reduce MP pollution in rural landscapes, such as reducing plastic use, ditch maintenance, and improved road runoff management.

Keywords: microplastics; drainage ditches; ponds; environmental pollution; microplastic shape parameters

Citation: Lotz, T.; Chen, W.; Su, S. Microplastic Transport and Accumulation in Rural Waterbodies: Insights from a Small Catchment in East China. *Toxics* **2024**, *12*, 761. https://doi.org/10.3390/toxics12100761

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

Received: 2 October 2024
Revised: 17 October 2024
Accepted: 18 October 2024
Published: 19 October 2024

1. Introduction

The presence of microplastics (MP), i.e., plastic particles of a size below 5 mm, in the environment has gained significant attention, particularly in the last decade [1,2]. MP pollution poses various environmental risks. MP particles have been shown to be ingested by marine and freshwater fish, invertebrates, and zooplankton, which leads to them consuming less natural prey, with negative effects on their growth, reproduction, and survival [3–5]. Similar effects have been observed in terrestrial habitats, where MP pollution negatively affected springtails, nematodes, and earthworms [6,7]. Negative effects on plants and photosynthetic organisms have been found in aquatic and terrestrial environments [8,9]. Through ingestion by plants and animals, MP particles move up the food chain and can eventually be consumed by humans. Other human exposure modes to MP pollution are food, bottled water, and even air [10]. MP has been discovered in various human body tissues and may cause adverse health effects from inflammatory reactions

and disturbed gut microbiota to autoimmune reactions [11,12]. Furthermore, MP particles interact with other pollutants in water and soil [13,14] and can alter bacterial communities, potentially affecting antibiotic resistance in human and animal pathogens [15,16].

Many human activities introduce MP into the environment from both point sources, including industrial production and waste disposal, and non-point sources, ranging from cosmetics, fabrics, and agriculture to road traffic. The specific combination of MP sources depends on the respective environment [17,18]. The type of MP (material and shape) allows for the estimation of the source of the particles [19]. MP transport and accumulation are two closely related processes that dynamically interact with each other [20–22]. Depending on size and material, MP transport from the source occurs through different vectors. Small MP particles can be transported by wind [23] and are dispersed in the soil by biota [24]. Most MP particles are carried by moving water, either mixed with sediments or floating in the water [25,26]. To tackle the environmental and health-related issues caused by MP, it is necessary to better understand MP sources and transport and accumulation processes [27].

In rural areas, agriculture is a significant source of MP in terrestrial and aquatic ecosystems [28]. MP is introduced into the environment through agricultural practices, including the use of biosolids, mulching films, and other agricultural plastics, which are often left to decay in the soil [29,30]. Despite recent progress in investigating MP in different environments, the behavior of MP in agricultural systems remains poorly understood [31,32]. The amount and mobility of MP in agricultural fields depend on a series of factors, including MP properties, soil properties, and farming activities [33]. While research has shed light on these individual contributors to MP presence in the agricultural context [34,35], the comprehensive understanding of how these factors interact remains limited. Current studies often isolate variables, neglecting the complex, interconnected dynamics that characterize agricultural ecosystems. MP transport in agricultural fields occurs along with eroded soil in surface runoff, wind transport, and soil biota [36], leading to their subsequent movement into ditches and ponds, primarily via surface runoff [17,37].

Rural ponds and drainage ditches are two widespread types of waterbodies in the agricultural areas of East China. Rural ponds are used for water storage and low-intensity food production and are often vital to everyday countryside life, local hydrology, and agriculture [38]. The quality of rural pond water has been directly linked to the source and volume of inflowing water, the effects of the surrounding landscape [39], and agricultural land cover [40]. MP in the water and sediment of ponds may harm populations using the water for irrigation or consuming food grown in the ponds [41], highlighting the need to improve the understanding of MP transport and accumulation processes [27]. Previous MP studies in ponds mainly focused on those used for crab and fish farming [42,43]. However, very little is known about MP in the much more common rural ponds, leading to shortcomings in understanding the role of these ponds in the transport and accumulation processes of MP. Drainage ditches have been shown to serve as pathways for transporting phosphorus, nitrogen, herbicides, and heavy metals [44,45]. Previous studies linked pollutant transport in ditches to management practices, catchment properties, and hydrological connectivity [46–49]. This study investigates the distribution and sources of MPs within a small agricultural catchment in East China, examining how connectivity with rural ponds and factors such as land cover, soil properties, and agricultural activities influence MP concentrations in ditch sediments. By integrating these factors, the study aims to provide a more comprehensive understanding of MP transport and accumulation in rural waterbodies, addressing the knowledge gap regarding the interactions between ditches, rural ponds, and the surrounding landscape in MP pollution dynamics.

2. Materials and Methods

2.1. Study Site

The research catchment is situated east of Nanjing, in Jiangsu province, China (Figure 1). It is part of the western end of the Tangshan Mountain area. The catchment covers an area of 20.3 km^2, of which 76% is forest, 7% is water, 7% is built-up (urban and paved

areas), 5% is grassland or range, and 5% is agricultural land [manual classification]. In a preliminary GIS study, all landscape elements in the research area have been identified using a manual classification of Sentinel-2 imagery. The resulting dataset contains all the research area's water bodies, villages, roads, and other relevant elements. Most of the water area is concentrated in two large reservoirs in the northern and southern parts of the research area. The mountains form a U-shape around the study area, providing a demarcation that can potentially minimize the introduction of external materials through wind transport and other mechanisms.

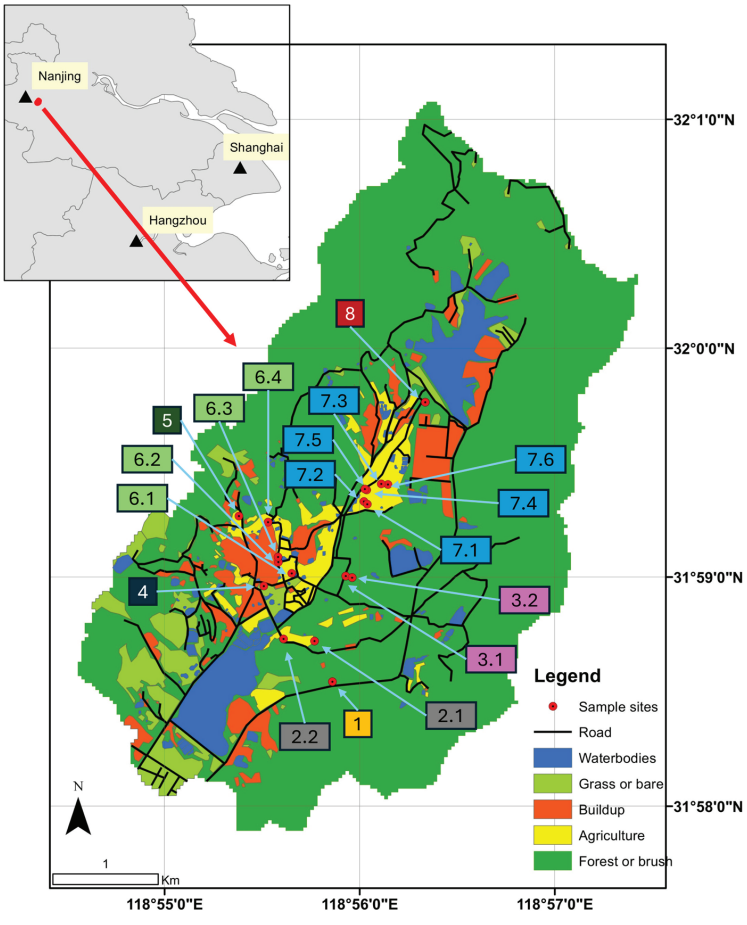


Figure 1. A land cover map of the research area, the sample sites, and an indication of its position near Nanjing. Label color indicates the different sampling complexes.

2.2. Microplastic Sampling

The sample sites were organized into eight complexes based on location and hydrological connection (Table 1). Complex 1 included Site 1, located in a forested area with no pond connection. Complex 2 comprised Site 2.1 in an agricultural area and Site 2.2 in a horticultural area, both without pond connections, representing typical agricultural and horticultural land covers. Complex 3 included Site 3.1 in a forested area with an "Above" pond connection and Site 3.2 in a forested area with a "Below" pond connection, allowing for assessing pond connection influence in forested settings. Complex 4 consisted of Site 4 in an urban area with no pond connection, representing urban land cover. Complex 5

included Site 5 in a horticultural area without a pond connection. Complex 6 was more diverse, with Site 6.1 in a horticultural area with an "Above" pond connection, Site 6.2 with a "Below" pond connection, Site 6.3 with an "Above" pond connection, and Site 6.4 without any pond connection, allowing for a detailed comparison within horticultural settings. Complex 7 included multiple agricultural sites with varying pond connections: Site 7.1 with an "Above" pond connection, Site 7.2 with a "Below" pond connection, Site 7.3 with a "Below" pond connection, Site 7.4 with an "Above" pond connection, Site 7.5 with a "Below" pond connection, and Site 7.6 with an "Above" pond connection. This complex enabled the assessment of MP contamination in agricultural areas with different pond connections. Lastly, Complex 8 included Site 8 in an urban area without a pond connection, providing another urban comparison point.

Table 1. The complexes with their associated sample sites, as well as the land cover type and pond connection of each sampling site.

Complex	Site	Land Cover	Pond Connection
1	1	Forest	None
2	2.1	Agriculture	None
	2.2	Horticulture	None
3	3.1	Forest	Above
	3.2	Forest	Below
4	4	Urban	None
5	5	Horticulture	None
6	6.1	Horticulture	Above
	6.2	Horticulture	Below
	6.3	Horticulture	Above
	6.4	Horticulture	None
7	7.1	Agriculture	Above
	7.2	Agriculture	Below
	7.3	Agriculture	Below
	7.4	Agriculture	Above
	7.5	Agriculture	Below
	7.6	Agriculture	Above
8	8	Urban	None

At each of the 18 sample sites, five samples were extracted 1 m apart along the ditch bottom using new disposable wooden spoons. The sample was collected from the top centimeter of the sediment. The five samples for each site were then mixed in a glass tube, which was immediately sealed with aluminum foil.

2.3. MP Identification

An MP extraction methodology suitable for the research questions was applied to the ditch and pond sediments [50–52]. Non-plastic consumables and equipment were employed throughout the experiment. Utilized glass and metal items were washed with ultrapure water produced by Milli-Q SQ2 (Millipore, Merck KGaA, Darmstadt, Germany) and dried in an infrared fast drying box WA70-1 (Hangzhou Qiwei Instrument Co. Ltd., Hangzhou, China). Items were stored in a sealed glass box before use. Working surfaces were wiped down regularly with paper towels and ethanol alcohol, and cotton coats were worn by everyone handling the samples. Windows and doors were closed during sample handling to reduce air circulation in the laboratory.

Exactly 20 g of soil sample was combined with 60 mL of $ZnCl_2$ solution (1.7–1.8 kg/L) in a 100 mL beaker, stirred for 10 min, and left to stand overnight to aid in the flotation of MP by increasing the solution's density. Subsequently, the mixture was transferred to a new beaker, and 60 mL of 30% hydrogen peroxide (H_2O_2) was added to digest organic impurities. After thorough mixing, the solution was left for 24 h to allow a complete reaction of H_2O_2 with organic materials. The supernatant underwent vacuum filtration,

and the filter membrane (0.22 μm polytetrafluoroethylene membrane) was subjected to 30 min of ultrasonication at 40 KHz in a JP-060S ultrasonic machine (Shenzhen Jiemeng Co., Ltd., Shenzhen, China) in ethanol to disperse adhered substances. After ultrasonication, the filter membrane was repeatedly rinsed with ethanol, and the solution was concentrated in the drier. The solution was then dripped on highly reflective glass slides (MY2108LD34 by Agilent Technologies Ltd., Santa Clara, CA, USA). The extracted particles were identified using laser-directed infrared spectroscopy (LDIR) utilizing an Agilent 8700 LDIR machine (Agilent Technologies Ltd., Santa Clara, CA, USA) with a 20–500 μm detection range. LDIR imaging combines infrared spectroscopy principles with high-resolution imaging capabilities, allowing for visualization of the spatial distribution of chemical compositions within the sample. This method maps heterogeneous samples with high sensitivity and spatial resolution, making it particularly useful in MP studies [53,54]. LDIR enables the counting of MP particles in a sample and the determination of the type of plastic of each particle, which is invaluable for estimating the source and transport path of the MP particles. MP particles with a match score of >0.65 were included in this study, consistent with established methodologies [55–58]. The MP counts were calculated as the MP particle count per kilogram, i.e., p/kg [59].

2.4. Physical and Chemical Sediment Analysis

Soil particle size distribution was determined using the Mastersizer 2000 laser particle size analyzer (Malvern Panalytical, Malvern, UK) [60]. First, to remove organic matter, approximately 0.5 g of soil sample was placed in a 150 mL glass flask and was pre-treated with 10 mL H_2O_2 (10%) on a heating plate under continuous washing of the flask wall to prevent foam from sticking to it. If necessary, additional H_2O_2 was added until no reaction was visible. This was followed by a treatment with 10 mL hydrochloric acid (HCl, 10%) at 50 °C to eliminate carbonates. Distilled water was added to reach a neutral pH, and the flask was left to stand for 24 h, after which the clear liquid was siphoned off. The remaining sample was then dispersed using 10 mL of sodium hexametaphosphate (1 mol/L) and treated for 10 min in the ultrasonic cleaning device. Then, the sample was analyzed using the laser particle size analyzer at a refractive index of 1.52. For the analysis, soil particle size classes D10, D50, and D90 were used. Each describes a diameter bigger than 10%, 50%, or 90% of the particles in the sample, respectively.

Dried soil sample was used for the determination of total organic carbon (OC). To remove inorganic carbon (IC), 10% HCl was added to the soil sample, and phosphoric acid was slowly added until there was no visible reaction. After the inorganic carbon was removed, the sample was analyzed using a Total Organic Carbon Analyzer TOC-L (Shimadzu, Kyoto, Japan). The soil's organic matter was combusted at 900 °C inside the TOC-L, converting it to carbon dioxide (CO_2), which was then carried by high-purity oxygen gas and detected using a non-dispersive infrared (NDIR) detector inside the TOC-L. This method ensured the accurate measurement of the total organic carbon content in the soil [61].

Organic nitrogen was calculated following the Kjeldahl methodology [62], which involves determining total nitrogen (TN) and subtracting the measured inorganic forms of nitrogen, i.e., nitrate and ammonium, from the total nitrogen content. For the determination of total nitrogen (TN), 0.5 g of air-dried soil (sieved to 0.25 mm) was placed in a Kjeldahl digestion flask (Foss Analytics, Hillerød, Denmark). After wetting the sample with a small amount of water, 4 mL of pure sulfuric acid (H_2SO_4) was added, and the mixture was left to soak overnight. The following day, 0.5 g of pentahydrate sodium thiosulfate was added, and the sample was heated until smoke appeared. After cooling, 1.1 g of a catalyst composed of potassium sulfate, copper sulfate pentahydrate, and titanium dioxide was added and mixed. The digestion continued until the mixture turned gray, and then it was further digested for 1 h. The digestion flask was connected to a Kjeldahl nitrogen analyzer Foss Kjeltec 8100 (Foss Analytics, Hillerød, Denmark), and the distillate was absorbed in 20 mL boric acid solution (323.47 mmol/L). After distillation, the distillate was titrated

using standard hydrochloric acid solution, and the total nitrogen content was calculated based on the amount of acid consumed.

For nitrate nitrogen (NO_3^--N) determination, 50 g of soil (sieved to 2 mm) was placed in a 500 mL bottle, and 0.5 g calcium sulfate and 250 mL of water were added. The bottle was then shaken for 30 min. Afterward, 25 mL of the sample solution (SS) was placed in an evaporating dish, and 0.05 g of carbonate was added. The solution was evaporated to dryness in a water bath, and after cooling, 2 mL of disulfonic acid reagent (1.18 mol/L) was added. The solution was stirred to ensure full contact with the dried material, then left to stand for 10 min. Afterward, 20 mL of water was added, and the mixture was stirred until all the dried material dissolved. While stirring, 3.71 mol/L ammonium hydroxide (1:1 ammonia solution) was slowly added until the solution became slightly alkaline (turning yellow), and an additional 2 mL of ammonia was added to ensure excess. The solution was transferred into a 100 mL volumetric flask and diluted to the mark. The nitrate nitrogen concentration was measured using a UV1800 UV-Visible Spectrophotometer (Shanghai Jinghua Science & Technology Instruments Co., Ltd., Shanghai, China) at a wavelength of 420 nm.

For ammonium nitrogen (NH_4^+-N) analysis, 5 mL of SS was transferred into a 50 mL volumetric flask and diluted to 10 mL with potassium chloride solution (2 mol/L). Then, 5 mL of phenol solution (10 g phenol and 100 mg sodium nitroprusside dissolved in water, diluted to 1 L) and 5 mL of alkaline sodium hypochlorite solution (10 g sodium hydroxide, 7.06 g disodium hydrogen phosphate, 31.8 g sodium phosphate dissolved in water with 10 mL of 705.25 mmol/L sodium hypochlorite solution, diluted to 1 L) was added. The mixture was shaken and left at room temperature (around 20 °C) for 1 h. After this, 1 mL of a masking agent (40 g potassium sodium tartrate and 10 g disodium EDTA dissolved in water, then mixed in equal parts) was added to dissolve any potential precipitate, and the solution was diluted to the mark with water. The ammonium nitrogen concentration was determined by measuring absorbance at a wavelength of 625 nm using the UV1800 UV-Visible Spectrophotometer.

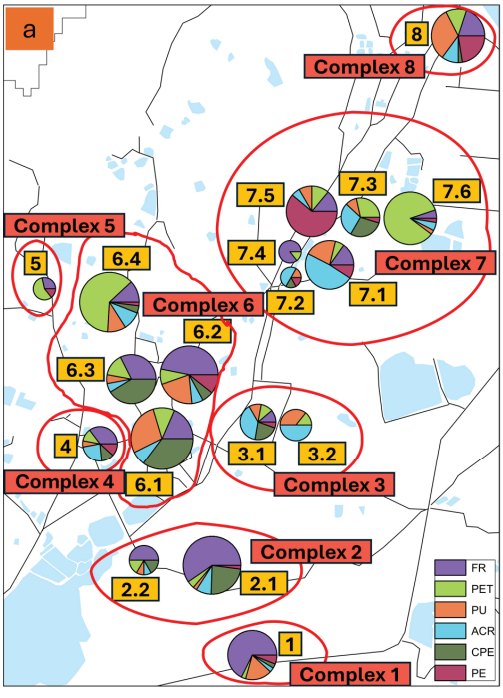
2.5. Software and Data

Statistical analyses were performed in Python (version 3.9, Python Software Foundation) and the statistical programming language R (version 4.3.1, R Core Team, Vienna, Austria) in the RStudio environment (version 2024.04.1-748, RStudio, Boston, MA, USA). ArcGIS (version 10.4, Esri, Redlands, CA, USA) was utilized to create maps. The digital elevation model used for delineating the catchment area was the SRTM 90 m DEM [63–66]. Landcover classification was performed in ArcGIS using Landsat data (Landsat-9 image courtesy of the U.S. Geological Survey).

3. Results

3.1. MP Distribution in the Research Area

In total, 922 MP particles from 31 different materials were identified from the sediment samples (full data in Supplementary Table S1, exemplary LDIR scans in Supplementary Figures S1–S4). The six most common materials were fluororubber (FR), polyethylene terephthalate (PET), polyurethane (PU), acrylonitrile (ACR), chlorinated polyethylene (CPE), and polyethylene (PE). Figure 2 shows the distribution of these six MP over the research area (a) and the particle counts per site and material (b). The distribution of microplastics across the study area showed significant (p = 0.028) variations based on material type, as evidenced by the results from the different sites and complexes.



b

Complex	Site	Particle Count [p/kg] FR	PET	PU	ACR	CPE	PE
1	1	1,150	50	300	50	50	100
2	2.1	1,400	100	50	200	550	50
	2.2	300	100	50	50	100	0
3	3.1	100	100	100	350	200	50
	3.2	0	100	250	350	0	0
4	4	300	100	50	200	100	100
5	5	100	200	0	0	0	50
6	6.1	500	300	750	200	950	0
	6.2	1,100	200	500	150	150	250
	6.3	550	250	100	100	700	0
	6.4	300	1,550	250	250	100	50
7	7.1	250	100	350	800	0	150
	7.2	0	0	50	150	50	50
	7.3	0	300	100	300	300	50
	7.4	300	50	0	0	0	0
	7.5	250	200	150	100	0	1,100
	7.6	100	1,650	50	100	0	50
8	8	400	250	650	200	50	450

Figure 2. (**a**) The MP particle composition of the six most common materials for all sample sites. The diagram size indicates the overall MP count per site. (**b**) The data show the particle count for the six most common materials for all sites.

FR was the most dominant microplastic overall and in several locations, particularly at Site 1 (1150 p/kg) in Complex 1, a forested area. High FR counts were also observed at Site 2.1 (1400 p/kg) in Complex 2, an agricultural area, and Site 6.2 (1100 p/kg) in Complex 6, a horticultural area. In contrast, FR was absent in Sites 3.2, 7.2, and 7.3, indicating variability in its presence across different locations. Sites 7.1 (250 p/kg), 7.4 (300 p/kg), and 7.5 (250 p/kg) in Complex 7 showed moderate levels of FR, while Site 8, an urban area in Complex 8, contained 400 p/kg of FR. PET showed the highest counts in Site 6.4 (1550 p/kg) within Complex 6, a horticultural area, and Site 7.6 (1650 p/kg) in Complex 7, an agricultural site. PET was also detected in smaller quantities across multiple locations, including Sites 2.1 and 2.2 in Complex 2, Sites 3.1 and 3.2 in Complex 3, and Site 4 in Complex 4, each with 100 p/kg. PU was found in notable amounts at Site 6.1 in Complex 6, with 750 p/kg, and Site 8 in Complex 8, with 650 p/kg. PU was also present at Site 7.1 in Complex 7 (350 p/kg), while smaller quantities were detected at other locations, such as Sites 3.1 and 3.2 in Complex 3 and Sites 2.1 and 2.2 in Complex 2. PU was absent at Sites 7.4 and 7.5 in Complex 7. ACR was most prevalent at Site 7.1 in Complex 7, where 800 p/kg were found. It was also present at Sites 3.1 and 3.2 in Complex 3, with 350 p/kg each, and at Site 4 in Complex 4 (200 p/kg). Other sites, including Sites 2.1, 6.1, and 8, showed smaller amounts of ACR. CPE exhibited notable concentrations at Site 6.1 in Complex 6, with 950 p/kg, and at Site 6.3, also in Complex 6, with 700 p/kg. Other sites, such as 2.1 in Complex 2 and 3.1 in Complex 3, had smaller amounts of CPE (550 p/kg and 200 p/kg, respectively). CPE was absent at several sites, including Sites 7.4 and 7.5 in Complex 7. PE was most abundant at Site 7.5 in Complex 7, where 1100 p/kg were recorded. PE was also present at Site 8 in Complex 8 (450 p/kg) and Site 7.1 (150 p/kg). In contrast, many sites, including 6.1 in Complex 6 and 7.4 in Complex 7, had no detectable PE particles.

3.2. Microplastic Particle Count Clustering

A k-means clustering analysis was performed to identify similar distribution patterns of different MP types. Acceptable clustering performances (measured by a silhouette score of >0.6) were obtained from clustering FR and PET particle counts and CPE and PU particle counts. Table 2 shows the clustering results for all 18 sampling sites.

Table 2. Clustering of FR/PET and CPE/PU particle counts for all sample sites.

Complex	**1**	**2**		**3**		**4**	**5**	**6**				**7**						**8**
Site	1	2.1	2.2	3.1	3.2	4	5	6.1	6.2	6.3	6.4	7.1	7.2	7.3	7.4	7.5	7.6	8
FR/PET	H/L	H/L	L/L	L/L	L/L	L/L	L/L	L/L	H/L	L/L	L/H	L/L	L/L	L/L	L/L	L/L	L/H	L/L
CPE/PU	L/L	H/H	L/L	L/L	L/L	L/L	L/L	H/H	L/L	H/H	L/L	L/L	L/L	L/L	L/L	L/L	L/L	L/L

Note: H = high, L = low, combination indicates which material is abundant at the respective site.

In the FR/PET clustering, most sites were grouped into either Cluster FR/PET_H/L or Cluster FR/PET_L/L, with fewer sites falling into Cluster FR/PET_L/H. Cluster FR/PET_H/L, characterized by high FR and low PET counts, included Site 1 and Site 2.1. At Site 1, located in a forested area, FR dominated with 1150 p/kg, while PET was minimal (50 p/kg). Similarly, Site 2.1, in an agricultural area, had 1400 p/kg and only 100 p/kg PET particles. Cluster FR/PET_L/L represented sites such as 2.2, 3.1, 3.2, and 4, where both FR and PET were found in moderate to low concentrations. For instance, Site 2.2 had 300 p/kg FR particles and 100 p/kg PET, while Site 3.1 and Site 3.2, located in forested areas with pond connections, each had 100 p/kg PET but a smaller number of FR particles (100 p/kg and 0 p/kg, respectively). Site 4, representing an urban area, also showed low levels of both FR (300 p/kg) and PET (100 p/kg). Cluster FR/PET_L/H, comprising Sites 6.4 and 7.6, exhibited the opposite pattern, with high-PET counts and low FR counts. Site 6.4, a horticultural area, contained 1550 p/kg PET and 300 p/kg FR, while Site 7.6, an agricultural site, showed 1650 p/kg PET and only 100 p/kg FR.

In the CPE/PU clustering, the sites were more evenly distributed between Cluster CPE/PU_H/H and Cluster CPE/PU_L/L. Cluster CPE/PU_H/H, with higher concentrations of CPE and PU, included Sites 2.1, 6.1, and 6.3. For instance, Site 2.1, located in an agricultural area, had 550 p/kg CPE and 50 p/kg PU. Similarly, horticultural sites such as 6.1 and 6.3 displayed elevated levels of CPE and PU, with Site 6.1 having 950 p/kg CPE and 750 p/kg PU. Cluster CPE/PU_L/L encompassed most other sites, including Sites 1, 2.2, 3.1, 3.2, and 4, which all had lower levels of CPE and PU. For example, Site 1 had 50 p/kg CPE and 300 p/kg PU, Site 2.2 had 100 p/kg CPE and 50 p/kg PU, and Sites 3.1 and 3.2 contained low quantities of both CPE and PU.

3.3. Interactions of MPs with Ponds

The distribution of MP in drainage ditches showed significant differences ($p < 0.001$) depending on their connection to ponds, with differences observed between sites sampled directly above ponds, below ponds, and those without significant pond connections (Table 3). These variations offer insights into how ponds influence MP retention and transport.

Table 3. Pond connection information for all sample sites.

Pond Connection	**Sites**	**Total MP Particle Count**	**Average MP Count per Site [p/kg]**
Above	3.1, 6.1, 6.3, 7.1, 7.4, 7.6	204	1700
Below	3.2, 6.2, 7.2, 7.3, 7.5	105	1050
None	1, 2.1, 2.2, 4, 5, 6.4, 8	176	1250

Sites with "Above" pond connections generally exhibited higher MP concentrations than those with "Below" pond connections or sites without pond influence. For example,

Site 6.1 (horticulture, above a pond) displayed elevated levels of FR and CPE, with 500 p/kg and 950 p/kg particles, respectively. Similarly, Site 7.6 (agriculture, above a pond) exhibited a large amount of PET, with 1650 p/kg. The average MP count in these sites was 1700 p/kg, indicating that ditches above ponds accumulated higher concentrations of MP. In contrast, sites with "Below" pond connections, such as Sites 3.2, 6.2, and 7.2, showed lower MP concentrations, particularly for FR. For instance, Site 3.2 (forest, below a pond) had no detectable FR particles, and Site 7.2 (agriculture, below a pond) also showed an absence of FR particles. The average MP count in these sites was lower at 1050 p/kg. Sites without significant pond connections showed variable MP concentrations. For instance, Site 2.1 (agriculture, no pond connection) exhibited the highest concentration of FR (1400 p/kg), while Site 6.4 (horticulture, no pond connection) had a notably high concentration of PET (1550 p/kg). The average MP count in sites without pond connections was 1250 p/kg.

3.4. MP Relationship with Land Cover

The analysis showed a significant ($p < 0.001$) difference in the distribution of MP types across different land cover types, indicating that land use considerably influences MP distribution. The study examined four land cover types, consisting of agriculture, horticulture, forest, and urban. The data revealed distinct patterns in the presence of various MP types, including FR, PET, PU, ACR, CPE, and PE. Agricultural sites (e.g., 2.1, 7.1–7.6) exhibited notably higher concentrations of FR and PET, with FR counts reaching up to 1400 p/kg at Site 2.1 and up to 1650 p/kg at Site 7.6. Horticultural areas (Sites 6.1–6.4) also showed significant PET, PU, and CPE concentrations. In contrast, forested sites (1, 3.1, and 3.2) generally had lower MP counts, although Site 1 had a relatively high concentration of FR particles (1150 p/kg). Urban sites (4 and 8) exhibited a more diverse MP profile, with notable counts of PU and PE.

3.5. MP Shape Parameter Relationship with Soil Properties

The analysis of the relationship between MP shape parameters and soil properties revealed several statistically significant correlations ($p < 0.05$), highlighting potential associations between specific MP characteristics and soil features. Table 4 presents the ten strongest correlations observed between MP shape parameters—such as solidity (a measure of how filled in or compact a particle is), eccentricity (a measure of the elongation of the particle), circularity (a measure of how circular the particle is), diameter, area, width, longest side, and height—and soil properties, including D90 (the particle size at which 90% of the soil sample is finer), D50 (the median particle size), organic carbon (OC), and organic nitrogen (ON). A full table of correlations is available in Supplementary Table S2.

Table 4. The ten strongest, statistically significant correlations observed between MP particle shape parameters and soil parameters.

Material	Shape Parameter	Soil Parameter	Coefficient
ACR	Solidity	D90	0.58
	Eccentricity	D50	0.45
	Circularity	D90	0.42
PE	Diameter	OC	−0.39
	Area	OC	−0.39
	Diameter	ON	−0.39
	Area	ON	−0.39
	Width	ON	−0.38
	Longest Side	ON	−0.38
PU	Height	D90	0.37

Among the strongest correlations, ACR showed a positive relationship between solidity and the D90 soil particle size parameter (coefficient = 0.58). This suggests that MPs with more compact and filled shapes are more commonly associated with soils containing

larger particles. Similarly, ACR eccentricity and circularity were positively correlated with D50 (r = 0.45) and D90 (r = 0.42), respectively, indicating that elongated and more circular MPs are associated with specific soil texture characteristics. For PE, several shape parameters, such as diameter, area, and width, were negatively correlated with organic carbon (r = −0.39 for diameter and area) and organic nitrogen (r = −0.39 for diameter and area; r = −0.38 for width and longest side), implying that larger PE particles tend to occur in soils with lower organic content. Finally, a positive correlation (r = 0.37) was observed between PU height and D90, suggesting a relationship between PU particle height and larger soil particles.

4. Discussion

4.1. Sources of MP Particles

The distribution of MPs across the study area revealed distinct variations that correspond to different land use practices and local sources of pollution. These variations highlight how the environmental context—such as urban, agricultural, or forested areas—affects the prevalence of certain MP types with clear ties to human activities.

Rubber-based MPs like FR were predominantly found in areas affected by road traffic. This is consistent with the observation that FR is commonly released through tire wear during driving, which introduces MPs into the environment via road runoff and airborne dust [67]. In forested areas near roads, this explains the high levels of FR, which may be further exacerbated by industrial activities involving seals and gaskets [68]. These findings suggest that proximity to roads and industrial zones significantly determines FR prevalence, particularly in Complex 1. However, Complexes 4–7, although also bordered by roads, show significantly lower levels of fluororubber. This discrepancy could be influenced by several factors, including the distance from the road, the presence of barriers such as drainage ditches, or local environmental conditions like wind patterns and rainfall. These variations underscore the complexity of MP transport mechanisms and highlight the need for further investigation into how such factors influence MP deposition. Polymer-based MPs, including PET, PE, and CPE, show a strong association with both urban and agricultural land use. PET particles are often linked to synthetic textiles, such as polyester, which release fibers into wastewater during washing [69]. This pattern is particularly evident in agricultural areas where synthetic textiles, plastic packaging, and agricultural films contribute to PET contamination [70]. In Complexes 6 and 7, the abundance of PET suggests significant agricultural use of plastic-based products, such as mulching films and packaging, as well as wastewater pathways that introduce these MPs into the environment [71]. Similarly, PE MPs, prevalent in urban environments, likely originate from consumer products such as cosmetics [72] and the degradation of larger plastic items like bags and packaging. PE in urban areas like Complex 4 underscores the importance of plastic waste management in minimizing MP pollution. PE in cosmetic products and packaging explains its widespread presence in urban and agricultural settings, reflecting how everyday consumer products contribute to environmental contamination. PU plays a different role in agricultural settings, particularly due to its use in pesticide microcapsules and coatings [73]. The high levels of PU observed in Complexes 6 and 7 suggest that agricultural practices involving pesticides and synthetic coatings are a significant source of this MP. Additionally, PU is released by degrading materials such as synthetic leathers and road markings, which can further contribute to pollution in nearby environments [74]. CPE, often used in industrial applications like roofing membranes and hoses, is also associated with agricultural and urban areas. The presence of CPE in Complexes 2 and 6 highlights the impact of agricultural plastic materials and industrial runoff, where these products degrade and release MPs into the surrounding environment [75]. Similar to PE, the degradation of CPE through exposure to UV radiation and environmental factors contributes to its persistence in terrestrial and aquatic environments [76]. Lastly, ACR MPs are commonly linked to textile fibers, especially in areas with high human activity. The detection of ACR

in both rural and urban areas suggests that these MPs are widespread due to atmospheric deposition and runoff from synthetic clothing and coatings [77].

These findings show that MP pollution is highly context-dependent, with the type and concentration of MPs varying significantly based on local sources. Furthermore, the relatively high concentration of microplastics (MPs) in forest ditches points to the role of these ditches as accumulation zones. While site selection aimed to minimize the influence of adjacent areas, temporary hydrological connections between the forested ditches and neighboring regions (e.g., agricultural fields, urban areas, or roads) may lead to runoff carrying MPs into these supposedly isolated areas. This suggests that even forested areas can be affected by external sources of pollution, especially during certain environmental conditions such as heavy rain. The clear connection between road traffic and FR concentrations indicates the need for targeted mitigation efforts in locations near infrastructure. At the same time, the prevalence of PET and PE in both agricultural and urban areas underscores the impact of plastic use in everyday products and agricultural practices. Agricultural sites are particularly affected by PU and CPE, likely due to their role in pesticides and industrial products. This raises concerns about the long-term environmental impacts of these materials, especially as they persist in the soil and can interact with other pollutants [73]. In urban areas, the diversity of MP sources—including consumer products, textiles, and construction materials—highlights the complexity of addressing MP pollution. These findings emphasize the importance of managing plastic use in urban and rural settings. By understanding MPs' specific sources and pathways, targeted strategies can be developed to reduce their environmental impact, particularly in areas most affected by industrial, agricultural, or urban activities.

4.2. MP Source Patterns

The clustering results highlighted distinct patterns in the distribution of MPs across different land use types, demonstrating how source activities and transport processes interact to shape MP presence in the environment. These patterns, characterized by specific combinations of MP types such as FR, PET, CPE, and PU, provide insights into the complex dynamics between sources, environmental conditions, and MP transport.

In Cluster FR/PET_H/L (high FR, low PET), the high FR concentrations reflect road traffic's significant role in introducing FR MPs into the environment [67]. The low PET concentrations in these areas suggest that while road traffic is a dominant source of FR, it does not contribute significantly to PET pollution. The transport of FR via runoff and its retention in nearby soil or sediment further emphasize the role of roads as pathways for this type of MP, particularly in forested areas like Complex 1. The relative isolation of these environments from urban centers may also limit the introduction of PET from urban-related sources like synthetic textiles and packaging. Conversely, Cluster FR/PET_L/H (low FR, high PET) highlights the dominance of PET in agricultural and horticultural environments, where synthetic textiles, plastic packaging, and agricultural films are common [69,70]. The high PET concentrations in these areas, particularly in Complexes 6 and 7, point to the influence of both local sources—such as agricultural plastics—and transport mechanisms, including surface runoff from agricultural fields and wastewater pathways. The interaction between PET sources and these transport routes creates an environment where PET is more readily retained and accumulates over time. FR remains limited due to the absence of substantial road traffic or industrial activity.

The CPE/PU clusters reveal further complexities in MP source and transport interactions. Cluster CPE/PU_H/H (high CPE, high PU) predominantly features agricultural and horticultural sites, such as Sites 2.1, 6.1, and 6.3 high in CPE and PU. These materials are commonly used in products such as plastic membranes, hoses, and pesticide microcapsules [73,75], reflecting these sites' management and use practices. These MPs likely enter the environment through direct degradation and runoff, but local environmental factors like sediment characteristics and water flow influence their persistence in the soil. Agricultural practices that disturb the soil and alter water retention could enhance CPE

and PU deposition and retention, contributing to higher concentrations in these areas. CPE and PU in high quantities indicate how land use practices can co-locate different MP types through shared transport mechanisms, such as irrigation runoff and agricultural drainage systems. In contrast, Cluster CPE/PU_L/L (low CPE, low PU) represents areas with lower agricultural and industrial activity, including forested and urban environments where these materials are minimal, such as Site 1 and Site 4. These areas lack the direct sources of CPE and PU, and the transport processes present—such as natural surface runoff in forests—are less likely to carry and deposit these MPs. The absence of agricultural practices reduces the pathways through which these MPs could be introduced and retained, resulting in lower concentrations.

The clustering results emphasize how different MP source and transport processes interact to create the specific source patterns observed in different environmental contexts. These patterns are shaped not only by the presence of specific sources but also by the environmental conditions and transport mechanisms that determine where and how MPs are retained. These findings align with previous research showing that MP distribution is highly influenced by both land use and the interaction of transport processes with MP sources [22–25,37].

4.3. MP Interactions with Ponds

The results revealed that pond connections significantly influence the distribution and retention of microplastics in drainage ditches. In sites with "Above" pond connections, higher concentrations of MP, particularly FR and PET, were observed. These elevated MP counts suggest that ditches above ponds accumulate MPs from upstream sources, such as road runoff or agricultural inputs, and transport them toward ponds. The higher average MP count (1700 p/kg) in these areas indicates that ponds serve as critical points where MPs are likely to settle or be retained before entering downstream aquatic systems. In contrast, sites with "Below" pond connections exhibited lower MP concentrations, especially for heavier materials like FR. The absence of FR particles at Sites 3.2 and 7.2 suggests that ponds act as sinks, effectively capturing heavier microplastics such as FR. This reduction in MPs downstream of ponds aligns with findings from other studies, which have demonstrated the role of ponds in filtering and retaining specific pollutants and materials in agricultural landscapes [78,79]. The lower average MP count (1050 p/kg) in sites below ponds supports the hypothesis that ponds play a significant role in MP retention. It is noteworthy here that many MP particles have, depending on the material, a lower density than water [80], causing them to be buoyant in water. Therefore, two processes of MP particle retention in ponds are feasible. First, the weight of MP particles may be increased through biofilm formation and aggregation with inorganic and organic matter [81,82]. This process may already begin while larger plastic objects are breaking down in the soil, contributing to the eventual formation of MPs. A second process of MP particle retention in ponds may be the ingestion by fish and aquatic invertebrates [3–5] and adsorption and accumulation in aquatic vegetation [83,84]. For sites without pond connections, the results were more variable. While some sites, like 2.1, had high concentrations of FR, others, such as 6.4, showed elevated PET levels. This variability suggests that microplastics are less likely to be retained in the absence of ponds and may accumulate in ditches or be transported further downstream.

This study reinforces the broader role of ponds in agricultural ecosystems, not merely as passive water bodies but as active agents in reducing MP pollution. Ponds below ditches, in particular, demonstrate their capacity to trap heavier MPs, thereby limiting the dispersal of pollutants downstream. The contrast between "Above" and "Below" pond connections reveals a clear dynamic, where ditches funnel MPs toward ponds, and the ponds, in turn, significantly reduce the downstream movement of certain materials. This function of ponds as retention zones is vital for developing strategies to mitigate the environmental impacts of microplastic pollution in agricultural landscapes, echoing insights from related studies on pollutant management [85–88].

4.4. MP Interactions with Land Cover

Land use not only acts as a source of MP pollution but also influences the pathways through which these particles are transported and accumulated. Different land use types introduce various patterns of runoff, management practices, and human activities, all of which affect how MPs enter and persist in the environment [89,90]. For instance, agricultural and urban areas at Sites 4 and 7.1 may generate high volumes of runoff containing MPs from fertilizers, plastics, and road materials. In contrast, forested areas, such as Complexes 1 and 3, may experience less direct contamination but still collect MPs from surrounding infrastructure. These patterns are shaped by land use decisions, including water management, soil conservation practices, and the intensity of human activity [91,92]. In this context, land use becomes a multifaceted factor that contributes to the generation of MP pollution and dictates how these pollutants move through and impact different ecosystems. The significant variation in MP types across different land cover types observed in this study aligns with findings from previous research [93,94], highlighting the role of human activity not only in the initial sources of MP pollution but also in the fate of MP particles. These results indicate the need for more sustainable agricultural practices that reduce reliance on plastic-based materials. The horticultural sites 6.3 and 6.4, where PET, PU, and CPE were more prominent, illustrate another case where plastic use, particularly in irrigation systems, coverings, and pesticide containers, contributes to environmental contamination. The frequent use of plastic-based products in these settings likely accounts for the higher concentrations of these MPs, which could persist in the environment long after use. The forest Sites 3.1, 3.2, and 1 typically had lower MP concentrations, but the high FR levels at Site 1 suggest localized pollution, possibly from nearby road traffic. This highlights how even remote or natural areas are not immune to MP contamination, especially when close to infrastructure. Urban areas, with their varied MP profiles, reflect the complexity of sources contributing to pollution. The elevated levels of PU and PE at Site 8 suggest a mixture of inputs. This diversity of MP types in urban environments underscores the need for better waste management strategies and infrastructure to mitigate plastic pollution. The findings suggest that land use is critical in determining the type and concentration of MPs in the environment. These insights can inform targeted mitigation strategies based on specific land use practices and associated risks to reduce MP pollution.

4.5. MP Shape Parameter Interactions with Soil Properties

The correlations observed between MP shape parameters and soil properties provided valuable insights into potential relationships, although these findings do not imply causality. The strong positive correlation between ACR solidity and soil particle size (D90) suggests that more compact and filled ACR particles may preferentially accumulate in coarser soils. This relationship could indicate that the soil's physical structure affects the retention or movement of MP particles with simpler, more solid shapes. Similarly, the positive relationships between ACR eccentricity and circularity with soil particle size parameters (D50 and D90) suggest that elongated or circular MPs may persist in soils with specific textures, perhaps due to the interactions between soil particles and the shape of the MPs. Elsewhere, the reduced mobility and increased retention of larger MP particles in soils have been demonstrated [95,96]. The negative correlations between PE particle size parameters (e.g., diameter, area, width) and organic content (both organic carbon and nitrogen) suggest that larger PE particles are less likely to be found in soils with higher organic content. This may be due to the ability of organic matter to better retain smaller particles. Other studies have found a strong effect of OC on MP particle sorption [97]. Alternatively, organic matter may be associated with different sources or behaviors of MPs in the soil, leading to a lower prevalence of larger MP particles in organic-rich environments. Microplastic has even been demonstrated to bond with OC aggregates [98]. The positive correlation between PU height and D90 indicates that longer PU particles are more likely to be associated with larger soil particles. This could suggest that PU particles behave differently in coarser soils, potentially influenced by the soil structure, water movement, or other factors affecting MP retention

and movement. However, since these correlations point to relationships rather than causal mechanisms, further research is needed to explore the underlying processes driving these associations. Understanding how soil properties interact with MP characteristics is critical for predicting MP behavior in different environments and could inform more effective management practices for mitigating MP contamination.

4.6. Limitations

This study offers insights into microplastic (MP) distribution and sources in a small agricultural catchment in East China, but some limitations should be noted. The limited scope of sample sites may affect the generalizability of our results to regions with different environmental conditions or land use practices. Furthermore, the sampling methodology focused only on the top 1 cm of sediment, which may not capture the full vertical distribution of MPs, especially in areas with high sedimentation rates. Additionally, temporal variability was not considered, as samples were collected at a single time point. Seasonal changes and varying agricultural practices could influence MP concentrations over time. Lastly, MP particles in environmental samples undergo aging processes, often contain chemical additives, and need chemical treatment to be isolated. Therefore, similar studies employing the same equipment for soil and sediment studies include particles with an LDIR matching score of >0.65. This means there may be some uncertainty in the chemical identification of particles within this range, potentially leading to misclassification.

5. Conclusions

This study provides critical insights into the distribution, sources, and transport dynamics of MP in drainage ditches in an East China catchment. The findings show significant variation in MP concentrations across different land uses, with agricultural and horticultural areas contributing the highest levels of PET, PU, and CPE. In contrast, forests and urban areas displayed a more diverse range of MP sources in this area. The influence of pond connectivity on MP transport was evident from the analyses, and ponds seem to act as sinks for certain MP. The specific processes associated with the accumulation of MP particles in ponds, such as biofilm formation and adsorption by aquatic plants, require further study. The analysis highlighted patterns, linking MP types to local sources such as road traffic, agricultural machinery, and plastic use in farming practices. The correlations between soil properties and MP shape parameters further suggest that soil composition plays a role in the retention and mobility of MP in agricultural landscapes. The findings of this study highlight the need for targeted management strategies to reduce MP pollution in agricultural landscapes. One key implication for the research area is the importance of improving agricultural practices, such as reducing the use of plastic materials in farming (e.g., mulching films and plastic packaging) and promoting biodegradable alternatives. Furthermore, the results showed that MP pollution is highly site-specific and dependent on multiple factors, which still need intense study. Additionally, regular maintenance of drainage ditches and rural ponds, including sediment removal, can help mitigate the accumulation of MP. Road runoff management, particularly near agricultural areas, should also be prioritized to minimize MP input from tire wear and industrial materials.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/toxics12100761/s1, Supplementary Table S1: Full data set of soil properties and microplastic particle counts in particles per kilogram [p/kg]; Supplementary Table S2: The statistically significant correlations observed between MP particle shape parameters and soil parameters. Supplementary Figure S1: The LDIR identification image of the sample from Site 6.3; Supplementary Figure S2: The LDIR identification image of the sample from Site 6.4; Supplementary Figure S3: A fluororubber (FR) particle identified in the sample from Site 7.1; Supplementary Figure S4: A polyethylene (PE) particle identified in the sample from Site 6.2.

Author Contributions: Conceptualization, T.L.; methodology, T.L., W.C. and S.S.; software, T.L. and S.S.; validation, T.L., W.C. and S.S.; formal analysis, T.L.; investigation, T.L. and W.C.; resources, W.C.

and S.S.; data curation, T.L. and W.C.; writing—original draft preparation, T.L.; writing—review and editing, T.L., W.C. and S.S.; visualization, T.L.; supervision, T.L. and S.S.; project administration, T.L; funding acquisition, T.L., W.C. and S.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the scientific research start-up fund for high-level talents of Jinling Institute of Technology [Grant No. jit-b-202139]; the National Natural Science Foundation of China [Grant No. 42101476], and the "Qing Lan" Project and Natural Science Research Project of Higher Education Institutions of Jiangsu Province [Grant No. 21KJB170025].

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article or Supplementary Material. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Kvale, K.; Oschlies, A. Recovery from microplastic-induced marine deoxygenation may take centuries. *Nat. Geosci.* **2022**, *16*, 10–12. [CrossRef]
2. Waldschläger, K.; Brückner, M.Z.M.; Carney Almroth, B.; Hackney, C.R.; Adyel, T.M.; Alimi, O.S.; Belontz, S.L.; Cowger, W.; Doyle, D.; Gray, A.; et al. Learning from natural sediments to tackle microplastics challenges: A multidisciplinary perspective. *Earth-Sci. Rev.* **2022**, *228*, 104021. [CrossRef]
3. Foley, C.J.; Feiner, Z.S.; Malinich, T.D.; Höök, T.O. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. *Sci. Total Environ.* **2018**, *631–632*, 550–559. [CrossRef] [PubMed]
4. Ziajahromi, S.; Kumar, A.K.A.; Neale, P.A.; Leusch, F.D.L. Environmentally relevant concentrations of polyethylene microplastics negatively impact the survival, growth and emergence of sediment-dwelling invertebrates. *Environ. Pollut.* **2018**, *236*, 425–431. [CrossRef]
5. Cole, M.; Lindeque, P.K.; Fileman, E.S.; Halsband, C.; Goodhead, R.M.; Moger, J.; Galloway, T.S. Microplastic ingestion by zooplankton. *Environ. Sci. Technol.* **2013**, *47*, 6646–6655. [CrossRef]
6. Ji, Z.; Huang, Y.; Feng, Y.; Johansen, A.; Xue, J.; Tremblay, L.A.; Li, Z. Effects of pristine microplastics and nanoplastics on soil invertebrates: A systematic review and meta-analysis of available data. *Sci. Total Environ.* **2021**, *788*, 147784. [CrossRef]
7. Ju, H.; Zhu, D.; Qiao, M. Effects of polyethylene microplastics on the gut microbial community, reproduction and avoidance behaviors of the soil springtail, Folsomia candida. *Environ. Pollut.* **2019**, *247*, 890–897. [CrossRef]
8. Ge, J.; Li, H.; Liu, P.; Zhang, Z.; Ouyang, Z.; Guo, X. Review of the toxic effect of microplastics on terrestrial and aquatic plants. *Sci. Total Environ.* **2021**, *791*, 148333. [CrossRef]
9. Larue, C.; Sarret, G.; Castillo-Michel, H.; Pradas Del Real, A.E. A Critical Review on the Impacts of Nanoplastics and Microplastics on Aquatic and Terrestrial Photosynthetic Organisms. *Small* **2021**, *17*, e2005834. [CrossRef]
10. Zhang, Q.; Xu, E.G.; Li, J.; Chen, Q.; Ma, L.; Zeng, E.Y.; Shi, H. A Review of Microplastics in Table salt, Drinking Water, and Air: Direct Human Exposure. *Environ. Sci. Technol.* **2020**, *54*, 3740–3751. [CrossRef]
11. Hirt, N.; Body-Malapel, M. Immunotoxicity and intestinal effects of nano- and microplastics: A review of the literature. *Part. Fibre Toxicol.* **2020**, *17*, 57. [CrossRef]
12. Koelmans, A.A.; Redondo-Hasselerharm, P.E.; Nor, N.H.M.; de Ruijter, V.N.; Mintenig, S.M.; Kooi, M. Risk assessment of microplastic particles. *Nat. Rev. Mater.* **2022**, *7*, 138–152. [CrossRef]
13. Castan, S.; Henkel, C.; Hüffer, T.; Hofmann, T. Microplastics and nanoplastics barely enhance contaminant mobility in agricultural soils. *Commun. Earth Environ.* **2021**, *2*, 193. [CrossRef]
14. Liu, Y.; Zhang, K.; Xu, S.; Yan, M.; Tao, D.; Chen, L.; Wei, Y.; Wu, C.; Liu, G.; Lam, P.K.S. Heavy metals in the "plastisphere" of marine microplastics: Adsorption mechanisms and composite risk. *Gondwana Res.* **2022**, *108*, 171–180. [CrossRef]
15. Zhang, M.; Zhao, Y.; Qin, X.; Jia, W.; Chai, L.; Huang, M.; Huang, Y. Microplastics from mulching film is a distinct habitat for bacteria in farmland soil. *Sci. Total Environ.* **2019**, *688*, 470–478. [CrossRef]
16. Wu, X.; Pan, J.; Li, M.; Li, Y.; Bartlam, M.; Wang, Y. Selective enrichment of bacterial pathogens by microplastic biofilm. *Water Res.* **2019**, *165*, 114979. [CrossRef]
17. Lwanga, E.H.; Beriot, N.; Corradini, F.; Silva, V.; Yang, X.; Baartman, J.; Rezaei, M.; van Schaik, L.; Riksen, M.; Geissen, V. Review of microplastic sources, transport pathways and correlations with other soil stressors: A journey from agricultural sites into the environment. *Chem. Biol. Technol. Agric.* **2022**, *9*, 20. [CrossRef]
18. Osman, A.I.; Hosny, M.; Eltaweil, A.S.; Omar, S.; Elgarahy, A.M.; Farghali, M.; Yap, P.S.; Wu, Y.S.; Nagandran, S.; Batumalaie, K.; et al. Microplastic sources, formation, toxicity and remediation: A review. *Environ. Chem. Lett.* **2023**, *21*, 1–41. [CrossRef]
19. Zhang, K.; Hamidian, A.H.; Tubic, A.; Zhang, Y.; Fang, J.K.H.; Wu, C.; Lam, P.K.S. Understanding plastic degradation and microplastic formation in the environment: A review. *Environ. Pollut.* **2021**, *274*, 116554. [CrossRef]

20. Drummond, J.D.; Schneidewind, U.; Li, A.; Hoellein, T.J.; Krause, S.; Packman, A.I. Microplastic accumulation in riverbed sediment via hyporheic exchange from headwaters to mainstems. *Sci. Adv.* **2022**, *8*, eabi9305. [CrossRef]
21. Besseling, E.; Quik, J.T.K.; Sun, M.; Koelmans, A.A. Fate of nano- and microplastic in freshwater systems: A modeling study. *Environ. Pollut.* **2017**, *220*, 540–548. [CrossRef] [PubMed]
22. Koutnik, V.S.; Leonard, J.; Alkidim, S.; DePrima, F.J.; Ravi, S.; Hoek, E.M.V.; Mohanty, S.K. Distribution of microplastics in soil and freshwater environments: Global analysis and framework for transport modeling. *Environ. Pollut.* **2021**, *274*, 116552. [CrossRef] [PubMed]
23. Evangeliou, N.; Grythe, H.; Klimont, Z.; Heyes, C.; Eckhardt, S.; Lopez-Aparicio, S.; Stohl, A. Atmospheric transport is a major pathway of microplastics to remote regions. *Nat. Commun.* **2020**, *11*, 3381. [CrossRef] [PubMed]
24. Rillig, M.C.; Ziersch, L.; Hempel, S. Microplastic transport in soil by earthworms. *Sci. Rep.* **2017**, *7*, 1362. [CrossRef] [PubMed]
25. Nizzetto, L.; Bussi, G.; Futter, M.N.; Butterfield, D.; Whitehead, P.G. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. *Environ. Sci. Process Impacts* **2016**, *18*, 1050–1059. [CrossRef] [PubMed]
26. Watkins, L.; McGrattan, S.; Sullivan, P.J.; Walter, M.T. The effect of dams on river transport of microplastic pollution. *Sci. Total Environ.* **2019**, *664*, 834–840. [CrossRef]
27. Meng, Y.; Kelly, F.J.; Wright, S.L. Advances and challenges of microplastic pollution in freshwater ecosystems: A UK perspective. *Environ. Pollut.* **2020**, *256*, 113445. [CrossRef]
28. Weithmann, N.; Moller, J.N.; Loder, M.G.J.; Piehl, S.; Laforsch, C.; Freitag, R. Organic fertilizer as a vehicle for the entry of microplastic into the environment. *Sci. Adv.* **2018**, *4*, eaap8060. [CrossRef]
29. Yang, L.; Zhang, Y.; Kang, S.; Wang, Z.; Wu, C. Microplastics in soil: A review on methods, occurrence, sources, and potential risk. *Sci. Total Environ.* **2021**, *780*, 146546. [CrossRef]
30. Huang, Y.; Liu, Q.; Jia, W.; Yan, C.; Wang, J. Agricultural plastic mulching as a source of microplastics in the terrestrial environment. *Environ. Pollut.* **2020**, *260*, 114096. [CrossRef]
31. Qi, R.; Jones, D.L.; Li, Z.; Liu, Q.; Yan, C. Behavior of microplastics and plastic film residues in the soil environment: A critical review. *Sci. Total Environ.* **2020**, *703*, 134722. [CrossRef] [PubMed]
32. Jin, T.; Tang, J.; Lyu, H.; Wang, L.; Gillmore, A.B.; Schaeffer, S.M. Activities of Microplastics (MPs) in Agricultural Soil: A Review of MPs Pollution from the Perspective of Agricultural Ecosystems. *J. Agric. Food Chem.* **2022**, *70*, 4182–4201. [CrossRef] [PubMed]
33. Rillig, M.C.; Ingraffia, R.; de Souza Machado, A.A. Microplastic Incorporation into Soil in Agroecosystems. *Front. Plant Sci.* **2017**, *8*, 1805. [CrossRef] [PubMed]
34. van den Berg, P.; Huerta-Lwanga, E.; Corradini, F.; Geissen, V. Sewage sludge application as a vehicle for microplastics in eastern Spanish agricultural soils. *Environ. Pollut.* **2020**, *261*, 114198. [CrossRef]
35. Cao, L.; Wu, D.; Liu, P.; Hu, W.; Xu, L.; Sun, Y.; Wu, Q.; Tian, K.; Huang, B.; Yoon, S.J.; et al. Occurrence, distribution and affecting factors of microplastics in agricultural soils along the lower reaches of Yangtze River, China. *Sci. Total Environ.* **2021**, *794*, 148694. [CrossRef]
36. He, D.; Luo, Y.; Lu, S.; Liu, M.; Song, Y.; Lei, L. Microplastics in soils: Analytical methods, pollution characteristics and ecological risks. *TrAC Trends Anal. Chem.* **2018**, *109*, 163–172. [CrossRef]
37. Rehm, R.; Zeyer, T.; Schmidt, A.; Fiener, P. Soil erosion as transport pathway of microplastic from agriculture soils to aquatic ecosystems. *Sci. Total Environ.* **2021**, *795*, 148774. [CrossRef]
38. Chen, W.; He, B.; Nover, D.; Lu, H.; Liu, J.; Sun, W.; Chen, W. Farm ponds in southern China: Challenges and solutions for conserving a neglected wetland ecosystem. *Sci. Total Environ.* **2019**, *659*, 1322–1334. [CrossRef]
39. Bichsel, D.; De Marco, P.; Bispo, A.Â.; Ilg, C.; Dias-Silva, K.; Vieira, T.B.; Correa, C.C.; Oertli, B. Water quality of rural ponds in the extensive agricultural landscape of the Cerrado (Brazil). *Limnology* **2016**, *17*, 239–246. [CrossRef]
40. Zongo, B.; Zongo, F.; Toguyeni, A.; Boussim, J.I. Water quality in forest and village ponds in Burkina Faso (western Africa). *J. For. Res.* **2017**, *28*, 1039–1048. [CrossRef]
41. Ramsperger, A.; Narayana, V.K.B.; Gross, W.; Mohanraj, J.; Thelakkat, M.; Greiner, A.; Schmalz, H.; Kress, H.; Laforsch, C. Environmental exposure enhances the internalization of microplastic particles into cells. *Sci. Adv.* **2020**, *6*, eabd1211. [CrossRef] [PubMed]
42. Bordos, G.; Urbanyi, B.; Micsinai, A.; Kriszt, B.; Palotai, Z.; Szabo, I.; Hantosi, Z.; Szoboszlay, S. Identification of microplastics in fish ponds and natural freshwater environments of the Carpathian basin, Europe. *Chemosphere* **2019**, *216*, 110–116. [CrossRef] [PubMed]
43. Xiong, X.; Liu, Q.; Chen, X.; Wang, R.; Duan, M.; Wu, C. Occurrence of microplastic in the water of different types of aquaculture ponds in an important lakeside freshwater aquaculture area of China. *Chemosphere* **2021**, *282*, 131126. [CrossRef] [PubMed]
44. Domagalski, J.L.; Ator, S.; Coupe, R.; McCarthy, K.; Lampe, D.; Sandstrom, M.; Baker, N. Comparative study of transport processes of nitrogen, phosphorus, and herbicides to streams in five agricultural basins, USA. *J. Environ. Qual.* **2008**, *37*, 1158–1169. [CrossRef]
45. King, K.W.; Williams, M.R.; Macrae, M.L.; Fausey, N.R.; Frankenberger, J.; Smith, D.R.; Kleinman, P.J.; Brown, L.C. Phosphorus transport in agricultural subsurface drainage: A review. *J. Environ. Qual.* **2015**, *44*, 467–485. [CrossRef]
46. Wang, J.; Yin, W.; Wang, L.X.; Li, N.X.; Hao, R.; Xu, J.F.; Xiao, H.B.; Zhong, Z.M.; Zhang, Y.P.; Shi, Z.H. Unraveling the effects of hydrological connectivity and landscape characteristics on reservoir water quality. *J. Hydrol.* **2022**, *613*, 128410. [CrossRef]

47. Liu, W.; Tan, Q.; Chu, Y.; Chen, J.; Yang, L.; Ma, L.; Zhang, Y.; Wu, Z.; He, F. An integrated analysis of pond ecosystem around Poyang Lake: Assessment of water quality, sediment geochemistry, phytoplankton and benthic macroinvertebrates diversity and habitat condition. *Aquat. Ecol.* **2022**, *56*, 775–791. [CrossRef]
48. Meng, C.; Liu, H.; Li, Y.; Shen, J.; Li, X.; Wu, J. Effects of environmental and agronomic factors on pond water quality within an intensive agricultural landscape in subtropical southern China. *Agric. Water Manag.* **2022**, *274*, 107953. [CrossRef]
49. Lin, T.; Yu, H.; Wang, Q.; Hu, L.; Yin, J. Surface water quality assessment based on the Integrated Water Quality Index in the Maozhou River basin, Guangdong, China. *Environ. Earth Sci.* **2021**, *80*, 368. [CrossRef]
50. Möller, J.N.; Löder, M.G.J.; Laforsch, C. Finding Microplastics in Soils: A Review of Analytical Methods. *Environ. Sci. Technol.* **2020**, *54*, 2078–2090. [CrossRef]
51. Prata, J.C.; da Costa, J.P.; Duarte, A.C.; Rocha-Santos, T. Methods for sampling and detection of microplastics in water and sediment: A critical review. *TrAC Trends Anal. Chem.* **2019**, *110*, 150–159. [CrossRef]
52. Shim, W.J.; Hong, S.H.; Eo, S.E. Identification methods in microplastic analysis: A review. *Anal. Methods* **2017**, *9*, 1384–1391. [CrossRef]
53. Wang, Z.; Wang, X.; Hu, C.; Ge, T.; Wang, L.; Xing, J.; He, X.; Zhao, Y. Comparative Evaluation of Analytical Techniques for Quantifying and Characterizing Polyethylene Microplastics in Farmland Soil Samples. *Agriculture.* **2024**, *14*, 554. [CrossRef]
54. Jia, W.; Karapetrova, A.; Zhang, M.; Xu, L.; Li, K.; Huang, M.; Wang, J.; Huang, Y. Automated identification and quantification of invisible microplastics in agricultural soils. *Sci. Total Environ.* **2022**, *844*, 156853. [CrossRef]
55. Deng, L.; Xi, H.; Wan, C.; Fu, L.; Wang, Y.; Wu, C. Is the petrochemical industry an overlooked critical source of environmental microplastics? *J. Hazard. Mater.* **2023**, *451*, 131199. [CrossRef]
56. Huang, S.; Huang, X.; Bi, R.; Guo, Q.; Yu, X.; Zeng, Q.; Huang, Z.; Liu, T.; Wu, H.; Chen, Y.; et al. Detection and Analysis of Microplastics in Human Sputum. *Environ. Sci. Technol.* **2022**, *56*, 2476–2486. [CrossRef]
57. Cui, J.; Chen, C.; Gan, Q.; Wang, T.; Li, W.; Zeng, W.; Xu, X.; Chen, G.; Wang, L.; Lu, Z.; et al. Indoor microplastics and bacteria in the atmospheric fallout in urban homes. *Sci. Total Environ.* **2022**, *852*, 158233. [CrossRef]
58. Hildebrandt, L.; Fischer, M.; Klein, O.; Zimmermann, T.; Fensky, F.; Siems, A.; Zonderman, A.; Hengstmann, E.; Kirchgeorg, T.; Pröfrock, D. An analytical strategy for challenging members of the microplastic family: Particles from anti-corrosion coatings. *J. Hazard. Mater.* **2024**, *470*, 134173. [CrossRef]
59. Zhang, G.S.; Liu, Y.F. The distribution of microplastics in soil aggregate fractions in southwestern China. *Sci. Total Environ.* **2018**, *642*, 12–20. [CrossRef]
60. Callesen, I.; Keck, H.; Andersen, T.J. Particle size distribution in soils and marine sediments by laser diffraction using Malvern Mastersizer 2000—Method uncertainty including the effect of hydrogen peroxide pretreatment. *J. Soils Sediments* **2018**, *18*, 2500–2510. [CrossRef]
61. Dhillon, G.S.; Amichev, B.Y.; de Freitas, R.; Van Rees, K. Accurate and Precise Measurement of Organic Carbon Content in Carbonate-Rich Soils. *Commun. Soil. Sci. Plant Anal.* **2015**, *46*, 2707–2720. [CrossRef]
62. Sáez-Plaza, P.; Navas, M.J.; Wybraniec, S.; Michałowski, T.; Asuero, A.G. An Overview of the Kjeldahl Method of Nitrogen Determination. Part II. Sample Preparation, Working Scale, Instrumental Finish, and Quality Control. *Crit. Rev. Anal. Chem.* **2013**, *43*, 224–272. [CrossRef]
63. Farr, T.G.; Rosen, P.A.; Caro, E.; Crippen, R.; Duren, R.; Hensley, S.; Kobrick, M.; Paller, M.; Rodriguez, E.; Roth, L.; et al. The Shuttle Radar Topography Mission. *Rev. Geophys.* **2007**, *45*, 1–33. [CrossRef]
64. Farr, T.G.; Kobrick, M. Shuttle radar topography mission produces a wealth of data. *Eos Trans. Am. Geophys. Union.* **2011**, *81*, 583–585. [CrossRef]
65. Rosen, P.A.; Hensley, S.; Joughin, I.R.; Li, F.K.; Madsen, S.N.; Rodriguez, E.; Goldstein, R.M. Synthetic aperture radar interferometry. *Proc. IEEE* **2000**, *88*, 333–382. [CrossRef]
66. NASA. NASA Shuttle Radar Topography Mission (SRTM) (2013). Shuttle Radar Topography Mission (SRTM) Global. Distributed by Open Topography. Available online: https://portal.opentopography.org/datasetMetadata?otCollectionID=OT.042013.4326.1 (accessed on 11 May 2024).
67. Abbasi, S.; Keshavarzi, B.; Moore, F.; Turner, A.; Kelly, F.J.; Dominguez, A.O.; Jaafarzadeh, N. Distribution and potential health impacts of microplastics and microrubbers in air and street dusts from Asaluyeh County, Iran. *Environ. Pollut.* **2019**, *244*, 153–164. [CrossRef]
68. Leslie, H.A.; Brandsma, S.H.; van Velzen, M.J.; Vethaak, A.D. Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. *Environ. Int.* **2017**, *101*, 133–142. [CrossRef]
69. Browne, M.A.; Crump, P.; Niven, S.J.; Teuten, E.; Tonkin, A.; Galloway, T.; Thompson, R. Accumulation of microplastic on shorelines woldwide: Sources and sinks. *Environ. Sci. Technol.* **2011**, *45*, 9175–9179. [CrossRef]
70. Schymanski, D.; Goldbeck, C.; Humpf, H.U.; Furst, P. Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles from different packaging into mineral water. *Water Res.* **2018**, *129*, 154–162. [CrossRef]
71. Hamidian, A.H.; Ozumchelouei, E.J.; Feizi, F.; Wu, C.; Zhang, Y.; Yang, M. A review on the characteristics of microplastics in wastewater treatment plants: A source for toxic chemicals. *J. Clean. Prod.* **2021**, *295*, 126480. [CrossRef]
72. Napper, I.E.; Bakir, A.; Rowland, S.J.; Thompson, R.C. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. *Mar. Pollut. Bull.* **2015**, *99*, 178–185. [CrossRef] [PubMed]

73. Zhang, P.; Huang, P.; Sun, H.; Ma, J.; Li, B. The structure of agricultural microplastics (PT, PU and UF) and their sorption capacities for PAHs and PHE derivates under various salinity and oxidation treatments. *Environ. Pollut.* **2020**, *257*, 113525. [CrossRef]
74. Gaylarde, C.C.; Neto, J.A.B.; da Fonseca, E.M. Paint fragments as polluting microplastics: A brief review. *Mar. Pollut. Bull.* **2021**, *162*, 111847. [CrossRef] [PubMed]
75. Loncarski, M.; Tubic, A.; Kragulj-Isakovski, M.; Jovic, B.; Apostolovic, T.; Nikic, J.; Agbaba, J. Modelling of the adsorption of chlorinated phenols on polyethylene and polyethylene terephthalate microplastic. *J. Serbian Chem. Soc.* **2020**, *85*, 697–709. [CrossRef]
76. Kleinteich, J.; Seidensticker, S.; Marggrander, N.; Zarfl, C. Microplastics Reduce Short-Term Effects of Environmental Contaminants. Part II: Polyethylene Particles Decrease the Effect of Polycyclic Aromatic Hydrocarbons on Microorganisms. *Int. J. Environ. Res. Public Health* **2018**, *15*, 287. [CrossRef] [PubMed]
77. Klein, M.; Fischer, E.K. Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. *Sci. Total Environ.* **2019**, *685*, 96–103. [CrossRef]
78. Teichert-Coddington, D.R.; Rouse, D.B.; Potts, A.; Boyd, C.E. Treatment of harvest discharge from intensive shrimp ponds by settling. *Aquac. Eng.* **1999**, *19*, 147–161. [CrossRef]
79. Xiong, H.F. Removal Effect of Ditch Pond Wetlands on Non-Point Source Pollutants from Agricultural Drainage. *Adv. Mater. Res.* **2014**, *955–959*, 2513–2516. [CrossRef]
80. Lenaker, P.L.; Baldwin, A.K.; Corsi, S.R.; Mason, S.A.; Reneau, P.C.; Scott, J.W. Vertical Distribution of Microplastics in the Water Column and Surficial Sediment from the Milwaukee River Basin to Lake Michigan. *Environ. Sci. Technol.* **2019**, *53*, 12227–12237. [CrossRef]
81. Semcesen, P.; Wells, M.G. Biofilm growth on buoyant microplastics leads to changes in settling rates: Implications for microplastic retention in the Great Lakes. *Mar. Pollut. Bull.* **2021**, *170*, 112573. [CrossRef]
82. Leiser, R.; Wu, G.M.; Neu, T.R.; Wendt-Potthoff, K. Biofouling, metal sorption and aggregation are related to sinking of microplastics in a stratified reservoir. *Water Res.* **2020**, *176*, 115748. [CrossRef] [PubMed]
83. Peller, J.R.; Nevers, M.B.; Byappanahalli, M.N.; Nelson, C.; Ganesh Babu, B.; Evans, M.A.; Kostelnik, E.; Keller, M.; Johnston, J.W.J.; Shidler, S. Sequestration of microfibers and other microplastics by green algae, Cladophora, in the US Great Lakes. *Environ. Pollut.* **2021**, *276*, 116695. [CrossRef] [PubMed]
84. Kalčíková, G. Aquatic vascular plants—A forgotten piece of nature in microplastic research. *Environ. Pollut.* **2020**, *262*, 114354. [CrossRef] [PubMed]
85. Sun, C.; Chen, L.; Liu, H.B.; Zhu, H.; Lü, M.Q.; Chen, S.B.; Wang, Y.W.; Shen, Z.Y. New Modeling Framework for Describing the Pollutant Transport and Removal of Ditch-Pond System in an Agricultural Catchment. *Water Resour. Res.* **2021**, *57*, 1–21. [CrossRef]
86. Jia, Z.; Chen, C.; Luo, W.; Zou, J.; Wu, W.; Xu, M.; Tang, Y. Hydraulic conditions affect pollutant removal efficiency in distributed ditches and ponds in agricultural landscapes. *Sci. Total Environ.* **2019**, *649*, 712–721. [CrossRef]
87. Wang, Z.; Yao, L.; Liu, G.; Liu, W. Heavy metals in water, sediments and submerged macrophytes in ponds around the Dianchi Lake, China. *Ecotoxicol. Environ. Saf.* **2014**, *107*, 200–206. [CrossRef]
88. Robotham, J.; Old, G.; Rameshwaran, P.; Sear, D.; Gasca-Tucker, D.; Bishop, J.; Old, J.; McKnight, D. Sediment and Nutrient Retention in Ponds on an Agricultural Stream: Evaluating Effectiveness for Diffuse Pollution Mitigation. *Water* **2021**, *13*, 1640. [CrossRef]
89. Pinon-Colin, T.J.; Rodriguez-Jimenez, R.; Rogel-Hernandez, E.; Alvarez-Andrade, A.; Wakida, F.T. Microplastics in stormwater runoff in a semiarid region, Tijuana, Mexico. *Sci. Total Environ.* **2020**, *704*, 135411. [CrossRef]
90. Liu, F.; Vianello, A.; Vollertsen, J. Retention of microplastics in sediments of urban and highway stormwater retention ponds. *Environ. Pollut.* **2019**, *255*, 113335. [CrossRef]
91. Horton, A.A.; Dixon, S.J. Microplastics: An introduction to environmental transport processes. *WIREs Water* **2017**, *5*, e1268. [CrossRef]
92. Townsend, K.R.; Lu, H.C.; Sharley, D.J.; Pettigrove, V. Associations between microplastic pollution and land use in urban wetland sediments. *Environ. Sci. Pollut. Res. Int.* **2019**, *26*, 22551–22561. [CrossRef] [PubMed]
93. Utama, I.K.A.P.; Darusman, D.; Syakur, S.; Zaitun, Z.; Ramli, I.; Warmadewanthi, I.D.A.A.; Nurhayati, E. Microplastic characteristics in several land covers in Banda Aceh and Aceh Besar. *BIO Web Conf.* **2024**, *89*, 01011. [CrossRef]
94. Luo, S.; Wu, H.; Xu, J.; Wang, X.; He, X.; Li, T. Effects of lakeshore landcover types and environmental factors on microplastic distribution in lakes on the Inner Mongolia Plateau, China. *J. Hazard. Mater.* **2024**, *465*, 133115. [CrossRef] [PubMed]
95. O'Connor, D.; Pan, S.; Shen, Z.; Song, Y.; Jin, Y.; Wu, W.M.; Hou, D. Microplastics undergo accelerated vertical migration in sand soil due to small size and wet-dry cycles. *Environ. Pollut.* **2019**, *249*, 527–534. [CrossRef]
96. Zhang, X.; Chen, Y.; Li, X.; Zhang, Y.; Gao, W.; Jiang, J.; Mo, A.; He, D. Size/shape-dependent migration of microplastics in agricultural soil under simulative and natural rainfall. *Sci. Total Environ.* **2022**, *815*, 152507. [CrossRef]

97. Luo, Y.; Zhang, Y.; Xu, Y.; Guo, X.; Zhu, L. Distribution characteristics and mechanism of microplastics mediated by soil physicochemical properties. *Sci. Total Environ.* **2020**, *726*, 138389. [CrossRef]
98. Rillig, M.C. Microplastic Disguising As Soil Carbon Storage. *Environ. Sci. Technol.* **2018**, *52*, 6079–6080. [CrossRef]

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Review

Sustainable Wastewater Treatment Strategies in Effective Abatement of Emerging Pollutants

Hafiz Waqas Ahmad [1], Hafiza Aiman Bibi [2], Murugesan Chandrasekaran [3], Sajjad Ahmad [4,*] and Grigorios L. Kyriakopoulos [5,*]

[1] Department of Food Engineering, Faculty of Agricultural Engineering & Technology, University of Agriculture Faisalabad, Faisalabad 38000, Pakistan; hafiz_waqasahmad@yahoo.com
[2] Department of Agriculture Sciences, Faculty of Agriculture, University of Agriculture Faisalabad, Faisalabad 38000, Pakistan; aimanalishair@yahoo.com
[3] Department of Food Science and Biotechnology, Sejong University, 209 Neundong-ro, Gwangjin-gu, Seoul 05006, Republic of Korea; chandrubdubio@sejong.ac.kr
[4] Environmental Sustainability & Health Institute (ESHI), School of Food Science & Environmental Health, Technological University Dublin, City Campus, Grangegorman Lower, GW212-35 Dublin, Ireland
[5] School of Electrical and Computer Engineering, Zografou Campus, National Technical University of Athens, 15780 Athens, Greece
* Correspondence: sajjad.ahmad@tudublin.ie (S.A.); gregkyr@chemeng.ntua.gr (G.L.K.)

Abstract: The fundamental existence of any living organism necessitates the availability of pure and safe water. The ever-increasing population has led to extensive industrialization and urbanization, which have subsequently escalated micropollutants and water contamination. The environmental impact on various life forms poses a dire need for research in effective environmental management. Versatile technologies involving multiple approaches, including physiochemical and biological bioremediation strategies, draw insights from environmental biology. Metabolic annihilation mediated by microbes shows significant potential in the bioconversion of toxic micropollutants to tolerable limits. Environmentally friendly, cost-effective, and sustainable strategies are envisaged for efficient environmental protection. Phytoremediation technology, especially floating wetland treatments, facilitates micropollutant elimination, landscape management, ecosystem conservation, and aesthetic enhancement in diverse environments. The incorporation of nanomaterials in the bioremediation of toxic micropollutants augments novel and innovative strategies for water pollution abatement. This paper offers a novel strategy that combines nanomaterials to improve micropollutant degradation with bioremediation techniques, particularly the creative application of phytoremediation technologies like floating wetlands. Combining these techniques offers a novel viewpoint on long-term, affordable approaches to reducing water pollution. Additionally, the review proposes a forward-looking strategic framework that addresses the accumulation and refractory nature of micropollutants, which has not been thoroughly explored in previous literature.

Keywords: micropollutants; toxicity; wastewater; floating wetlands; bioremediation

Citation: Ahmad, H.W.; Bibi, H.A.; Chandrasekaran, M.; Ahmad, S.; Kyriakopoulos, G.L. Sustainable Wastewater Treatment Strategies in Effective Abatement of Emerging Pollutants. *Water* **2024**, *16*, 2893. https://doi.org/10.3390/w16202893

Academic Editor: William Frederick Ritter

Received: 30 August 2024
Revised: 30 September 2024
Accepted: 4 October 2024
Published: 11 October 2024

1. Introduction

Water is a natural resource on Earth, and its accessibility in a pure state is indispensable for human beings and other living creatures, as the concept of life is unimaginable without it [1]. Increasing industrialization, urbanization, and the unchecked nature of human activities are directly correlated with the release of hazardous substances, often criticized as the foremost sources of pollution in aquatic ecosystems [2]. The controlled or uncontrolled discharge of industrially contaminated wastewater introduces toxic agents into groundwater, surface water, and subsurface soils [3]. The presence of dye pollutants, pharmaceuticals, personal care products (such as plastic bottles, children's toys, cosmetics, toothpaste, detergents, and drugs like hormones, anti-inflammatory drugs,

antiepileptics, statins, antidepressants, beta-blockers, antibiotics, and contrast agents), toxic metals, volatile organic compounds, pesticides, and petroleum hydrocarbons in industrial wastewater severely compromises the quality of drinking water and accumulates in the food chain. This poses a severe threat to human beings and aquatic creatures, attracting increasing scholarly attention [4–7] (Figure 1). A subclass of organic chemicals gradually being detected in our water bodies has been categorized as emerging pollutants (EPs), also known as "contaminants of emerging concern" or micropollutants (MPs) [8]. These hazardous substances deplete water quality and gravely affect water for drinking and domestic purposes. Recently, a United Nations report stated that purified and freshwater availability is a worldwide issue and has become a challenge in the 21st century because living organisms are not safe with contaminated water [9].

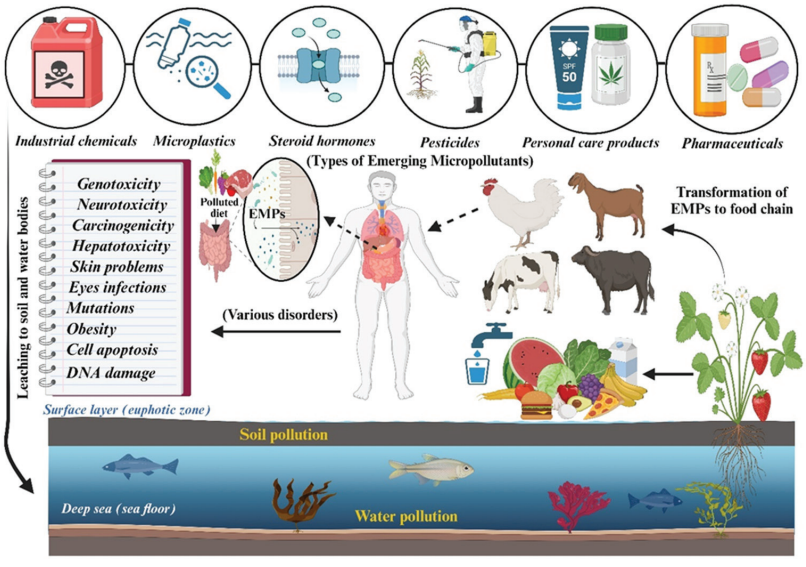

Figure 1. Various emerging pollutants and their negative effects on the ecosystem.

Emerging pollutants are divided into three main categories: the first includes newly synthesized compounds released into the ecosystem. The second category comprises compounds that have existed in the environment for a long time but are only recently being recognized. The third category includes compounds that have been detected for a long time, but their significant impact on the environment and human health has only recently been documented, such as hormones [10,11]. These emerging pollutants fundamentally alter human behavior, landscapes, water bodies, and demography through developing technologies, microbial adaptation, climate changes, increased travel, and more [12]. Some of these compounds cause endocrine disruption and are defined as exogenic agents that interfere with the synthesis, secretion, transport, binding, or elimination of natural hormones in the human body responsible for homeostasis, reproduction, development, and behavior [13]. Furthermore, the presence of these compounds is responsible for many internal and external diseases in living organisms, such as effects on embryo growth, sexual differentiation, metabolism, reproductive system fluctuations, the sexually dimorphic neuroendocrine system, endogenous steroid levels, diabetes, cardiovascular problems, abnormal neuronal behavior, and obesity [14–17]. These emerging contaminants extensively affect the atmosphere and pollute air quality and marine zones [18]. Due to the high accumulation and persistence of heavy metals, ecotoxicological effects on non-target organisms appear rapidly and pose severe threats to aquatic life [19]. The presence of toxic

metals in fish has revealed tissue damage, pathological effects on blood cell indices, and disrupted hormone regulation and enzymatic activities [20].

The low concentration of emerging pollutants in water bodies can cause severe effects on humans, mammals, and beneficial flora and fauna, such as immune suppression, cancer, organ damage, and nervous system damage, potentially leading to death [21]. The genotoxicity of textile dyes is considered a major source of long-term health injuries in humans and other living organisms [22]. For example, Alaguprathana et al. [23] studied the genotoxic effects of textile dye contaminants in *Allium cepa* root cells. The findings revealed that the consumption of polluted *Allium cepa* caused chromosomal aberrations in humans. Consequently, approaches for their practical and rapid degradation have become the primary focus of governments worldwide, academic research groups, and environmental protection agencies. A wide range of remediation policies has been developed to efficiently remove and eradicate these organic and inorganic toxic pollutants from wastewater and other water systems. Various physical and chemical techniques, including adsorption, sedimentation, degasification, filtration, aeration, chemical precipitation, flocculation and coagulation, ion exchange, and ozonation, have been attempted to remove pollutants from wastewater [24–28]. Although these approaches can perform smoothly for remediation purposes, they are associated with various limitations and negative impacts, such as high costs, production of secondary pollutants, use of toxic chemicals and solvents, inefficiency, and time consumption [29–32].

However, researchers have been paying widespread and urgent attention to alternative approaches that are fundamentally smart, inexpensive, easy to handle, efficient, greener, and environmentally friendly. Biological methods are considered relatively novel and environmentally sustainable for the remediation of emerging pollutants from aquatic systems [33]. In this approach, different microbial species such as bacteria, fungi, yeast, algae, and archaea are developed for the remediation of contaminants from water systems. These microbial species use pollutants as substrates to enhance their enzymatic machinery, leading to the enzymatic breakdown of recalcitrant emerging pollutants into less toxic or non-toxic compounds [4]. Additionally, microbial cell cultures, components, biomolecules, and various plant extracts and seeds are utilized to synthesize sustainable nanoparticles [34]. These nanoparticles can serve as outstanding candidates in the biodegradation of different pollutants by immobilizing and facilitating the extracellular synthesis of microbial degrading enzymes and genes [35]. Nanoparticles are excellent adsorbents and catalysts extensively applied in extreme environmental conditions in both laboratory and pilot-scale studies due to their novel characteristics such as large specific surface area, modifiability at various temperatures, customizable pore size, reduced interparticle diffusion width, and diverse shapes [36].

In contrast to all the above conventional energy-intensive treatment technologies, phytoremediation, which utilizes plant species for the removal of even lower concentrations/dosages of pollutants in the aquatic system, is considered one of the most efficient, environment-friendly, low-cost, easy-to-perform, and effective approaches. This method offers engineering solutions including constructed wetlands and floating treatment wetlands [37]. In constructed and floating wetlands, plant materials uptake all pollutants from the water and utilize them as nutrients, which then accumulate in the plant biomass [38]. Hence, the ultimate aim of this review is to provide new insights for the remediation of emerging pollutants from different water systems and to understand the novel concepts and interactions with microbial species, plant species, different solvents and chemicals, physiochemical methods, and nanomaterials that are considered efficient facilitators for environmental clean-up. Moreover, this review also elucidates the impact and mechanisms of physical, chemical, and biological methods in the degradation of emerging pollutants from wastewater, establishing a myriad of promising approaches for potential ecosystem monitoring. This manuscript also highlights the cutting-edge methods for growing, gathering, and effectively cleaning up various pollutants using microbes. This assessment

explores the current advancements as well as how they might fit with the objectives of global sustainability.

2. Biological Methods for Wastewater Treatment

The biological methods removed various emerging pollutants more efficiently. Using biological methods aims to establish a system for effectively eliminating pollutants from the contaminated environment [39]. Biological methods are widely utilized in both laboratory and field-scale environments, and they are considered to be less expensive, more efficient, reliable, and eco-friendly compared to physical and chemical treatments [40]. This method applied normal cellular processes that depend on different microbial species such as nematodes, bacteria, fungi, or other microorganisms involved in breaking emerging pollutants [41] (Table 1). These toxic pollutants retarded the growth of microbial species.

Table 1. Source of microbial species and their degradation efficiency for wastewater treatment.

Name of Emerging Pollutant	Name of Microbial Species	Source of Isolation	Culture Conditions	Degradation Efficiency %	References
Dyes	*Pseudomonas fluorescens*, *Bacillus* sp., and *Escherichia coli*	Dye contaminated soil	Temperature 30 ± 1 °C Nutrient agar Shaken 110 rpm	43, 15, 90	[42]
Azo dyes	*Enterobacter hormaechei* SKB 16	Textile-effluent-polluted soil	Temperature 37 °C Nutrient agar Shaken 115 rpm	98	[43]
Methyl red	*Vibrio logei* and *Pseudomonas nitroreducens*	Wastewater	Temperature 25 °C Minimal medium Shaken 110 rpm	65–82	[44]
Monoazo and diazo dyes	*Acinetobacter* sp. and *Klebsiella* sp.	Activated sludge	Temperature 30 °C Minimal medium Shaken 120 rpm	80	[45]
Carmoisine	*Saccharomyces cerevisiae* ATCC 9763	Commercial	Temperature 30 °C Yeast extract peptone dextrose Shaken 180 rpm	100	[46]
Copper, nickel, manganese, cobalt, and dichromate	*Bacillus* sp., *Shewanella* sp., *Lysinibacillus* sp., and *Acinetobacter* sp.	Sludge	Temperature 30 °C Luria broth medium Shaken 120 rpm	90–100	[47]
Iron, copper, zinc, cadmium, manganese, nickel, and lead	*Bacillus* sp. PS-6	Industrial wastewater	Temperature 35 °C Luria broth medium Shaken 120 rpm	44.12–89.46	[48]
Nickel, chromium, and textile dyes	*Lysinibacillus* sp.	Wastewater	Temperature 30 °C Nutrient broth medium Shaken 150 rpm	70, 58, 82	[49]
Zinc, cobalt, nickel, lead, copper, chromium, mercury, arsenic, and silver	*Rhodococcus* sp. AQ5-07	Oil-polluted soil	Temperature 10 °C Tween-peptone agar Shaken 150 rpm	80–100	[50]
Chromium, lead, iron, cobalt, nickel, manganese, zinc, copper, and aluminum	*Agaricus bisporus*	Commercial	Temperature 25 °C	80–98	[51]

Table 1. *Cont.*

Name of Emerging Pollutant	Name of Microbial Species	Source of Isolation	Culture Conditions	Degradation Efficiency %	References
Sulfamethoxazole	*Escherichia coli* JM109 and *Chlorella sorokiniana*	Fish breeding tank	Temperature 37 °C Temperature 28 °C	54.34	[52]
Erythromycin	*Geobacter* sp. and *Acetoanaerobium* sp.	Wastewater	-	99	[53]
Sulfamethoxazole	*Shewanella* sp. *Alcaligenes* sp., *Pseudomonas* sp., and *Achromobacter* sp.	Wastewater	Temperature 30 °C	85.1	[54]
Tetracycline	*Shewanella* sp., *Bacillus* sp., and *Pseudomonas* sp.	Seed sludge	Temperature 30 °C Luria–Bertani medium Agitation 150 rpm	95	[55]
Ciprofloxacin	*Lactobacillus gesseri*, *Enterobacter* sp., *Bacillus* sp., *Bacillus subtilius*, and *Micrococcus luteus*	Hospital effluent water	Temperature 28 °C Luria–Bertani medium Agitation 100 rpm	100	[56]
Tetracycline	*Bacillus velezensis* strain Al-Dhabi 140	Municipal soil sludge	Temperature 37 °C Minimal medium Agitation 150 rpm	100	[57]
Triazophos, methamidophos, and carbofuran	*Enterobacter* sp. strain Z1	Wastewater	Temperature 37 °C, pH 7	100, 100, 98.7	[58]
Fludioxonil	*Betaproteobacteria* sp., *Chloroflexi* sp., *Planctomycete* sp., *Firmicutes* sp., *Empedobacter* sp., *Sphingopyxis* sp., and *Rhodopseudomonas* sp.	Fungicide wastewater	Room temperature Agitation 120 rpm	95.4	[59]
Chlorpyriphos, oxadiazon, and cypermethrin	*Chlorella* sp. and *Scenedesmus* sp.	Contaminated semiopen photobioreactor	Temperature 25 °C pH 7.5 Agitation 120 rpm	97, 88, 74	[60]
Deltamethrin, cyfluthrin, cypermethrin, permethrin, and lambda-cyhalothrin	*Enterobacter ludwigii*	Industrial wastewater	Temperature 30 °C Saline condition pH 7	90	[61]
Allethrin	*Sphingomonas trueperi*	Wastewater sludge	Temperature 30 °C pH 7.0 Inoculum concentration 100 mg/L	93	[62]
Anthracene, phenanthrene, fluorene, naphthalene, pyrene, benzo(e)pyrene, benzo(k)fluoranthene, and benzo(a)pyrene	*Ochrobactrum* sp., *Bacillus* sp., *Marinobacter* sp., *Pseudomonas* sp., *Martelella* sp., *Stenotrophomonas* sp., and *Rhodococcus* sp.	Wastewater	Temperature 55 °C pH 9 Agitation 150 rpm Salt concentration 100 g/L	100, 100, 100, 100, 93, 60, 55, 51	[63]

Table 1. *Cont.*

Name of Emerging Pollutant	Name of Microbial Species	Source of Isolation	Culture Conditions	Degradation Efficiency %	References
Phenanthrene and fluorene	*Ochrobactrum halosaudis* strain CEES1, *Stenotrophomonas maltophilia* CEES2, *Achromobacter xylosoxidans* CEES3 and *Mesorhizobium halosaudis* CEES4	Red Sea saline water and sediment samples	Temperature 37 °C pH 7	90	[64]
Naphthalene, phenanthrene, fluoranthene, pyrene, total petroleum hydrocarbons, and phenolic compounds	*Stenotrophomonas* sp. S1VKR-26	Polluted Damanganga river	Temperature 37 °C pH 7 Incubation time 7 days	93, 86, 92, 98.3, 72.33, 93.06	[65]
Petroleum hydrocarbons	*Paramecium* sp., *Vorticella* sp., *Epistylis* sp. and *Opercularia* sp.	Wastewater	Temperature 25 °C pH 7 Incubation time 16 days	70	[66]
Crude oil, crude oil alkanes, pristane, and phytane	*Pseudomonas* sp. and *Bacillus* sp.	Oil-polluted sediment	Temperature 30 °C pH 7 Incubation time 14 days	80.64, 76.30, 46.75, 78.23	[67]
Naphthalene	*Bordetella avium*	Petroleum refinery wastewater	Temperature 30 °C pH 7.5 Naphthalene concentration 100 to 500 mg/L Incubation time 10 days	100	[68]

In most cases, various mechanisms and co-metabolisms boosted the microbial species and promoted the growth of microbial species and the degradation rate of pollutants from the contaminated sites [69]. Apart from the application of microbial species, plants are also considered superior agents which play a vital role in the clean-up of the environment, more specifically known as phytoremediation [70]. Phytoremediation properties of plants are highly beneficial for soil properties and the environment. The plants degrade parent toxic compounds into less toxic products such as carbon dioxide, water, and ammonia, which are further taken up by plants, improving the quality of soil, enhancing the growth of plants, and increasing environmental sustainability [71,72]. In this paper, biological treatment in two broad categories, microbial biodegradation and phytoremediation, have been discussed.

2.1. Microbial Biodegradation of Emerging Pollutants

Chemical and physical treatments for removing emerging pollutants are extensively applied and considered efficient technologies. However, these techniques are related to severe drawbacks such as cost, efficiency, reliability, and production of secondary pollutants [73,74]. So, biological treatment processes are more effective and are considered an efficient approach for the clean-up of the environment. It is well known that the efficiency of microbial species for various treatment processes primarily relies on the activity

and interaction of microbial species and pollutant removal efficiency is correlated with microbial species and their diversity [75] (Figure 2). The adoption of microbial species strongly depended on their ability to transfer electrons from substrates to the anode. For biological and environmental stability, different microbial species and their single and mixed cells were isolated from various contaminated places, such as marine sediments, wastewater, and polluted soil, and extensively applied to remove emerging pollutants from the environment [76,77].

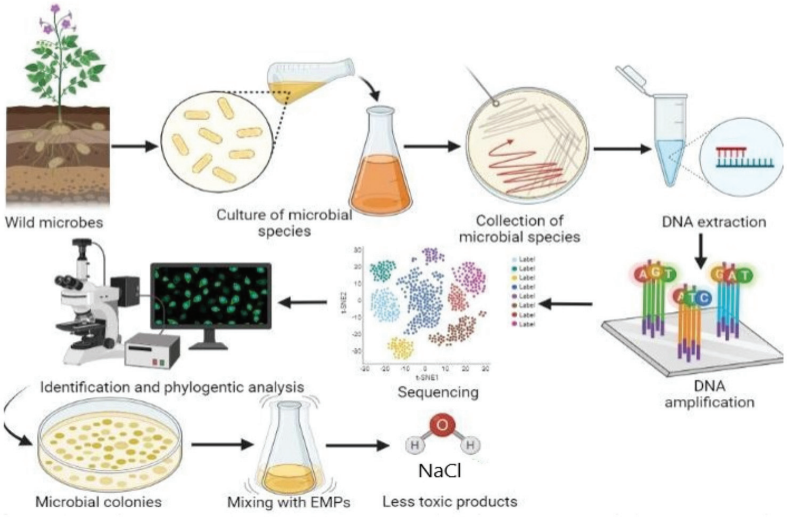

Figure 2. Schematic diagram of microbial degradation for the removal of pollutants. Source: Authors own study, based on [75].

Ferreira et al. [78] isolated a bacterial strain from contaminated marine sediment, and after physiochemical, morphological, and genetic identification, this strain showed a remarkable resemblance to *Bacillus thuringiensis*. The capability of the bacterial strain was tested for the degradation of various pesticides and polycyclic aromatic hydrocarbons. Results of this study indicated that after 10 days of incubation, microbial strains could degrade 97.3% of polycyclic aromatic hydrocarbons, and for pesticides, the findings revealed that approximately 78% was degraded after 11 days of culture. Recently, Li et al. [79] isolated a manganese-oxidizing bacterium to remove ofloxacin from the aquatic environment. Morphological and genetic findings showed that the bacterial strain revealed the highest similarity to *Pseudomonas* sp. and was named the F2 strain. Results of the degradation experiment demonstrated that bacteria could completely degrade ofloxacin with a concentration of 5 μg/L. The effective removal of the two most recalcitrant pharmaceutical compounds, carbamazepine and diatrizoate, by using microbes was investigated in laboratory batch experiments. In this study, mixed microbial culture was initially isolated from polluted soil and identified as *Acinetobacter* sp., *Bacillus halodurans*, and *Pseudomonas putida* and their degradation efficiency against the pollutants was examined. Results of this study revealed that after 12 days, mixed microbial culture could degrade 43.2% of diatrizoate and 60% of carbamazepine with a concentration of 100 μg/L. This study concluded that indigenous microbial species are a superior agent for removing emerging pollutants from the contaminated environment [80].

For the degradation of microplastics from the polluted environment, Lwanga et al. [81] obtained earthworms and identified them as *Lumbricus terrestris*. The degradation efficiency of the earthworm was tested against low-density polyethylene and isolated bacteria from the earthworm's gut. Results showed that the bacteria isolated from the gut were Gram-positive and showed a high resemblance to the phyla Actinobacteria and Firmicutes. The

isolated bacteria were used to treat gamma-sterilized soil and low-density polyethylene microplastics. The results of this study demonstrated that in the soil treated with bacteria the size of microplastics was significantly decreased compared to the control soil. Recently, the removal of the heavy metal cadmium and antibiotic sulfamethoxazole by bacteria was studied. The novel bacterial species was screened from the polluted soil and identified as *Achromobacter* sp. L3. The optimum conditions were set, and was it found that for the degradation of sulfamethoxazole, this strain performed efficiently at a pH range of 6–8, temperature range of 25–30 °C, and initial concentration of sulfamethoxazole of 10–40 mg/L. The maximum removal rate was 91.98%. Meanwhile, the optimum conditions for removing cadmium were pH 7–9, temperature 25–30 °C, and cadmium concentration 10–30 mg/L, and complete degradation was achieved. This study added valuable insights into removing emerging pollutants from wastewater, polluted soil, and sediments [82].

In another study, for the remediation of total petroleum hydrocarbons, alkanes, and polycyclic aromatic hydrocarbons by using mixed microbial cultures (*Pseudomonas stutzeri* GQ-4 strain KF453954, *Pseudomonas* SZ-2 strain KF453956, and *Bacillus* SQ2 strain KF453961) a microcosm experiment was carried out. Findings indicated a linear correlation between the total petroleum hydrocarbons and alkane degradation rates. Moreover, this study explained that the petroleum hydrocarbon-degrading microbial population measured by the most probable number was significantly correlated with metabolic activity in the biology assay [83]. The unique ability of microalgae to remove heavy metals and nutrients (carbon, phosphorus, and nitrogen) from wastewater has drawn a lot of interest in the past decade [84]. Oxygen-evolving organisms known as microalgae are able to absorb significant amounts of nutrients (carbon, nitrogen, and phosphorus) from wastewater in order to reproduce and grow [85]. They are mostly photoautotrophic organisms, making efficient use of CO_2 and sunlight. Additionally, depending on the energy and carbon sources that are available in their surroundings, they can effectively display both heterotrophic and mixotrophic modes [86]. They offer an additional choice because they are adaptable in a variety of climatic and environmental circumstances, economically efficient, and environmentally proficient for wastewater treatment [87]. Furthermore, the concentrated biomass recovered from microalgae-based wastewater treatment systems can be utilized as a biofertilizer or as a starting point for the synthesis of bioenergy [88].

Following a chemical adaption procedure, the degradation of sulfamethazine and sulfamethoxazole at various concentrations by *S. obliquus* was evaluated. High removals for high concentrations were observed (31.4–62.3% in 0.025–0.25 mg/L of sulfamethazine, and 27.7–46.8% in 0.025–0.25 mg/L of sulfamethoxazole) [89]. One of the potential algal strains for heavy metal biodegradation is *Phacus* sp. This strain is highly resistant to heavy metals. *Phacus spp.* exhibited a 75.17% absorption rate of the heavy metal nickel (Ni) at a concentration of 5 mg/L. Furthermore, the strain reduced the concentration of the heavy metal aluminum (Al) by 19% from 9.94 mg/L. Moreover, the concentration of the heavy metal lead (Pb) was decreased from 1 mg/L by 96.7 percent [90]. It has been established that the green filamentous algae *Cladophora* sp. can degrade arsenic from water at quantities of up to 6 mg/L, with incredible results. Because of their great sorption ability, they have been demonstrated to be able to absorb arsenic in inorganic forms (like As III and As V), organic forms (such methyl methacrylate and DMA), and arsenosugar forms [91]. Manganese (Mn) from tainted water was remedied by *Spirogyra* sp. and *Richeterella* sp. When compared to *Spirogyra* sp., the experimental investigation showed that *Richeterella* sp. had the best capacity to accumulate [92]. The degradation of industrial metals such as Cd and Zn from the brown alga *Sargassum polycystum* was investigated through optimization experiments. Under optimal conditions, the highest removal efficiencies of Cd and Zn are 86.20% and 92.90%, respectively [93].

However, the removal of various pollutants using indigenous microbial species to clean up the environment is considered an efficient method and supplying various kinds of nutrients by biostimulation and bioaugmentation processes stimulates the microbial species for the degradation of emerging pollutants [94,95].

2.2. Installation of Floating Wetlands for Wastewater Treatment

Floating treatment wetlands are extensively applied and well known due to their functional benefits, such as being highly efficient, easy to install, inexpensive, and ecofriendly for treating polluted water [96]. They contain macrophytes developing in a floating structure that keeps the plant roots in direct contact with the effluent regardless of the water flow variation over time, allowing the removal of pollutants by various processes [97]. The shoots and crowns of macrophytes develop at the water level while their roots are expanded beneath the water column [98]. A dangling setup of roots, rhizomes, and supported biofilm forms below the floating mat, which supplies a biologically active surface area for biochemical processes and physical processes, such as filtering and entrapment of particulates [99] (Figure 3). Floating treatment wetland technologies are designed and applied to remove various kinds of emerging pollutants from water, such as nutrients, petroleum hydrocarbons, and pharmaceutical products [100]. More interestingly, the treated water can be applied for irrigation purposes to crops and provide a sustainable and ecofriendly approach which is beneficial for the long-term removal of contaminants with minimal ecological involvement [101] (Table 2). To treat domestic sewage, constructed wetlands were installed by applying macrophyte *Typha domingensis* Pers. For one year, the average degradation of organic matter was investigated by chemical oxygen demand, 5-day biochemical oxygen demand, and total suspended solids. The results of this study revealed that 41% of nutrients and 31% of total phosphorus were removed efficiently. Finally, this study concluded that investigating various parameters in the floating wetlands treatment technique provides valuable data in enhancing the removal capability of macrophytes and improving the quality of water bodies [102]. On a large scale, nutrients and agrochemicals in the floating wetlands were removed for 16 weeks. Two different plant species (duckweed and water hyacinth) were applied, and their growth parameters and climatic conditions were measured. Five different pesticides, imidacloprid, thiacloprid, myclobutanil, chlorpyriphos, and dimethomorph, were used with the volumes of 0.75 mL, 0.63 mL, 3.3 mL, 0.31 mL, and 0.3 mL. In contrast, the volume of nutrients was 68.9 g, used in the tank to pollute the water artificially. The results of this study showed that both plant species could remove pesticides and nutrients in the ranges of 27.4% to 83.6% and 12.4% to 42.7%, respectively [103].

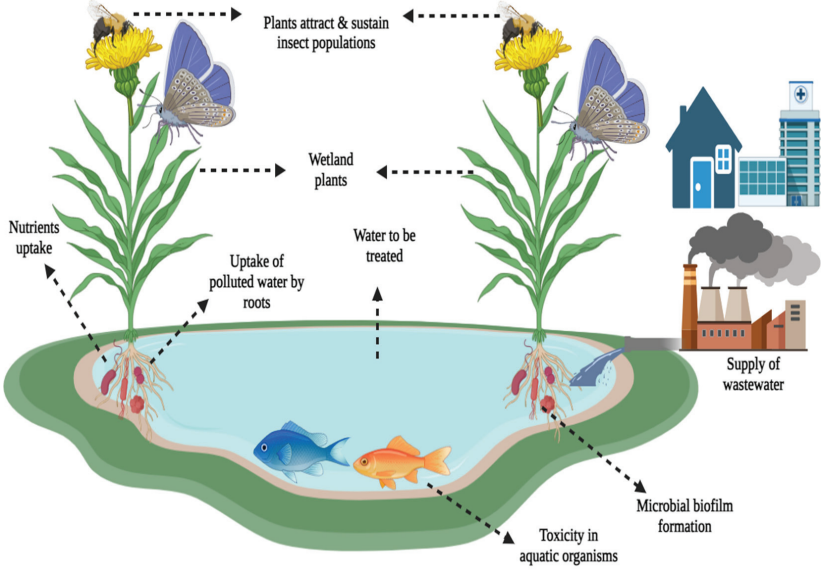

Figure 3. Installation of constructed floating wetlands for wastewater treatment.

Steroid hormones and biocides are considered emerging sources of pollution in the urban environment, which are extensively applied and cause severe threats to living organisms in aquatic and terrestrial environments [104]. An integrated constructed wetland system was recently installed to treat wastewater with steroid hormones and biocides. The results of this study revealed that five kinds of steroid hormones and four biocides could be detected within a constructed wetland system. The concentrations of the effluents were 30.5 ± 1.25 ng/L to 105 ± 5.14 ng/L and from 63.4 ± 2.85 ng/L to 515 ± 19.7 ng/L, respectively. By installing a floating treatment wetland system, 97.4% of steroid hormones and 92.4% of biocides were removed from the wastewater efficiently [105]. Constructed wetland systems are applied to remove a wide range of pollutants from the wastewater, such as livestock wastewater, organic matter, nutrients, and metals called emerging pollutants [106]. A microcosm of *Phragmites australis* was used to clean livestock wastewater with 100 µg/L of enrofloxacin, ceftiofur, and two antibiotics commonly applied in the livestock industry. Results of this study demonstrated that after 8 weeks of installation, floating constructed wetlands could remove 90% of pollutants from the environment [107]. In another study, a constructed floating wetland system was built to remove various kinds of pollutants, including petroleum hydrocarbons, from wastewater. The plant *Typha latifolia* was grown for three months and various plant growth indexes, biofilm formation, and degradation rate of pollutants were measured. Results of this study indicated that the floating wetland mediated with the plant was able to remove petroleum hydrocarbon, phenol, oil and grease, chemical oxygen demand, and total suspended solids within the ranges of 45–99%, 99–100%, 70–80%, 45–91%, and 46–88%, respectively. Finally, this study concluded that *Typha latifolia* is a superior candidate that could be further used to remove other emerging pollutants from aquatic systems and save the diversity of living organisms in water bodies [108].

Table 2. Application of potential plant species for remediation of emerging pollutants in wastewater.

Name of Pollutants	Plant Species	Water Matrix	Degradation %	References
Iron, nickel, manganese, lead, and chromium	*Phragmites australis* and *Brachia mutica*	Polluted river water	79.05, 91.4, 91.8, 36.14, and 85.19	[109]
Ammonia, chromium, and total ammonia nitrogen	*Chrysopogon zizanioides* L.	Industrial wastewater	40.29–50	[110]
Sewage and industrial wastewater	*Brachiaria mutica*, *Cannabis indica*, *Leptochloa fusca*, *Phragmites australis*, *Rhaphiolepis indica*, and *Typha domingensis*	Ponds and industrial wastewater	60 and 40	[111]
Hexavalent chromium	*Brachiaria mutica*	Industrial wastewater	53	[112]
Copper, nickel, manganese, zinc, lead, and iron	*Phragmites australis*	Textile wastewater	77.5, 73.3, 89.7, 81, 70, and 65.5	[113]
COD, TN, ammoniacal nitrogen, nitrate nitrogen, TP, and phosphate ion	*Canna* sp.	Synthetic wastewater	91.3. 58.3, 58.3, 92, 79.5, and 67.7	[114]
COD, BOD, ammonia nitrogen, and orthophosphate	*Cyperus* sp. and *Heliconia* sp.	Polluted fishpond water	33.96, 29.41, 27.80, and 28.44	[115]
Nitrogen, phosphorus, organic matter, and coliform	*Phragmites* sp.	Domestic wastewater	93, 100, 99.6, and 99.9	[116]

Table 2. *Cont.*

Name of Pollutants	Plant Species	Water Matrix	Degradation %	References
COD, BOD, and TSS	*Eichhornia crassipes*, *Eichhornia paniculate*, *polygonum ferrugineum*, and *Borreria scabiosoides*	Dairy wastewater	74.8, 86.4, and 84.8	[117]
Hydrocarbons, COD, BOD, TOC, and phenol	*Phragmites australis*	Diesel-oil-contaminated water	95.8, 98.6, 97.7, 95.2, and 98.9	[118]
COD, BOD, colors, and trace metals	*Phragmites australis*	Textile industry wastewater	92, 91, 86, and 87	[119]
Oil, COD, and BOD	*Brachiara mutica* and *Phragmites australis*	Oil field wastewater	97, 93, and 97	[120]
BOD, TSS, nitrogen and phosphorus	*Carex virgata*	Domestic wastewater	100, 100, 93, and 93	[121]
Nitrogen and phosphorus	*Agrostis alba*, *Canna generalis*, *Carex stricta*, *Iris ensata*, and *Panicum virgatum*	Nursery wastewater	59.6 and 64.7	[122]
TP, TSS, BOD, TOC, turbidity, and DOC	*Juncus maritimus*	Saline wastewater	86, 82, 78, 55, 53, and 19	[123]
Total phosphorus (TP) and total nitrogen (TN)	*Pontederia cordata* and *Juncus effusus*	Agricultural runoff	90 and 84	[124]
Phenolic compounds, TOC, and TN	*Cyperus alternifolius* and *Vetiveria zizanioides*	Olive mill wastewater	98.8, 95.3, 82.7, and 98.8	[125]
Total nitrogen and total phosphorus	*Iris wilsonii*	Municipal wastewater	57.6 and 46.7	[126]
Ammonium, BOD, TN, TP, iron, lead, copper, and nickel	*Eichhornia crassipes*	Polluted lake water	97.4, 75, 82, 84.2, 62.5, 88.9, 81.7, and 80.4	[127]
BOD, COD, TN, ammonium, nitrate, phosphate, and sulfate	*Spirodela polyrhiza*	Septage effluent	68.43, 64.29, 66.41, 81.87, 58.02, 60.48, and 64.45	[128]

3. Physiochemical Methods for Wastewater Treatment

Different strategies are involved in remediating hazardous substances in water. The suitability of these techniques can be analyzed by various parameters such as the ability of a method to remove toxic contaminants from wastewater to an acceptable level, the stability and shelf life of the method, and, most importantly, its compatibility with the environment and human resources [129]. Physiochemical approaches for the remediation of emerging micropollutants in water matrices are mentioned in Table 3.

3.1. Physical Method

3.1.1. Sedimentation

This physical method is considered one of the most prominent for wastewater treatment. The principle of this strategy is to disperse waste particles from the liquid based on gravity and decrease the velocity of water, favoring the suspension of particles in motionless situations. After that, gravitational forces are used to separate the particles [130]. The efficiency of the sedimentation process depends on various parameters such as the size of wastewater particles, their density, the velocity of subsidence, and the volume of wastewater particles [131]. The water flow velocity plays a crucial role in removing suspended solids. In the primary sedimentation process, the travel time of pollutants is adjusted to be slower than the travel time of water. Suspended solids with low density

or smaller size and greater density are efficiently removed in the primary sedimentation process [132].

This technique is also highly effective against various pollutants, including antibiotic-resistant bacteria and antibiotic-resistant genes. Anthony et al. [133] demonstrated the removal of antibiotic-resistant bacteria and genes using three distinct plants in an experiment. The study indicated that the primary treatment, which involves the remediation of suspended solids, could have been more efficient, while the secondary treatment process efficiently degraded antibiotic-resistant bacteria and genes. Furthermore, the sludge sedimentation technique effectively removed antibiotic-resistant bacteria and genes in all three plants [133]. An investigation was conducted to remove three different pollutants (phenolic compounds, turbidity, and suspended solids) from wastewater using the sedimentation process. The results revealed that sedimentation significantly reduced contaminant levels by up to 75% and lowered chemical oxygen demand and discoloration by about 90% [134]. In another study, the same method was applied to remediate antibiotics from pharmaceutical wastewater through sedimentation. The results showed that color reduction, chemical oxygen demand, and suspended solids were reduced by approximately 97.3%, 96.9%, and 86.7%, respectively, under optimal conditions [135]. Recently, Zhou et al. [136] developed a novel wastewater treatment method that combines chemically enhanced primary sedimentation with acidogenic sludge fermentation. This novel sedimentation technique not only recovers valuable resources like phosphorus and organics but also efficiently removes various pollutants in sewage sludge under laboratory conditions.

3.1.2. Degasification

In this method, the exclusion of suspended harmful gases and biological nutrients is achieved through physical methods, a process known as degasification [137]. The effectiveness of degasification depends on factors such as the temperature of the wastewater solution, the volume of the tank, ultrasonic power, and their frequency [138]. Degasification membranes have been widely utilized for removing various toxic gases from wastewater, offering an efficient alternative to vacuum towers, forced draft deaerators, and oxygen scavengers across multiple sectors including microelectronics, pharmaceuticals, power generation, food and beverages, photography, ink production, and analytical industries [139]. For instance, in the removal of CH_4 from water, a degassing membrane was used in conjunction with granular sludge for anaerobic wastewater treatment [140].

A study conducted by Saidou et al. [141] investigated pH and airflow factors for the removal of phosphorus gas. This study utilized the dissolved carbon dioxide degasification technique, where precipitation occurred due to carbon dioxide and atmospheric air. Below a pH of 6.5, no precipitation was recorded. Conversely, at pH levels above 6.5, phosphorus elimination reached approximately 78%, with precipitation observed upon increasing the airflow volume by about 25 L/min. In another study focusing on phosphorus removal from animal manure wastewater, carbon dioxide degasification and a continuous U-shaped bioreactor system were employed. The results indicated that without magnesium, the remediation rate exceeded 50%, whereas in the presence of magnesium, phosphorus removal ranged from 80% to 86% [142].

3.1.3. Filtration

There are two primary kinds of filtration processes used for wastewater treatment: particle filtration and membrane filtration processes [143]. Filtration is essential in wastewater treatment for removing solid contaminants up to several microns in size, and it depends on parameters such as the size, texture, quantity, and density of contaminated particles [144,145]. Membrane filtration is a highly complex method extensively used to remove various pollutants. It is often integrated with other physical, chemical, and biological removal methods to enhance overall efficiency [146]. Direct membrane filtration systems are compact, requiring a small footprint, and do not involve an additional activated sludge process, thereby reducing energy consumption [147]. Recently, a study investigated the

filtration method for removing organic matter, suspended solid particles, nutrients, and pathogens from domestic wastewater. In this study, a pilot-scale reactor system with an up-flow anaerobic sludge blanket operated at 19 °C for six hours of hydraulic retention time. The results showed that the filtration reactor system effectively removed suspended solid particles by 93%, reduced chemical oxygen demand by up to 87%, and decreased fecal coliform levels by nearly 93% in the combined system. Furthermore, the study highlighted that combining the filtration system with an anaerobic reactor is a more efficient and cost-effective method for removing toxic pollutants in developing countries [148]. Many studies have concluded that wastewater treatment through filtration processes is effective due to reduced time consumption, filtering water with lower concentrations of solids and turbidity, and efficiently removing colloidal particles from liquid waste [149].

3.1.4. Aeration

The aeration technique involves bringing air and water into close contact for the remediation of emerging pollutants [150]. This method utilizes air blowers in combination with an air support system to supply oxygen to the aeration tank, facilitating the conversion of pollutants into various non-toxic or less toxic compounds such as ammonia, water, and carbon dioxide [151]. Another critical component is the pump system, supported by the air system, which consumes over 70% of electricity in wastewater treatment processes [152]. Aeration has proven effective in removing toxic gases, heavy metals, and volatile organic compounds and reducing odors [153]. Uggetti et al. [154] conducted a pilot-scale study on the aeration method for wastewater treatment. The results demonstrated that under anoxic and aerobic conditions, the aeration technique removed 66% of chemical oxygen demand, 99% of ammonium, and 79% of total nitrogen. Continuous aeration further accelerated pollutant removal, achieving 99% removal of ammonium starting from an initial concentration of 27 mg/L. The study highlighted the potential of intermittent aeration to enhance pollutant removal efficiency, offering new insights into energy and cost consumption. Many studies have emphasized that aeration is the primary large-scale physical treatment method for wastewater treatment, historically preceding chemical methods in efficiency for removing nutrients from domestic wastewater [155,156]. Even biological wastewater treatment in aerobic environments requires pure oxygen aeration [157]. Therefore, the aeration technique stands out as a superior physical treatment widely applicable on an industrial scale [158].

3.2. Chemical Methods

3.2.1. Adsorption

There are two kinds of adsorption processes: physical adsorption and chemical adsorption. In the physical adsorption process, van der Waals forces are applied to non-dependent substrates to enhance the adsorbate concentration. In the chemical adsorption process, different chemical reactions occur between the adsorbate and adsorbent, stimulating covalent and ionic bonding [159]. For the chemical process, adsorbents based on activated carbons are extensively applied for the remediation of toxic metals due to their distinguishing characteristics, such as well-established porous structures, large specific surface area, and various functional groups [160].

Recently, two nanocomposites (kaolin and kaolin zinc oxide) were studied to remove heavy metals, including chromium, iron, and chloride, and their chemical and biological oxygen demands in wastewater were investigated. Different characteristics were analyzed to optimize the batch adsorption experiment, including temperature, adsorbent concentration, and contact time. This study showed that the batch adsorption process significantly removes the pollutants within 15 min. Specifically, the action of both composites resulted in complete chromium degradation. In contrast, under similar conditions using only kaolin, chromium removal was 78%, iron 91%, chloride 73%, and chemical and biological oxygen demand were 91% and 89%, respectively [161].

In another study, a biomass adsorbent was used to remove dye contaminants from water. The adsorption capability of biomass such as graphene oxide aminated lignin aerogels was compared with malachite green by investigating various parameters such as pH, temperature, aerogel concentration, and contact time. The results of this study demonstrated that after optimizing the adsorbents, they were responsible for removing 91.72% of dyes with the highest adsorption efficiency of 113.5 mg/g [162].

3.2.2. Chemical Precipitation

The chemical precipitation method is often used and considered one of the most efficient techniques for removing different pollutants from wastewater, especially for eliminating heavy metals [163]. A study was conducted to remove sulfide from petroleum-refined wastewater using a chemical precipitation technique. Two coagulants, $FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$, were used as partial precipitants, along with coagulant aids, $Ca(OH)_2$ and $CaCO_3$. The results showed that when Fe^{3+} ions were used, sulfide removal ranged from 62% to 95%, and chemical oxygen demand (COD) reduction ranged from 45% to 75%, depending on the pH of the treated water. In contrast, the combination of Fe^{2+} and $Ca(OH)_2$ under similar conditions achieved sulfide removal of 96% to 99% and COD reduction of 50% to 80% [164]. In another study, chemical precipitation is used to remove fluoride, phosphate, and total ammonia nitrogen simultaneously from semiconductor wastes. The outcomes of the laboratory experiment showed that the use of magnesium salts to remove fluoride led to good performance. The investigation conducted on a pilot scale showed that a two-stage precipitation procedure was capable of elimination of 97% of the phoapproach has a treatment cost of about 1.58 USD/m^3, according to an economic analysisphate, 58% of the total ammonia nitrogen, and 91% of the fluoride from semiconductor wastewater. The suggested s [165].

Recently, a study investigated the application of chemical precipitation of calcium oxide and calcium hydroxide to remove chromium, sulfates, and chemical oxygen demand from industrial tannery wastewater. Low to high alkali concentrations were applied, and chromium was theoretically removed using 0.3–3.2 g alkali $(Cr^{+3})^{-1}$. The precipitation was conducted at room temperature, with vigorous stirring of 10 min at 200 rotations per minute for 1 day. The results of this study revealed that using a high concentration of alkalis boosts the removal of chromium and sulfates while the removal of chemical oxygen demand was not affected. The removal rate using calcium oxide precipitation for the removal of Cr, SO_4^{2-}, $ZnSO_4$, $FeSO_4$, CN^{-1}, $NiSO_4$, and Fe^{+2} [Fe $(CN)_6$] was 99.8%, 66.9%, 99.6%, 21.4%, 70.9%, 52.8% and 76.4%, respectively. In comparison, by using the precipitation of calcium hydroxide, 99.8%, 61.6%, 99.9%, 7.1%, 84.0%, 54.4%, and 90.5% were recorded, respectively [166].

3.2.3. Flocculation and Coagulation

Undermined contaminated elements are clustered into bigger particles by mechanical agitation for the flocculation method. In contrast, for the coagulation process, some materials stabilize the colloidal suspensions by neutralizing their charges, leading to the accumulation of minor particles [167,168]. Recently, the method of flocculation and coagulation was adopted to remove microplastics from secondary wastewater. In this study, two different sizes of polystyrene spheres, 1 μm and 6.3 μm, with wastewater effluent were spiked and the removal rate in a wide pH range was investigated. Moreover, ferric chloride, poly aluminum chloride, and polyamine were used as organic and inorganic coagulants. The results of this study revealed that coagulants play a vital role in removing microplastics. Ferric chloride and poly aluminum chloride efficiently almost completely removed microplastics compared to polyamine [169]. In another, a batch experiment was conducted for textile wastewater treatment. It evaluated the efficiency of four different coagulants, ferric sulfate, aluminum chloride, aluminum sulfate, and ferric chloride, at various ranges of pH (1–11) for the elimination of different pollutants such as chemical oxygen demand, total suspended solids, color, total nitrogen, and turbidity from industrial wastewater. Results

of this study demonstrated that by using a high concentration of ferric chloride at pH 9, a fast-mixing speed of 150 rpm for 1 min, and a slow mixing speed of 30 rpm for 20 min with 30 min of settlement time, almost 90% of the pollutants were eliminated [170]. Heterogeneous photocatalysis was used to optimize the traditional coagulation–flocculation process and finish the removal of natural organic matter from drinking water. TiO_2-P25 suspended catalyst and TiO_2-P25/β-SiC supported materials were used in heterogeneous photocatalytic experiments [171]. Coagulation–flocculation and heterogeneous photocatalysis have been used alone and in combination to determine the most effective method for breaking down the most significant number of organic molecules. Total organic carbon, specific UV absorbance (SUVA254nm), and UV absorbance at 254 nm (UV254nm) were evaluated to quantify the number of humic compounds eliminated from each treatment. Findings indicated that at pH 5 for 110 mg/L of coagulant dosage, the coagulation–flocculation process conditions were optimized, and at this pH, 70% of humic compounds were eliminated. After 220 min of irradiation, the coupling of coagulation–flocculation and the supported photocatalytic process (with TiO_2-P25/β-SiC supported catalyst) reveals that around 80% of the mineralization of humic compounds is still present in water treated by coagulation–flocculation. As a result, these two procedures together eliminate around 90% of humic compounds [172].

3.2.4. Ion Exchange

The alteration of ions present in the wastewater for their treatment is called ion exchange, and the constituents used to detect ion pollutants are known as resins [173,174] (Figure 4). Mainly two types of resins are used for the ion-exchange process: synthetic and natural resins. Due to their distinguished characteristics and good efficiency, synthetic resins are extensively used [175]. Natural resins include clays, polysaccharides, proteins, and carbon materials. Synthetic resins used for ion exchange are heavy metal silicates, formaldehyde resins, Sephadex, and acrylic acid co-polymers [176].

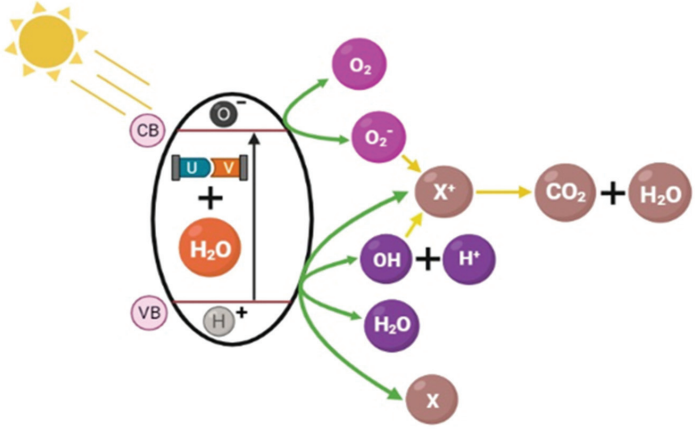

Figure 4. Ion-exchange mechanism for removal of pollutants in wastewater.

Recently, for removing nitrite ions from the tailwater of dyeing wastewater, an ion-exchange polymer and modified carbonization bacterial cellulose were made using different concentrations of ion-exchange polymers such as glutaric acid and sulfosuccinate acid. Furthermore, the ion-exchange polymers are manufactured within an asymmetric capacitive deionization unit, and the efficiency of NO^{-2} electroadsorption was investigated. Results of the study showed that the activated carbon and carbonization bacterial cellulose group efficiently showed adsorption capacity of salts upto 14.56 mg/g and effectively eliminated nitrates at the rate of 71.01% as compared to the activated carbonization bacterial cellulose and glutaric acid (10.72 mg/g, 47.83%) and activated carbon-based (4.81 mg/g, 12.74%)

groups [177]. A study was carried out on removing the heavy metal chromium from wastewater using an ion-exchange technique. In this study, the resin used for ion exchange was Lewatit S 100. Results of the study showed that at lower pH (3.5), resin adsorption was maximum and performed efficiently to remove chromium metal [178].

3.2.5. Ozonation

Due to the excellent oxidation and disinfection efficiency of ozone, the ozonation process is extensively applied for wastewater treatment [179]. The most prominent characteristic of ozone gas is that it is unstable, which is why removing pollutants from wastewater can be performed directly by molecular ozone and indirectly via hydroxyl radicals' formation [180]. Generally, the most used ozonation method has been applied to detoxify contaminants in wastewater as a disinfector and to reduce waste sludge. In addition, this method is also mainly applied for the decolorization of dyes, removing micro- and nanoscale contaminants, and eliminating chemical oxygen demand [181] (Figure 5).

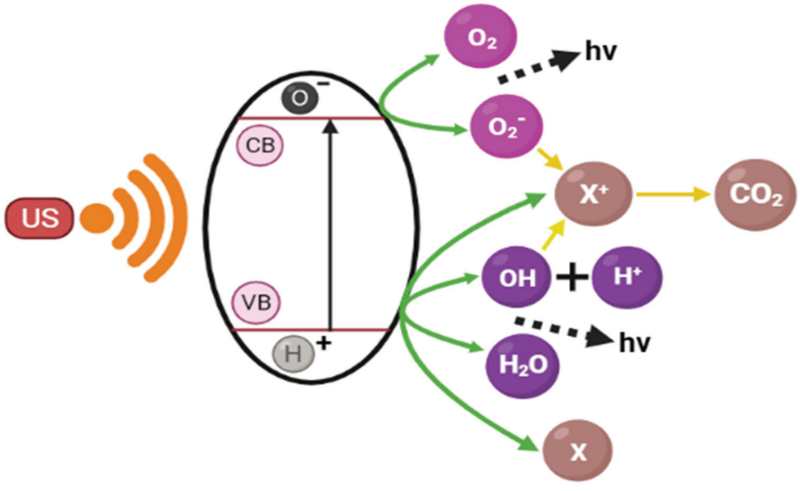

Figure 5. Ozonation and oxidation mechanism for wastewater treatment.

Pulp and paper wastewater treatment using the ozonation method at a large scale in a medium-potentiality wastewater treatment plant (143,000 population equivalent) was recently investigated. To evaluate the efficiency of the ozonation technique, single wastewater lines, including bleaching and processing wastewater, and the mixture of wastewater before and after secondary biological treatment were carried out. Ozone demonstrated considerably greater efficiency on pulp and paper wastewater mixture following biological treatment (up to 81% COD removal) rather than before biological process (46% mean COD abatement). Ozone effectiveness was more noticeable in process water (60% COD removal) than in bleaching water (28% COD removal). A good TSS abatement (up to 20–30 mg/L) was also noted, and the COD elimination effectiveness at a dosage of 100 mg O_3/L was comparable to the current physicochemical treatment (mean 50%). [182]. In another study, various methods were applied to treat oily gas wastewater, such as hybridization of the electrochemical coagulation method, ceramic microfiltration membrane, and ozonation, and their efficiencies were compared. This study investigated different parameters, such as hydraulic retention time, aeration, current density, method of supply, and pH of samples. Results of this study revealed that the ozonated reactor eliminates chemical oxygen demand by up to 53.1% compared to the aerated electromembrane reactor. Moreover, this study explained that the ozonation-assisted elec −romembrane hybrid plays a vital role in removing pollutants in wastewater [183]. An indicative profile of the physiochemical

characteristics for remediation of emerging micropollutants in water matrices is presented in Table 3.

Table 3. Physiochemical approaches for remediation of emerging micropollutants in water matrices.

Name of Treatment	Micropollutants	Water Matrix	Removal Efficiency (%)	References
Sedimentation	Phosphorus Nitrogen	Municipal wastewater	72.43 98.63	[184]
	Oils	Oily wastewater	82	[185]
	Phenolic compounds	Olive mill wastewater	76.2	[186]
	Phosphorus Volatile fatty acids	Organic wastewater	31	[187]
	Ferric chloride Phosphorus Nitrogen	Sewage effluent	80 70 40	[188]
	Colors	Antibiotic fermentation wastewater	97.3	[137]
	Toxic phenolic compounds	Olive mill wastewater	71	[186]
Degasification	Methane Hydrogen sulfide	Anerobic treated wastewater	94 88	[189]
	Phosphate	Animal manure wastewater	80–86	[142]
	Dust Carbon monoxide Nitrogen oxides	Industrial wastewater	20 59.4 55.1	[190]
	Nitrogen	Coal gasification wastewater	81.23	[138]
	Organic compounds	Anaerobic wastewater	90	[191]
	Chromium	Synthetic and industrial wastewater	92.6	[192]
	Organic matter	Sugar industry wastewater	79	[193]
Filtration	Phenol Sodium sulfate Ferrous sulfate Sulfuric acid Sodium hydroxide Potassium titanium	Synthetic and industrial wastewater	100	[143]
	Conventional pollutants	Swine wastewater	99	[194]
	Color Total Nitrogen	Textile wastewater	98.4 86.1	[195]
	Microplastics	Sewage wastewater	96	[144]
	Phosphorus Organic carbon Heavy metals	Urban road runoff	84.1–97.4	[145]
	Copper	Acid mine drainage	100	[146]
	Dye/salt mixtures	Textile wastewater	99.84	[147]
	p-chloroaniline	Industrial wastewater	50	[149]
	Free DNA Antibiotic resistance genes	Domestic wastewater	99.80	[196]
Adsorption	Manganese	Agricultural wastewater	99	[197]

Table 3. *Cont.*

Name of Treatment	Micropollutants	Water Matrix	Removal Efficiency (%)	References
	Heavy metal ions	Domestic wastewater	99	[198]
	Dyes (basic violet and red)	Textile wastewater	77 and 93	[199]
	TetrabromobispenolA	Industrial wastewater	90	[200]
	Bisphenol A	Hospital effluents	100	[201] [202]
	Estrone 17β-estradiol 17α--ethinylestradiol	Laboratory wastewater	86 94 94	[203]
	Cadmium	Industrial wastewater	86	[204]
	Chromium	Industrial wastewater	96	[205]
	Lead	Tannery wastewater	99.12	[206]
	Zinc	Domestic wastewater	93.3	[21]
	Copper Iron Lead Nickel Cadmium	Agricultural and industrial wastewater	98.54 99.25 87.17 96.95 73.54	[207]
Chemical precipitation	Chromium Copper Lead Zinc	Contaminated river water	99.8	[208]
	Zinc	Industrial wastewater	99–99.3	[209]
	Fluoride Ammonia nitrogen Phosphate	Synthetic wastewater	91 58 97	[165]
	Lead	Industrial wastewater	99.4	[210]
	Silicon	Pulping whitewater	93–95	[211]
	Polycyclic aromatic hydrocarbons Micropollutants	Domestic wastewater	80–100	[212]
	Copper	Textile wastewater	80.2	[213]
	Lead	Contaminated river water	94	[214]
	Cobalt	Industrial wastewater	99.9	[215]
	Copper	Textile wastewater	92	[216]
Flocculation and coagulation	Iron, phosphorus, and aluminum	Tannery wastewater	99	[217]
	Reactive and acid	Dye bath effluents	98	[218]
	Sulfur	Industrial dying wastewater	100	[219]
	Arsenic Mercury Lead	Mature landfill leachate	46 9 85	[220]
	Microplastics Humic acid	Synthetic wastewater	98.2 97.9	[221]
	Colors	Tannery wastewater	95	[222]
	Total organic carbon Color	Textile effluents	82 70	[223]

Table 3. *Cont.*

Name of Treatment	Micropollutants	Water Matrix	Removal Efficiency (%)	References
	Turbidity Total organic carbon	Vegetable oil refinery wastewater	100 98	[224]
Ion exchange	Arsenic	Domestic wastewater	100	[225]
	Nickel Zinc	Synthetic wastewater	98	[226]
	Chromium	Synthetic wastewater	93	[227]
	Chromium	Tannery wastewater	95	[228]
	Nickel Vanadium	Hospital effluents	98	[229]
	Hexavalent chromium	Tannery wastewater	98.5	[230]
	Cadmium Lead	Mango peel wastewater	72.46 76.26	[231]
	Thallium Chloride	Industrial wastewater	98 90	[232]
	Methylene blue	Textile wastewater	97.02	[233]
Ozonation	Colors	Tannery wastewater	100	[234]
	Nitrogenous heterocyclic compounds Total nitrogen	Coal gasification wastewater	95.6 80.6	[235]
	Ibuprofen	Synthetic wastewater	99	[236]
	Proteins Polysaccharides	Organic wastewater	100 42	[237]
	Non-polar pollutants	Synthetic wastewater	95	[238]
	Metolachlor	Organic wastewater	82	[239]
	Atrazine Metolachlor Nonylphenol	Organic wastewater	75 78 100	[240]
	Diclofenac Sulfamethoxazole	Pharmaceutical industrial wastewater	100 95	[241]
	Diclofenac Sulfamethoxazole 17-α-Ethynylestradiol	Pharmaceutical industrial wastewater	100	[242]
	Ibuprofen Ciprofloxacin	Pharmaceutical industrial wastewater	100 88	[243]
	2,4–Dichlorophenol 2,4,6–Trichlorophenol Phenazone	Synthetic wastewater	98 98 79	[244]

4. Nanotechnology for Wastewater Treatment

Clean and safe water is one of the most fundamental elements needed for the survival of life on the planet [245]. The increasing population, industrialization, urbanization, and an imprudent number of agriculture procedures produce dirty and deadly wastewater [246,247]. Every year millions of people are affected due to harmful pathogens through the consumption of drinking water worldwide [248]. However, in the last few years, many techniques have been applied to wastewater treatment. However, their applications are confined by various limitations such as the use of chemicals, formation of secondary pollutants, time consumption, and high prices [249].

Nanoparticles incorporate a high surface-to-volume ratio, high sensitivity and reactivity, a high adsorption capacity, and ease of functionalization, making them suitable for wastewater treatment [250]. Nanoparticles have been proven efficient in removing different toxic contaminants from wastewater, such as heavy metals, organic and inorganic compounds, dyes, biological toxins, and pathogens that spread lethal diseases [251] (Figure 6).

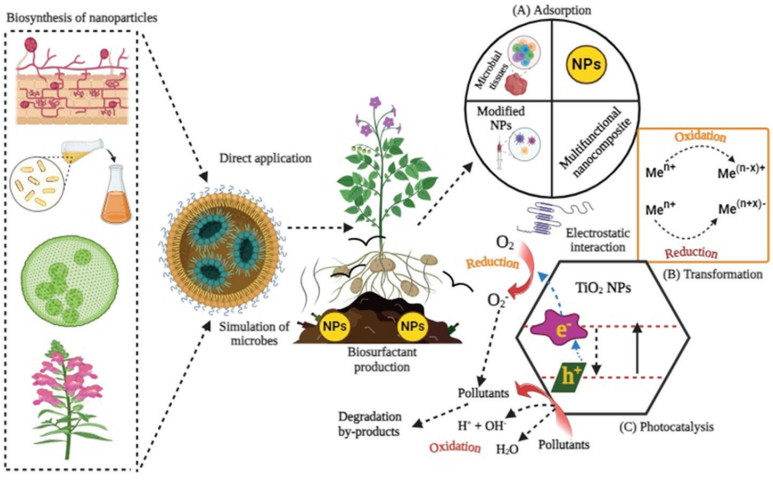

Figure 6. Synthesis of nanoparticles and their contribution to remediating pollutants.

Recently, Shkir et al. [252] investigated applications of novel Ni-mediated ZnO nanomaterials for removing methylene blue and tetracycline from wastewater. Ni- and ZnO-doped nanoparticles were synthesized at the wt% of 0.0, 0.5, 1.0, 2.0, and 3.0 by the high-combustion method at 550 °C. The morphology, homogeneity, and particle size were evaluated by scanning electron microscope analysis, and it was found that both particles were braced efficiently with each other at the range of 30–60 nm. In addition, this study explained that 3.0% wt of Ni doping in ZnO nanoparticles is more suitable, effective, and more rapidly removed both pollutants from the wastewater and reused several times. In another study, various kinds of emerging pollutants were removed from wastewater with the synthesis of silver and gold nanoparticles by using an extract of *Crinum latifolium*. The results of this study explained that the average diameters of unique and multishaped silver and gold nanoparticles were 20.5 and 17.6 nm, respectively. Moreover, the biosynthesized metallic nanoparticles efficiently removed dyes and antibiotics from the wastewater [253]. Nanoparticles and their preferential role in degrading different toxic compounds in wastewater are mentioned in Table 4. To remove secondary effluents from municipal wastewater, a comprehensive investigation was proposed and synthesized magnetic and silver nanoparticles had average dimensions of 41 and 34 nm, respectively, and the saturation magnetization was recorded as 62 and 67 emu/g, respectively. The findings of this study revealed that the dose of nanoparticles at 105 mg/L with a contact time of 70 min was able to effectively remove 55% of toxic pollutants and 30.40% of chemical oxygen demand. In addition, this study explained that increasing the dose of nanomaterials and contact time and decreasing the concentration of pollutants enhanced the removal rate, and nanoparticles could be reused several times [254]. For the removal of various cationic and anionic dyes such as malachite green, Congo red, methylene blue, and eosin Y from wastewater, the biosynthesis of flower-shaped zinc oxide (ZnO) nanoparticles from the extract of sea buckthorn fruit was carried out, and their external and internal properties were examined by using different microscopes. This study showed that by a photocatalyst mechanism, almost 99% removal of malachite green, methyl blue, Congo red, and eosin Y

was achieved at the concentration of 15 mg/L with a contact time of 70, 70, 80, and 90 min, respectively. In addition, using 5 mg/L of green synthesized nanoparticles, all dyes were completely degraded with a contact time of 23, 25, 28, and 30 min, respectively. This study concluded that biosynthesized zinc oxide nanoparticles are faster, inexpensive, easy to synthesize, efficient, recyclable, ecofriendly, and non-toxic agents to degrade various kinds of emerging pollutants from wastewater [255].

Heavy metals are extensively released into the ecosystem by various industries, and their accumulation causes severe threats to terrestrial as well as aquatic organisms [256]. To remove chromium from tannery wastewater, green synthesis of titanium oxide nanoparticles (TiO_2 NPs) from *Jatropha curcas* leaf extract was carried out, and their efficiency was evaluated. The findings of this study explained that by photocatalytic treatment, complete removal of chromium metal and chemical oxygen demand (COD) was achieved, while by applying TiO_2 NPs in a parabolic trough reactor, 82.26% of COD and 76.48% of chromium were removed from tannery wastewater. This study concluded that biosynthesized TiO_2 NPs performed rapidly and could be used to remove other kinds of emerging pollutants from the environment at a pilot scale [257]. Another study studied the formation of novel magnetic nanoparticles modified by organodisulfide polymer to remove highly toxic metals, including lead, mercury, and cadmium, from high-salinity wastewater. The results of adsorption kinetics and isotherm thermodynamics followed the pseudo-second-order model. The Freundlich equation revealed that, by using inorganic salt in high-salinity water, removing all metals was efficiently achieved by applying nanoparticles for twenty seconds and they could be recycled five times [258]. The imprudent applications of pesticides and other organic chemicals in the agriculture system cause various diseases in living organisms, reaching the groundwater and contaminating drinking water. Due to the imperative need for a healthy natural environment, researchers are constantly seeking to maintain biodiversity by removal of toxic pesticide residues worldwide [259].

Recently, the removal of chlorpyriphos residues from the wastewater synthesis of green nanoparticles from the leaf extract of *Moringa olivera* was carried out and their efficiency was investigated. To gain the best degradation rate of chlorpyriphos residues in wastewater, wide ranges of chlorpyriphos concentrations (10–80 g/mL), doses of nanoparticles (0.05–0.5 g), pH (5–9), contact time (10–1440 min), and temperature (25–45 °C) were used. The outcomes of this study indicated that 81% removal of chlorpyriphos residues at the concentration of 80 µg/mL was achieved within 30 min at pH 7 and a temperature of 35 °C. The CPF sorption process was shown to be exothermic and spontaneous by the thermodynamic characteristics.

This study concluded that a novel nanobiosorbent could be an effective, inexpensive, ecofriendly, non-toxic, and superior candidate for removing chlorpyriphos and other organophosphate pesticides from polluted water [260].

Antibiotics are one of the fundamental components of pharmaceuticals that are extensively applied as a growth factor in fish farms, livestock, and other living organisms, including humans, for the treatment of various infectious diseases [261]. The secondary metabolites of various microbial species, such as bacteria, actinomycetes, archaea, fungi, and algae, could synthesize antibiotics. However, in recent years due to high demand, the synthesis of antibiotics changed and extended to synthetic and semisynthetic compounds [262]. Due to their injudicious use and high applications, their toxic residues are often detected in different compartments of water, including wastewater, surface water, groundwater, and even potable water, causing severe threats to non-target organisms [263,264]. To remove flumequine antibiotics from wastewater, green synthesis of zinc oxide nanoparticles (ZnO NPs) from pomegranate seed molasses was carried out and their external and internal characteristics were analyzed by using a scanning electron microscope, spectrometry, fluorimetry, and Raman spectroscopy. Findings revealed that green synthesized ZnO NPs indicated narrowed band gaps and more excellent absorption of visible photons that allow the maximum density of hydroxyl radicals during the solar illumination process and efficiently degraded 97.6% of flumequine from the wastewater.

This study provides new insights into the biosynthesis of various nanoparticles to remove different emerging pollutants from the environment [265]. In another study, the biosynthesis of novel magnetic iron oxide nanoparticles (Fe_3O_4 NPs) from the extract of *Excoecaria cochinchinensis* to remove rifampicin antibiotics from polluted water and their degradation efficiency as well as other physiochemical properties were investigated. The outcomes of this study revealed that at the initial concentration of 20 mL of rifampicin, 98.4% removal was achieved by a 10 mg dose of Fe_3O_4 NPs at a temperature of 303 K. The scanning electron microscope analysis revealed that the size of nanoparticles was 20–30 nm, and the N_2 adsorption/desorption isotherms explained that the surface area of nanomaterials was 111.8 m^2/g. In addition, this study demonstrated that after five reuses, Fe_3O_4 NPs could degrade 61.5% of rifampicin with a concentration of 20 mL [266].

Table 4. Nanoparticles and their preferential role to degrade different toxic compounds in wastewater.

Name of Emerging Pollutant	Name of Nanomaterials	Characteristics	Removal Efficiency %	References
Organic dyes	PVA/PAA/GO-COOH@AgNPs	High catalytic activity, easy to recycle, perform efficiently at room temperature, inexpensive	99.8	[267]
Dyes	Bismuth oxychloride	Controllable shape, perform at various temperatures (low–high), large surface area, ecofriendly	85.31	[268]
4-nitrophenol and 2-nitroaniline	PVA/PAA/Fe_3O_4/MXene@AgNP	Excellent structure, high thermal stability and good magnetic properties, able to be reused and high catalytic activity	72.55 and 88.8	[269]
Phosphorus and nitrogen	Carbon-based nanomaterials	Easy to synthesize, ecofriendly, high adsorption capacity, high enzymatic and catalytic activity	24.1–42.7	[270]
Organic matter and personal care products	TiO_2 and ZnO	Diverse range of particle sizes, grow in clusters, inexpensive, high sorption capacity, and perform in different temperatures efficiently	43.8–55.3	[271]
Methylene blue	Rod-shaped manganese oxide	High adsorption capacity, perform efficiently at pH 8.0 and room temperature, ecofriendly, inexpensive, high degradation ability	99.8	[272]
Congo red dye	Silica composite (Si-IL) and silica-coated magnetite (Fe_3O_4-Si-IL) composites	Excellent adsorption capacity, high catalytic activity, diverse range of sizes, good magnetic and thermal properties	100	[273]
Chromium, arsenic, and lead	Single-walled carbon nanotubes	Reduced pore size, smoother surface, and high rejection ability	96.8, 87.6, and 30.3	[274]
Diazinon, phosalone, and chlorpyrifos	Modified magnetic chitosan nanoparticles based on mixed hemimicelle of sodium dodecyl sulfate	Excellent absorbance, easy to synthesize, inexpensive, ecofriendly, and easy to recycle	99, 98, and 96	[275]

Table 4. *Cont.*

Name of Emerging Pollutant	Name of Nanomaterials	Characteristics	Removal Efficiency %	References
Methomyl	$Cu/Cu_2O/CuO$ hybrid nanoparticles	Efficient in extreme environmental conditions, good reusability, ecofriendly, high adsorption ability, good catalytic activity, and easy to synthesize	91	[276]
Chlorpyriphos	ZnO	Highly dependent on pH, good thermodynamic properties, economical, and environmentally friendly	56	[277]
Ciprofloxacin	Fe_3O_4/red mud nanoparticles	High removal efficiency, depend on pH, contact time, and temperature, high adsorption capacity, and able to reuse	30–100	[278]
Naproxen	Silica and magnetic nanoparticle-decorated graphene oxide (GO-MNPs-SiO_2)	Perform efficiently in optimum conditions, good adsorption ability, inexpensive, and environmentally friendly	83–94	[279]
Lead	TiO_2	High catalytic activity, average crystalline size, large surface area, and easy to recycle	82.53	[280]
Dimethoate	Graphene-oxide-supported graphitic carbon nitride microflowers decorated by silver nanoparticles	Grow in crystals, high adsorption ability, good removal efficiency, and long reaction time	93	[281]

5. Conclusions and Future Perspectives

Water systems around the world are facing significant accumulations of micropollutants. These primary contaminants largely stem from human activities, such as industrialization, urbanization, and poor wastewater management. The harmful effects of micropollutants on both terrestrial and aquatic ecosystems are unavoidable, with dire consequences for life in these environments. The heavy accumulation and biotoxicity of these pollutants pose severe environmental hazards. As a result, environmentalists emphasize the need for environmentally friendly, cost-effective, and biologically reliable strategies. Conventional physiochemical and biotechnological methods for removing micropollutants from aquatic environments are being re-evaluated, as biological approaches offer advantages in terms of energy efficiency and low costs, improving the efficacy of pollutant removal. Additionally, the integration of nanomaterials has proven to be a significant adjunct in sustainable wastewater management and effective micropollutant abatement. Furthermore, bio-monitoring methods utilizing microbiology, bioinformatics, omics technologies, big data analysis, and systems biology approaches are critical for the sustainable management of micropollutants. The use of novel microbes, innovative nanomaterial strategies, and advanced data management is essential for enhancing the efficiency of micropollutant removal. Holistic approaches, such as sustainable wastewater treatment using floating wetlands, show promise for practical application. Future research should focus on comprehensive assessments of technical stability, from pilot-scale to long-term implementations, for effective micropollutant removal and water body conservation. Additionally, practical applicability and economic feasibility analyses are necessary to develop a sustainable and appropriate wastewater management strategy

Author Contributions: H.W.A. wrote the initial draft, conceptualized and developed the document. H.A.B., S.A. and M.C. provided critical feedback, reviewed the manuscript. H.W.A. and H.A.B. revised the manuscript. S.A. and G.L.K. supervised the manuscript. S.A. and G.L.K. validated the manuscript. G.L.K. provided the funding for publication. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: We would like to express our sincere gratitude to G.L.K. for providing the funding that made this publication possible. His support has been invaluable to our research, and we are truly thankful for his generosity.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Yaqoob, A.A.; Parveen, T.; Umar, K.; Mohamad Ibrahim, M.N. Role of Nanomaterials in the Treatment of Wastewater: A Review. *Water* **2020**, *12*, 495. [CrossRef]
2. Ahmad, S.; Dongming, C.; Zhong, G.; Liu, J. Microbial Technologies Employed for Biodegradation of Neonicotinoids in the Agroecosystem. *Front. Microbiol.* **2021**, *12*, 759439. [CrossRef]
3. Rasheed, T.; Shafi, S.; Bilal, M.; Hussain, T.; Sher, F.; Rizwan, K. Surfactants-Based Remediation as an Effective Approach for Removal of Environmental Pollutants—A Review. *J. Mol. Liq.* **2020**, *318*, 113960. [CrossRef]
4. Ahmad, S.; Ahmad, H.W.; Bhatt, P. Microbial Adaptation and Impact into the Pesticide's Degradation. *Arch. Microbiol.* **2022**, *204*, 288. [CrossRef] [PubMed]
5. Nazir, M.S.; Mahdi, A.J.; Bilal, M.; Sohail, H.M.; Ali, N.; Iqbal, H.M. Environmental Impact and Pollution-Related Challenges of Renewable Wind Energy Paradigm—A Review. *Sci. Total Environ.* **2019**, *683*, 436–444. [CrossRef] [PubMed]
6. Wang, W.; Gao, H.; Jin, S.; Li, R.; Na, G. The Ecotoxicological Effects of Microplastics on Aquatic Food Web, from Primary Producer to Human: A Review. *Ecotoxicol. Environ. Saf.* **2019**, *173*, 110–117. [CrossRef]
7. Gavrilescu, M.; Demnerová, K.; Aamand, J.; Agathos, S.; Fava, F. Emerging Pollutants in the Environment: Present and Future Challenges in Biomonitoring, Ecological Risks, and Bioremediation. *New Biotechnol.* **2015**, *32*, 147–156. [CrossRef]
8. Teodosiu, C.; Gilca, A.-F.; Barjoveanu, G.; Fiore, S. Emerging Pollutants Removal Through Advanced Drinking Water Treatment: A Review on Processes and Environmental Performances Assessment. *J. Clean. Prod.* **2018**, *197*, 1210–1221. [CrossRef]
9. Hodges, B.C.; Cates, E.L.; Kim, J.-H. Challenges and Prospects of Advanced Oxidation Water Treatment Processes Using Catalytic Nanomaterials. *Nat. Nanotechnol.* **2018**, *13*, 642–650. [CrossRef]
10. Bilal, M.; Adeel, M.; Rasheed, T.; Zhao, Y.; Iqbal, H.M. Emerging Contaminants of High Concern and Their Enzyme-Assisted Biodegradation—A Review. *Environ. Int.* **2019**, *124*, 336–353. [CrossRef]
11. Rasheed, T.; Bilal, M.; Nabeel, F.; Adeel, M.; Iqbal, H.M. Environmentally-Related Contaminants of High Concern: Potential Sources and Analytical Modalities for Detection, Quantification, and Treatment. *Environ. Int.* **2019**, *122*, 52–66. [CrossRef] [PubMed]
12. Kumar, M.; Borah, P.; Devi, P. Priority and emerging pollutants in water. In *Inorganic Pollutants in Water*; Kumar, M., Borah, P., Devi, P., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 33–49. [CrossRef]
13. Vargas-Berrones, K.; Bernal-Jácome, L.; de León-Martínez, L.D.; Flores-Ramírez, R. Emerging Pollutants (EPs) in Latin América: A Critical Review of Under-Studied EPs, Case of Study—Nonylphenol. *Sci. Total Environ.* **2020**, *726*, 138493. [CrossRef] [PubMed]
14. Ahmad, S.; Chandrasekaran, M.; Ahmad, H.W. Investigation of the Persistence, Toxicological Effects, and Ecological Issues of S-Triazine Herbicides and Their Biodegradation Using Emerging Technologies: A Review. *Microorganisms* **2023**, *11*, 2558. [CrossRef] [PubMed]
15. Arlos, M.J.; Parker, W.J.; Bicudo, J.R.; Law, P.; Hicks, K.A.; Fuzzen, M.L.; Andrews, S.A.; Servos, M.R. Modeling the Exposure of Wild Fish to Endocrine Active Chemicals: Potential Linkages of Total Estrogenicity to Field-Observed Intersex. *Water Res.* **2018**, *139*, 187–197. [CrossRef] [PubMed]
16. Mackuľak, T.; Cverenkárová, K.; Vojs Staňová, A.; Fehér, M.; Tamáš, M.; Škulcová, A.B.; Gál, M.; Naumowicz, M.; Špalková, V.; Bírošová, L. Hospital Wastewater—Source of Specific Micropollutants, Antibiotic-Resistant Microorganisms, Viruses, and Their Elimination. *Antibiotics* **2021**, *10*, 1070. [CrossRef]
17. Vilela, C.L.S.; Bassin, J.P.; Peixoto, R.S. Water Contamination by Endocrine Disruptors: Impacts, Microbiological Aspects and Trends for Environmental Protection. *Environ. Pollut.* **2018**, *235*, 546–559. [CrossRef]
18. Bilal, M.; Iqbal, H.M.; Barceló, D. Mitigation of Bisphenol A Using an Array of Laccase-Based Robust Bio-Catalytic Cues—A Review. *Sci. Total Environ.* **2019**, *689*, 160–177. [CrossRef]
19. Bao, Q.; Huang, L.; Xiu, J.; Yi, L.; Ma, Y. Study on the Treatment of Oily Sludge in Oil Fields with Lipopeptide/Sophorolipid Complex Bio-Surfactant. *Ecotoxicol. Environ. Saf.* **2021**, *212*, 111964. [CrossRef]

20. Al-Asgah, N.A.; Abdel-Warith, A.-W.A.; Younis, E.-S.M.; Allam, H.Y. Haematological and Biochemical Parameters and Tissue Accumulations of Cadmium in *Oreochromis niloticus* Exposed to Various Concentrations of Cadmium Chloride. *Saudi J. Biol. Sci.* **2015**, *22*, 543–550. [CrossRef]
21. Meena, A.K.; Mishra, G.; Rai, P.; Rajagopal, C.; Nagar, P. Removal of Heavy Metal Ions from Aqueous Solutions Using Carbon Aerogel as an Adsorbent. *J. Hazard. Mater.* **2005**, *122*, 161–170. [CrossRef]
22. Ahmad, H.W.; Ahmad, S.; Maqsood, U. Sustainable Application of Microwave-Assisted Extraction for the Recovery of Bioactive Components from Agro-Waste. In Proceedings of the 2nd International Conference on Precision and Sustainable Agriculture Under Climate Change, Rahim Yar Khan, Pakistan, 22–24 February 2024.
23. Alaguprathana, M.; Poonkothai, M.; Al-Ansari, M.M.; Al-Humaid, L.; Kim, W. Cytogenotoxicity assessment in *Allium cepa* roots exposed to methyl orange treated with *Oedogonium subplagiostomum* AP1. *Environ. Res.* **2022**, *2013*, 113612. [CrossRef] [PubMed]
24. Tang, Y.; Chen, Z.; Wen, Q.; Yang, B.; Pan, Y. Evaluation of a hybrid process of magnetic ion-exchange resin treatment followed by ozonation in secondary effluent organic matter removal. *Sci. Total Environ.* **2021**, *754*, 142361. [CrossRef] [PubMed]
25. Fan, R.; Tian, H.; Wu, Q.; Yi, Y.; Yan, X.; Liu, B. Mechanism of Bio-Electrokinetic Remediation of Pyrene Contaminated Soil: Effects of an Electric Field on the Degradation Pathway and Microbial Metabolic Processes. *J. Hazard. Mater.* **2021**, *422*, 126959. [CrossRef] [PubMed]
26. Kasiotis, K.M.; Zafeiraki, E.; Manea-Karga, E.; Kouretas, D.; Tekos, F.; Skaperda, Z.; Doumpas, N.; Machera, K. Bioaccumulation of Organic and Inorganic Pollutants in Fish from Thermaikos Gulf: Preliminary Human Health Risk Assessment Assisted by a Computational Approach. *J. Xenobiot.* **2024**, *14*, 701–716. [CrossRef] [PubMed]
27. Polińska, W.; Kotowska, U.; Kiejza, D.; Karpińska, J. Insights into the Use of Phytoremediation Processes for the Removal of Organic Micropollutants from Water and Wastewater; A Review. *Water* **2021**, *13*, 2065. [CrossRef]
28. Piai, L.; Blokland, M.; van der Wal, A.; Langenhoff, A. Biodegradation and Adsorption of Micropollutants by Biological Activated Carbon from a Drinking Water Production Plant. *J. Hazard. Mater.* **2020**, *388*, 122028. [CrossRef]
29. Popova, S.; Tsenter, I.; Garkusheva, N.; Beck, S.E.; Matafonova, G.; Batoev, V. Evaluating (Sono)-Photo-Fenton-Like Processes with High-Frequency Ultrasound and UVA LEDs for Degradation of Organic Micropollutants and Inactivation of Bacteria Separately and Simultaneously. *J. Environ. Chem. Eng.* **2021**, *9*, 105249. [CrossRef]
30. Ouarda, Y.; Tiwari, B.; Azaïs, A.; Vaudreuil, M.-A.; Ndiaye, S.D.; Drogui, P.; Tyagi, R.D.; Sauvé, S.; Desrosiers, M.; Buelna, G. Synthetic Hospital Wastewater Treatment by Coupling Submerged Membrane Bioreactor and Electrochemical Advanced Oxidation Process: Kinetic Study and Toxicity Assessment. *Chemosphere* **2018**, *193*, 160–169. [CrossRef]
31. Di Marcantonio, C.; Chiavola, A.; Bains, A.; Singhal, N. Effect of Oxic/Anoxic Conditions on the Removal of Organic Micropollutants in the Activated Sludge Process. *Environ. Technol. Innov.* **2020**, *20*, 101161. [CrossRef]
32. Adar, E.; Ilhan, F.; Aygun, A. Different Methods Applied to Remove Pollutants from Real Epoxy Paint Wastewater: Modeling Using the Response Surface Method. *Sep. Sci. Technol.* **2022**, *57*, 492–507. [CrossRef]
33. Nabgan, W.; Jalil, A.A.; Nabgan, B.; Ikram, M.; Ali, M.W.; Lakshminarayana, P. A State of the Art Overview of Carbon-Based Composites Applications for Detecting and Eliminating Pharmaceuticals Containing Wastewater. *Chemosphere* **2022**, *288*, 132535. [CrossRef] [PubMed]
34. Varsha, M.; Kumar, P.S.; Rathi, B.S. A Review on Recent Trends in the Removal of Emerging Contaminants from Aquatic Environment Using Low-Cost Adsorbents. *Chemosphere* **2022**, *287*, 132270. [CrossRef] [PubMed]
35. Morin-Crini, N.; Lichtfouse, E.; Fourmentin, M.; Ribeiro, A.R.L.; Noutsopoulos, C.; Mapelli, F.; Fenyvesi, É.; Vieira, M.G.A.; Picos-Corrales, L.A.; Moreno-Piraján, J.C. Removal of Emerging Contaminants from Wastewater Using Advanced Treatments: A Review. *Environ. Chem. Lett.* **2022**, *20*, 43. [CrossRef]
36. Fiorenza, R.; Di Mauro, A.; Cantarella, M.; Iaria, C.; Scalisi, E.M.; Brundo, M.V.; Gulino, A.; Spitaleri, L.; Nicotra, G.; Dattilo, S. Preferential Removal of Pesticides from Water by Molecular Imprinting on TiO_2 Photocatalysts. *Chem. Eng. J.* **2020**, *379*, 122309. [CrossRef]
37. Mapelli, F.; Scoma, A.; Michoud, G.; Aulenta, F.; Boon, N.; Borin, S.; Kalogerakis, N.; Daffonchio, D. Biotechnologies for Marine Oil Spill Cleanup: Indissoluble Ties with Microorganisms. *Trends Biotechnol.* **2017**, *35*, 860–870. [CrossRef]
38. Arslan, M.; Afzal, M.; Anjum, N.A. Constructed and Floating Wetlands for Sustainable Water Reclamation. *Sustainability* **2022**, *14*, 1268. [CrossRef]
39. Mani, S.; Chowdhary, P.; Zainith, S. Microbes Mediated Approaches for Environmental Waste Management. In *Microorganisms for Sustainable Environment and Health*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 17–36. [CrossRef]
40. Khalid, M.Z.; Ahmad, S.; Ngegba, P.M.; Zhong, G. Role of Endocrine System in the Regulation of Female Insect Reproduction. *Biology* **2021**, *10*, 614. [CrossRef]
41. Ahmad, S.; Bhatt, P.; Ahmad, H.W.; Cui, D.; Guo, J.; Zhong, G. Enzymes Involved in the Bioremediation of Pesticides. In *Industrial Applications of Microbial Enzymes*; CRC Press: Boca Raton, FL, USA, 2022; pp. 133–168. [CrossRef]
42. Sriram, N.; Reetha, D.; Saranraj, P. Biological Degradation of Reactive Dyes by Using Bacteria Isolated from Dye Effluent Contaminated Soil. *Middle-East J. Sci. Res.* **2013**, *17*, 1695–1700. [CrossRef]
43. Thangaraj, S.; Bankole, P.O.; Sadasivam, S.K. Microbial Degradation of Azo Dyes by Textile Effluent Adapted *Enterobacter hormaechei* Under Microaerophilic Condition. *Microbiol. Res.* **2021**, *250*, 126805. [CrossRef]
44. Adedayo, O.; Javadpour, S.; Taylor, C.; Anderson, W.; Moo-Young, M. Decolourization and Detoxification of Methyl Red by Aerobic Bacteria from a Wastewater Treatment Plant. *World J. Microbiol. Biotechnol.* **2004**, *20*, 545–550. [CrossRef]

45. Meerbergen, K.; Willems, K.A.; Dewil, R.; Van Impe, J.; Appels, L.; Lievens, B. Isolation and Screening of Bacterial Isolates from Wastewater Treatment Plants to Decolorize Azo Dyes. *J. Biosci. Bioeng.* **2018**, *125*, 448–456. [CrossRef] [PubMed]
46. Kiayi, Z.; Lotfabad, T.B.; Heidarinasab, A.; Shahcheraghi, F. Microbial Degradation of Azo Dye Carmoisine in Aqueous Medium Using Saccharomyces cerevisiae ATCC 9763. *J. Hazard. Mater.* **2019**, *373*, 608–619. [CrossRef] [PubMed]
47. Cai, X.; Zheng, X.; Zhang, D.; Iqbal, W.; Liu, C.; Yang, B.; Zhao, X.; Lu, X.; Mao, Y. Microbial Characterization of Heavy Metal Resistant Bacterial Strains Isolated from an Electroplating Wastewater Treatment Plant. *Ecotoxicol. Environ. Saf.* **2019**, *181*, 472–480. [CrossRef] [PubMed]
48. Sharma, P.; Tripathi, S.; Chaturvedi, P.; Chaurasia, D.; Chandra, R. Newly Isolated *Bacillus* sp. PS-6 Assisted Phytoremediation of Heavy Metals Using Phragmites communis: Potential Application in Wastewater Treatment. *Bioresour. Technol.* **2021**, *320*, 124353. [CrossRef]
49. San Keskin, N.O.; Celebioglu, A.; Sarioglu, O.F.; Uyar, T.; Tekinay, T. Encapsulation of Living Bacteria in Electrospun Cyclodextrin Ultrathin Fibers for Bioremediation of Heavy Metals and Reactive Dye from Wastewater. *Colloids Surf. B Biointerfaces* **2018**, *161*, 169–176. [CrossRef]
50. Ibrahim, S.; Zulkharnain, A.; Zahri, K.N.M.; Lee, G.; Convey, P.; Gomez Fuentes, C.; Sabri, S.; Khalil, K.; Alias, S.; Gonzales-Rocha, G. Effect of Heavy Metals and Other Xenobiotics on Biodegradation of Waste Canola Oil by Cold-Adapted *Rhodococcus* sp. AQ5-07. *Rev. Mex. Ing. Quim.* **2020**, *19*, 1041–1052. [CrossRef]
51. Corral-Bobadilla, M.; González-Marcos, A.; Vergara-González, E.P.; Alba-Elías, F. Bioremediation of Waste Water to Remove Heavy Metals Using the Spent Mushroom Substrate of *Agaricus bisporus*. *Water* **2019**, *11*, 454. [CrossRef]
52. Da Silva, A.P.A.; De Oliveira, C.D.L.; Quirino, A.M.S.; Da Silva, F.D.M.; De Aquino Saraiva, R.; Silva-Cavalcanti, J.S. Endocrine Disruptors in Aquatic Environment: Effects and Consequences on the Biodiversity of Fish and Amphibian Species. *Aquat. Sci. Technol.* **2018**, *6*, 35–51. [CrossRef]
53. Hua, T.; Li, S.; Li, F.; Ondon, B.S.; Liu, Y.; Wang, H. Degradation Performance and Microbial Community Analysis of Microbial Electrolysis Cells for Erythromycin Wastewater Treatment. *Biochem. Eng. J.* **2019**, *146*, 1–9. [CrossRef]
54. Xue, W.; Li, F.; Zhou, Q. Degradation Mechanisms of Sulfamethoxazole and Its Induction of Bacterial Community Changes and Antibiotic Resistance Genes in a Microbial Fuel Cell. *Bioresour. Technol.* **2019**, *289*, 121632. [CrossRef]
55. Wang, Q.; Li, X.; Yang, Q.; Chen, Y.; Du, B. Evolution of Microbial Community and Drug Resistance During Enrichment of Tetracycline-Degrading Bacteria. *Ecotoxicol. Environ. Saf.* **2019**, *171*, 746–752. [CrossRef] [PubMed]
56. Liyanage, G.; Manage, P.M. Removal of Ciprofloxacin (CIP) by Bacteria Isolated from Hospital Effluent Water and Identification of Degradation Pathways. *Int. J. Med. Pharm. Drug Res.* **2018**, *2*, 37–47. Available online: http://dr.lib.sjp.ac.lk/handle/123456789/6985 (accessed on 10 April 2024). [CrossRef]
57. Al-Dhabi, N.A.; Esmail, G.A.; Arasu, M.V. Effective Degradation of Tetracycline by Manganese Peroxidase Producing *Bacillus velezensis* Strain Al-Dhabi 140 from Saudi Arabia Using Fibrous-Bed Reactor. *Chemosphere* **2020**, *268*, 128726. [CrossRef] [PubMed]
58. Zhang, Y.; Xu, Z.; Chen, Z.; Wang, G. Simultaneous Degradation of Triazophos, Methamidophos and Carbofuran Pesticides in Wastewater Using an Enterobacter Bacterial Bioreactor and Analysis of Toxicity and Biosafety. *Chemosphere* **2020**, *261*, 128054. [CrossRef]
59. Mavriou, Z.; Alexandropoulou, I.; Melidis, P.; Karpouzas, D.G.; Ntougias, S. Biotreatment and Bacterial Succession in an Upflow Immobilized Cell Bioreactor Fed with Fludioxonil Wastewater. *Environ. Sci. Pollut. Res.* **2021**, *28*, 3774–3786. [CrossRef]
60. Avila, R.; Peris, A.; Eljarrat, E.; Vicent, T.; Blánquez, P. Biodegradation of Hydrophobic Pesticides by Microalgae: Transformation Products and Impact on Algae Biochemical Methane Potential. *Sci. Total Environ.* **2021**, *754*, 142114. [CrossRef]
61. Ramya, K.; Vasudevan, N. Biodegradation of Synthetic Pyrethroid Pesticides Under Saline Conditions by a Novel Halotolerant *Enterobacter ludwigii*. *Desalin. Water Treat.* **2020**, *173*, 255–266. [CrossRef]
62. Bhatt, P.; Huang, Y.; Rene, E.R.; Kumar, A.J.; Chen, S. Mechanism of Allethrin Biodegradation by a Newly *Isolated Sphingomonas trueperi* Strain CW3 from Wastewater Sludge. *Bioresour. Technol.* **2020**, *305*, 123074. [CrossRef] [PubMed]
63. Al-Mur, B.A.; Pugazhendi, A.; Jamal, M.T. Application of Integrated Extremophilic (Halo-Alkalo-Thermophilic) Bacterial Consortium in the Degradation of Petroleum Hydrocarbons and Treatment of Petroleum Refinery Wastewater Under Extreme Condition. *J. Hazard. Mater.* **2021**, *413*, 125351. [CrossRef]
64. Jamal, M.T.; Pugazhendi, A. Degradation of Petroleum Hydrocarbons and Treatment of Refinery Wastewater Under Saline Condition by a Halophilic Bacterial Consortium Enriched from Marine Environment (Red Sea), Jeddah, Saudi Arabia. *3 Biotech* **2018**, *8*, 276. [CrossRef]
65. Patel, K.; Patel, M. Improving Bioremediation Process of Petroleum Wastewater Using Biosurfactants Producing *Stenotrophomonas* sp. S1VKR-26 and Assessment of Phytotoxicity. *Bioresour. Technol.* **2020**, *315*, 123861. [CrossRef] [PubMed]
66. Kachieng'a, L.; Momba, M. The Synergistic Effect of a Consortium of Protozoan Isolates (*Paramecium* sp., *Vorticella* sp., *Epistylis* sp. and *Opercularia* sp.) on the biodegradation of petroleum hydrocarbons in wastewater. *J. Environ. Chem. Eng.* **2018**, *6*, 4820–4827. [CrossRef]
67. Tian, X.; Wang, X.; Peng, S.; Wang, Z.; Zhou, R.; Tian, H. Isolation, Screening, and Crude Oil Degradation Characteristics of Hydrocarbons-Degrading Bacteria for Treatment of Oily Wastewater. *Water Sci. Technol.* **2018**, *78*, 2626–2638. [CrossRef] [PubMed]
68. Abo-State, M.; Riad, B.; Bakr, A.; Aziz, M.A. Biodegradation of naphthalene by *Bordetella avium* isolated from petroleum refinery wastewater in Egypt and its pathway. *J. Radiat. Res. Appl. Sci.* **2018**, *11*, 1–9. [CrossRef]

69. Carullo, D.; Abera, B.D.; Casazza, A.A.; Donsì, F.; Perego, P.; Ferrari, G.; Pataro, G. Effect of Pulsed Electric Fields and High Pressure Homogenization on the Aqueous Extraction of Intracellular Compounds from the Microalgae *Chlorella vulgaris*. *Algal Res.* **2018**, *31*, 60–69. [CrossRef]
70. Hejna, M.; Moscatelli, A.; Stroppa, N.; Onelli, E.; Pilu, S.; Baldi, A.; Rossi, L. Bioaccumulation of Heavy Metals from Wastewater Through a *Typha latifolia* and *Thelypteris palustris* Phytoremediation System. *Chemosphere* **2020**, *241*, 125018. [CrossRef]
71. Bhatt, P.; Ahmad, S.; Joshi, S.; Bhatt, K. Recent Advancement in Microbial Enzymes and Their Industrial Applications. In *Industrial Applications of Microbial Enzymes*; Bhatt, P., Ed.; CRC Press: Boca Raton, FL, USA, 2022; pp. 1–17. [CrossRef]
72. Dey, P.; Malik, A.; Mishra, A.; Singh, D.K.; von Bergen, M.; Jehmlich, N. Mechanistic Insight to Mycoremediation Potential of a Metal Resistant Fungal Strain for Removal of Hazardous Metals from Multimetal Pesticide Matrix. *Environ. Pollut.* **2020**, *262*, 114255. [CrossRef]
73. Morsi, R.; Bilal, M.; Iqbal, H.M.; Ashraf, S.S. Laccases and Peroxidases: The Smart, Greener and Futuristic Biocatalytic Tools to Mitigate Recalcitrant Emerging Pollutants. *Sci. Total Environ.* **2020**, *714*, 136572. [CrossRef]
74. Siddiqui, J.A.; Khan, M.M.; Bamisile, B.S.; Hafeez, M.; Qasim, M.; Rasheed, M.T.; Rasheed, M.A.; Ahmad, S.; Shahid, M.I. Role of Insect Gut Microbiota in Pesticide Degradation: A Review. *Front. Microbiol.* **2022**, *13*, 870462. [CrossRef]
75. Zhang, Y.; Carvalho, P.N.; Lv, T.; Arias, C.; Brix, H.; Chen, Z. Microbial Density and Diversity in Constructed Wetland Systems and the Relation to Pollutant Removal Efficiency. *Water Sci. Technol.* **2016**, *73*, 679–686. [CrossRef]
76. Munoz-Cupa, C.; Hu, Y.; Xu, C.; Bassi, A. An Overview of Microbial Fuel Cell Usage in Wastewater Treatment, Resource Recovery and Energy Production. *Sci. Total Environ.* **2021**, *754*, 142429. [CrossRef]
77. Slate, A.J.; Whitehead, K.A.; Brownson, D.A.; Banks, C.E. Microbial Fuel Cells: An Overview of Current Technology. *Renew. Sustain. Energy Rev.* **2019**, *101*, 60–81. [CrossRef]
78. Ferreira, L.; Rosales, E.; Danko, A.S.; Sanromán, M.A.; Pazos, M.M. Bacillus thuringiensis: A Promising Bacterium for Degrading Emerging Pollutants. *Process Saf. Environ. Prot.* **2016**, *101*, 19–26. [CrossRef]
79. Li, K.; Xu, A.; Wu, D.; Zhao, S.; Meng, T.; Zhang, Y. Degradation of Ofloxacin by a Manganese-Oxidizing Bacterium *Pseudomonas* sp. F2 and Its Biogenic Manganese Oxides. *Bioresour. Technol.* **2021**, *328*, 124826. [CrossRef] [PubMed]
80. Ha, H.; Mahanty, B.; Yoon, S.; Kim, C.-G. Degradation of the Long-Resistant Pharmaceutical Compounds Carbamazepine and Diatrizoate Using Mixed Microbial Culture. *J. Environ. Sci. Health A* **2016**, *51*, 467–471. [CrossRef] [PubMed]
81. Lwanga, E.H.; Thapa, B.; Yang, X.; Gertsen, H.; Salánki, T.; Geissen, V.; Garbeva, P. Decay of Low-Density Polyethylene by Bacteria Extracted from Earthworm's Guts: A Potential for Soil Restoration. *Sci. Total Environ.* **2018**, *624*, 753–757. [CrossRef]
82. Liang, D.H.; Hu, Y. Application of a Heavy Metal-Resistant *Achromobacter* sp. for the simultaneous immobilization of cadmium and degradation of sulfamethoxazole from wastewater. *J. Hazard. Mater.* **2021**, *402*, 124032. [CrossRef]
83. Wu, M.; Li, W.; Dick, W.A.; Ye, X.; Chen, K.; Kost, D.; Chen, L. Bioremediation of Hydrocarbon Degradation in a Petroleum-Contaminated Soil and Microbial Population and Activity Determination. *Chemosphere* **2017**, *169*, 124–130. [CrossRef]
84. Jaffar, S.; Ahmad, S.; Lu, Y. Contribution of insect gut microbiota and their associated enzymes in insect physiology and biodegradation of pesticides. *Front. Microbiol.* **2022**, *13*, 979383. [CrossRef]
85. Chandrasekaran, M.; Paramasivan, M.; Ahmad, S. Review on arbuscular mycorrhizal fungi mediated alleviation of arsenic stress. *Int. Biodeterior. Biodegrad.* **2024**, *194*, 105872. [CrossRef]
86. Akram, M.A.; Asad, M.J.; Mahmood, R.T.; Shah, M.B.; Nazir, S.; Khan, J.; Rashid, K.; Aslam, S.; Rawalpindi, P. First Report on the Biodegradation of Direct Flavine 5-G and Reactive Red S3B Textile Dyes by *Piptoporus betulinus*. *Pak. J. Sci. Ind. Res. Ser. A Phys. Sci.* **2023**, *66*, 138–143.
87. Uqaili, A.A.; Usman, G.; Bhatti, U.; Nasir, H.; Zia, R.; Akram, M.A.; Jawad, F.A.; Farid, A.; Abdelgawwad, M.R.; Almutairi, S.M.; et al. Bioinformatics, RNA sequencing, and targeted bisulfite sequencing analyses identify the role of PROM2 as a diagnostic and prognostic biomarker. *Am. J. Transl. Res.* **2023**, *15*, 5389. [PubMed]
88. Li, J.; Shaikh, S.N.; Uqaili, A.A.; Nasir, H.; Zia, R.; Akram, M.A.; Jawad, F.A.; Sohail, S.; Abdelgawwad, M.R.; Almutairi, S.M.; et al. A pan-cancer analysis of pituitary tumor-transforming 3, pseudogene. *Am. J. Transl. Res.* **2023**, *15*, 5408.
89. Xiong, J.Q.; Govindwar, S.; Kurade, M.B.; Paeng, K.J.; Roh, H.S.; Khan, M.A.; Jeon, B.H. Toxicity of sulfamethazine and sulfamethoxazole and their removal by a green microalga, *Scenedesmus obliquus*. *Chemosphere* **2019**, *218*, 551–558. [CrossRef]
90. Asiandu, A.P.; Wahyudi, A. Phycoremediation: Heavy metals green-removal by microalgae and its application in biofuel production. *J. Environ. Treat. Tech.* **2021**, *9*, 647–656.
91. Jasrotia, S.; Kansal, A.; Kishore, V.V.N. Arsenic phyco-remediation by *Cladophora algae* and measurement of arsenic speciation and location of active absorption site using electron microscopy. *Microchem. J.* **2014**, *114*, 197–202. [CrossRef]
92. Abioye, O.P.; Keji, Y.D.; Aransiola, S.A.; Oyewole, O. Phycoremediation of manganese by *Spirogyra* and *Richterella* species isolated from pond. *J. Glob. Agric. Ecol.* **2015**, *2*, 78–83.
93. Jayakumar, V.; Govindaradjane, S.; Rajamohan, N.; Rajasimman, M. Biosorption potential of brown algae, *Sargassum polycystum*, for the removal of toxic metals, cadmium and zinc. *Environ. Sci. Pollut. Res.* **2021**, *29*, 41909–41922. [CrossRef]
94. Wu, M.; Dick, W.A.; Li, W.; Wang, X.; Yang, Q.; Wang, T.; Xu, L.; Zhang, M.; Chen, L. Bioaugmentation and Biostimulation of Hydrocarbon Degradation and the Microbial Community in a Petroleum-Contaminated Soil. *Int. Biodeterior. Biodegrad.* **2016**, *107*, 158–164. [CrossRef]

95. Tirpak, R.A.; Tondera, K.; Tharp, R.; Borne, K.E.; Schwammberger, P.; Ruppelt, J.; Winston, R.J. Optimizing Floating Treatment Wetland and Retention Pond Design through Random Forest: A Meta-Analysis of Influential Variables. *J. Environ. Manag.* **2022**, *312*, 114909. [CrossRef]
96. Landmann, J.; Hammer, T.C.; Günther, H.; Hildebrandt, A. Large-Scale Investigation of Wave Dampening Characteristics of Organic, Artificial Floating Islands. *Ecol. Eng.* **2022**, *181*, 106691. [CrossRef]
97. Oliveira, G.A.; Colares, G.S.; Lutterbeck, C.A.; Dell'Osbel, N.; Machado, Ê.L.; Rodrigues, L.R. Floating Treatment Wetlands in Domestic Wastewater Treatment as a Decentralized Sanitation Alternative. *Sci. Total Environ.* **2021**, *773*, 145609. [CrossRef] [PubMed]
98. Pavlineri, N.; Skoulikidis, N.T.; Tsihrintzis, V.A. Constructed Floating Wetlands: A Review of Research, Design, Operation, and Management Aspects, and Data Meta-Analysis. *Chem. Eng. J.* **2017**, *308*, 1120–1132. [CrossRef]
99. Rottle, N.; Bowles, M.; Andrews, L.; Engelke, J. Constructed Floating Wetlands: A "Safe-to-Fail" Study with Multi-Sector Participation. *Restor. Ecol.* **2022**, *31*, e13672. [CrossRef]
100. Chance, L.M.G.; Van Brunt, S.C.; Majsztrik, J.C.; White, S.A. Short- and Long-Term Dynamics of Nutrient Removal in Floating Treatment Wetlands. *Water Res.* **2019**, *159*, 153–163. [CrossRef]
101. Magwaza, S.T.; Magwaza, L.S.; Odindo, A.O.; Mditshwa, A. Hydroponic Technology as Decentralised System for Domestic Wastewater Treatment and Vegetable Production in Urban Agriculture: A Review. *Sci. Total Environ.* **2020**, *698*, 134154. [CrossRef]
102. Benvenuti, T.; Hamerski, F.; Giacobbo, A.; Bernardes, A.M.; Zoppas-Ferreira, J.; Rodrigues, M.A. Constructed Floating Wetland for the Treatment of Domestic Sewage: A Real-Scale Study. *J. Environ. Chem. Eng.* **2018**, *6*, 5706–5711. [CrossRef]
103. Pavlidis, G.; Zotou, I.; Karasali, H.; Marousopoulou, A.; Bariamis, G.; Tsihrintzis, V.A.; Nalbantis, I. Performance of Pilot-Scale Constructed Floating Wetlands in the Removal of Nutrients and Pesticides. *Water Resour. Manag.* **2021**, *36*, 399–416. [CrossRef]
104. Han, W.; Luo, G.; Luo, B.; Yu, C.; Wang, H.; Chang, J.; Ge, Y. Effects of Plant Diversity on Greenhouse Gas Emissions in Microcosms Simulating Vertical Constructed Wetlands with High Ammonium Loading. *J. Environ. Sci.* **2019**, *77*, 229–237. [CrossRef]
105. Chen, J.; Liu, Y.-S.; Deng, W.-J.; Ying, G.-G. Removal of Steroid Hormones and Biocides from Rural Wastewater by an Integrated Constructed Wetland. *Sci. Total Environ.* **2019**, *660*, 358–365. [CrossRef]
106. Gholipour, A.; Zahabi, H.; Stefanakis, A.I. A Novel Pilot and Full-Scale Constructed Wetland Study for Glass Industry Wastewater Treatment. *Chemosphere* **2020**, *247*, 125966. [CrossRef] [PubMed]
107. Almeida, C.M.R.; Santos, F.; Ferreira, A.C.F.; Lourinha, I.; Basto, M.C.P.; Mucha, A.P. Can Veterinary Antibiotics Affect Constructed Wetlands Performance during Treatment of Livestock Wastewater? *Ecol. Eng.* **2017**, *102*, 583–588. [CrossRef]
108. Mustapha, H.I.; Van Bruggen, H.J.J.A.; Lens, P.N. Vertical Subsurface Flow Constructed Wetlands for the Removal of Petroleum Contaminants from Secondary Refinery Effluent at the Kaduna Refining Plant (Kaduna, Nigeria). *Environ. Sci. Pollut. Res.* **2018**, *25*, 30451–30462. [CrossRef]
109. Shahid, M.J.; Arslan, M.; Siddique, M.; Ali, S.; Tahseen, R.; Afzal, M. Potentialities of Floating Wetlands for the Treatment of Polluted Water of River Ravi, Pakistan. *Ecol. Eng.* **2019**, *133*, 167–176. [CrossRef]
110. Effendi, H.; Margaretha, J.; Krisanti, M. Reducing Ammonia and Chromium Concentration in Batik Wastewater by Vetiver (*Chrysopogon zizanioides* L.) grown in floating wetland. *Appl. Ecol. Environ. Res.* **2018**, *16*, 2947–2956. [CrossRef]
111. Afzal, M.; Arslan, M.; Müller, J.A.; Shabir, G.; Islam, E.; Tahseen, R.; Anwar-ul-Haq, M.; Hashmat, A.J.; Iqbal, S.; Khan, Q.M. Floating Treatment Wetlands as a Suitable Option for Large-Scale Wastewater Treatment. *Nat. Sustain.* **2019**, *2*, 863–871. [CrossRef]
112. Akram, A.; Tara, N.; Khan, M.A.; Abbasi, S.A.; Irfan, M.; Arslan, M.; Afzal, M. Enhanced Remediation of Cr^{6+} in Bacterial-Assisted Floating Wetlands. *Water Environ. J.* **2020**, *34*, 970–978. [CrossRef]
113. Nawaz, N.; Ali, S.; Shabir, G.; Rizwan, M.; Shakoor, M.B.; Shahid, M.J.; Afzal, M.; Arslan, M.; Hashem, A.; Abd_Allah, E.F.; et al. Bacterial Augmented Floating Treatment Wetlands for Efficient Treatment of Synthetic Textile Dye Wastewater. *Sustainability* **2020**, *12*, 3731. [CrossRef]
114. Gupta, P.; Ann, T.-W.; Lee, S.-M. Use of Biochar to Enhance Constructed Wetland Performance in Wastewater Reclamation. *Environ. Eng. Res.* **2016**, *21*, 36–44. [CrossRef]
115. Somprasert, S.; Mungkung, S.; Kreetachat, N.; Imman, S.; Homklin, S. Implementation of an Integrated Floating Wetland and Biofilter for Water Treatment in *Nile Tilapia* Aquaculture. *J. Ecol. Eng.* **2021**, *22*, 146–152. [CrossRef]
116. Saeed, T.; Afrin, R.; Al-Muyeed, A.; Miah, M.J.; Jahan, H. Bioreactor Septic Tank for On-Site Wastewater Treatment: Floating Constructed Wetland Integration. *J. Environ. Chem. Eng.* **2021**, *9*, 105606. [CrossRef]
117. Queiroz, R.d.C.S.d.; Lôbo, I.P.; Ribeiro, V.d.S.; Rodrigues, L.B.; Almeida Neto, J.A.d. Assessment of Autochthonous Aquatic Macrophytes with Phytoremediation Potential for Dairy Wastewater Treatment in Floating Constructed Wetlands. *Int. J. Phytoremediat.* **2020**, *22*, 518–528. [CrossRef] [PubMed]
118. Fahid, M.; Arslan, M.; Shabir, G.; Younus, S.; Yasmeen, T.; Rizwan, M.; Siddique, K.; Ahmad, S.R.; Tahseen, R.; Iqbal, S. *Phragmites australis* in Combination with Hydrocarbons Degrading Bacteria Is a Suitable Option for Remediation of Diesel-Contaminated Water in Floating Wetlands. *Chemosphere* **2020**, *240*, 124890. [CrossRef] [PubMed]
119. Tara, N.; Arslan, M.; Hussain, Z.; Iqbal, M.; Khan, Q.M.; Afzal, M. On-Site Performance of Floating Treatment Wetland Macrocosms Augmented with Dye-Degrading Bacteria for the Remediation of Textile Industry Wastewater. *J. Clean. Prod.* **2019**, *217*, 541–548. [CrossRef]
120. Rehman, K.; Imran, A.; Amin, I.; Afzal, M. Inoculation with Bacteria in Floating Treatment Wetlands Positively Modulates the Phytoremediation of Oil Field Wastewater. *J. Hazard. Mater.* **2018**, *349*, 242–251. [CrossRef]

121. Park, J.B.; Sukias, J.P.; Tanner, C.C. Floating Treatment Wetlands Supplemented with Aeration and Biofilm Attachment Surfaces for Efficient Domestic Wastewater Treatment. *Ecol. Eng.* **2019**, *139*, 105582. [CrossRef]
122. Spangler, J.T.; Sample, D.J.; Fox, L.J.; Albano, J.P.; White, S.A. Assessing Nitrogen and Phosphorus Removal Potential of Five Plant Species in Floating Treatment Wetlands Receiving Simulated Nursery Runoff. *Environ. Sci. Pollut. Res.* **2019**, *26*, 5751–5768. [CrossRef]
123. Cicero-Fernandez, D.; Expósito-Camargo, J.; Peña-Fernandez, M. Efficacy of *Juncus maritimus* Floating Treatment Saltmarsh as Anti-Contamination Barrier for Saltwater Aquaculture Pollution Control. *Water Sci. Technol.* **2022**, *85*, 2811–2826. [CrossRef]
124. Spangler, J.T.; Sample, D.J.; Fox, L.J.; Owen, J.S., Jr.; White, S.A. Floating treatment wetland aided nutrient removal from agricultural runoff using two wetland species. *Ecol. Eng.* **2019**, *127*, 468–479. [CrossRef]
125. Goren, A.Y.; Yucel, A.; Sofuoglu, S.C.; Sofuoglu, A. Phytoremediation of Olive Mill Wastewater with *Vetiveria zizaniodes* (L.) Nash and *Cyperus alternifolius* L. *Environ. Technol. Innov.* **2021**, *24*, 102071. [CrossRef]
126. Huang, Z.; Kong, F.; Li, Y.; Xu, G.; Yuan, R.; Wang, S. Advanced Treatment of Effluent from Municipal Wastewater Treatment Plant by Strengthened Ecological Floating Bed. *Bioresour. Technol.* **2020**, *309*, 123358. [CrossRef] [PubMed]
127. Gaballah, M.S.; Ismail, K.; Aboagye, D.; Ismail, M.M.; Sobhi, M.; Stefanakis, A.I. Effect of Design and Operational Parameters on Nutrients and Heavy Metal Removal in Pilot Floating Treatment Wetlands with *Eichhornia crassipes* Treating Polluted Lake Water. *Environ. Sci. Pollut. Res.* **2021**, *28*, 25664–25678. [CrossRef] [PubMed]
128. Parihar, P.; Chand, N.; Suthar, S. Septage Effluent Treatment Using Floating Constructed Wetland with *Spirodela polyrhiza*: Response of Biochar Addition in the Support Matrix. *Nat.-Based Solut.* **2022**, *2*, 100020. [CrossRef]
129. Ezzatahmadi, N.; Millar, G.J.; Ayoko, G.A.; Zhu, J.; Zhu, R.; Liang, X.; He, H.; Xi, Y. Degradation of 2,4-Dichlorophenol Using Palygorskite-Supported Bimetallic Fe/Ni Nanocomposite as a Heterogeneous Catalyst. *Appl. Clay Sci.* **2019**, *168*, 276–286. [CrossRef]
130. Samal, S. Effect of Shape and Size of Filler Particle on the Aggregation and Sedimentation Behavior of the Polymer Composite. *Powder Technol.* **2020**, *366*, 43–51. [CrossRef]
131. Kim, H.; Shin, M.; Jang, D.; Jung, S.; Jin, J. Study of Flow Characteristics in a Secondary Clarifier by Numerical Simulation and Radioisotope Tracer Technique. *Appl. Radiat. Isot.* **2005**, *63*, 519–526. [CrossRef]
132. Zhang, X.; Chen, J.; Li, J. The Removal of Microplastics in the Wastewater Treatment Process and Their Potential Impact on Anaerobic Digestion Due to Pollutants Association. *Chemosphere* **2020**, *251*, 126360. [CrossRef]
133. Anthony, E.T.; Ojemaye, M.O.; Okoh, O.O.; Okoh, A.I. A Critical Review on the Occurrence of Resistomes in the Environment and Their Removal from Wastewater Using Apposite Treatment Technologies: Limitations, Successes, and Future Improvement. *Environ. Pollut.* **2020**, *263*, 113791. [CrossRef]
134. Amosa, M.K.; Jami, M.S.; Alkhatib, M.A.F.R.; Tajari, T.; Jimat, D.N.; Owolabi, R.U. Turbidity and suspended solids removal from high-strength wastewater using high surface area adsorbent: Mechanistic pathway and statistical analysis. *Cogent Eng.* **2016**, *3*, 1162384. [CrossRef]
135. Chen, H.; Zhang, M. Effects of Advanced Treatment Systems on the Removal of Antibiotic Resistance Genes in Wastewater Treatment Plants from Hangzhou, China. *Environ. Sci. Technol.* **2013**, *47*, 8157–8163. [CrossRef]
136. Zhou, G.-J.; Lin, L.; Li, X.-Y.; Leung, K.M.Y. Removal of emerging contaminants from wastewater during chemically enhanced primary sedimentation and acidogenic sludge fermentation. *Water Res.* **2020**, *175*, 115646. [CrossRef] [PubMed]
137. Xing, Z.-P.; Sun, D.-Z. Treatment of Antibiotic Fermentation Wastewater by Combined Polyferric Sulfate Coagulation, Fenton, and Sedimentation Process. *J. Hazard. Mater.* **2009**, *168*, 1264–1268. [CrossRef] [PubMed]
138. Ma, W.; Han, Y.; Ma, W.; Han, H.; Zhu, H.; Xu, C.; Li, K.; Wang, D. Enhanced Nitrogen Removal from Coal Gasification Wastewater by Simultaneous Nitrification and Denitrification (SND) in an Oxygen-Limited Aeration Sequencing Batch Biofilm Reactor. *Bioresour. Technol.* **2017**, *244*, 84–91. [CrossRef]
139. Chen, H.; Lu, Z.; Cheng, Y.; Drioli, E.; Wang, Z.; Zhang, F.; Cui, Z. Development and emerging application of membrane degassing technology. *Adv. Membr.* **2023**, *3*, 100076. [CrossRef]
140. Bandara, W.M.; Satoh, H.; Sasakawa, M.; Nakahara, Y.; Takahashi, M.; Okabe, S. Removal of residual dissolved methane gas in an upflow anaerobic sludge blanket reactor treating low-strength wastewater at low temperature with degassing membrane. *Water Res.* **2011**, *45*, 3533–3540. [CrossRef]
141. Saidou, H.; Korchef, A.; Moussa, S.B.; Amor, M.B. Struvite precipitation by the dissolved CO_2 degasification technique: Impact of the airflow rate and pH. *Chemosphere* **2009**, *74*, 338–343. [CrossRef]
142. Zhang, T.; Li, P.; Fang, C.; Jiang, R. Phosphate recovery from animal manure wastewater by struvite crystallization and CO_2 degasification reactor. *Ecol. Chem. Eng.* **2014**, *21*, 89. [CrossRef]
143. Sun, M.; An, J.; Pan, Z.; Feng, G.; Fan, X.; Song, C.; Wang, T. Enhanced Organic Wastewater Treatment Performance in Electrochemical Filtration Process of Coal-Based Carbon Membrane via the Simple Fe^{2+} Addition. *Sep. Purif. Technol.* **2021**, *276*, 119418. [CrossRef]
144. Shen, M.; Hu, T.; Huang, W.; Song, B.; Zeng, G.; Zhang, Y. Removal of Microplastics from Wastewater with Aluminosilicate Filter Media and Their Surfactant-Modified Products: Performance, Mechanism, and Utilization. *Chem. Eng. J.* **2021**, *421*, 129918. [CrossRef]

145. Lee, H.-S.; Lim, B.-R.; Hur, J.; Kim, H.-S.; Shin, H.-S. Combined Dual-Size Foam Glass Media Filtration Process with Micro-Flocculation for Simultaneous Removal of Particulate and Dissolved Contaminants in Urban Road Runoff. *J. Environ. Manag.* **2021**, *277*, 111475. [CrossRef]
146. Menzel, K.; Barros, L.; García, A.; Ruby-Figueroa, R.; Estay, H. Metal Sulfide Precipitation Coupled with Membrane Filtration Process for Recovering Copper from Acid Mine Drainage. *Sep. Purif. Technol.* **2021**, *270*, 118721. [CrossRef]
147. Jiang, M.; Ye, K.; Deng, J.; Lin, J.; Ye, W.; Zhao, S.; Van der Bruggen, B. Conventional Ultrafiltration as an Effective Strategy for Dye/Salt Fractionation in Textile Wastewater Treatment. *Environ. Sci. Technol.* **2018**, *52*, 10698–10708. [CrossRef] [PubMed]
148. Lohani, S.P.; Khanal, S.N.; Bakke, R. A simple anaerobic and filtration combined system for domestic wastewater treatment. *Water-Energy Nexus* **2020**, *3*, 41–45. [CrossRef]
149. Zheng, J.; Wang, Z.; Ma, J.; Xu, S.; Wu, Z. Development of an Electrochemical Ceramic Membrane Filtration System for Efficient Contaminant Removal from Waters. *Environ. Sci. Technol.* **2018**, *52*, 4117–4126. [CrossRef]
150. Ghernaout, D. The hydrophilic/hydrophobic ratio vs. dissolved organics removal by coagulation—A review. *J. King Saud Univ. Sci.* **2014**, *26*, 169–180. [CrossRef]
151. Asadi, A.; Verma, A.; Yang, K.; Mejabi, B. Wastewater treatment aeration process optimization: A data mining approach. *J. Environ. Manag.* **2017**, *203*, 630–639. [CrossRef] [PubMed]
152. Zhang, Z.; Kusiak, A.; Zeng, Y.; Wei, X. Modeling and optimization of a wastewater pumping system with data-mining methods. *Appl. Energy* **2016**, *164*, 303–311. [CrossRef]
153. Ghernaout, D. Aeration Process for Removing Radon from Drinking Water—A Review. *Appl. Eng.* **2019**, *3*, 32–45.
154. Uggetti, E.; Hughes-Riley, T.; Morris, R.H.; Newton, M.I.; Trabi, C.L.; Hawes, P.; Puigagut, J.; García, J. Intermittent aeration to improve wastewater treatment efficiency in pilot-scale constructed wetland. *Sci. Total Environ.* **2016**, *559*, 212–217. [CrossRef]
155. Iskurt, C.; Keyikoglu, R.; Kobya, M.; Khataee, A. Treatment of coking wastewater by aeration assisted electrochemical oxidation process at controlled and uncontrolled initial pH conditions. *Sep. Purif. Technol.* **2020**, *248*, 117043. [CrossRef]
156. Jehawi, O.H.; Abdullah, S.R.S.; Kurniawan, S.B.; Ismail, N.I.; Idris, M.; Al Sbani, N.H.; Muhamad, M.H.; Hasan, H.A. Performance of pilot Hybrid Reed Bed constructed wetland with aeration system on nutrient removal for domestic wastewater treatment. *Environ. Technol. Innov.* **2020**, *19*, 100891. [CrossRef]
157. Skouteris, G.; Rodriguez-Garcia, G.; Reinecke, S.; Hampel, U. The use of pure oxygen for aeration in aerobic wastewater treatment: A review of its potential and limitations. *Bioresour. Technol.* **2020**, *312*, 123595. [CrossRef] [PubMed]
158. Ahmed, S.; Mofijur, M.; Nuzhat, S.; Chowdhury, A.T.; Rafa, N.; Uddin, M.A.; Inayat, A.; Mahlia, T.; Ong, H.C.; Chia, W.Y. Recent developments in physical, biological, chemical, and hybrid treatment techniques for removing emerging contaminants from wastewater. *J. Hazard. Mater.* **2021**, *416*, 125912. [CrossRef] [PubMed]
159. Singh, N.; Gupta, S. Adsorption of heavy metals: A review. *Int. J. Innov. Res. Sci. Eng. Technol.* **2016**, *5*, 2267–2281.
160. Deliyanni, E.A.; Kyzas, G.Z.; Triantafyllidis, K.S.; Matis, K.A. Activated carbons for the removal of heavy metal ions: A systematic review of recent literature focused on lead and arsenic ions. *Open Chem.* **2015**, *13*, 699–708. [CrossRef]
161. Mustapha, S.; Tijani, J.; Ndamitso, M.; Abdulkareem, S.; Shuaib, D.; Mohammed, A.; Sumaila, A. The role of kaolin and kaolin/ZnO nanoadsorbents in adsorption studies for tannery wastewater treatment. *Sci. Rep.* **2020**, *10*, 13068. [CrossRef] [PubMed]
162. Chen, H.; Liu, T.; Meng, Y.; Cheng, Y.; Lu, J.; Wang, H. Novel graphene oxide/aminated lignin aerogels for enhanced adsorption of malachite green in wastewater. *Colloids Surf. A Physicochem. Eng. Asp.* **2020**, *603*, 125281. [CrossRef]
163. Son, D.-J.; Kim, W.-Y.; Jung, B.-R.; Chang, D.; Hong, K.-H. Pilot-scale anoxic/aerobic biofilter system combined with chemical precipitation for tertiary treatment of wastewater. *J. Water Process Eng.* **2020**, *35*, 101224. [CrossRef]
164. Altaş, L.; Büyükgüngör, H. Sulfide removal in petroleum refinery wastewater by chemical precipitation. *J. Hazard. Mater.* **2008**, *153*, 462–469. [CrossRef]
165. Huang, H.; Liu, J.; Zhang, P.; Zhang, D.; Gao, F. Investigation on the simultaneous removal of fluoride, ammonia nitrogen and phosphate from semiconductor wastewater using chemical precipitation. *Chem. Eng. J.* **2017**, *307*, 696–706. [CrossRef]
166. Reyes-Serrano, A.; López-Alejo, J.E.; Hernández-Cortázar, M.A.; Elizalde, I. Removing contaminants from tannery wastewater by chemical precipitation using CaO and Ca $(OH)_2$. *Chin. J. Chem. Eng.* **2020**, *28*, 1107–1111. [CrossRef]
167. Nyström, F.; Nordqvist, K.; Herrmann, I.; Hedström, A.; Viklander, M. Removal of metals and hydrocarbons from stormwater using coagulation and flocculation. *Water Res.* **2020**, *182*, 115919. [CrossRef]
168. Sun, Y.; Zhou, S.; Pan, S.-Y.; Zhu, S.; Yu, Y.; Zheng, H. Performance evaluation and optimization of flocculation process for removing heavy metal. *Chem. Eng. J.* **2020**, *385*, 123911. [CrossRef]
169. Rajala, K.; Grönfors, O.; Hesampour, M.; Mikola, A. Removal of microplastics from secondary wastewater treatment plant effluent by coagulation/flocculation with iron, aluminum and polyamine-based chemicals. *Water Res.* **2020**, *183*, 116045. [CrossRef]
170. Karam, A.; Bakhoum, E.S.; Zaher, K. Coagulation/flocculation process for textile mill effluent treatment: Experimental and numerical perspectives. *Int. J. Sustain. Eng.* **2021**, *14*, 983–995. [CrossRef]
171. John, D.; Yesodharan, S.; Achari, V.S. Integration of coagulation-flocculation and heterogeneous photocatalysis for the treatment of pulp and paper mill effluent. *Environ. Technol.* **2022**, *43*, 443–459. [CrossRef]
172. Ayekoe, C.Y.P.; Robert, D.; Lanciné, D.G. Combination of coagulation-flocculation and heterogeneous photocatalysis for improving the removal of humic substances in real treated water from Agbô River (Ivory-Coast). *Catal. Today* **2017**, *281*, 2–13. [CrossRef]

173. Liu, Z.; Lompe, K.M.; Mohseni, M.; Bérubé, P.R.; Sauvé, S.; Barbeau, B. Biological ion exchange as an alternative to biological activated carbon for drinking water treatment. *Water Res.* **2020**, *168*, 115148. [CrossRef]
174. Martins, V.L.; Ogden, M.D.; Jones, M.R.; Trowsdale, S.A.; Hall, P.J.; Jensen, H.S. Opportunities for coupled electrochemical and ion-exchange technologies to remove recalcitrant micropollutants in water. *Sep. Purif. Technol.* **2020**, *239*, 116522. [CrossRef]
175. Amini, A.; Kim, Y.; Zhang, J.; Boyer, T.; Zhang, Q. Environmental and economic sustainability of ion exchange drinking water treatment for organics removal. *J. Clean. Prod.* **2015**, *104*, 413–421. [CrossRef]
176. Carolin, C.F.; Kumar, P.S.; Saravanan, A.; Joshiba, G.J.; Naushad, M. Efficient techniques for the removal of toxic heavy metals from aquatic environment: A review. *J. Environ. Chem. Eng.* **2017**, *5*, 2782–2799. [CrossRef]
177. Li, D.; Ning, X.-a.; Yuan, Y.; Hong, Y.; Zhang, J. Ion-exchange polymers modified bacterial cellulose electrodes for the selective removal of nitrite ions from tail water of dyeing wastewater. *J. Environ. Sci.* **2020**, *91*, 62–72. [CrossRef] [PubMed]
178. Gode, F.; Pehlivan, E. Removal of chromium (III) from aqueous solutions using Lewatit S 100: The effect of pH, time, metal concentration and temperature. *J. Hazard. Mater.* **2006**, *136*, 330–337. [CrossRef]
179. Guo, Y.; Zhu, S.; Wang, B.; Huang, J.; Deng, S.; Yu, G.; Wang, Y. Modelling of emerging contaminant removal during heterogeneous catalytic ozonation using chemical kinetic approaches. *J. Hazard. Mater.* **2019**, *380*, 120888. [CrossRef] [PubMed]
180. Grace Pavithra, K.; Jaikumar, V.; Kumar, P.S.; SundarRajan, P. A review on cleaner strategies for chromium industrial wastewater: Present research and future perspective. *J. Clean. Prod.* **2019**, *228*, 580–593. [CrossRef]
181. Malik, S.N.; Ghosh, P.C.; Vaidya, A.N.; Mudliar, S.N. Hybrid ozonation process for industrial wastewater treatment: Principles and applications: A review. *J. Water Process Eng.* **2020**, *35*, 101193. [CrossRef]
182. Mainardis, M.; Buttazzoni, M.; De Bortoli, N.; Mion, M.; Goi, D. Evaluation of ozonation applicability to pulp and paper streams for a sustainable wastewater treatment. *J. Clean. Prod.* **2020**, *258*, 120781. [CrossRef]
183. Khalifa, O.; Banat, F.; Srinivasakannan, C.; AlMarzooqi, F.; Hasan, S.W. Ozonation-assisted electro-membrane hybrid reactor for oily wastewater treatment: A methodological approach and synergy effects. *J. Clean. Prod.* **2021**, *289*, 125764. [CrossRef]
184. He, W.; Wang, Q.; Zhu, Y.; Wang, K.; Mao, J.; Xue, X.; Shi, Y. Innovative technology of municipal wastewater treatment for rapid sludge sedimentation and enhancing pollutants removal with nano-material. *Bioresour. Technol.* **2021**, *324*, 124675. [CrossRef]
185. Rubi, H.; Fall, C.; Ortega, R. Pollutant removal from oily wastewater discharged from car washes through sedimentation–coagulation. *Water Sci. Technol.* **2009**, *59*, 2359–2369. [CrossRef]
186. Khoufi, S.; Feki, F.; Sayadi, S. Detoxification of olive mill wastewater by electrocoagulation and sedimentation processes. *J. Hazard. Mater.* **2007**, *142*, 58–67. [CrossRef] [PubMed]
187. Lin, L.; Li, R.-h.; Yang, Z.-y.; Li, X.-y. Effect of coagulant on acidogenic fermentation of sludge from enhanced primary sedimentation for resource recovery: Comparison between $FeCl_3$ and PACl. *Chem. Eng. J.* **2017**, *325*, 681–689. [CrossRef]
188. Poon, C.S.; Chu, C. The use of ferric chloride and anionic polymer in the chemically assisted primary sedimentation process. *Chemosphere* **1999**, *39*, 1573–1582. [CrossRef]
189. Lee, E.; Rout, P.R.; Kyun, Y.; Bae, J. Process optimization and energy analysis of vacuum degasifier systems for the simultaneous removal of dissolved methane and hydrogen sulfide from anaerobically treated wastewater. *Water Res.* **2020**, *182*, 115965. [CrossRef] [PubMed]
190. Shchokin, V.; Ezhov, V.; Shchokina, O.; Chasova, E. Degasification and removal of dust at mass explosions in pits using a humate reagent in the internal and external storage. *Ukr. J. Ecol.* **2021**, *11*, 132–138.
191. Bandara, W.; Ikeda, M.; Satoh, H.; Sasakawa, M.; Nakahara, Y.; Takahashi, M.; Okabe, S. Introduction of a degassing membrane technology into anaerobic wastewater treatment. *Water Environ. Res.* **2013**, *85*, 387–390. [CrossRef]
192. Dias, D.; Lapa, N.; Bernardo, M.; Ribeiro, W.; Matos, I.; Fonseca, I.; Pinto, F. Cr (III) removal from synthetic and industrial wastewaters by using co-gasification chars of rice waste streams. *Bioresour. Technol.* **2018**, *266*, 139–150. [CrossRef]
193. Satoh, H.; Bandara, W.M.; Sasakawa, M.; Nakahara, Y.; Takahashi, M.; Okabe, S. Enhancement of organic matter degradation and methane gas production of anaerobic granular sludge by degasification of dissolved hydrogen gas. *Bioresour. Technol.* **2017**, *244*, 768–775. [CrossRef] [PubMed]
194. Liang, C.; Wei, D.; Zhang, S.; Ren, Q.; Shi, J.; Liu, L. Removal of antibiotic resistance genes from swine wastewater by membrane filtration treatment. *Ecotoxicol. Environ. Saf.* **2021**, *210*, 111885. [CrossRef]
195. Badawi, A.K.; Zaher, K. Hybrid treatment system for real textile wastewater remediation based on coagulation/flocculation, adsorption and filtration processes: Performance and economic evaluation. *J. Water Process Eng.* **2021**, *40*, 101963. [CrossRef]
196. Slipko, K.; Reif, D.; Woegerbauer, M.; Hufnagl, P.; Krampe, J.; Kreuzinger, N. Removal of extracellular free DNA and antibiotic resistance genes from water and wastewater by membranes ranging from microfiltration to reverse osmosis. *Water Res.* **2019**, *164*, 114916. [CrossRef] [PubMed]
197. Rudi, N.N.; Muhamad, M.S.; Te Chuan, L.; Alipal, J.; Omar, S.; Hamidon, N.; Hamid, N.H.A.; Sunar, N.M.; Ali, R.; Harun, H. Evolution of adsorption process for manganese removal in water via agricultural waste adsorbents. *Heliyon* **2020**, *6*, e05049. [CrossRef] [PubMed]
198. Mao, M.; Yan, T.; Shen, J.; Zhang, J.; Zhang, D. Selective Capacitive Removal of Heavy Metal Ions from Wastewater over Lewis Base Sites of S-Doped Fe–N–C Cathodes via an Electro-Adsorption Process. *Environ. Sci. Technol.* **2021**, *55*, 7665–7673. [CrossRef]
199. Wakkel, M.; Khiari, B.; Zagrouba, F. Textile wastewater treatment by agro-industrial waste: Equilibrium modelling, thermodynamics and mass transfer mechanisms of cationic dyes adsorption onto low-cost lignocellulosic adsorbent. *J. Taiwan Inst. Chem. Eng.* **2019**, *96*, 439–452. [CrossRef]

200. Zhang, W.; Chen, J.; Hu, Y.; Fang, Z.; Cheng, J.; Chen, Y. Adsorption characteristics of tetrabromobisphenol A onto sodium bisulfite reduced graphene oxide aerogels. *Colloids Surf. A Physicochem. Eng. Asp.* **2018**, *538*, 781–788. [CrossRef]
201. Zhao, Y.; Cho, C.-W.; Cui, L.; Wei, W.; Cai, J.; Wu, G.; Yun, Y.-S. Adsorptive removal of endocrine-disrupting compounds and a pharmaceutical using activated charcoal from aqueous solution: Kinetics, equilibrium, and mechanism studies. *Environ. Sci. Pollut. Res.* **2019**, *26*, 33897–33905. [CrossRef]
202. Heo, J.; Yoon, Y.; Lee, G.; Kim, Y.; Han, J.; Park, C.M. Enhanced adsorption of bisphenol A and sulfamethoxazole by a novel magnetic $CuZnFe_2O_4$–biochar composite. *Bioresour. Technol.* **2019**, *281*, 179–187. [CrossRef]
203. Al-Khateeb, L.A.; Obaid, A.Y.; Asiri, N.A.; Salam, M.A. Adsorption behavior of estrogenic compounds on carbon nanotubes from aqueous solutions: Kinetic and thermodynamic studies. *J. Ind. Eng. Chem.* **2014**, *20*, 916–924. [CrossRef]
204. Karnib, M.; Kabbani, A.; Holail, H.; Olama, Z. Heavy metals removal using activated carbon, silica and silica activated carbon composite. *Energy Procedia* **2014**, *50*, 113–120. [CrossRef]
205. Owalude, S.O.; Tella, A.C. Removal of hexavalent chromium from aqueous solutions by adsorption on modified groundnut hull. *Beni-Suef Univ. J. Basic Appl. Sci.* **2016**, *5*, 377–388. [CrossRef]
206. Kumar, P.S.; Ramalingam, S.; Abhinaya, R.; Thiruvengadaravi, K.; Baskaralingam, P.; Sivanesan, S. Lead (II) adsorption onto sulphuric acid treated cashew nut shell. *Sep. Sci. Technol.* **2011**, *46*, 2436–2449. [CrossRef]
207. Hegazi, H.A. Removal of heavy metals from wastewater using agricultural and industrial wastes as adsorbents. *HBRC J.* **2013**, *9*, 276–282. [CrossRef]
208. Meunier, N.; Drogui, P.; Montané, C.; Hausler, R.; Blais, J.-F.; Mercier, G. Heavy metals removal from acidic and saline soil leachate using either electrochemical coagulation or chemical precipitation. *J. Environ. Eng.* **2006**, *132*, 545–554. [CrossRef]
209. Ghosh, P.; Samanta, A.N.; Ray, S. Reduction of COD and removal of Zn^{2+} from rayon industry wastewater by combined electro-Fenton treatment and chemical precipitation. *Desalination* **2011**, *266*, 213–217. [CrossRef]
210. Matlock, M.M.; Howerton, B.S.; Atwood, D.A. Chemical precipitation of lead from lead battery recycling plant wastewater. *Ind. Eng. Chem. Res.* **2002**, *41*, 1579–1582. [CrossRef]
211. Huuha, T.S.; Kurniawan, T.A.; Sillanpää, M.E. Removal of silicon from pulping whitewater using integrated treatment of chemical precipitation and evaporation. *Chem. Eng. J.* **2010**, *158*, 584–592. [CrossRef]
212. Ates, H.; Argun, M.E. Removal of PAHs from leachate using a combination of chemical precipitation and Fenton and ozone oxidation. *Water Sci. Technol.* **2018**, *78*, 1064–1070. [CrossRef]
213. Wang, T.; Wang, Q.; Soklun, H.; Qu, G.; Xia, T.; Guo, X.; Jia, H.; Zhu, L. A green strategy for simultaneous Cu (II)-EDTA decomplexation and Cu precipitation from water by bicarbonate-activated hydrogen peroxide/chemical precipitation. *Chem. Eng. J.* **2019**, *370*, 1298–1309. [CrossRef]
214. Meunier, N.; Drogui, P.; Montané, C.; Hausler, R.; Mercier, G.; Blais, J.-F. Comparison between electrocoagulation and chemical precipitation for metals removal from acidic soil leachate. *J. Hazard. Mater.* **2006**, *137*, 581–590. [CrossRef]
215. Dhiman, S.; Gupta, B. Partition studies on cobalt and recycling of valuable metals from waste Li-ion batteries via solvent extraction and chemical precipitation. *J. Clean. Prod.* **2019**, *225*, 820–832. [CrossRef]
216. Wang, Q.; Yu, J.; Chen, X.; Du, D.; Wu, R.; Qu, G.; Guo, X.; Jia, H.; Wang, T. Non-thermal plasma oxidation of Cu (II)-EDTA and simultaneous Cu (II) elimination by chemical precipitation. *J. Environ. Manag.* **2019**, *248*, 109237. [CrossRef] [PubMed]
217. Anouzla, A.; Abrouki, Y.; Souabi, S.; Safi, M.; Rhbal, H. Colour and COD removal of disperse dye solution by a novel coagulant: Application of statistical design for the optimization and regression analysis. *J. Hazard. Mater.* **2009**, *166*, 1302–1306. [CrossRef] [PubMed]
218. Golob, V.; Vinder, A.; Simonič, M. Efficiency of the coagulation/flocculation method for the treatment of dyebath effluents. *Dye. Pigment.* **2005**, *67*, 93–97. [CrossRef]
219. Bidhendi, G.N.; Torabian, A.; Ehsani, H.; Razmkhah, N. Evaluation of industrial dyeing wastewater treatment with coagulants and polyelectrolyte as a coagulant aid. *J. Environ. Health Sci. Eng.* **2007**, *4*, 29–36.
220. Vedrenne, M.; Vasquez-Medrano, R.; Prato-Garcia, D.; Frontana-Uribe, B.A.; Ibanez, J.G. Characterization and detoxification of a mature landfill leachate using a combined coagulation–flocculation/photo Fenton treatment. *J. Hazard. Mater.* **2012**, *205*, 208–215. [CrossRef]
221. Peydayesh, M.; Suta, T.; Usuelli, M.; Handschin, S.; Canelli, G.; Bagnani, M.; Mezzenga, R. Sustainable removal of microplastics and natural organic matter from water by coagulation–flocculation with protein amyloid fibrils. *Environ. Sci. Technol.* **2021**, *55*, 8848–8858. [CrossRef]
222. Irfan, M.; Butt, T.; Imtiaz, N.; Abbas, N.; Khan, R.A.; Shafique, A. The removal of COD, TSS and colour of black liquor by coagulation–flocculation process at optimized pH, settling and dosing rate. *Arab. J. Chem.* **2017**, *10*, S2307–S2318. [CrossRef]
223. Torres, N.H.; Souza, B.S.; Ferreira, L.F.R.; Lima, A.S.; Dos Santos, G.N.; Cavalcanti, E.B. Real textile effluents treatment using coagulation/flocculation followed by electrochemical oxidation process and ecotoxicological assessment. *Chemosphere* **2019**, *236*, 124309. [CrossRef]
224. Khouni, I.; Louhichi, G.; Ghrabi, A.; Moulin, P. Efficiency of a coagulation/flocculation–membrane filtration hybrid process for the treatment of vegetable oil refinery wastewater for safe reuse and recovery. *Process Saf. Environ. Prot.* **2020**, *135*, 323–341. [CrossRef]
225. Rathi, B.S.; Kumar, P.S.; Ponprasath, R.; Rohan, K.; Jahnavi, N. An effective separation of toxic arsenic from aquatic environment using electrochemical ion exchange process. *J. Hazard. Mater.* **2021**, *412*, 125240. [CrossRef]

226. Alyüz, B.; Veli, S. Kinetics and equilibrium studies for the removal of nickel and zinc from aqueous solutions by ion exchange resins. *J. Hazard. Mater.* **2009**, *167*, 482–488. [CrossRef] [PubMed]
227. Edebali, S.; Pehlivan, E. Evaluation of Amberlite IRA96 and Dowex 1× 8 ion-exchange resins for the removal of Cr (VI) from aqueous solution. *Chem. Eng. J.* **2010**, *161*, 161–166. [CrossRef]
228. Rengaraj, S.; Yeon, K.-H.; Moon, S.-H. Removal of chromium from water and wastewater by ion exchange resins. *J. Hazard. Mater.* **2001**, *87*, 273–287. [CrossRef] [PubMed]
229. Al-Jaser, Z.A.; Hamoda, M.F. Removal of nickel and vanadium from desalination brines by ion-exchange resins. *Desalin. Water Treat* **2019**, *157*, 148–156. [CrossRef]
230. Alvarado, L.; Torres, I.R.; Chen, A. Integration of ion exchange and electrodeionization as a new approach for the continuous treatment of hexavalent chromium wastewater. *Sep. Purif. Technol.* **2013**, *105*, 55–62. [CrossRef]
231. Iqbal, M.; Saeed, A.; Zafar, S.I. FTIR spectrophotometry, kinetics and adsorption isotherms modeling, ion exchange, and EDX analysis for understanding the mechanism of Cd^{2+} and Pb^{2+} removal by mango peel waste. *J. Hazard. Mater.* **2009**, *164*, 161–171. [CrossRef]
232. Li, H.; Chen, Y.; Long, J.; Jiang, D.; Liu, J.; Li, S.; Qi, J.; Zhang, P.; Wang, J.; Gong, J. Simultaneous removal of thallium and chloride from a highly saline industrial wastewater using modified anion exchange resins. *J. Hazard. Mater.* **2017**, *333*, 179–185. [CrossRef] [PubMed]
233. Pathania, D.; Sharma, G.; Thakur, R. Pectin@ zirconium (IV) silicophosphate nanocomposite ion exchanger: Photo catalysis, heavy metal separation and antibacterial activity. *Chem. Eng. J.* **2015**, *267*, 235–244. [CrossRef]
234. Poznyak, T.; Bautista, G.L.; Chaírez, I.; Córdova, R.I.; Ríos, L.E. Decomposition of toxic pollutants in landfill leachate by ozone after coagulation treatment. *J. Hazard. Mater.* **2008**, *152*, 1108–1114. [CrossRef]
235. Zhu, H.; Han, Y.; Ma, W.; Han, H.; Ma, W. Removal of selected nitrogenous heterocyclic compounds in biologically pretreated coal gasification wastewater (BPCGW) using the catalytic ozonation process combined with the two-stage membrane bioreactor (MBR). *Bioresour. Technol.* **2017**, *245*, 786–793. [CrossRef]
236. Quero-Pastor, M.; Garrido-Perez, M.; Acevedo, A.; Quiroga, J. Ozonation of ibuprofen: A degradation and toxicity study. *Sci. Total Environ.* **2014**, *466*, 957–964. [CrossRef] [PubMed]
237. Wu, J.; Ma, L.; Chen, Y.; Cheng, Y.; Liu, Y.; Zha, X. Catalytic ozonation of organic pollutants from bio-treated dyeing and finishing wastewater using recycled waste iron shavings as a catalyst: Removal and pathways. *Water Res.* **2016**, *92*, 140–148. [CrossRef] [PubMed]
238. Santiago-Morales, J.; Gómez, M.J.; Herrera-López, S.; Fernández-Alba, A.R.; García-Calvo, E.; Rosal, R. Energy efficiency for the removal of non-polar pollutants during ultraviolet irradiation, visible light photocatalysis and ozonation of a wastewater effluent. *Water Res.* **2013**, *47*, 5546–5556. [CrossRef]
239. Restivo, J.; Órfão, J.; Armenise, S.; Garcia-Bordejé, E.; Pereira, M. Catalytic ozonation of metolachlor under continuous operation using nanocarbon materials grown on a ceramic monolith. *J. Hazard. Mater.* **2012**, *239*, 249–256. [CrossRef] [PubMed]
240. Restivo, J.; Órfão, J.J.; Pereira, M.F.; Garcia-Bordejé, E.; Roche, P.; Bourdin, D.; Houssais, B.; Coste, M.; Derrouiche, S. Catalytic ozonation of organic micropollutants using carbon nanofibers supported on monoliths. *Chem. Eng. J.* **2013**, *230*, 115–123. [CrossRef]
241. Martins, R.C.; Cardoso, M.; Dantas, R.F.; Sans, C.; Esplugas, S.; Quinta-Ferreira, R.M. Catalytic studies for the abatement of emerging contaminants by ozonation. *J. Chem. Technol. Biotechnol.* **2015**, *90*, 1611–1618. [CrossRef]
242. Pocostales, P.; Álvarez, P.; Beltrán, F. Catalytic ozonation promoted by alumina-based catalysts for the removal of some pharmaceutical compounds from water. *Chem. Eng. J.* **2011**, *168*, 1289–1295. [CrossRef]
243. Yang, L.; Hu, C.; Nie, Y.; Qu, J. Surface acidity and reactivity of β-FeOOH/Al_2O_3 for pharmaceuticals degradation with ozone: In situ ATR-FTIR studies. *Appl. Catal. B Environ.* **2010**, *97*, 340–346. [CrossRef]
244. Lv, A.; Hu, C.; Nie, Y.; Qu, J. Catalytic ozonation of toxic pollutants over magnetic cobalt-doped Fe_3O_4 suspensions. *Appl. Catal. B Environ.* **2012**, *117*, 246–252. [CrossRef]
245. Cheriyamundath, S.; Vavilala, S.L. Nanotechnology-based wastewater treatment. *Water Environ. J.* **2021**, *35*, 123–132. [CrossRef]
246. Jain, K.; Patel, A.S.; Pardhi, V.P.; Flora, S.J.S. Nanotechnology in wastewater management: A new paradigm towards wastewater treatment. *Molecules* **2021**, *26*, 1797. [CrossRef] [PubMed]
247. Kokkinos, P.; Mantzavinos, D.; Venieri, D. Current Trends in the Application of Nanomaterials for the Removal of Emerging Micropollutants and Pathogens from Water. *Molecules* **2020**, *25*, 2016. [CrossRef] [PubMed]
248. Mazhar, M.A.; Khan, N.A.; Ahmed, S.; Khan, A.H.; Hussain, A.; Changani, F.; Yousefi, M.; Ahmadi, S.; Vambol, V. Chlorination disinfection by-products in municipal drinking water—A review. *J. Clean. Prod.* **2020**, *273*, 123159. [CrossRef]
249. Zheng, A.L.T.; Phromsatit, T.; Boonyuen, S.; Andou, Y. Synthesis of silver nanoparticles/porphyrin/reduced graphene oxide hydrogel as dye adsorbent for wastewater treatment. *FlatChem* **2020**, *23*, 100174. [CrossRef]
250. Kariim, I.; Abdulkareem, A.; Abubakre, O. Development and characterization of MWCNTs from activated carbon as adsorbent for metronidazole and levofloxacin sorption from pharmaceutical wastewater: Kinetics, isotherms and thermodynamic studies. *Sci. Afr.* **2020**, *7*, e00242. [CrossRef]
251. Pan, Y.; Liu, X.; Zhang, W.; Liu, Z.; Zeng, G.; Shao, B.; Liang, Q.; He, Q.; Yuan, X.; Huang, D. Advances in photocatalysis based on fullerene C60 and its derivatives: Properties, mechanism, synthesis, and applications. *Appl. Catal. B Environ.* **2020**, *265*, 118579. [CrossRef]

252. Shkir, M.; Palanivel, B.; Khan, A.; Kumar, M.; Chang, J.-H.; Mani, A.; AlFaify, S. Enhanced photocatalytic activities of facile auto-combustion synthesized ZnO nanoparticles for wastewater treatment: An impact of Ni doping. *Chemosphere* **2022**, *291*, 132687. [CrossRef] [PubMed]
253. Vo, T.-T.; Nguyen, T.T.-N.; Huynh, T.T.-T.; Vo, T.T.-T.; Nguyen, T.T.-N.; Nguyen, D.-T.; Dang, V.-S.; Dang, C.-H.; Nguyen, T.-D. Biosynthesis of silver and gold nanoparticles using aqueous extract from *Crinum latifolium* leaf and their applications forward antibacterial effect and wastewater treatment. *J. Nanomater.* **2019**, *2019*, 8385935. [CrossRef]
254. Najafpoor, A.; Norouzian-Ostad, R.; Alidadi, H.; Rohani-Bastami, T.; Davoudi, M.; Barjasteh-Askari, F.; Zanganeh, J. Effect of magnetic nanoparticles and silver-loaded magnetic nanoparticles on advanced wastewater treatment and disinfection. *J. Mol. Liq.* **2020**, *303*, 112640. [CrossRef]
255. Rupa, E.J.; Kaliraj, L.; Abid, S.; Yang, D.-C.; Jung, S.-K. Synthesis of a zinc oxide nanoflower photocatalyst from sea buckthorn fruit for degradation of industrial dyes in wastewater treatment. *Nanomaterials* **2019**, *9*, 1692. [CrossRef]
256. Fouda, A.; Hassan, S.E.-D.; Abdel-Rahman, M.A.; Farag, M.M.; Shehal-deen, A.; Mohamed, A.A.; Alsharif, S.M.; Saied, E.; Moghanim, S.A.; Azab, M.S. Catalytic degradation of wastewater from the textile and tannery industries by green synthesized hematite (α-Fe_2O_3) and magnesium oxide (MgO) nanoparticles. *Curr. Res. Biotechnol.* **2021**, *3*, 29–41. [CrossRef]
257. Goutam, S.P.; Saxena, G.; Singh, V.; Yadav, A.K.; Bharagava, R.N.; Thapa, K.B. Green synthesis of TiO_2 nanoparticles using leaf extract of *Jatropha curcas* L. for photocatalytic degradation of tannery wastewater. *Chem. Eng. J.* **2018**, *336*, 386–396. [CrossRef]
258. Huang, X.; Yang, J.; Wang, J.; Bi, J.; Xie, C.; Hao, H. Design and synthesis of core–shell Fe_3O_4@ PTMT composite magnetic microspheres for adsorption of heavy metals from high salinity wastewater. *Chemosphere* **2018**, *206*, 513–521. [CrossRef]
259. Bhatt, P.; Chaudhary, P.; Ahmad, S.; Bhatt, K.; Chandra, D.; Chen, S. Recent advances in the application of microbial inoculants in the phytoremediation of xenobiotic compounds. In *Unravelling Plant-Microbe Synergy*; Chandra, D., Bhatt, P., Eds.; Elsevier: Amsterdam, The Netherlands, 2023; pp. 37–48. [CrossRef]
260. Hamadeen, H.M.; Elkhatib, E.A.; Badawy, M.E.; Abdelgaleil, S.A. Green low cost nanomaterial produced from *Moringa oleifera* seed waste for enhanced removal of chlorpyrifos from wastewater: Mechanism and sorption studies. *J. Environ. Chem. Eng.* **2021**, *9*, 105376. [CrossRef]
261. Guzmán-Trampe, S.; Ceapa, C.D.; Manzo-Ruiz, M.; Sánchez, S. Synthetic biology era: Improving antibiotic's world. *Biochem. Pharmacol.* **2017**, *134*, 99–113. [CrossRef]
262. Su, H.-C.; Liu, Y.-S.; Pan, C.-G.; Chen, J.; He, L.-Y.; Ying, G.-G. Persistence of antibiotic resistance genes and bacterial community changes in drinking water treatment system: From drinking water source to tap water. *Sci. Total Environ.* **2018**, *616*, 453–461. [CrossRef]
263. Azanu, D.; Styrishave, B.; Darko, G.; Weisser, J.J.; Abaidoo, R.C. Occurrence and risk assessment of antibiotics in water and lettuce in Ghana. *Sci. Total Environ.* **2018**, *622*, 293–305. [CrossRef]
264. Fang, T.; Wang, H.; Cui, Q.; Rogers, M.; Dong, P. Diversity of potential antibiotic-resistant bacterial pathogens and the effect of suspended particles on the spread of antibiotic resistance in urban recreational water. *Water Res.* **2018**, *145*, 541–551. [CrossRef]
265. Essawy, A.A.; Alsohaimi, I.H.; Alhumaimess, M.S.; Hassan, H.M.; Kamel, M.M. Green synthesis of spongy Nano-ZnO productive of hydroxyl radicals for unconventional solar-driven photocatalytic remediation of antibiotic enriched wastewater. *J. Environ. Manag.* **2020**, *271*, 110961. [CrossRef]
266. Cai, W.; Weng, X.; Chen, Z. Highly efficient removal of antibiotic rifampicin from aqueous solution using green synthesis of recyclable nano-Fe_3O_4. *Environ. Pollut.* **2019**, *247*, 839–846. [CrossRef]
267. Liu, Y.; Hou, C.; Jiao, T.; Song, J.; Zhang, X.; Xing, R.; Zhou, J.; Zhang, L.; Peng, Q. Self-assembled AgNP-containing nanocomposites constructed by electrospinning as efficient dye photocatalyst materials for wastewater treatment. *Nanomaterials* **2018**, *8*, 35. [CrossRef] [PubMed]
268. Wu, Z.; Luo, W.; Zhang, H.; Jia, Y. Strong pyro-catalysis of shape-controllable bismuth oxychloride nanomaterial for wastewater remediation. *Appl. Surf. Sci.* **2020**, *513*, 145630. [CrossRef]
269. Huang, X.; Wang, R.; Jiao, T.; Zou, G.; Zhan, F.; Yin, J.; Zhang, L.; Zhou, J.; Peng, Q. Facile preparation of hierarchical AgNP-loaded MXene/Fe_3O_4/polymer nanocomposites by electrospinning with enhanced catalytic performance for wastewater treatment. *ACS Omega* **2019**, *4*, 1897–1906. [CrossRef] [PubMed]
270. Yang, X.; He, Q.; Guo, F.; Sun, X.; Zhang, J.; Chen, Y. Impacts of carbon-based nanomaterials on nutrient removal in constructed wetlands: Microbial community structure, enzyme activities, and metabolism process. *J. Hazard. Mater.* **2021**, *401*, 123270. [CrossRef] [PubMed]
271. Choi, S.; Johnston, M.; Wang, G.-S.; Huang, C. A seasonal observation on the distribution of engineered nanoparticles in municipal wastewater treatment systems exemplified by TiO_2 and ZnO. *Sci. Total Environ.* **2018**, *625*, 1321–1329. [CrossRef]
272. Xu, Y.; Ren, B.; Wang, R.; Zhang, L.; Jiao, T.; Liu, Z. Facile preparation of rod-like MnO nanomixtures via hydrothermal approach and highly efficient removal of methylene blue for wastewater Treatment. *Nanomaterials* **2018**, *9*, 10. [CrossRef]
273. Atta, A.M.; Moustafa, Y.M.; Ezzat, A.O.; Hashem, A.I. Novel magnetic silica-ionic liquid nanocomposites for wastewater treatment. *Nanomaterials* **2019**, *10*, 71. [CrossRef]
274. Gupta, S.; Bhatiya, D.; Murthy, C. Metal removal studies by composite membrane of polysulfone and functionalized single-walled carbon nanotubes. *Sep. Sci. Technol.* **2015**, *50*, 421–429. [CrossRef]

275. Bandforuzi, S.R.; Hadjmohammadi, M.R. Modified magnetic chitosan nanoparticles based on mixed hemimicelle of sodium dodecyl sulfate for enhanced removal and trace determination of three organophosphorus pesticides from natural waters. *Anal. Chim. Acta* **2019**, *1078*, 90–100. [CrossRef]
276. Tony, M.; Mansour, S.A. Microwave-assisted catalytic oxidation of methomyl pesticide by $Cu/Cu_2O/CuO$ hybrid nanoparticles as a Fenton-like source. *Int. J. Environ. Sci. Technol.* **2020**, *17*, 161–174. [CrossRef]
277. ul Haq, A.; Saeed, M.; Usman, M.; Naqvi, S.A.R.; Bokhari, T.H.; Maqbool, T.; Ghaus, H.; Tahir, T.; Khalid, H. Sorption of chlorpyrifos onto zinc oxide nanoparticles impregnated Pea peels (*Pisum sativum* L): Equilibrium, kinetic and thermodynamic studies. *Environ. Technol. Innov.* **2020**, *17*, 100516. [CrossRef]
278. Aydin, S.; Aydin, M.E.; Beduk, F.; Ulvi, A. Removal of antibiotics from aqueous solution by using magnetic Fe_3O_4/red mud-nanoparticles. *Sci. Total Environ.* **2019**, *670*, 539–546. [CrossRef] [PubMed]
279. Mohammadi Nodeh, M.K.; Radfard, M.; Zardari, L.A.; Rashidi Nodeh, H. Enhanced removal of naproxen from wastewater using silica magnetic nanoparticles decorated onto graphene oxide; parametric and equilibrium study. *Sep. Sci. Technol.* **2018**, *53*, 2476–2485. [CrossRef]
280. Sethy, N.K.; Arif, Z.; Mishra, P.K.; Kumar, P. Green synthesis of TiO_2 nanoparticles from *Syzygium cumini* extract for photo-catalytic removal of lead (Pb) in explosive industrial wastewater. *Green Process. Synth.* **2020**, *9*, 171–181. [CrossRef]
281. Deng, X.; Chen, R.; Zhao, Z.; Cui, F.; Xu, X. Graphene oxide-supported graphitic carbon nitride microflowers decorated by sliver nanoparticles for enhanced photocatalytic degradation of dimethoate via addition of sulfite: Mechanism and toxicity evolution. *Chem. Eng. J.* **2021**, *425*, 131683. [CrossRef]

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Retention, Degradation, and Runoff of Plastic-Coated Fertilizer Capsules in Paddy Fields in Fukushima and Miyagi Prefectures, Japan: Consistency of Capsule Degradation Behavior and Variations in Carbon Weight and Stable Carbon Isotope Abundance

Shigeki Harada [1,*], Itsuki Yajima [2], Keitaro Fukushima [1] and Youji Nitta [1]

[1] Faculty of Food and Agricultural Sciences, Fukushima University, 1 Kanayagawa, Fukushima City 960-1296, Fukushima, Japan
[2] North Alps Regional Development Bureau, 1058-2 Ohmachi, Ohmachi City 398-8602, Nagano, Japan
* Correspondence: harada.shigeki@agri.fukushima-u.ac.jp

Abstract: Paddy field runoff containing plastic capsules that are used to coat fertilizers has been receiving increased attention. However, the behavior of these capsules, especially their degradation behavior, has not been extensively investigated. We divided the capsules in runoff into two categories: "floating capsules after ploughing" and "floating capsules discharged via pipes at the exits of paddy fields". The behaviors of the capsules in both types of runoff were monitored in 2022 and 2023 at four paddy fields in Fukushima and Miyagi prefectures in northern Japan. Sampling of capsules in paddy biomass and soil, and comparisons of capsule weight to biomass weight showed that a decrease in plastic capsule weight reflected a decrease in capsule runoff. However, the emergence of clear effects showed a delay of 2 to 3 years, as explained by carbon isotopic analyses. The decrease in the weight of the plastic capsules could be attributed to a combination of capsule degradation and the release of urea inside the capsules, which was also explained by carbon isotopic analyses. Three types of degraded capsules were found: shrunken, broken, and spherical. Statistically significant differences among the weights of each type found.

Keywords: microplastics; coated fertilizer; plastic capsule; paddy field; isotopic analyses; statistical analyses; scanning electron microscopes

Citation: Harada, S.; Yajima, I.; Fukushima, K.; Nitta, Y. Retention, Degradation, and Runoff of Plastic-Coated Fertilizer Capsules in Paddy Fields in Fukushima and Miyagi Prefectures, Japan: Consistency of Capsule Degradation Behavior and Variations in Carbon Weight and Stable Carbon Isotope Abundance. *Water* **2024**, *16*, 1782. https://doi.org/10.3390/w16131782

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

Received: 15 May 2024
Revised: 16 June 2024
Accepted: 20 June 2024
Published: 23 June 2024

1. Introduction

Plastic-coated fertilizers (Figure 1), which are widely used in Japan [1], consist of manure encapsulated within plastic shells [2–4]. This design facilitates the gradual release of nutrients into paddy fields [5–7]. Applying these fertilizers to paddy fields in spring obviates the need for additional fertilizer application in summer [5–7]. The method offers significant advantages in terms of paddy field management [5–7], especially given the diminishing labor force [5–7] and the reticence of younger individuals to return to the exclusion zone following the Fukushima Daiichi nuclear disaster [8,9]. However, concerns have been raised regarding the plastic capsules being transported from the paddy fields in runoff [2–4], especially since these capsules account for 15% of the total microplastics discharged from the catchment into the marine environment [10].

The increase in the plastic runoff into oceans has been identified as an issue of global concern [11–14]. Although the present study does not directly address the severity of this problem, its eco-toxicological impact is important to note [15–17].

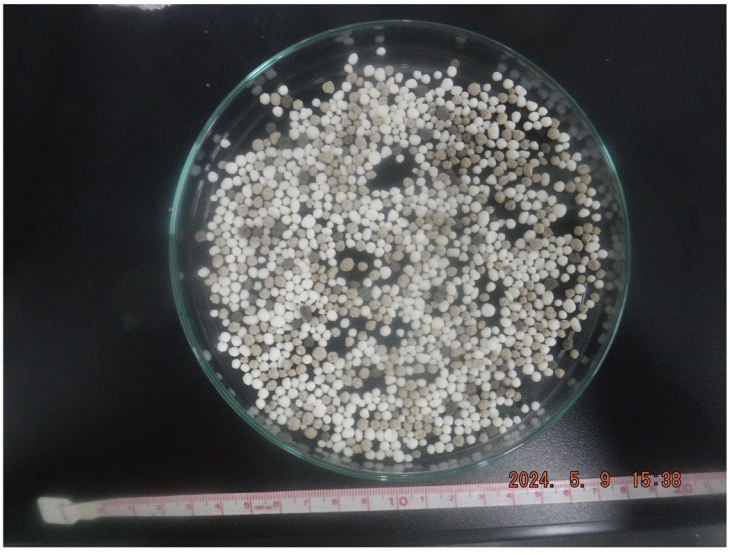

Figure 1. Coated fertilizer (white) and normal fertilizer (dark) capsules used in paddy fields C1 and C2 in this study (see Table 1).

Table 1. Characteristics of the four paddy fields in Fukushima and Miyagi prefectures examined in this study.

Field	Prefecture	Area (Acres)
A	Fukushima	30
B	Miyagi	20
C1	Fukushima	13
C2	Fukushima	13

Microplastics are generally defined as plastic particles measuring <5 mm [18,19]. Of these particles, primary microplastics are derived from domestic and industrial sources [18,19] and frequently pass through sewerage treatment systems [15]. Secondary microplastics are derived from waste that has been crushed and fragmented by ultraviolet rays and physical abrasion [18,19].

The plastic capsules derived from agricultural applications are classified as primary microplastics. However, because they are an example of a non-point-source of pollution, they do not pass through sewage treatment systems, which are relatively effective in trapping such microplastics. In cases where the use of sludge on farmland is planned, countermeasures against runoff are necessary [20].

Such countermeasures include placing simple microplastic traps on site [5–7]. Examples of these have been developed by the authors and are described elsewhere [6]. Briefly, the authors' technique utilizes porous concrete to filter runoff and capture the discharged plastic capsules from the paddy fields [21–25].

Previous studies [2–7] have shown that plastic capsules persist for several years in paddy fields. Capsule longevity is affected mainly by the following three factors: the proportion of capsules that float relative to the total volume of capsules in the paddy soil after ploughing, the fraction of these capsules that enter runoff or are retained within the rice paddy as a proportion of the total volume of floating capsules during discharge after ploughing, and the capsule degradation rate during retention inside the paddy field.

Understanding the lifespan and behavior of these capsules is important; however, this requires a modeling approach, which is a goal for future research.

In 2022 and 2023, we performed surveys, including the monitoring and sampling of capsules in samples of biomass and mud, as well as floating capsules in runoff that were discharged via drainage pipes from the paddy fields after ploughing. In this study, four paddy fields (A, B, C1, and C2; Tables 1 and 2) in Fukushima and Miyagi prefectures were sampled (Figures 2 and 3).

Table 2. Timing of sampling relative to ploughing and fertilizer application in this study.

Field	Timing	Coated Fertilizer Type and Volume *,**
A	Before ploughing	X, 1.0 kg/acre in 2022 Y, 1.0 kg/acre in 2023
B	After ploughing	Z, 0.6 kg/acre in 2022 and 2023
C1	Before ploughing	W, about 0.87 kg/acre (see also Table 3)
C2	Before ploughing	

* Weights represent total nitrogen, ** X, Y, Z, and W represent commodity names.

Figure 2. Typical appearance of floating fertilizer after ploughing (C2 in 2023).

Figure 3. Typical appearance of plastic capsules in floating biomass after ploughing (C2, 2023).

This paper reports the results of our surveys and subsequent physical measurements (capsule weight and density), scanning electron microscopy (SEM) analyses, and chemical (C and $\delta^{13}C$) measurements.

The 2022 surveys examined the differences in capsule behavior at the four paddy fields. Preliminary results showed general tendencies in capsule behaviors, including retention within paddy fields. The authors subsequently focused on variations in the capsule weights, capsule depth in the paddy soil, and chemical measurement results in two adjacent paddy fields (C1 and C2 in Table 1) under identical conditions, except for the timing of coated fertilizer application. The temporal differences between the proportions and weights of the floating capsules in C2, where the same coated fertilizer (Figure 1) was used in 2019–2023 (Table 3), and those in C1 (a coated fertilizer was not used in 2021 and 2023) provide information about the degradation behavior of the capsules.

Table 3. Application timing and type of fertilizer used in fields C1 and C2 in Fukushima Prefecture.

Date	C1	C2
April 2019	Coated fertilizer	Coated fertilizer
April 2020	Coated fertilizer	Coated fertilizer
April 2021	Normal fertilizer	Coated fertilizer
April 2022	Coated fertilizer	Coated fertilizer
April 2023	Normal fertilizer	Coated fertilizer

We confirmed that a negligible proportion of the capsules were transported in runoff on rainy days. In addition, nearly 100% of the total capsule runoff load from paddy field B during the rice harvesting period occurred shortly after ploughing. This was confirmed by placing a fine mesh net (2 mm mesh) at the discharge pipe of paddy field B in 2022 (Table 1 and Figure 4). Thus, the main focus of this study was to investigate the behavior of the capsules during the ploughing period.

Figure 4. Deployment of 2 mm mesh at the drainage pipe exiting paddy field B (Table 1).

2. Materials and Methods

2.1. Study Sites and Paddy Conditions

The study was conducted in four paddy fields (fields A, B, C1, and C2) at three sites in Fukushima and Miyagi prefectures; three paddy fields in Fukushima Prefecture and one in Miyagi Prefecture (Table 1). Table 1 shows the characteristics of each field. Table 2 shows the differences in the fertilizer application methods and/or timing employed in the paddy fields. "Before ploughing" means that fertilizer was applied when the paddy soil was dry before ploughing (Table 2). "After ploughing" means that the fertilizer was applied at the same time as rice planting (Table 2).

Paddy fields C1 and C2 were adjacent to each other, and the types of fertilizer applied to these fields are shown in Table 3.

2.2. Monitoring and Sampling

In 2022, samples of floating and discharged capsules mixed with biomass and soil particles were collected from rice field ridges at the four paddy fields and at the drainage pipes. The methods were the same as those shown in Figure 4, but the sampling duration was shorter (5 min). The timing of the sampling was after ploughing in early May (fields A and B) and mid-May (fields C1 and C2). Collected samples were dried at room temperature. Following drying, the sediments were sorted using screens with mesh sizes of 2 and 3 mm. Subsequently, capsules were manually extracted from the biomass mixtures (Figure 5).

Figure 5. Hand sorting of capsules in the 2–3 mm and >3 mm fractions.

During the 2023 survey, we focused on capsules in paddy fields C1 and C2, the collection and weighing of floating coated fertilizers in C1 and C2, and the C and $\delta^{13}C$ measurements (as described in Section 2.3 below).

2.3. Analyses of Capsule Behavior

The authors evaluated the variations in the mass (weight) of the plastic capsules relative to biomass fragments > 3 mm in the floated and discharged mixtures in 2022. First, the floating capsules were categorized into three distinct shapes: shrunken (Figure 6), broken (Figure 7), and spherical (Figure 8). Subsequently, the weight distributions of floating capsules in fields C1 and C2 were compared with those of unused coated fertilizer capsules.

Figure 6. Shrunken plastic capsule.

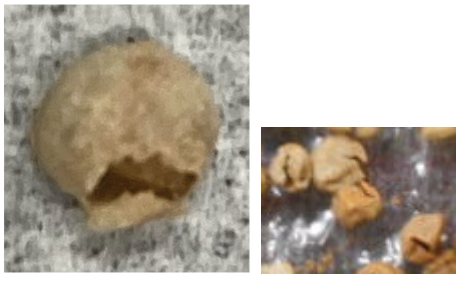

Figure 7. Broken capsule.

Figure 8. Spherical capsule.

Randomly selected floating capsules of the three types were obtained from fields C1 and C2 in 2022 (50 capsules of each, except for C2, from which only 25 capsules were collected). Then, 100 unused coated fertilizer capsules were weighed, and the average was calculated. Separately, the densities of these capsules were measured. The statistical significance of the differences among the average weights of the unused coated fertilizer capsules, and those among the floated capsules in C1 and C2, were analyzed by the t-test. Also, the statistical differences among the average weights of the three types of floated capsules in C1 and C2 were analyzed by variance analysis.

2.4. Extent of Coated Fertilizer Capsules in Paddy Fields

To assess the distribution of coated fertilizer capsules in paddy fields, capsules were collected by inserting transparent acrylic pipes (inner diameter: 180 mm) into the soil at several locations in paddies C1 and C2 (Figures 9–11).

Figure 9. Insertion of an acrylic pipe into the paddy soil (to a depth of 15 cm) to collect fertilizer capsules.

Figure 10. Placement of a soil sample on a 2 mm mesh.

Figure 11. Washing a sample to remove soil and count the capsules in the sample.

2.5. Chemical Analyses and Sample Preparation

All of the capsules were washed thoroughly with Milli-Q water (Millipore) in an ultrasonic cleaner. Scanning electron microscope (SEM) images were captured after the capsules had been sectioned using a microtome and mounted. The segments were then coated with platinum using a sputtering machine (JEC-3000FC; JEOL, Tokyo, Japan). The outer and inner surfaces, as well as the freshly cut cross-sections of these segments, were examined using SEM (JCM7000; JEOL, Japan).

Carbon concentration and carbon isotopic analyses were performed using a continuous flow system with an elemental analyzer coupled with an isotope ratio mass spectrometer (EA-IRMS; Delta-V equipped with Flash EA 2000, Thermo Fisher Scientific, Waltham, MA, USA) at Fukushima University. The methods used were based on protocols used by the authors previously [26–29]. The isotopic composition was expressed in ‰ and calculated using the following equation:

$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where R_{sample} is the $^{13}C/^{12}C$ ratio in the sample, and $R_{standard}$ is the $^{13}C/^{12}C$ ratio in the standard [30]. The standard reference materials are carbonate in Vienna PeeDee Belemnite (PDB). We used DL-α-alanine (Shoko Science Co., Ltd., Yokohama, Japan), L-leucine (CERKU-04), and L-threonine (CERKU-05) as internal standards to calibrate the $\delta 1^3C$ of the samples [30]. The analytical precision, expressed as the standard deviation for our $\delta^{13}C$ analyses, was within ±0.15‰.

3. Results and Discussion

3.1. Proportion of Floating Capsule Weight to the >3-mm Biomass Fraction

The authors compared the variations in the mass (weight) of the plastic capsules relative to those of >3 mm fragments screened from floating biomass and discharged mixtures in 2022 (Tables 4 and 5). The trends in the proportions shown in Table 4 across four paddy fields appeared to reflect the trends in total nitrogen usage at the sites. At field B, where farmers are trying to reduce the fertilizer volume, the smallest proportion of floating capsules was observed among the four fields. A comparison of the proportions shown in Tables 4 and 5 suggested similar trends, leading to the conclusion that the reduction in capsule runoff from the paddy fields reflected the reduction in the volume of floating

capsules. This conclusion should be verified through a comparison of the total floating and total discharged loads. However, collecting all of the floating and discharged loads is quite difficult and the authors were unable to accomplish this within the scope of the current study. This limitation is an important area for future research.

Table 4. Proportion of floating capsule weight to weight of >3 mm biomass fragments in 2022.

Parameter	A	B	C1	C2
>3-mm biomass (g)	849.31	750.52	641.32	167.42
Capsule (g)	68.03	11.07	14.38	6.45
Proportion (%)	8.01	1.47	2.24	3.85

Table 5. Proportion of discharged capsule weight to weight of >3 mm biomass fragments in 2022.

Parameter	A	B	C1	C2
>3-mm biomass (g)	21.08	625.93	654.40	1261.48
Capsule (g)	1.14	12.93	23.20	43.15
Proportion (%)	5.40	2.07	3.54	3.42

3.2. Comparison of Floating Capsule Weight in Fields C1 and C2

Table 4 shows that the proportion of the floating capsule weight in C1 corresponded to 78.6% of that in C2 in 2022, suggesting that the discharge of capsules from C1 is smaller than that from C2 because the farmers did not use coated fertilizer in C1 in 2021. The same comparison in 2022 indicates that the proportion of floating capsules in C1 corresponded to 17.6% of that in C2 in 2023. The results suggest that the decrease in the floating capsule weight after the use of coated fertilizer was stopped in 2021 proceeded more rapidly for the 3-year period from 2020 to 2023 than for the 2-year period from 2020 to 2022. These results are compared with carbon isotopic analyses below.

3.3. Presence of Plastic-Coated Fertilizer within Paddy Soil

Table 6 shows that the depth profile of the coated fertilizer capsules exhibited a clear peak at 5–10 cm. This pattern can be attributed to the density of the coated fertilizers, which exceeds unity (>1.3 g/cm^3), causing the fertilizer to penetrate deeper into the paddy soil. The Ministry of Agriculture reported that coated fertilizers typically accumulate within 10 cm of the surface [1]. Our analysis at 5 cm intervals showed more granular insights. The age of the fertilizer capsules buried in the soil was estimated based on their color; white capsules were initially presumed to be newer, although recent findings have identified some older white capsules. Consequently, while the results shown in Table 6 are considered to be qualitatively accurate, a more rigorous approach is needed to obtain more quantitative data.

Table 6. Number of solid coated fertilizer capsules present in different depths of paddy soil.

Field	0–5 cm	5–10 cm	10–15 cm
C1	0	0.33	0
C2	2.00	5.67	2.00

3.4. Carbon Isotopic Analyses

Figure 12 shows the variations in δ^{13}C for the samples collected from field C1 in 2022 and 2023 and from field C2 in 2023 (hereafter referred to as 2022.C1, 2023.C1, and 2023.C2, respectively). Given that the floating capsules are a mixture of capsules of different ages, we analyzed the statistical significance of the differences in the average δ^{13}C values across these three groups. The comparison between the average δ^{13}C values of 2022.C1 and

2023.C1 assesses whether a population that is 1 year older exhibits a higher average ^{13}C abundance; the findings demonstrated that this is the case ($p = 0.0246$). Furthermore, the comparison between the average $\delta^{13}C$ values of 2023.C1 and 2023.C2 investigates whether the population with the higher proportion of older capsules demonstrates a higher average ^{13}C abundance; the findings demonstrated that this is the case ($p = 2 \times 10^{-6}$). These results verify that the cessation of coated fertilizer application leads to increases in ^{13}C abundance due to an increase in the proportion of older capsules.

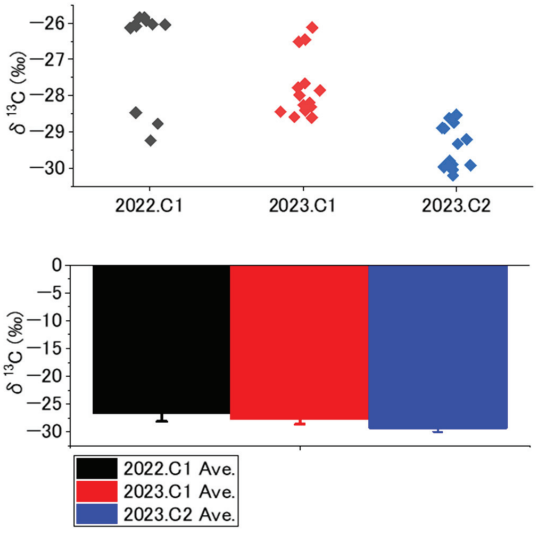

Figure 12. Variations in $\delta^{13}C$ in floating capsules from field C1 in 2022 and 2023 and from field C2 in 2023.

3.5. SEM Analyses and Additional Factors Affecting ^{13}C Abundance

Figure 13 shows an SEM image of the outer surface of a floating capsule. Since no mud or biota is present on the surface, contamination of the capsule exterior is not of concern. However, the SEM image of the interior of a floating capsule (Figure 14) shows that some materials, probably crystallized fertilizer, are present.

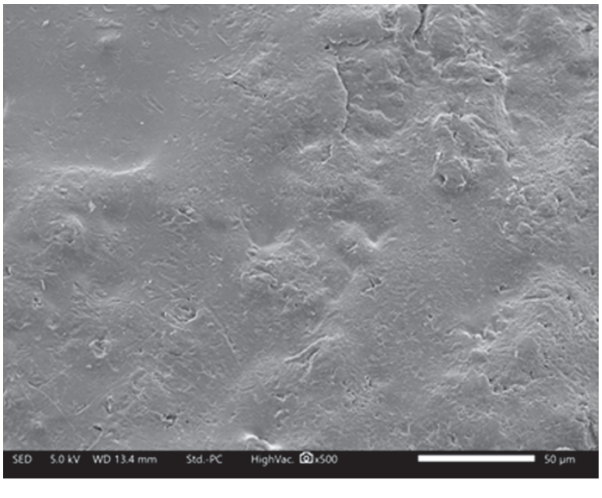

Figure 13. SEM image of the outer surface of a floating capsule.

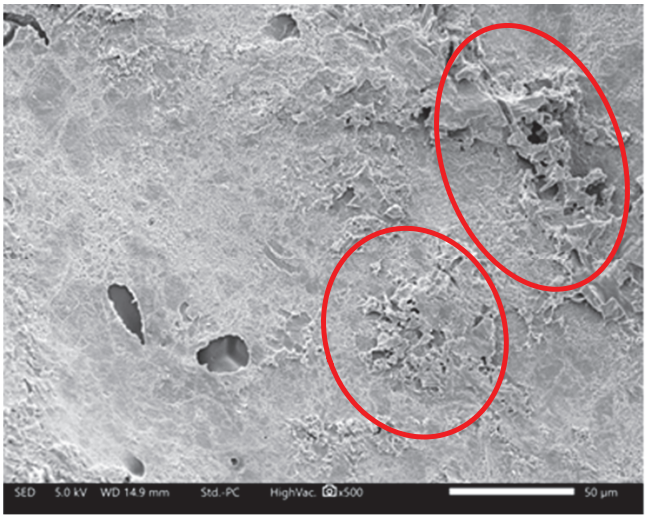

Figure 14. SEM image of the interior surface of a floating capsule, with red circles indicating the presence of materials such as crystalized fertilizer.

We measured ^{13}C abundance in capsules from unused fertilizer that were rinsed in Milli-Q water (Millipore) (Figure 15). The results show that the rinsed capsules exhibited higher $\delta^{13}C$ abundance. Analysis of the fertilizer content within the capsules revealed the presence of urea, which has a very low ^{13}C abundance. These results indicate that the release of urea from the coated fertilizer increases ^{13}C abundance. This mechanism not only increases ^{13}C abundance, but also accelerates the degradation of the capsule itself. We concluded that the observed variation in the ^{13}C abundance, as shown in Figure 16, reflects the combined effect of capsule degradation and the release of urea, resulting in significant differences in the average ^{13}C abundance.

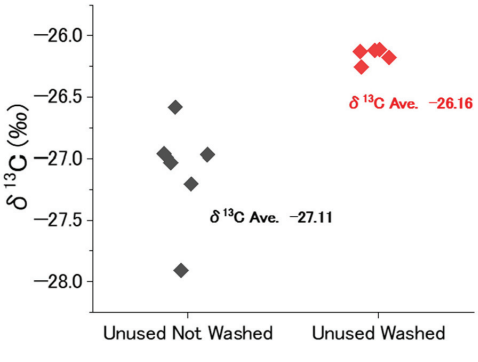

Figure 15. $\delta^{13}C$ abundance in capsules detached from unused fertilizer (capsules washed and those not washed).

Further analyses were performed on the differences in the average weight of unused fertilizer capsules, the 2022.C1 population, and the 2022.C2 population (Figure 16). The figure shows the weight distribution and average of randomly selected three types of capsules obtained from C1 and C2 in 2022 (50 capsules each, but 25 capsules in the case of the spherical capsules from C2) and that of unused coated fertilizer (100 capsules). The results indicate that the urea constituted a large part of the unused fertilizer by weight. Also, the average weights of the 2022.C1 and 2022.C2 samples were significantly different

(p = 0.0013), which might be due to the increased proportion of older capsules in the 2022.C1 samples. Regarding the differences in the three types of floating capsules in 2022.C1 and 2022.C2 (Figures 17 and 18, respectively), a statistically significant difference ($p < 0.05$) was observed for both the 2022.C1 and 2022.C2 samples, as estimated by variance analysis.

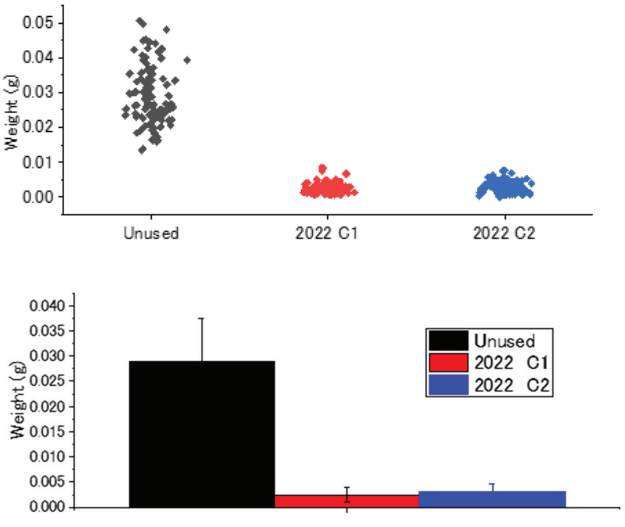

Figure 16. Weights of unused coated fertilizer capsules and floating capsules from fields C1 and C2 (2022).

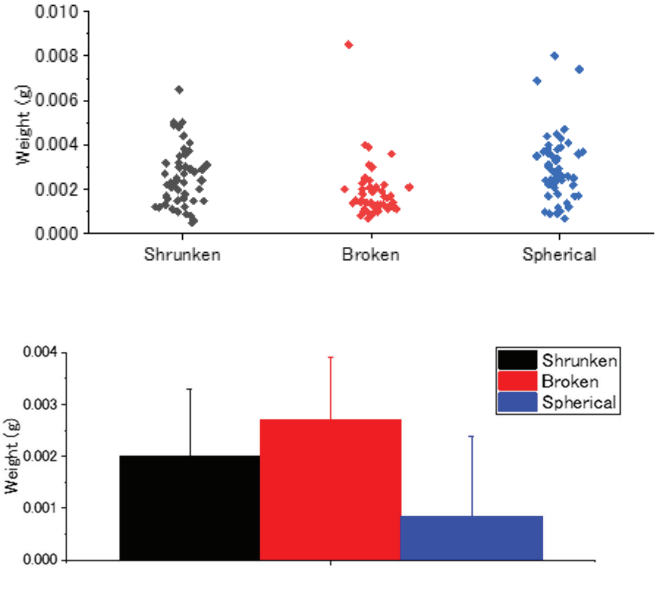

Figure 17. Weights of three types of floating capsules from field C1 (2022).

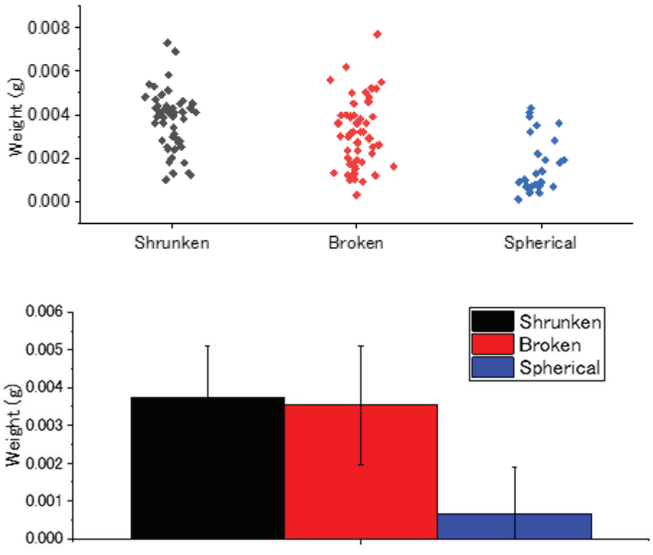

Figure 18. Weights of three types of floating capsules from field C2 (2022).

4. Conclusions and Future Work

- Intensive surveys were conducted in four paddy fields Fukushima and Miyagi prefectures in 2022 and 2023 to investigate the behavior of floating and discharged plastic capsules, originally used as coated fertilizer and degraded over time. Understanding the floating behaviors of these capsules after ploughing is critical for assessing their role in the serious issue of plastic runoff. The capsules collected from the paddy fields represent a mixture of different ages, each having been subjected to a variety of different processes, such as degradation and leaching. Therefore, the primary objective of the present study was to clarify the average behaviors of different capsule populations.
- Comparisons of the proportion of the floating capsules to >3 mm biomass fragments between two distinct populations were conducted. Population 1 consisted of floating capsules from a field with continuous use of coated fertilizer, while Population 2 consisted of floating capsules from a field with intermittent coated fertilizer application. The results revealed that significant degradation of the capsules typically occurred more than 2 years after the application of the coated fertilizer to the paddy fields.
- Marked differences in the proportions as well as the variations in $\delta^{13}C$ values were observed between the two populations after 3 years. The enhancement in $\delta^{13}C$ values can be attributed to both the degradation of the capsules in the paddy soils and the release of urea from within the coated fertilizers, which collectively contribute to increasing $\delta^{13}C$ values over time.
- The weight of each capsule was measured, revealing statistically significant differences between the two populations, in addition to variations in $\delta^{13}C$. A high degree of consistency was observed in the variation in the variations concerning the weight proportion of the floating capsules relative to that of >3 mm biomass, the weight of individual floating capsules, and $\delta^{13}C$ values.

For future work, it is essential to quantify the capsules that float, the capsules that are discharged, and the capsules that are retained within the paddy soils, and to develop a mass balance model. Key parameters for this model might include the mechanical efficiency of ploughing, the ratios of discharged to floating capsule weights, and the degradation rates of capsules under various conditions. Conducting both full-scale and controlled indoor experiments are considered necessary to validate the model.

The indoor experiments are expected to quantify the degradation rates of the capsules under different physical, chemical, biological, and ecological conditions. While the approach employed in this study to estimate the average behavior of each population effectively clarified the role of phenomena occurring in the actual paddy field environment, a more precise approach is required to analyze the factors affecting the individual phenomena. Therefore, controlled indoor experiments are strongly recommended to provide detailed insights into these complex interactions.

Subsequent full-scale monitoring is expected to elucidate the variations in parameters and the causal relationships underlying these variations. For instance, one observation in Field B in 2022 after ploughing involved collecting all of the discharged biomass with capsules at the end of the discharge pipe, as shown in Figure 4. It was observed that the quantity of discharged capsules and biomass was significantly smaller than that which floated after ploughing (Figure 19). This reduction was due to the farmer's efforts to mitigate the environmental impact of paddy discharge. Contrasting results were observed in other fields, where the volume of discharged biomass and capsules was affected by the water level at the discharge pipe, which varied due to farmers' operations. Attention must be paid to the differences in water levels across paddy fields, as well as to discharge velocities. The parameter "the ratio of discharged capsule weight to that of floating capsules" is quite effective for analyzing capsule behavior and for developing capsule runoff reduction methodologies in close collaboration between farmers and researchers.

Figure 19. Comparison of all discharged biomass and capsules with a portion of floating biomass and capsules after ploughing Field B (see Table 1), during reduced discharge volume.

Another important aspect of understanding capsule behavior involves assessing the differences in degradation and urea release rates among the three capsule types, i.e., shrunken, broken, and spherical. Marked differences among these types suggest potential differences in urea release rates. Ongoing SEM analyses are anticipated to better clarify the differences among the three types of capsules.

Moreover, the chemical analyses, especially the $\delta^{15}N$ analyses coupled with CN composition assessments, are expected to provide a more precise understanding of the degradation and urea release behaviors of various capsules.

Author Contributions: Conceptualization, S.H. and I.Y.; Methodology, S.H., I.Y., K.F. and Y.N.; Validation, S.H.; Formal analysis, S.H.; Investigation, S.H. and I.Y.; Data curation, S.H., I.Y., K.F. and Y.N.; Writing—original draft, S.H.; Writing—review & editing, S.H.; Visualization, S.H.; Supervision, S.H.; Project administration, S.H.; Funding acquisition, S.H. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by Fukushima University Research Funds (262Q006, 23RG001) and Takahashi Industrial and Economic Research Foundation (14-003-383).

Data Availability Statement: Data is contained within the article.

Acknowledgments: At the time of this study, Itsuki Yajima was an undergraduate student at the Faculty of Food and Agricultural Sciences, Fukushima University.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ministry of Agriculture, Japan. Available online: https://www.maff.go.jp/j/seisan/sien/sizai/s_hiryo/attach/pdf/jigyo-2.pdf (accessed on 15 May 2024).
2. Katsumi, N.; Kusube, T.; Nagao, S.; Okochi, H. The role of coated fertilizer used in paddy fields as a source of microplastics in the marine environment. *Mar. Pollut. Bull.* **2021**, *161*, 111727. [CrossRef] [PubMed]
3. Katsumi, N.; Kusube, T.; Nagao, S.; Okochi, H. Accumulation of microcapsules derived from coated fertilizer in paddy fields. *Chemosphere* **2021**, *267*, 129185. [CrossRef] [PubMed]
4. Katsumi, N.; Kusube, T.; Nagao, S.; Okochi, H. The input-output balance of microplastics derived from coated fertilizer in paddy fields and the timing of their discharge during the irrigation season. *Chemosphere* **2021**, *279*, 130574. [CrossRef] [PubMed]
5. Harada, S.; Ishii, H.; Hayashi, K.; Kubota, Y. Monitoring of pollution runoff while ploughing and irrigating rice fields, specifically plastic capsules of coating fertilizer. In Proceedings of the 25th Symposium of Japan Society on Water Environment, Tokyo, Japan, 6–7 September 2022; Volume 25, pp. 107–108. (In Japanese).
6. Oikawa, H.; Harada, S. Analyses on the behavior of plastic capsule of coated fertilizer during ploughing and irrigating the rice fields, and indoor experiments for designing the runoff reduction systems. *Appl. Hydrol. (Symp. Proc.)* **2023**, *35*, 65–74. (In Japanese)
7. Harada, S. A priority research project of Fukushima University adopted in the fiscal year 2022: Implementation of the on-site infiltration and retention techniques for non-point contamination reduction due to farming and daily humane activities. *J. Community Future Des. Cent. Fukushima Univ.* **2023**, *34*, 1–4. (In Japanese)
8. Harada, S. Questionnaires for Fukushima university students and subjects belonging to other societies outside the university aiming to clarify the conditions and opportunities to visit Iitate-mura village. *J. Reconstr. Agric. Sci.* **2023**, *3*, 10–23. (In Japanese)
9. Harada, S. Index list for the youth to realize farm village residence; factor analyses; subjective importance of each index; questionnaire. *J. Jpn. Soc. Civ. Eng. Ser. G. (Environ. Res.) (Symp. Proc.)* **2023**, *51*, 39–44. (In Japanese)
10. The Mainichi. Available online: https://mainichi.jp/articles/20220621/ddm/013/040/008000c (accessed on 15 May 2024).
11. Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K.L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347*, 768–771. [CrossRef] [PubMed]
12. Hurley, R.; Horton, A.; Lusher, A.; Nizzetto, L. Chapter 7—Plastic waste in the terrestrial environment. In *Plastic Waste and Recycling Environmental Impact, Societal Issues, Prevention, and Solutions*; Academic Press: New York, NY, USA, 2020; pp. 163–193. [CrossRef]
13. Kosior, E.; Crescenzi, I. Chapter 16—Solutions to the plastic waste problem on land and in the oceans. In *Plastic Waste and Recycling Environmental Impact, Societal Issues, Prevention and Solutions*; Academic Press: New York, NY, USA, 2020; pp. 415–446. [CrossRef]
14. Laurent, C.M.; Lebreton, J.Z.; Damsteeg, J.-W.; Boyan, S.; Andrady, A.; Reisser, J. River plastic emissions to the world's oceans. *Nat. Commun.* **2017**, *8*, 15611. [CrossRef]
15. Takada, H. Reduction of single-use plastics is first priority considering hazardous chemicals in plastics and view pf global material cycles. *Environ. Inf. Sci.* **2019**, *48*, 12–16. (In Japanese)
16. Hahladakis, J.N.; Velis, C.A.; Weber, R.; Iacovidou, E.; Purnell, P. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. *J. Hazard. Mater.* **2018**, *344*, 179–199. [CrossRef] [PubMed]
17. Hermabessiere, L.; Dehaut, A.; Paul-Pont, I.; Lacroix, C.; Jezequel, R.; Soudant, P.; Duflos, P. Occurrence and effects of plastic additives on marine environments and organisms: A review. *Chemosphere* **2017**, *182*, 781–793. [CrossRef] [PubMed]
18. National Institute for Environmental Studies, Japan. Available online: https://www-cycle.nies.go.jp/magazine/kenkyu/201902.html (accessed on 15 May 2024).
19. Ohtsuka, Y.; Takada, H.; Nihei, Y.; Kameda, Y.; Nishikawa, K. Current status and issues of microplastic pollution research. *J. Jpn. Soc. Water Environ.* **2021**, *44*, 35–42. (In Japanese) [CrossRef]

20. Xu, K.; Chen, X.; Wang, J.; Liu, X. Current status and issues of microplastic pollution in China. *Irrig. Wastewater* **2020**, *62*, 55–68. (In Japanese)
21. Harada, S.; Kim, J. Application of infiltration technic for residential zone in agricultural area. *Water Land Environ. Eng.* **2014**, *82*, 301–304. (In Japanese)
22. Harada, S.; Komuro, Y. Decrease of heavy metal non-point runoff using porous concrete. *Trans. JSIDRE* **2009**, *264*, 81–82. (In Japanese)
23. Harada, S.; Komuro, Y. Decrease of non-point zinc runoff using porous concrete. *Chemosphere* **2010**, *78*, 488–491. [CrossRef]
24. Harada, S.; Yanbe, M. Adsorption by and artificial release of zinc and lead from porous concrete for recycling of adsorbed zinc and lead and of porous concrete to reduce non-point heavy metal runoff. *Chemosphere* **2018**, *197*, 451–456. [CrossRef] [PubMed]
25. Harada, S. Application of porous concrete infiltration techniques to street stormwater inlets that simultaneously mitigate against non-point heavy metal pollution and stormwater runoff reduction in urban areas: Catchment-scale evaluation of the potential of discrete and small-scale techniques. *Water* **2023**, *15*, 1998. [CrossRef]
26. Koshikawa, H.; Harada, S.; Watanabe, M.; Sato, K.; Aketaha, K. Relative contribution of bacterial and photosynthetic production to metazooplankton as carbon sources. *J. Plankton Res.* **1996**, *18*, 2269–2281. [CrossRef]
27. Koshikawa, H.; Harada, S.; Watanabe, M.; Kogure, K.; Ioriya, T.; Kohata, K.; Kimura, T.; Sato, K.; Akehata, T. Influence of plankton community structure on the contribution of bacterial production to metazooplankton in a coastal mesocosm. *Mar. Ecol. Prog. Ser.* **1999**, *186*, 31–42. [CrossRef]
28. Koshikawa, H.; Harada, S.; Watanabe, M. Carbon transfer from dissolved organic matter to higher order organisms via microbial loop. *Bull. Plankton Soc. Jpn.* **1999**, *46*, 78–87. (In Japanese)
29. Harada, S.; Koshikawa, H.; Watanabe, M.; Ioriya, T. Implications for top-down control of phytoplankton species succession within a large coastal mesocosm. *J. Glob. Environ. Eng.* **2006**, *11*, 17–27.
30. Tayasu, I.; Hirasawa, R.; Ogawa, N.O.; Ohkochi, N.; Yamada, K. New organic reference materials for carbon- and nitrogen-stable isotope ratio measurements provided by Center for Ecological Research, Kyoto University, and Institute of Biogeosciences, Japan Agency for Marine-Earth Science and Technology. *Limnology* **2011**, *12*, 261–266. [CrossRef]

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Sorption-Based Removal Techniques for Microplastic Contamination of Tap Water

Natalya S. Salikova [1], Almagul R. Kerimkulova [2], Javier Rodrigo-Ilarri [3,*], Kulyash K. Alimova [4], María-Elena Rodrigo-Clavero [3] and Gulzhanat A. Kapbassova [1]

[1] Department of Ecology, Life Safety and Environmental Protection, Abay Myrzakhmetov Kokshetau University, Kokshetau 020000, Kazakhstan; natsal66@mail.ru (N.S.S.); askerbaevna_82@mail.ru (G.A.K.)
[2] Department of Chemical Physics and Materials Science, Al-Farabi Kazakh National University, Almaty 050000, Kazakhstan; almusha_84@mail.ru
[3] Instituto de Ingeniería del Agua y del Medio Ambiente (IIAMA), Universitat Politècnica de València, 46022 Valencia, Spain; marodcla@upv.es
[4] Department of Engineering Systems and Networks, K.I. Satpaev Kazakh National Research Technical University, Almaty 050000, Kazakhstan; kkalimova@mail.ru
* Correspondence: jrodrigo@upv.es

Abstract: This study investigates the presence of microplastics in tap drinking water and evaluates the efficacy of various sorbents for their removal in the context of Kazakhstan's water treatment system. Water samples taken in the cities of Kokshetau and Krasny Yar (Akmola region) were analyzed. Microplastics were detected in all samples, with concentrations ranging from 2.0×10^{-2} to 6.0×10^{-2} particles/dm^3, predominantly in fiber form (74.1%). Outdated technologies and non-compliance with treatment regimens contribute to poor water quality, including high turbidity (87% of samples), color deviations (40% of samples), and acidity issues (20% of samples). To address these challenges, the study examined the sorption efficiency of different sorbents, with results indicating high retention rates (82.7–97.8%) for microplastic particles. Notably, aliphatic structures like PE and PP exhibited higher retention than PET. Among the sorbents tested, the synthesized carbon sorption material (CSM) demonstrated the highest efficiency in both microplastic retention and improvement in water quality parameters, making it a promising option for water treatment facilities and household filters.

Keywords: microplastic; tap water; Kazakhstan water treatment technologies; drinking water quality; microplastic sorption

Citation: Salikova, N.S.; Kerimkulova, A.R.; Rodrigo-Ilarri, J.; Alimova, K.K.; Rodrigo-Clavero, M.-E.; Kapbassova, G.A. Sorption-Based Removal Techniques for Microplastic Contamination of Tap Water. *Water* **2024**, *16*, 1363. https://doi.org/10.3390/w16101363

Academic Editors: Grigorios L. Kyriakopoulos, Antonis A. Zorpas, María Rocío Rodríguez Barroso and Vassilis J. Inglezakis

Received: 18 April 2024
Revised: 2 May 2024
Accepted: 9 May 2024
Published: 11 May 2024

1. Introduction

Microplastics, particles smaller than 5 mm, pervade various environmental objects, as evidenced by ongoing scientific investigations worldwide. Documentation exists on their presence in natural waters [1,2], soils [3,4], atmospheric air [5,6], wastewater [7], and municipal solid waste landfills [8]. Initial studies exploring microplastic contamination of drinking water date back to 2017, encompassing examinations of raw and treated water from treatment facilities, tap water, and bottled water [9,10]. Disparities emerge in microplastic concentrations between natural and treated waters, attributed to the lesser susceptibility of groundwater to anthropogenic influences [11].

Nevertheless, the quantification of microplastics in drinking water yields variable data. For instance, in ref. [12], no microplastic particles were discerned, despite employing an analytical method with a relatively high detection limit (60 μm). Conversely, ref. [11] identified minute concentrations, averaging 0.7 microplastics/m^3.

The concentration of microplastic fragments increases inversely with particle size, with the regular fragmentation contributing to heightened levels in smaller particles. Consequently, analyses of microplastics smaller than 10 μm in mineralized drinking water [13]

unveiled elevated concentrations (656.8 μg/L). Notably, nanomicroplastics, ranging from 0.7 to 20 μm, were detected in Barcelona tap water at notable concentrations, up to 19 μg/L. Diverse polymers such as polyethylene (PE), polypropylene (PP), polyisoprene (PI), polybutadiene (PBD), polystyrene (PS), polyamide (PA), and polydimethylsiloxanes (PDMS) have been identified [14].

Furthermore, studies commonly report higher concentrations of microplastics in bottled water compared to tap water, potentially linked to contamination from plastic packaging materials (e.g., caps and bottles) [15]. Researchers also explore the size distribution of microplastics in drinking water. Disparities in particle size between raw and treated water are documented in [16], where particles larger than 125 μm predominate in raw water, while those between 20 nm and 125 μm are prevalent in treated water.

Understanding the origins of microplastic contamination of drinking water is also a subject of investigation. The primary source of contamination of treated drinking water may be the materials used in containers (e.g., water pipes, bottles) [17]. Additionally, potential secondary contamination may arise from the erosion of plastic pipes within the water supply system [17,18]. The choice of pipe materials is assumed to significantly influence the migration of microplastics during water conveyance and storage. Notably, tap water in contact with polymer pipes exhibits the highest average concentrations of microplastics (polyvinyl chloride (PVC), polyamide (PA), polypropylene (PP), polyethylene (PE), and polyethylene terephthalate (PET)).

The primary forms of microplastic content in drinking water include PET, PP, and PE fibers [16], as well as films [19]. Concerns arise regarding the presence of microplastics in natural waters (potential sources of drinking water) and treated drinking water [19,20], particularly due to the potential ingestion of microplastics through swimming or drinking. Moreover, published data highlight the detection of microplastics in aquatic organisms [21,22], food items [23], and human organs [24,25], suggesting a potential trophic transfer of microplastics through the food chain [26], thereby raising concerns about the potential toxic risks to human health associated with the ingestion of microplastics in food and water [27–29].

Reports documenting the presence of microplastics in water are widespread across various regions worldwide, including North and South America [30,31], Asia [32], and Europe [10,33]. However, there has been no systematic monitoring of microplastics in the environment in Kazakhstan. Kazakhstan, ranked 93rd out of 180 countries in the Environmental Performance Index [34] in 2022 and 76th out of 85 in 2023 [35], faces significant environmental quality challenges, particularly concerning the quality of drinking water [36,37]. Consequently, efforts by Kazakhstani authorities and scientists primarily focus on achieving drinking water quality according to standardized indicators, which currently do not include microplastics. Kazakhstani scientists initiated the study of microplastic distribution in human-contact media only in 2022, funded by the Ministry of Science and Higher Education. The widespread use and unregulated disposal of plastics, coupled with a deficient waste management system, pose significant risks of plastic waste pollution in Kazakhstan's natural environment, potentially serving as sources of microplastics in the soil and natural waters [38,39]. The absence of standards and quality control measures for drinking water regarding microplastic content heightens the likelihood of these pollutants entering the Kazakhstani population's bodies through drinking water consumption.

The aim of this study is to assess the presence of microplastics in tap water and, by analyzing the current water treatment infrastructure, identify optimal filtration materials for microplastic removal in Kazakhstan. However, purifying water from microplastics presents a formidable challenge. Microplastics represent a diverse group of emerging pollutants with varying structures, sizes, and densities, compounded by their inert properties, rendering them resistant to removal from water [40,41]. Existing water treatment systems exhibit variable efficacy in trapping microplastics, contingent upon factors such as size, nature, and technological sophistication [7,42]. A straightforward approach in drinking water treatment involves passing water through filter media, where the effectiveness of

microplastic removal hinges on the pore size of the filter medium. In resource-constrained settings like Kazakhstan, quartz sand serves as a readily available and cost-effective filtering material in drinking water treatment systems. Nonetheless, zeolites and coals, including those indigenous to Kazakhstan, demonstrate notable sorption capabilities against organic contaminants, heavy metals, and radionuclides [43–46]. Published findings suggest that sand filters could be replaced effectively by aluminosilicate filter media [47], and zeolites have shown promise in microplastic treatment in wastewater [48], while granular coals, when combined with ion exchange and microfiltration, have demonstrated efficacy in removing certain microplastic types [49].

However, research on microplastic water treatment primarily focuses on wastewater and is limited to specific types and sizes of microplastics. Moreover, studies on water treatment for microplastics are comparatively scant, falling short of evidence substantiating their presence in water. There is a dearth of sorption studies for domestically produced plastics in the Kazakhstani market utilizing locally sourced sorbents. Consequently, investigating microplastic content in local tap water as an indicator of water treatment system efficiency in Kazakhstan and evaluating the viability of incorporating local zeolites, activated carbon sorbents (derived from local organic waste) and widely available ion-exchange resins into the sorption complex represents novel and pertinent research. The outcomes of this study will contribute to a better understanding of drinking water quality and offer guidance for effective management of water treatment systems in Kazakhstan in terms of microplastic content.

2. Materials and Methods

2.1. Methods of Sampling and Analyzing Tap Water

To assess microplastic particles and physicochemical parameters in tap water, 15 water samples were collected over the course of one month from various buildings in Kokshetau city and Krasny Yar village, located in the Akmola region of Kazakhstan. These buildings exhibit varying degrees of water supply system degradation, including flats with centralized water supply in multistory buildings and individual houses with water supply pipes constructed between 1960 and 2008.

Figure 1 illustrates the geographical location of Kokshetau and Krasny Yar within the Akmola region, while Figure 2 and Table 1 provide details regarding the specific sampling points for tap water within these two cities.

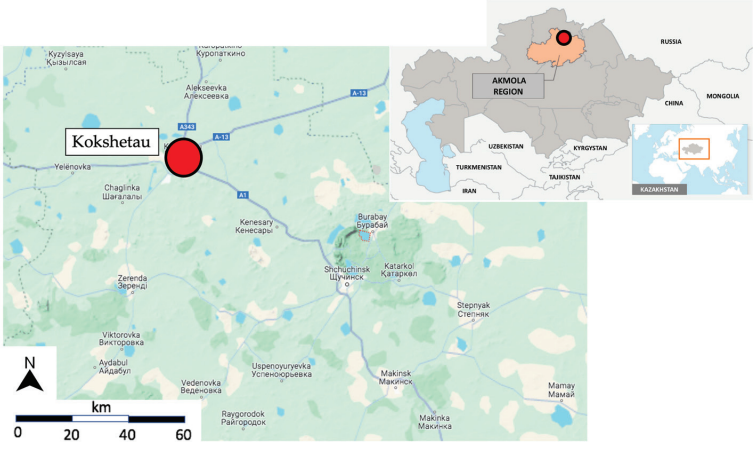

Figure 1. Location of Kokshetau city inside the Akmola region in Kazakhstan.

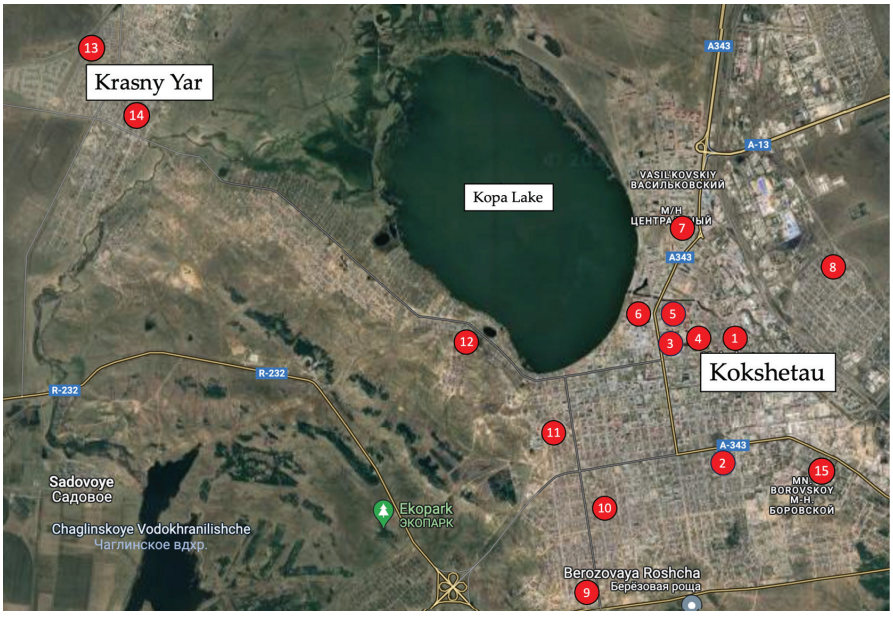

Figure 2. Location of tap water sampling points inside Kokshetau and Krasny Yar.

Table 1. Universal Transverse Mercator (UTM) coordinates of the sampling points.

Sample No.	UTM X	UTM Y	Sample No.	UTM X	UTM Y
1	53.289008	69.404590	9	53.252827	69.359687
2	53.274207	69.405828	10	53.264980	69.371940
3	53.289204	69.391170	11	53.277500	69.361754
4	53.289488	69.392308	12	53.292318	69.336518
5	53.293338	69.386138	13	53.329990	69.253996
6	53.292478	69.386359	14	53.322229	69.264094
7	53.306466	69.389917	15	53.271033	69.428809
8	53.302045	69.427693			

Tap water sampling for microplastic analysis was conducted directly at the water source following the protocol outlined in [50]. Microplastic extraction from water was accomplished using a custom-designed filtration device, which comprised a plastic slip-on coupling with sealing gaskets measuring 110 mm in diameter, featuring a transition from polypropylene. This transition tightly joined with the coupling, as detailed in [51], and connected to a Sefar polyamide filter with a diameter of 120 mm and a mesh size of 300 µm. During tap water sampling, 100 dm^3 of water was passed through the polyamide filter. The volume of water drawn was controlled using a metal bucket with a capacity of 10 dm^3. Before each filtration, the bucket and filtering device underwent thorough rinsing with distilled water. Subsequently, the filters were placed in labeled glass petri dishes and transported to the laboratory for further analysis.

The surface of the filters was washed with distilled water, and the filter washes were collected in glass heat-resistant beakers. The collected samples underwent non-destructive oxidation for plastics, followed by density separation and the collection of microplastics on filters for subsequent optical identification [52–55]. To prevent microplastic degradation, the filters were dried in a desiccator at a temperature not exceeding 35 °C within closed petri dishes [56]. The dried filters were then examined under a DTX 500 LCD Levenhuk microscope with photoregistration at magnifications ranging from 100 to 500 [57–59]. The

concentration of particles/dm^3 was calculated based on the volume of water filtered through the filter [60].

In the study, measures were taken to prevent cross-contamination of samples with microplastic according to quality criteria [61–63]. The estimation of microplastic particle losses at different stages of the study was carried out by introducing a mixture of microplastics of different compositions into blank samples and further extraction of a known number of colored particles. According to the results of the positive control, the recovery rate of microplastics of the 170–300 μm fraction was 80.32 ± 6.01%.

Analysis of physicochemical parameters of water was carried out according to State mandatory standards 31868-2012 [64], 3351-74 [65], 26449.1-85 [66] and ISO standard 4316-2019 [67].

2.2. Methodology for Sorption Treatment of Water from Microplastics

The investigation into the removal of microplastics from drinking water was conducted using model waters representing the most common types of plastics (PET, PP, PE) utilized for transporting or storing drinking water in Kazakhstan. To generate plastic microparticles, PET bottles and water pipes made of PP and PE were crushed using a homemade crusher, grinding equipment, laboratory mill, and other methods. The resulting shredded particles were then sieved using a Lab-VIBSIEVE-8 electric vibrating screen (Bessaiman Group LLP, Almaty, Kazakhstan) to obtain fractions with sizes of 0.45 mm, 0.2 mm, 0.105 mm, and 0.0063 mm.

Sorption materials were chosen based on their availability and widespread application in water treatment in Kazakhstan and globally [68–71]:

- Carbon sorption material (CSM), obtained by carbonizing apricot pits, an annually renewable waste material from plant sources.
- Zeolite sourced from the Chankanaisky zeolite deposit in the Kerbulak district of the Almaty region, Republic of Kazakhstan. This zeolite is a medium-porous material with a brown hue, featuring particle sizes not exceeding 2–4 mm. Its generalized formula is $K_{x/n}$ [Al_x Si O_{y2} (x + y)] × pH_2O, where K represents alkali and alkaline-earth metal cations, ammonium, etc., p denotes cation charge, y/x = 1:6, and p/x = 1:4.
- A complex comprising activated carbon sorption material (CSM) and ion exchange resins, specifically anionite Ecotar-B and cationite KU-2-8, utilized in household filters for drinking water treatment at a ratio of 1:1:1 (CSM:anionite:cationite).

The carbon sorption material was derived from the carbonization of apricot pits, which are agricultural industry by-products. Dried apricot pits were crushed into particles ranging from 2 to 4 mm in size and then placed in a carbonization reactor. The carbonization process was conducted under precisely controlled isothermal conditions (at a temperature of 850 °C) within a rotating reactor, and the environment was filled with an inert gas (argon), supplied into the reactor at a constant rate of 50 cm^3/min. Following carbonization, the samples were activated using a potassium hydroxide solution in a 1:2 ratio.

Electron microscopy was employed to examine the morphological and structural characteristics of the synthesized carbon sorption materials (CSMs) using a Quanta 3D 200iDualSystem (FEI company, Hillsboro, OR, USA), an FEI double-beam microscope that integrates scanning electron microscopy (SEM) and ion scanning microscopy. Additionally, it features an integrated energy-dispersive microanalysis system. The specific surface area of the sorbents and the specific pore volume were determined on a Sorbtometer-M analyzer manufactured by Katakon LLP (Novosibirsk, Russia) using the Brunauer–Emmet–Teller low-temperature nitrogen adsorption method [72]. This method is associated with the evacuation of air and moisture from the sample by heating (to 200 °C) under high vacuum, which makes it impossible to determine the specific surface area and specific pore volume of sorption materials of organic origin, such as ion exchange resins.

The elemental composition of the sorbents, including a sample of carbon sorption material and zeolite, was determined using an energy dispersive spectrometer, which serves

as an auxiliary device of the Quanta 3D 200iDualSystem scanning electron microscope (refer to Tables 2 and 3 for detailed results).

Table 2. Elemental composition of CSM (%).

Chemical Element	C	O	Si	K	Ca
Share (%)	92.45	6.87	0.06	0.17	0.44

Table 3. Elemental composition of zeolite (%).

Chemical Element	C	O	Na	Mg	Al	Si	K	Ca	Fe
Share (%)	8.52	60.09	0.52	1.15	6.91	19.29	0.68	1.62	1.22

As shown in Table 2, 92.45% of the composition of CSM is carbon. The EDX spectrum of carbon sorption material is shown in Figure 3. The energy-dispersive spectrum of elemental composition of zeolite is presented in Figure 4. The main element in the composition of zeolites is oxygen (60.09% of the total composition), as shown in Table 3.

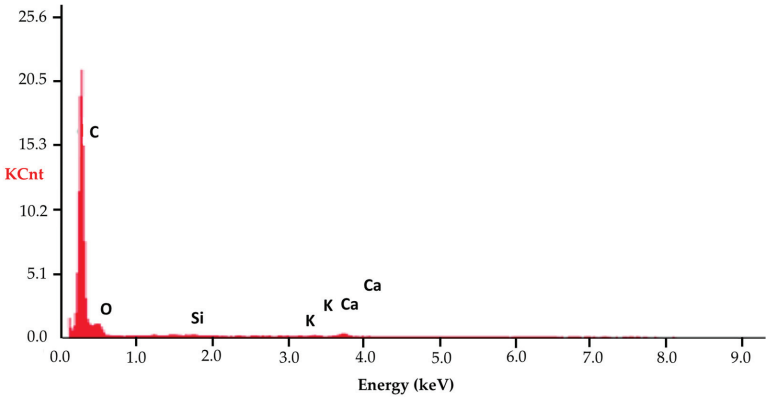

Figure 3. EDX spectrum of carbon sorption material.

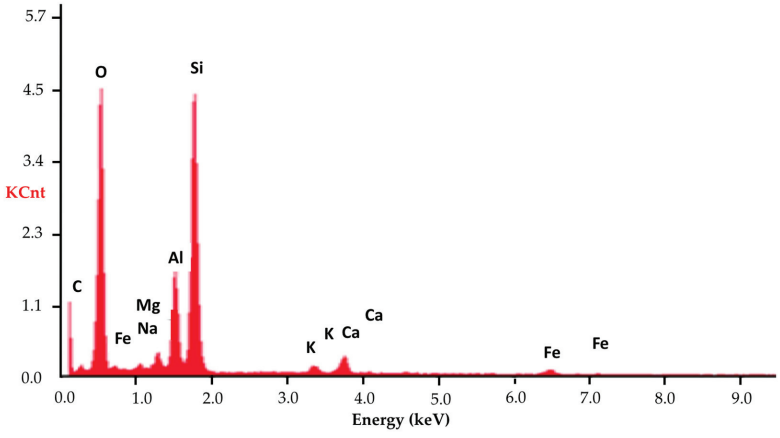

Figure 4. EDX spectrum of the zeolite.

The energy-dispersive spectrum of the elemental composition of the sorbent (ion exchange resins) was established using an energy-dispersive X-ray analyzer built into a

JSM-6490LA scanning electron microscope (JEOL Ltd., Tokyo, Japan) and is presented in Figure 5.

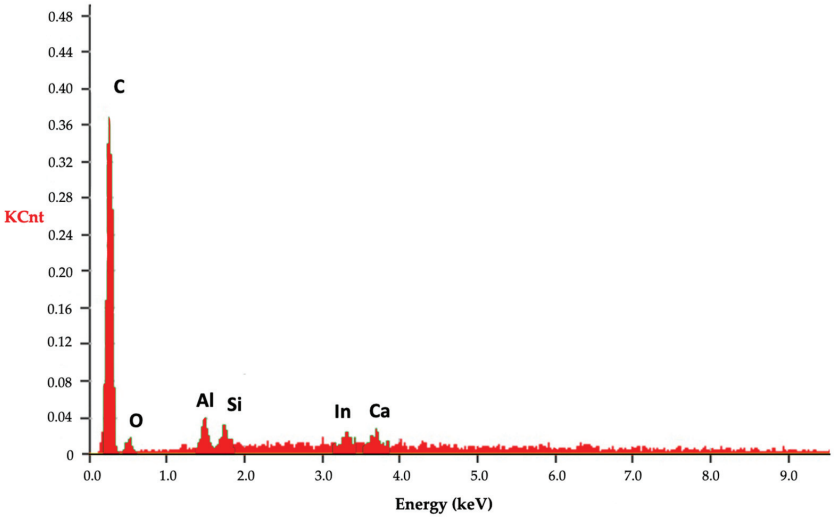

Figure 5. EDX spectrum of the sorbent (CSM + ion exchange resins).

The main element in the composition of sorbent (CSM + ion exchange resins) is carbon (84.06%), as shown in Table 4. There is a decrease in carbon content and an increase in oxygen content in comparison with the original CSM.

Table 4. Elemental composition of the sorbent (CSM + ion exchange resins), %.

Chemical Element	C	O	Al	Si	Ca	In
Share (%)	84.06	14.03	0.75	0.61	0.35	0.20

Glass columns containing sorbent materials (CSM, zeolite, complex of CSM + ion-exchange resins) were prepared for purifying water from plastic microparticles. Prior to sorption, the columns were rinsed with distilled water (300 dm^3 volume) to remove any fine dust-like particles of sorbents. Model solutions of polymers (PET, PP, PE) were created by mixing 0.2 g of microplastics of a specific fraction in 10 dm^3 of distilled water. The mass of microplastics before and after sorption was measured using a RADWAG 220R2 analytical scale (RADWAG Wagi Elektroniczne, Radom, Poland) with an accuracy of ±0.0001 g.

The prepared model solutions of microplastics were then passed through the sorbent columns, followed by washing the columns with 200 mL of distilled water. The water that passed through the columns was collected for subsequent optical analysis.

Samples of the initial plastics used to prepare the model solutions were analyzed using infrared (IR) spectroscopy on a Shimadzu IR-Prestige 21 instrument (Shimadzu Corporation, Kyoto, Japan) in the wavelength range of 4000–400 cm^{-1}. The IR spectra were obtained without special sample preparation using the DuraSampl IR II (Smiths detection, Danbury, CT, USA) broken total internal reflection attachment with single reflection (prism material diamond on ZnSe substrate). To identify polymers, we analyzed absorption bands caused by stretching and bending vibrations of groups characteristic of certain types of polymers. IR spectra were compared with library databases such as IRs Polymer2, Polymer, T-Polymer and T-Organic in order to identify the structures of polymers that are difficult to interpret (PET, microplastics found in tap water).

3. Results and Discussion

3.1. Analysis of Technological Processes of Water Treatment in Kazakhstan

Surface water sources constitute the primary source of drinking water for the population of cities in Kazakhstan, accounting for 96.1% of supply, with centralized water supply serving 94.7% of the population. Among these, 14.6% of the population utilizes standpipes [73].

The construction of water supply systems in Kazakhstani cities began in the early 1950s, with significant development occurring between 1960 and 1970. During this period, extensive water supply and sewerage infrastructure was established, resulting in a total urban water supply network length of 23.44 thousand kilometers. However, prolonged operation without adequate maintenance and timely renovation has led to considerable wear and tear of the fixed assets within water supply and sewerage systems. Consequently, the technical condition of water supply and drainage systems in most cities and towns of Kazakhstan is deemed critical [74]. The fundamental processes of drinking water treatment technology within the water supply system are depicted schematically in Figure 6.

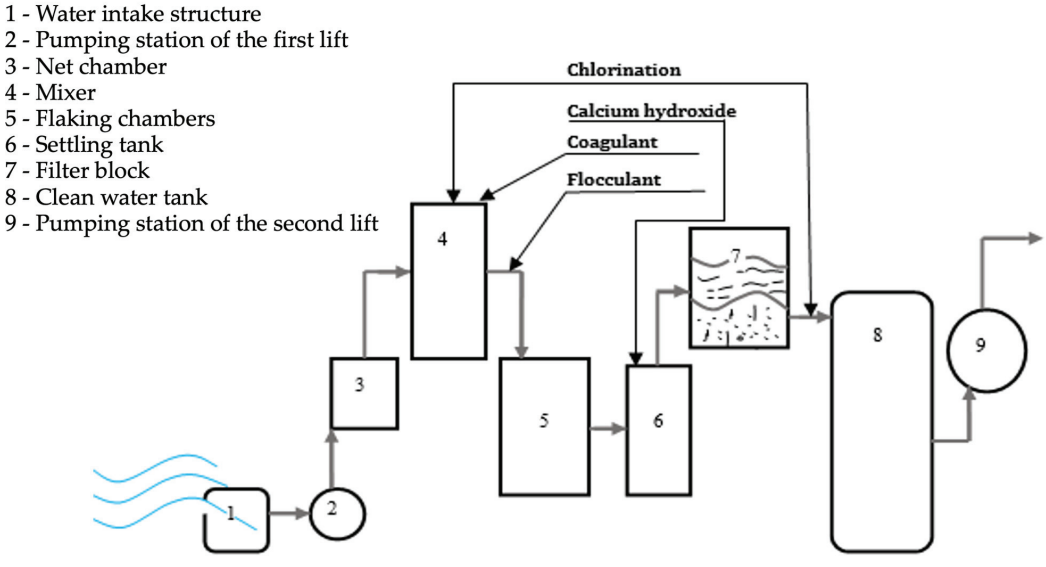

Figure 6. Technological scheme of water intake and treatment of water treatment facilities in Kazakhstan.

A conventional purification scheme is implemented to clarify and treat water entering the municipal water supply, encompassing the following processes.

- Mechanical cleaning: Coarse debris is removed through screening or gridding.
- Settling: Preliminary removal of suspended solids occurs in settling tanks (radial or horizontal).
- Coagulation: Reagents such as iron chloride or aluminum sulfate are used to induce coagulation.
- Flocculation: Aggregation of particles into larger masses for easier removal.
- Alkalization: Water may be alkalinized with a calcium hydroxide solution if necessary.
- Clarification: Large impurities precipitate and settle in settling tanks for 3–4 h after coagulation and flocculation.
- Filtration: Final clarification and removal of bacteria, and small impurities are achieved through rapid filtration. Quartz sand (Kokshetau) or a combination of quartz sand and silica (Almaty) serve as filtering materials.

- Disinfection: Water is disinfected by chlorination to maintain residual free chlorine content in the supplied water at 0.3–0.5 mg/L. Currently, water disinfection is accomplished using sodium hypochlorite (NaClO) solution produced from table salt at electrolysis plants.
- Fluoridation: Fluoride content in drinking water is adjusted at the fluorator unit when it falls below 0.5 mg/L, raising it to a concentration of 0.9–1.2 mg/L. Sodium fluoride is typically used as the reagent.

The treated water is conveyed to clean drinking water tanks, from where it is distributed to consumers through gravity and pumping stations within the distribution network. Quality control of treated drinking water is conducted to ensure compliance with sanitary norms [75], which presently do not stipulate limits on the content of microplastic particles.

Analysis of the technological process of drinking water treatment in Kazakhstan has enabled an assessment of its potential efficacy in retaining particles smaller than 5 mm. The disinfection stage, particularly chlorination, may impact the structure of polymers, indirectly influencing their sorption and sedimentation capacity. However, there is no definitive consensus on the effect of chlorination on the degree of microplastic removal from drinking water [76,77]. Due to the unique physicochemical properties of microplastics, characterized by a small surface area and minimal porosity, their removal from water is primarily influenced by mechanical processes such as filtration and sedimentation, as well as the physicochemical process of coagulation [78–80].

In Kazakhstan, mechanical filtration of treated water is performed through screens and grids featuring vertical or inclined metal bars spaced at regular intervals (15–20 mm or more) to create a mesh-like structure (Figure 7a). This design anticipates that natural water pretreatment on these grids can effectively capture macroplastic particles larger than 15–20 mm. Subsequently, water coarse-filtered from larger impurities on the meshes/grids undergoes further treatment in sedimentation tanks (Figure 7b), engineered to retain suspended solids larger than 0.2–1 mm [81].

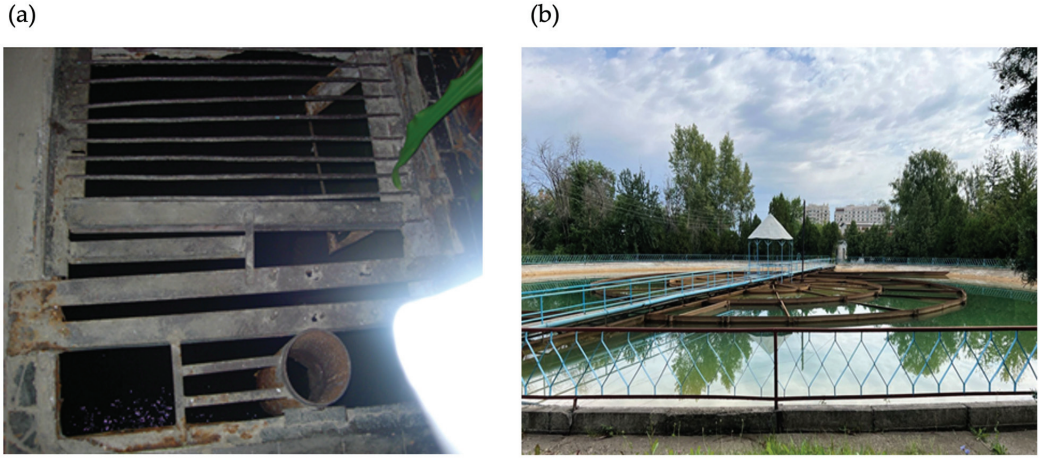

Figure 7. (**a**) Grate system for coarse debris removal at Kokshetau, and (**b**) radial settling tank for fines removal at Almaty.

The potential efficacy of the coagulation stage within the Kazakhstani water treatment system can be assessed based on findings from published studies. For instance, in ref. [82], the efficiency of microplastics removal using coagulants derived from Al and Fe salts in the presence of sodium dodecylbenzenesulfonate was reported as 95.92% and 98.9%, respectively. However, the specific size range of effectively removed microplastic particles was not specified in the study. Conversely, in [83], it was observed that with the coagulant

$Al_2(SO_4)_3$, the deposition rate of pure plastic remained below 2.0% for all plastic particle sizes. Even with the addition of the coagulant PolyDADADMAC, the maximum removal achieved was only 13.6% for particle sizes ranging from 45–53 μm. The authors highlighted that the critical size at which microplastic removal efficiency significantly decreased was within the range of 10–20 μm. However, the sequential combination of coagulation and sand filtration was found to completely remove microplastics larger than 45 μm [84].

Thus, by adhering to the prescribed technological process regimes of drinking water treatment in Kazakhstan, it is feasible to retain microplastic particles larger than 45 microns. However, the non-compliance with water treatment protocols in Kokshetau city, such as the absence of coagulation and flotation units, coupled with a limited number of sand filters with low productivity, results in the presence of microplastics in tap water samples from Kokshetau city, particularly in sizes exceeding 300 μm (refer to Section 3.2).

3.2. Microplastics in Tap Water

The results of the physicochemical analysis of 15 water samples for color, turbidity, pH, acidity, and microplastic content are summarized in Table 5.

Table 5. Results of physicochemical analysis of the tap water samples.

Sample No.	Color (Degrees)	Turbidity (mg/dm^3)	pH	Oxidization (mg/dm^3)	Microplastic Concentration ($Particles/dm^3$)
MPC	≤20	≤1.5	6–9	≤5	-
1	18	9.51	6.82	6.8	4.0×10^{-2}
2	11	4.52	6.81	5.4	4.0×10^{-2}
3	15	3.42	6.80	5.8	4.0×10^{-2}
4	14	4.13	6.83	4.7	2.0×10^{-2}
5	37	5.22	6.82	3.3	2.0×10^{-2}
6	23	2.64	7.01	3.3	6.0×10^{-2}
7	22	3.10	7.10	3.2	6.0×10^{-2}
8	32	3.13	7.12	3.2	6.0×10^{-2}
9	18	3.82	7.13	2.4	2.0×10^{-2}
10	35	2.91	7.04	2.9	4.0×10^{-2}
11	29	3.50	7.10	3.2	2.0×10^{-2}
12	16	1.90	7.03	2.8	2.0×10^{-2}
13	6	0.34	7.12	0.7	2.0×10^{-2}
14	7	0.46	7.10	0.8	4.0×10^{-2}
15	26	4.93	7.02	2.9	4.0×10^{-2}

Analysis of Table 5 reveals inconsistencies in the quality of drinking water across the majority of samples, potentially attributable to the degradation of water supply networks and the subpar quality of water provided by the Kokshetau Su Arnasy water treatment facilities. Exceedances of sanitary and hygienic quality standards for drinking water are observed in the following parameters.

- Turbidity: Exceedance in 87% of samples (ranging from 1.26 to 6.33 times the maximum allowable concentration).
- Color: Exceedance in 40% of samples (ranging from 1.10 to 1.85 times the maximum allowable concentration).
- Acidity: Exceedance in 20% of samples (ranging from 1.1 to 1.36 times the maximum allowable concentration).

Only two samples, collected from private houses in Krasny Yar village (samples 13 and 14), comply with sanitary–hygienic norms. These samples from Krasny Yar village met the standards for the studied indicators.

All tap water samples exhibit the presence of microplastics in various forms, as depicted in Figures 8 and 9. Fiber-shaped microplastics (acrylic, viscose, polyamide) comprise

the majority, accounting for 74.1% of the total number of microplastic particles, followed by fragments at 22.2% (polyethylene terephthalate, polypropylene, and polystyrene). Additionally, one particle (3.7%) in the form of a film (polyimide) was identified. These findings are consistent with published data, indicating that fibers dominate over fragments and other forms of microplastic in drinking water, often comprising up to 90% of the total microplastic content [85]. The Supplementary Materials presents FTIR spectra of the types of microplastics found in tap water and their identification in Figures S1–S14.

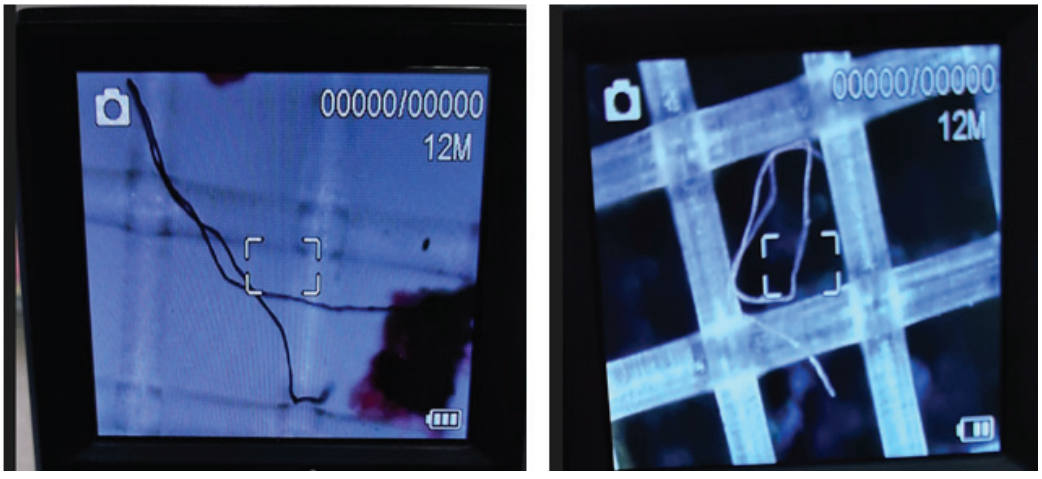

Figure 8. Examples of microplastic fibers found in tap water samples.

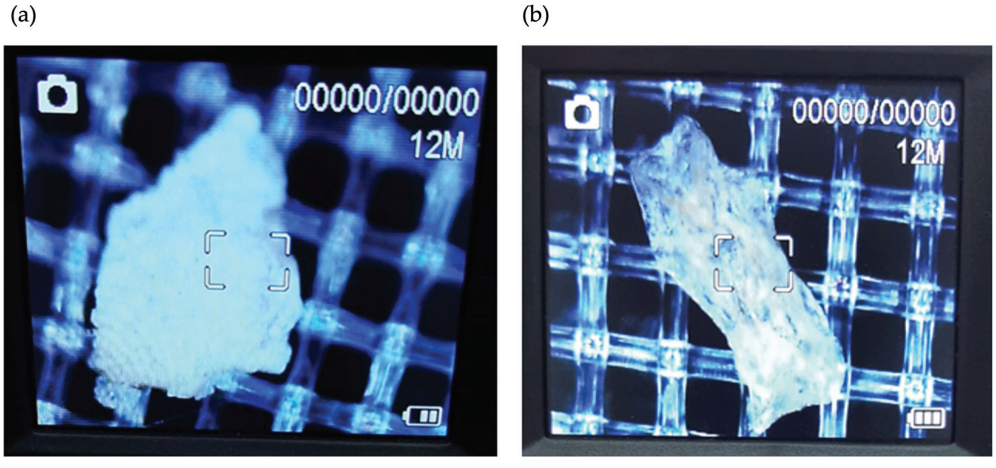

Figure 9. (**a**) Fragments, and (**b**) films of microplastics found in tap water samples.

The microplastic content in the analyzed tap water samples ranges from 2.0×10^{-2} to 6.0×10^{-2} particles/dm^3, aligning with published data worldwide, which typically ranges from 1×10^{-4} to 100 particles/dm^3 [11].

Table 6 presents a comparison between the concentration of microplastics observed in this study and those reported in several previous studies conducted at various sites worldwide.

Table 6. Comparison with results from previous research about microplastic concentration in tap water.

Reference	Location	Number of Samples	Microplastic Concentration (Particles/dm^3)
This study	Kokshetau and Krasny Yar (Kazakhstan)	15	4.0×10^{-2}–6.0×10^{-2}
[23]	Central region (Saudi Arabia)	2	1.8
[86]	Barcelona city (Spain)	21	0–5.0×10^{-2}
[40]	Tianjin (China)	1	13.23
[87]	Zahedan (Iran)	10	7.5×10^{-2}–40.0×10^{-2}
[88]	Mexico City (Mexico)	42	5.0–91.0
[11]	North-western region (Germany)	24	1×10^{-4}–100
[89]	England and Wales (UK)	39	0–2.4×10^{-2}
[90]	Baden-Wurttemberg (Germany)	2	0.6×10^{-2}–7.4×10^{-2}
[91]	Chongqing (Southwest China)	1	1.4
[92]	Gauteng (South Africa)	30	4.7–31
[93]	Denmark	17	8.0×10^{-2}–60.0×10^{-2}
[94]	Japan	28	29–45

The results shown in Table 6 demonstrate that the concentration of microplastics found in Kokshetau city and Krasny Yar village is similar to those found in Barcelona [86] and England and Wales [89]. It has been observed that the tap water quality in Kokshetau City and Krasny Yar village, in terms of microplastic concentration, is better than in the rest of the case studies found in the scientific literature. However, the detection of microplastic particles in 100% of the analyzed tap water samples, coupled with the unsatisfactory quality of water based on physical and chemical indicators, underscores the necessity to explore new technological solutions for the water treatment system in Kazakhstan, particularly focusing on sorption processes.

The detection of microplastic particles in 100% of the analyzed tap water samples, coupled with the unsatisfactory quality of water based on physical and chemical indicators, underscores the necessity to explore new technological solutions for the water treatment system in Kazakhstan, particularly focusing on sorption processes.

3.3. Treatment of Water from Microplastics by Sorption Methods

Our study departed from using standard polymer granules of known quality and instead employed grinding to mimic the forms of microplastics found in water more accurately. In identifying plastics, the study considered the infrared (IR) spectrum of PET, which exhibits absorption bands characteristic of out-of-plane and in-plane vibrations of the benzene group, stretching vibrations of the C=O bond of ester groups, the carbonyl group C=O conjugated with the benzene ring, and asymmetric stretching of the C-O-C group (at 721, 870, 1018, 1246, 1710, and 1095 cm^{-1}, respectively).

Polypropylene was identified based on the presence of absorption bands characteristic of stretching and bending vibrations of CH, CH_2, and CH_3 groups in its IR spectrum (at 2950, 2918, 2836, 1456, and 1376 cm^{-1}). Similarly, the IR spectrum of PE contains absorption bands (at 2916, 2846, 1468, and 717 cm^{-1}) arising from stretching and bending vibrations of the CH_2 group [95]. Furthermore, the complex structure of PET was further elucidated by comparing its FTIR spectrum with those in polymer library databases. The Supplementary Materials present the spectra of polymers used in the sorption process in Figures S15–S18.

The efficacy of the selected sorbents in retaining microplastics (PET, PP, PE) of various fractions was also assessed (Table 7).

Table 7. Sorption efficiency of microplastic by different sorbents (%).

Polymer	Fractional Size of Microplastic (mm)		
	0.105	**0.2**	**0.45**
Complex (CSM + ion exchange resins)			
PET	82.7	85.2	86.0
PE	91.7	92.1	93.6
PP	85.8	85.7	89.9
Average efficiency by sorbent	86.7	87.7	89.8
Zeolite			
PET	91.0	91.3	91.2
PE	90.2	90.8	94.3
PP	89.9	90.0	92.8
Average efficiency by sorbent	90.4	90.7	92.8
Carbon sorption material (CSM)			
PET	92.7	92.6	93.3
PE	95.3	97.2	97.8
PP	95.0	94.9	96.8
Average efficiency by sorbent	94.3	94.9	96.0

The sorption findings demonstrate a high retention of microplastic particles by the investigated sorbents, ranging from 82.7% to 97.8%. These results from weight analysis were corroborated by optical microscopy examinations of aqueous solutions before and after filtration through the sorbents. Figures 10–12 show microphotographs of water samples containing PET, PP, and PE microparticles (0.45 mm fraction as an example) after filtration through columns containing the examined sorbents.

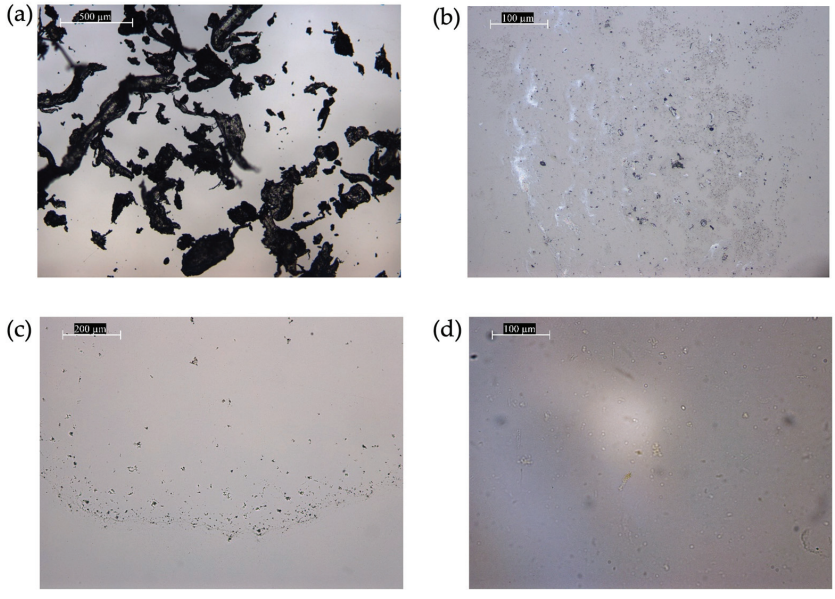

Figure 10. Microphotographs of water samples with 0.45 mm PET microparticles before and after sorption. (**a**) Model solution before sorption; (**b**) complex (CSM + ion exchange resins); (**c**) zeolite; (**d**) CSM.

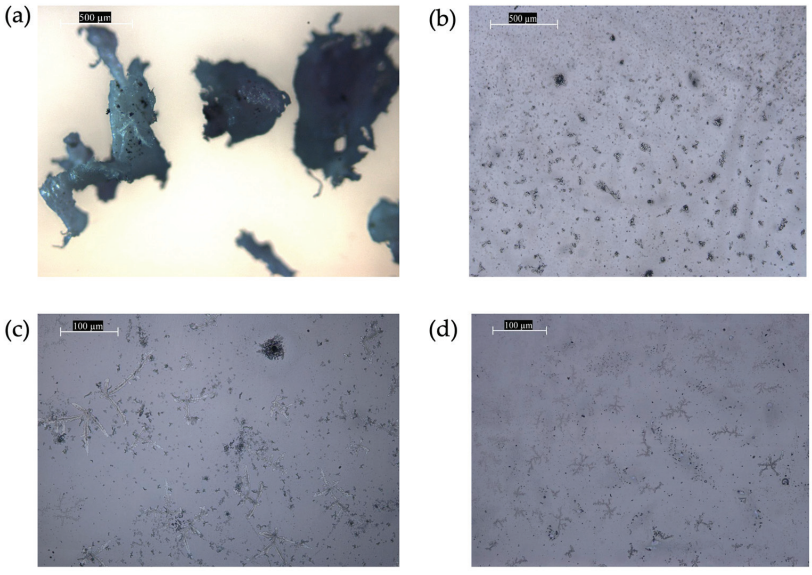

Figure 11. Microphotographs of water samples with PP microparticles of 0.45 mm fraction before and after sorption. (**a**) Model solution before sorption; (**b**) complex (CSM + ion exchange resins); (**c**) zeolite; (**d**) CSM.

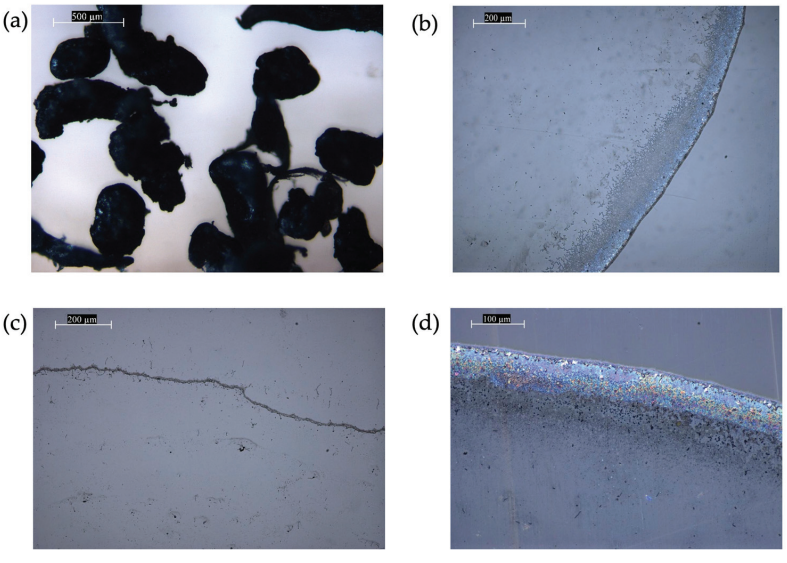

Figure 12. Microphotographs of water samples with 0.45 mm PE microparticles before and after sorption. (**a**) Model solution before sorption; (**b**) complex (CSM + ion exchange resins); (**c**) zeolite; (**d**) CSM.

Optical scanning microscope studies revealed that in all solutions filtered through the sorbents, there was virtually no microplastic content of any fraction. The utilization of zeolite for microplastic retention at filter stations in the Kazakhstani drinking water treatment system can be justified due to its high efficiency in retaining microplastic particles (PP, PE, PET), ranging from 90.4% to 92.8%. However, the denser structure of zeolite, as

opposed to the uneven and rough surface of highly porous CSM, as established by scanning microscopy (Figure 13), demonstrates lower microplastic retention. Specifically, the average efficiency of PP retention on zeolite is 91.3%, while on CSM it is 95.1%.

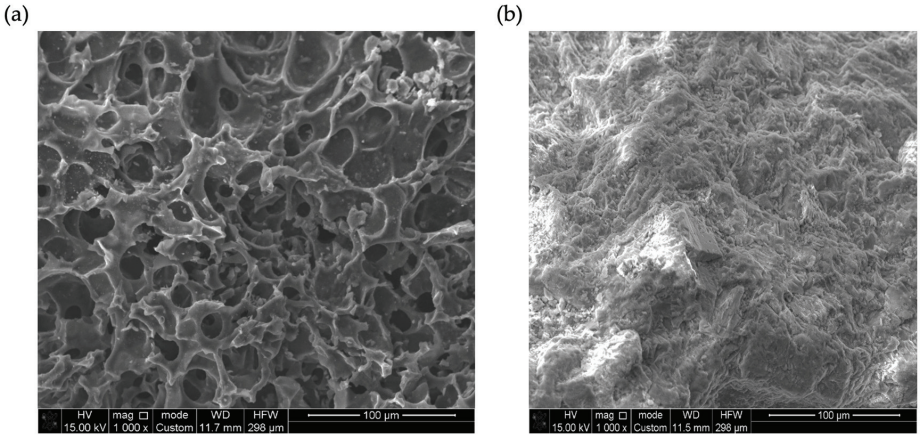

Figure 13. Electronic images of CSM structure (**a**) and zeolite structure (**b**).

The hydrophobic structure of CSM, with a high specific surface area (475 m^2/g) and its porosity (specific pore volume—0.0642 cm^3/g) create significantly more potential sites for the sorption of inert microplastics in comparison with zeolite (specific surface area—7 m^2/g, specific pore volume—0.0091 cm^3/g) [96,97].

The addition of ion exchange resins to the CSM diminishes its sorption capacity against all types and sizes of microplastics. It is likely that ion exchange sorbents are not effective against inert microplastic particles. Furthermore, ion exchange resins compete with microplastic particles during sorption on free cavity carbon sorbent (CSM). Consequently, the average efficiency on the mixed sorbent averages 88.1%, decreasing with decreasing microplastic fraction size from 89.8% (for 0.45 mm particles) to 86.7% (for 0.105 mm particles).

The highest degree of retention of PE particles was observed, with an average efficiency of sorption on the three sorbents reaching 93.7%. Following PE, PP exhibited the next-highest retention, with an average sorption efficiency on the three sorbents at 91.2%. PET showed a lesser degree of sorption retention (89.6%). These results may suggest the predominance of hydrophobic effects in the sorption of aliphatic compounds (PE, PP) over π–π donor–acceptor interactions, which are characteristic in the case of carbon affinity for compounds containing an aromatic ring (PET). Thus, based on their ability to retain microplastic particles, the studied sorbents can be ranked in the following order, according to the degree of efficiency reduction: carbon sorbent (CSM) > zeolite > complex (CSM + ion exchange resins).

The efficiency of drinking water treatment from microplastics should also ensure the improvement of the treated water quality according to standardized indicators, generally accepted in the world and Kazakhstan practice. This primarily concerns the content of suspended substances of organic and inorganic nature capable of sorbing microplastic particles, as determined in the study by color, turbidity, and oxidizability. The results of the study on the effect of selected sorbents on the physicochemical parameters of water—color, turbidity, and acidity—as well as on the ability of these sorption processes to alter the acid–base balance of treated water are presented in Table 8.

Table 8. Results of physicochemical analysis of water before and after sorption.

Sample No.	Color (Degrees)			Turbidity (mg/dm^3)			pH			Oxidization (mg/dm^3)		
	Initial	Final	EFFICIENCY (%)	Initial	Final	Efficiency (%)	Initial	Final	Efficiency (%)	Initial	Final	Efficiency (%)
Sorbent 1—Zeolite												
1	10	1	90.0	1.87	1.41	24.6	8.05	7.52	6.5	132.0	0.67	99.5
2	9	0	90.0	1.87	1.43	23.5	7.96	7.53	5.4	132.8	0.70	99.5
3	10	1	90.0	1.90	1.51	20.5	8.00	7.53	5.8	132.7	0.60	99.5
Sorbent 2—CSM												
1	10	0	100.0	1.87	0.50	73.3	8.05	7.38	8.3	132.0	0.32	99.8
2	9.0	0.0	100.0	1.87	0.46	75.4	7.96	7.38	7.2	132.8	0.25	99.8
3	10	0	100.0	1.90	0.48	74.7	8.00	7.39	7.6	132.7	0.30	99.8
Sorbent 3—(CSM + ion exchange resins)												
1	10	2	80.0	1.87	0.77	58.8	8.05	7.63	5.2	132.0	4.32	96.7
2	9	2	77.7	1.87	0.73	61.0	7.96	7.62	4.2	132.8	4.40	96.7
3	10	2	80.0	1.90	0.72	62.1	8.00	7.60	5.0	132.7	4.25	96.8

As evident from the results presented in Table 7, the sorption of suspended organic and inorganic impurities on the studied sorbents results in a reduction in water turbidity, a shift in pH towards a more neutral environment, and an improvement in color and acidity parameters.

The investigated sorbents exhibited the highest efficiency with respect to acidity and chromaticity. The average efficiency of sorbents in terms of water acidification was 99.8% for CSM, 99.5% for zeolite, and 96.7% for the complex (CSM + ion exchange resins).

Regarding the water color index, the average efficiency of sorbents was 100% for CSM, 90.0% for zeolite, and 79.2% for the complex (CSM + ion exchange resins). Additionally, the average efficiency of sorbents on the water turbidity index was 74.5% for CSM, 60.6% for the complex (CSM + ion exchange resins), and 22.9% for zeolite.

Furthermore, sorption on all sorbents resulted in a shift in pH of treated water towards a more neutral value, averaging 4.2–8.3%.

Our synthesized sorbent, CSM, demonstrated the highest efficiency among the investigated sorbents, both for microplastic particles (PP, PE, PET) and concerning all investigated water parameters (color, acidity, turbidity). Therefore, it can be recommended as an effective sorbent for treatment facilities in cities of Kazakhstan and in household filters for drinking water purification.

It is difficult to compare the sorption efficiency of microplastics in this study with other published works, due to the impossibility of ensuring equality of experimental conditions (types and shapes of microplastics, their size, and concentration). However, based on the efficiency value (%), it can be argued that the carbon sorption material we synthesized shows better results than new sorbents such as organic sponge materials (81.2% at pH 6–9) [98]. Modification of biochar adsorbents, Mg, and Zn demonstrates high efficiency rates (97.7%) against high-concentration polystyrene (0.1 g/mL) [99]. Synthesized Zn–Al layered double hydroxide shows high sorption efficiency (100%) at very low pH values (pH = 4), which are not found in natural and tap waters. Increasing the pH to 9 leads to a decrease in the efficiency of polystyrene sorption to 37% [100], which is significantly lower than the results obtained in our study.

A combination of sorption processes based on carbon sorption material and zeolite with microfiltration can improve the efficiency of removing microplastic particles. Complex methods of water purification, including carbon sorbents, were tested in [49] and showed sorption efficiency of PVC and PET (with particle sizes of 30–1000 microns) of 78–86% and 94–100%, respectively.

However, in the conditions of Kazakhstan, the use of physical barriers, such as membrane filters with a pore size of less than 1 micron, is unlikely in the near future due to their high cost of implementation. That is why our further research on purifying drinking

water from microplastics will relate to increasing the efficiency of existing water treatment technologies to minimize enterprise costs for their implementation.

4. Conclusions

The assessment of microplastic content in tap drinking water and its potential retention by the water treatment system in Kazakhstan is critical, particularly given that the centralized water supply is the main source of drinking water for the population. In our study, microplastic was detected in all drinking water samples, regardless of the service life of water pipes. The concentrations ranged from 2.0×10^{-2} to 6.0×10^{-2} particles/dm^3, with fibers being the predominant form (74.1%), followed by fragments (22.2%) and films (3.7%). These findings align with internationally published data.

The need to remove microplastics from water and improve the physical and chemical parameters of treated water prompted the search for new technologies and materials in the water treatment system. Analysis revealed that outdated technologies and noncompliance with treatment regimens result in the presence of microplastics in tap water samples of Kokshetau city and poor water quality in terms of turbidity (87% of samples), color (40% of samples), and acidity (20% of samples).

To address this, we studied the efficiency of sorption of microplastics and other pollutants on various sorbents. The results showed high retention of microplastic particles by the investigated sorbents (82.7–97.8%). Sorbents with larger pores and hydrophobic interactions exhibited greater retention efficiency. Specifically, aliphatic structures, such as PE and PP, showed higher retention (93.7% for PE, 91.2% for PP) compared to PET (89.6%), which has a more complicated structure.

The investigated sorbents were ranked based on their ability to retain microplastic particles: carbon sorbent (CSM) > zeolite > (CSM + ionites). Moreover, the sorption of suspended impurities on the studied sorbents led to a reduction in water turbidity, pH adjustment towards neutrality, and improvement in color and acidity parameters.

Among the studied sorbents, our synthesized sorbent, CSM, demonstrated the highest efficiency in retaining microplastic particles and improving water parameters. Therefore, it can be recommended for use in treatment facilities across cities in Kazakhstan and in household filters for drinking water purification.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w16101363/s1. Figure S1: FTIR spectrum of Polyamide fiber (experimental sample); Figure S2: Identification of Polyamide fiber by FTIR spectrum of library databases; Figure S3: FTIR spectrum of Acryl fiber (experimental sample); Figure S4: Identification of Acryl fiber by FTIR spectrum of library databases; Figure S5: FTIR spectrum of Viscose fiber, Bember (experimental sample); Figure S6: Identification of Viscose fiber (Bember) by FTIR spectrum of library databases; Figure S7: FTIR spectrum of polyethylene terephthalate (experimental sample); Figure S8: Identification of polyethylene terephthalate by FTIR spectrum of library databases; Figure S9: FTIR spectrum of polystyrene (experimental sample); Figure S10: Identification of polystyrene by FTIR spectrum of library databases; Figure S11: FTIR spectrum of polyimide film Kapton, (experimental sample); Figure S12: Identification of polyimide film (Kapton) by FTIR spectrum of library databases; Figure S13: FTIR spectrum of polypropylene (experimental sample); Figure S14: Identification of polypropylene by FTIR spectrum of library databases; Figure S15: FTIR spectrum of polypropylene (experimental sample); Figure S16: FTIR spectrum of polyethylene (experimental sample); Figure S17: FTIR spectrum of polyethylene terephthalate (experimental sample); Figure S18: Identification of polyethylene terephthalate by PET FTIR spectrum of library databases.

Author Contributions: Conceptualization, N.S.S. and J.R.-I.; methodology, N.S.S.; software, J.R.-I. and M.-E.R.-C.; validation, N.S.S.; formal analysis, N.S.S. and J.R.-I.; investigation, N.S.S. and J.R.-I.; resources, N.S.S. and J.R.-I.; data curation, N.S.S., A.R.K., K.K.A. and G.A.K.; writing—original draft preparation, N.S.S. and J.R.-I.; writing—review and editing, J.R.-I. and M.-E.R.-C.; visualization, J.R.-I. and M.-E.R.-C.; supervision, N.S.S. and J.R.-I.; project administration, N.S.S.; funding acquisition, N.S.S. and J.R.-I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan through the research project "Health risk modelling based on the identification of microplastics in water systems and the reasoning about actions to manage the water resources quality" (grant AP14869081).

Data Availability Statement: Data is contained within the article and Supplementary Material.

Acknowledgments: The authors thank Seythan Azat, Director of the Engineering Profile Laboratory at Satbayev University for providing the laboratory for sorption research, and Nurbol Appazov, Director of the Institute of Chemical Research and Technology at Korkyt Ata Kyzylorda University, for his leadership in the quantitative analysis of microplastics using FTIR analysis.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ma, H.; Chao, L.; Wan, H.; Zhu, Q. Microplastic Pollution in Water Systems: Characteristics and Control Methods. *Diversity* **2024**, *16*, 70. [CrossRef]
2. D'avignon, G.; Gregory-Eaves, I.; Ricciardi, A. Microplastics in lakes and rivers: An issue of emerging significance to limnology. *Environ. Rev.* **2022**, *30*, 228–244. [CrossRef]
3. He, D.; Luo, Y.; Lu, S.; Liu, M.; Song, Y.; Lei, L. Microplastics in soils: Analytical methods, pollution characteristics and ecological risks. *TrAC Trends Anal. Chem.* **2018**, *109*, 163–172. [CrossRef]
4. Wang, C.; Tang, J.; Yu, H.; Wang, Y.; Li, H.; Xu, S.; Li, G.; Zhou, Q. Microplastic Pollution in the Soil Environment: Characteristics, Influencing Factors, and Risks. *Sustainability* **2022**, *14*, 13405. [CrossRef]
5. Habibi, N.; Uddin, S.; Fowler, S.W.; Behbehani, M. Microplastics in the atmosphere: A review. *J. Environ. Exp. Asses.* **2022**, *1*, 6. [CrossRef]
6. Abad López, A.P.; Trilleras, J.; Arana, V.A.; García-Alzate, L.S.; Grande-Tovar, C.D. Atmospheric microplastics: Exposure, toxicity, and detrimental health effects. *RSC Adv.* **2023**, *13*, 7468–7489. [CrossRef] [PubMed]
7. Sun, J.; Dai, X.; Wang, Q.; van Loosdrecht, M.C.M.; Ni, B.J. Microplastics in wastewater treatment plants: Detection, occurrence and removal. *Water Res.* **2019**, *152*, 21–37. [CrossRef] [PubMed]
8. Calero, M.; Martín-Lara, M.A.; Godoy, V.; Quesada, L.; Martínez, D.; Peula, F.; Soto, J.M. Characterization of plastic materials present in municipal solid waste: Preliminary study for their mechanical recycling. *Detritus* **2018**, *4*, 104. [CrossRef]
9. Mintenig, S.M.; Int-Veen, I.; Löder, M.G.J.; Primpke, S.; Gerdts, G. Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. *Water Res.* **2017**, *108*, 365–372. [CrossRef]
10. Pivokonsky, M.; Cermakova, L.; Novotna, K.; Peer, P.; Cajthaml, T.; Janda, V. Occurrence of microplastics in raw and treated drinking water. *Sci. Total Environ.* **2018**, *643*, 1644–1651. [CrossRef]
11. Mintenig, S.M.; Löder, M.G.J.; Primpke, S.; Gerdts, G. Low numbers of microplastics detected in drinking water from ground water sources. *Sci. Total Environ.* **2019**, *648*, 631–635. [CrossRef] [PubMed]
12. Uhl, W.; Eftekhardadkhah, M.; Svendsen, C. Mapping Microplastic in Norwegian Drinking Water. Available online: www.norskvann.no (accessed on 11 April 2024).
13. Zuccarello, P.; Ferrante, M.; Cristaldi, A.; Copat, C.; Grasso, A.; Sangregorio, D.; Fiore, M.; Oliveri Conti, G. Exposure to microplastics (<10 μm) associated to plastic bottles mineral water consumption: The first quantitative study. *Water Res.* **2019**, *157*, 365–371. [CrossRef]
14. Vega-Herrera, A.; Llorca, M.; Borrel-Diaz, X.; Redondo-Hasselerharm, P.E.; Abad, E.; Villanueva, C.M.; Farré, M. Polymers of micro(nano) plastic in household tap water of the Barcelona Metropolitan Area. *Water Res.* **2022**, *220*, 118645. [CrossRef] [PubMed]
15. Oßmann, B.E.; Sarau, G.; Holtmannspötter, H.; Pischetsrieder, M.; Christiansen, S.H.; Dicke, W. Small-sized microplastics and pigmented particles in bottled mineral water. *Water Res.* **2018**, *141*, 307–316. [CrossRef]
16. Negrete Velasco, A.; Ramseier Gentile, S.; Zimmermann, S.; Le Coustumer, P.; Stoll, S. Contamination and removal efficiency of microplastics and synthetic fibres in a conventional drinking water treatment plant in Geneva, Switzerland. *Sci. Total Environ.* **2023**, *880*, 163270. [CrossRef]
17. Mohammadi, A.; Dobaradaran, S.; Schmidt, T.C.; Malakootian, M.; Spitz, J. Emerging contaminants migration from pipes used in drinking water distribution systems: A review of the scientific literature. *Environ. Sci. Pollut. Res.* **2022**, *29*, 75134–75160. [CrossRef]
18. Lam, T.W.L.; Ho, H.T.; Ma, A.T.H.; Fok, L. Microplastic Contamination of Surface Water-Sourced Tap Water in Hong Kong—A Preliminary Study. *Appl. Sci.* **2020**, *10*, 3463. [CrossRef]
19. Gambino, I.; Bagordo, F.; Grassi, T.; Panico, A.; De Donno, A. Occurrence of Microplastics in Tap and Bottled Water: Current Knowledge. *Int. J. Environ. Res. Public Health* **2022**, *19*, 5283. [CrossRef]
20. Weber, F.; Kerpen, J.; Wolff, S.; Langer, R.; Eschweiler, V. Investigation of microplastics contamination in drinking water of a German city. *Sci. Total Environ.* **2021**, *755*, 143421. [CrossRef]

21. Gray, A.D.; Weinstein, J.E. Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp (Palaemonetes pugio). *Environ. Toxicol. Chem.* **2017**, *36*, 3074–3080. [CrossRef]
22. Li, J.; Green, C.; Reynolds, A.; Shi, H.; Rotchell, J.M. Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. *Environ. Pollut.* **2018**, *241*, 35–44. [CrossRef]
23. Almaiman, L.; Aljomah, A.; Bineid, M.; Aljeldh, F.M.; Aldawsari, F.; Liebmann, B.; Lomako, I.; Sexlinger, K.; Alarfaj, R. The occurrence and dietary intake related to the presence of microplastics in drinking water in Saudi Arabia. *Environ. Monit. Assess.* **2021**, *193*, 1–13. [CrossRef] [PubMed]
24. Ragusa, A.; Svelato, A.; Santacroce, C.; Catalano, P.; Notarstefano, V.; Carnevali, O.; Papa, F.; Rangioletti, M.C.A.; Baiocco, F.; Draghi, S.; et al. Plasticenta: First evidence of microplastics in human placenta. *Environ. Int.* **2021**, *146*, 106274. [CrossRef]
25. Amato-Lourenço, L.F.; Carvalho-Oliveira, R.; Júnior, G.R.; dos Santos Galvão, L.; Ando, R.A.; Mauad, T. Presence of airborne microplastics in human lung tissue. *J. Hazard. Mater.* **2021**, *416*, 126124. [CrossRef]
26. Farrell, P.; Nelson, K. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). *Environ. Pollut.* **2013**, *177*, 1–3. [CrossRef] [PubMed]
27. Leslie, H.A.; Depledge, M.H. Where is the evidence that human exposure to microplastics is safe? *Environ. Int.* **2020**, *142*, 105807. [CrossRef] [PubMed]
28. Lee, Y.; Cho, J.; Sohn, J.; Kim, C. Health Effects of Microplastic Exposures: Current Issues and Perspectives in South Korea. *Yonsei Med. J.* **2023**, *64*, 301–308. [CrossRef]
29. Jadhav, E.B.; Sankhla, M.S.; Bhat, R.A.; Bhagat, D.S. Microplastics from food packaging: An overview of human consumption, health threats, and alternative solutions. *Environ. Nanotechnol. Monit. Manag.* **2021**, *16*, 100608. [CrossRef]
30. Eriksen, M.; Mason, S.; Wilson, S.; Boz, C.; Zellers, A.; Edwards, W.; Farley, H.; Amato, S. Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Mar. Pollut. Bull.* **2013**, *77*, 177–182. [CrossRef]
31. Nacaratte, F.; Cuevas, P.; Becerra-Herrera, M.; Manzano, C.A. Early screening of suspected microplastics in bottled water in the Santiago Metropolitan Region of Chile. *Environ. Pollut.* **2023**, *334*, 122118. [CrossRef]
32. Yuan, C.; Almuhtaram, H.; McKie, M.J.; Andrews, R.C. Assessment of microplastic sampling and extraction methods for drinking waters. *Chemosphere* **2022**, *286*, 131881. [CrossRef] [PubMed]
33. Horton, A.A.; Svendsen, C.; Williams, R.J.; Spurgeon, D.J.; Lahive, E. Large microplastic particles in sediments of tributaries of the River Thames, UK—Abundance, sources and methods for effective quantification. *Mar. Pollut. Bull.* **2017**, *114*, 218–226. [CrossRef]
34. Environmental Performance Index. Available online: https://epi.yale.edu/epi-results/2022/component/epi (accessed on 11 April 2024).
35. Most Sustainable Countries—U.S. News. Available online: https://www.usnews.com/news/best-countries/most-sustainable-countries (accessed on 11 April 2024).
36. Nurtazin, S.; Pueppke, S.; Ospan, T.; Mukhitdinov, A.; Elebessov, T. Quality of Drinking Water in the Balkhash District of Kazakhstan's Almaty Region. *Water* **2020**, *12*, 392. [CrossRef]
37. Salikova, N.S.; Rodrigo-Ilarri, J.; Alimova, K.K.; Rodrigo-Clavero, M.E. Analysis of the Water Quality of the Ishim River within the Akmola Region (Kazakhstan) Using Hydrochemical Indicators. *Water* **2021**, *13*, 1243. [CrossRef]
38. Salikova, N.S.; Rodrigo-Ilarri, J.; Rodrigo-Clavero, M.E.; Urazbayeva, S.E.; Askarova, A.Z.; Magzhanov, K.M. Environmental Assessment of Microplastic Pollution Induced by Solid Waste Landfills in the Akmola Region (North Kazakhstan). *Water* **2023**, *15*, 2889. [CrossRef]
39. Salikova, N.S.; Rodrigo-Ilarri, J.; Makeyeva, L.A.; Rodrigo-Clavero, M.E.; Tleuova, Z.O.; Makhmutova, A.D. Monitoring of Microplastics in Water and Sediment Samples of Lakes and Rivers of the Akmola Region (Kazakhstan). *Water* **2024**, *16*, 1051. [CrossRef]
40. Chu, X.; Zheng, B.; Li, Z.; Cai, C.; Peng, Z.; Zhao, P.; Tian, Y. Occurrence and distribution of microplastics in water supply systems: In water and pipe scales. *Sci. Total Environ.* **2022**, *803*, 150004. [CrossRef] [PubMed]
41. Wang, Z.; Lin, T.; Chen, W. Occurrence and removal of microplastics in an advanced drinking water treatment plant (ADWTP). *Sci. Total Environ.* **2020**, *700*, 134520. [CrossRef] [PubMed]
42. Acarer, S. Abundance and characteristics of microplastics in drinking water treatment plants, distribution systems, water from refill kiosks, tap waters and bottled waters. *Sci. Total Environ.* **2023**, *884*, 163866. [CrossRef]
43. Kuldeyev, E.I.; Orynbekov, Y.S.; Mansurov, Z.A.; Nurlybayev, R.E.; Zhumadilova, Z.O.; Murzagulova, A.A. Applicability of Zeolite from the Daubabinsk and Chankanai Deposits as a Sorbent for Natural Waters. *Water* **2023**, *15*, 2231. [CrossRef]
44. Sobczyk, M.; Nguyen Dihn, C.; Marzec, M.; Bazarkina, E.; Kvashnina, K.O.; Cwanek, A.; Lokas, E.; Bajda, E. Insights into uranium sequestration by coal fly-ash-derived zeolites: Understanding via wet chemistry, and advanced spectroscopies. *J. Clean Prod.* **2024**, *449*, 141206. [CrossRef]
45. Shilina, A.S.; Bakhtin, V.D.; Burukhin, S.B.; Askhadullin, S.R. Sorption of cations of heavy metals and radionuclides from the aqueous media by new synthetic zeolite-like sorbent. *Nucl. Energy Technol.* **2017**, *3*, 249–254. [CrossRef]
46. Marisela, A.; Franco, M.; Rojas García, E.; Medina, R.L.; Arturo, A.; Ramírez, C. Properties and applications of natural zeolites. *Braz. J. Develop.* **2024**, *10*, 1713–1799. [CrossRef]

47. Shen, M.; Hu, T.; Huang, W.; Song, B.; Zeng, G.; Zhang, Y. Removal of microplastics from wastewater with aluminosilicate filter media and their surfactant-modified products: Performance, mechanism and utilization. *J. Chem. Eng.* **2021**, *421*, 129918. [CrossRef]
48. Spacilova, M.; Dytrych, P.; Lexa, M.; Wimmerova, L.; Masin, P.; Kvacek, R.; Solcova, O. An Innovative Sorption Technology for Removing Microplastics from Wastewater. *Water* **2023**, *15*, 892. [CrossRef]
49. Cherian, A.G.; Liu, Z.; McKie, M.J.; Almuhtaram, H.; Andrews, R.C. Microplastic Removal from Drinking Water Using Point-of-Use Devices. *Polymers* **2023**, *15*, 1331. [CrossRef] [PubMed]
50. *ST RK GOST R 51593-2003*; Drinking Water. Sampling. State Standard of the Republic of Kazakhstan: Republic of Kazakhstan. Available online: https://online.zakon.kz/Document/?doc_id=30015917 (accessed on 11 April 2024).
51. Kondratyeva, E.M. Monitoring of surface waters of the southern Baikal in conditions of increasing anthropogenic load. *Innov. Sci. Educ.* **2021**, *35*, 60–64. Available online: https://elibrary.ru/item.asp?id=46324676 (accessed on 11 April 2024). (In Russian)
52. Oni, B.A.; Sanni, S.E. Occurrence of Microplastics in Borehole Drinking Water and Sediments in Lagos, Nigeria. *Environ. Toxicol. Chem.* **2022**, *41*, 1721–1731. [CrossRef] [PubMed]
53. Mattsson, K.; da Silva, V.H.; Deonarine, A.; Louie, S.M.; Gondikas, A. Monitoring anthropogenic particles in the environment: Recent developments and remaining challenges at the forefront of analytical methods. *Curr. Opin. Colloid Interface Sci.* **2021**, *56*, 101513. [CrossRef]
54. Shi, H.; Wang, X.; Zhu, L.; Li, D. Comprehensive Comparison of Various Microplastic Sampling Methods in Sea Water: Implications for Data Compilation. *Water* **2023**, *15*, 1035. [CrossRef]
55. Monteiro, S.S.; Rocha-Santos, T.; Prata, J.C.; Duarte, A.C.; Girao, A.V.; Lopes, P.; Cristovao, T.; da Costa, J.P. A straightforward method for microplastic extraction from organic-rich freshwater samples. *Sci. Total Environ.* **2022**, *815*, 152941. [CrossRef] [PubMed]
56. Munno, K.; Helm, P.A.; Jackson, D.A.; Rochman, C.; Sims, A. Impacts of temperature and selected chemical digestion methods on microplastic particles. *Environ. Toxicol. Chem.* **2018**, *37*, 91–98. [CrossRef] [PubMed]
57. Tirkey, A.; Upadhyay, L.S.B. Microplastics: An overview on separation, identification and characterization of microplastics. *Mar. Pollut. Bull.* **2021**, *170*, 112604. [CrossRef] [PubMed]
58. Faure, F.; Demars, C.; Wieser, O.; Kunz, M.; De Alencastro, L.F. Plastic pollution in Swiss surface waters: Nature and concentrations, interaction with pollutants. *Environ. Chem.* **2015**, *12*, 582–591. [CrossRef]
59. Cole, M.; Webb, H.; Lindeque, P.K.; Fileman, E.S.; Halsband, C.; Galloway, T.S. Isolation of microplastics in biota-rich seawater samples and marine organisms. *Sci. Rep.* **2014**, *4*, 1–8. [CrossRef] [PubMed]
60. Mendoza, L.M.R.; Balcer, M. Microplastics in freshwater environments: A review of quantification assessment. *TrAC Trends Anal. Chem.* **2019**, *113*, 402–408. [CrossRef]
61. Aminah, I.S.; Ikejima, K. Potential sources of microplastic contamination in laboratory analysis and a protocol for minimising contamination. *Environ. Monit. Assess.* **2023**, *195*, 1–9. [CrossRef] [PubMed]
62. Wesch, C.; Elert, A.M.; Wörner, M.; Braun, U.; Klein, R.; Paulus, M. Assuring quality in microplastic monitoring: About the value of clean-air devices as essentials for verified data. *Sci. Rep.* **2017**, *7*, 1–8. [CrossRef] [PubMed]
63. Hermsen, E.; Pompe, R.; Besseling, E.; Koelmans, A.A. Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. *Mar. Pollut. Bull.* **2017**, *122*, 253–258. [CrossRef]
64. Interstate Standard 31868-2012; Water. *Interstate Standard 31868-2012*; Water. Methods for Determining Color. Interstate Council for Standardization, Metrology and Certification: Moscow, Russia, 2019; p. 12. Available online: https://docs.cntd.ru/document/1200097407 (accessed on 31 March 2024).
65. *Interstate Standard 3351-74*; Drinking Water. Method for Determination of Odour, Taste, Color and Turbidity. Publishing House of Standards: Moscow, Russia, 2003; p. 8. Available online: https://meganorm.ru/Index2/1/4294850/4294850608.htm (accessed on 31 March 2024).
66. *State Mandatory Standard 26449.1-85*; Stationary Distillation and Desalination Plants. Methods for Chemical Analysis of Salt Waters. USSR State Committee on Standards: Moscow, Russia, 1985; p. 101. Available online: https://files.stroyinf.ru/Index2/1/4294850/4294850335.htm (accessed on 31 March 2024).
67. *ISO Standard 4316-2019*; Surfactants. Determination of pH of Aqueous Solutions. Potentiometric Method. Technical Committee ISO/TC91. International Organization for Standardization: Geneva, Switzerland, 1977. Available online: https://online.zakon.kz/Document/?doc_id=33823182&pos=30;-10#pos=30;-10 (accessed on 31 March 2024).
68. Kuldeyev, E.; Seitzhanova, M.; Tanirbergenova, S.; Tazhu, K.; Doszhanov, E.; Mansurov, Z.; Azat, S.; Nurlybaev, R.; Berndtsson, R. Modifying Natural Zeolites to Improve Heavy Metal Adsorption. *Water* **2023**, *15*, 2215. [CrossRef]
69. Tauanov, Z.; Azat, S.; Baibatyrova, A. A mini-review on coal fly ash properties, utilization and synthesis of zeolites. *Int. J. Coal Prep. Util.* **2022**, *42*, 1968–1990. [CrossRef]
70. Krstić, V. Role of zeolite adsorbent in water treatment. In *Handbook of Nanomaterials for Wastewater Treatment: Fundamentals and Scale up Issues*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 417–481. [CrossRef]
71. Calmon, C. Recent developments in water treatment by ion exchange. *React. Polym. Ion Exch. Sorbents* **1986**, *4*, 131–146. [CrossRef]
72. Sinha, P.; Datar, A.; Jeong, C.; Deng, X.; Chung, Y.G.; Lin, L.-C. Surface Area Determination of Porous Materials Using the Brunauer–Emmett–Teller (BET) Method: Limitations and Improvements. *J. Phys. Chem. C* **2019**, *123*, 20195–20209. [CrossRef]

73. Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan. Available online: https://old.stat.gov.kz/for_users/ecologic_indicators/ecologic_indicator/water_consumption (accessed on 11 April 2024).
74. Salikova, N.S.; Alimova, K.K. *Communal, Domestic and Industrial Water Supply in Kazakhstan: Interim Report on the Project IRN AP14869081 "Modelling of Health Risks Based on Identification of Microplastics in Water Systems and Justification of Actions to Manage the Quality of Water Resources"*; Abay Myrzakhmetov Kokshetau University: Kokshetau, Kazakhstan, 2022; 57p. Available online: https://kuam.edu.kz/sites/default/files/2022/salikova_n.s._alimova_k.k._communal_domestic_and_industrial_water_supply_in_kazakhstan._interim_report_on_the_project_irn_ap14869081.pdf (accessed on 1 May 2024).
75. Order of the Minister of Health of the Republic of Kazakhstan from 20 February 2023 №26 'Sanitary and Epidemiological Requirements for Water Sources, Places of Water Intake for Household and Drinking Purposes, Household and Drinking Water Supply and Places of Cultural and Domestic Water Use and Safety of Water Bodies'. 2023. Available online: https://adilet.zan.kz/rus/docs/V2300031934 (accessed on 11 April 2024).
76. Miao, M.; Liu, J.; Dou, Y.; Hao, H.; Cheng, X.; Zhang, M.; Li, Y. Effects of microplastics on DBPs formation under the chlorination of natural organic matters. *Chemosphere* **2022**, *296*, 134067. [CrossRef] [PubMed]
77. Miao, M.; Yu, B.; Cheng, X.; Hao, T.; Dou, Y.; Zhang, M.; Li, Y. Effects of chlorination on microplastics pollution: Physicochemical transformation and chromium adsorption. *Environ. Pollut.* **2023**, *323*, 121254. [CrossRef] [PubMed]
78. Rullander, G.; Lorenz, C.; Herbert, R.B.; Strömvall, A.-M.; Vollertsen, J.; Dalahmeh, S.S. How effective is the retention of microplastics in horizontal flow sand filters treating stormwater? *J. Environ. Manag.* **2023**, *344*, 118690. [CrossRef] [PubMed]
79. Azizi, N.; Pirsaheb, M.; Jaafarzadeh, N.; Nodehi, R.N. Microplastics removal from aquatic environment by coagulation: Selecting the best coagulant based on variables determined from a systematic review. *Heliyon* **2023**, *9*, e15664. [CrossRef] [PubMed]
80. Lange, K.; Magnusson, K.; Viklander, M.; Blecken, G.-T. Removal of rubber, bitumen and other microplastic particles from stormwater by a gross pollutant trap—bioretention treatment train. *Water Res.* **2021**, *202*, 117457. [CrossRef]
81. Prasad, K.Y. Sedimentation in Water and Used Water Purification. In *Handbook of Water and Used Water Purification*; Springer: Cham, Germany, 2019; pp. 1–27. [CrossRef]
82. Ziembowicz, S.; Kida, M.; Koszelnik, P. Efficient removal of polyethylene and polyvinyl chloride microplastics from water using a modified coagulation process supported by the addition of a surfactant. *Desalination Water Treat.* **2023**, *288*, 51–59. [CrossRef]
83. Zhang, Y.; Diehl, A.; Lewandowski, A.; Gopalakrishnan, K.; Baker, T. Removal efficiency of micro- and nanoplastics (180 nm–125 μm) during drinking water treatment. *Sci. Total Environ.* **2020**, *720*, 137383. [CrossRef]
84. Na, S.-H.; Kim, M.-J.; Kim, J.-T.; Jeong, S.; Lee, S.; Chung, J.; Kim, E.-J. Microplastic removal in conventional drinking water treatment processes: Performance, mechanism, and potential risk. *Water Res.* **2021**, *202*, 117417. [CrossRef] [PubMed]
85. Hossain, M.B.; Yu, J.; Banik, P.; Noman, A.; Nur, A.-A.U.; Haque, R.; Rahman, M.; Albeshr, M.F.; Arai, T. First evidence of microplastics and their characterization in bottled drinking water from a developing country. *Front. Environ. Sci.* **2023**, *11*, 1232931. [CrossRef]
86. Li, L.; Quinlivan, P.A.; Knappe, D.R. Effects of activated carbon surface chemistry and pore structure on the adsorption of organic contaminants from aqueous solution. *Carbon* **2002**, *40*, 2085–2100. [CrossRef]
87. Nguyen, T.H.; Cho, H.H.; Poster, D.L.; Ball, W.P. Evidence for a pore-filling mechanism in the adsorption of aromatic hydrocarbons to a natural wood char. *Environ. Sci. Technol.* **2007**, *41*, 1212–1217. [CrossRef] [PubMed]
88. Wang, Z.; Sun, C.; Li, F.; Chen, L. Fatigue resistance, re-usable and biodegradable sponge materials from plant protein with rapid water adsorption capacity for microplastics removal. *Chem. Eng. J.* **2021**, *415*, 129006. [CrossRef]
89. Wang, J.; Sun, C.; Huang, Q.-X.; Chi, Y.; Yan, J.-H. Adsorption and thermal degradation of microplastics from aqueous solutions by Mg/Zn modified magnetic biochars. *J. Hazard. Mater.* **2021**, *419*, 126486. [CrossRef] [PubMed]
90. Tiwari, E.; Singh, N.; Khandelwal, N.; Monikh, F.A.; Darbha, G.K. Application of Zn/Al layered double hydroxides for the removal of nano-scale plastic debris from aqueous systems. *J. Hazard. Mater.* **2020**, *397*, 122769. [CrossRef] [PubMed]
91. Dalmau-Soler, J.; Ballesteros-Cano, R.; Ferrer, N.; Boleda, M.R.; Lacorte, S. Microplastics throughout a tap water supply network. *Water Environ. J.* **2022**, *36*, 292–298. [CrossRef]
92. Taghipour, H.; Ghayebzadeh, M.; Ganji, F.; Mousavi, S.; Azizi, N. Tracking microplastics contamination in drinking water in Zahedan, Iran: From source to consumption taps. *Sci. Total Environ.* **2023**, *872*, 162121. [CrossRef]
93. Shruti, V.; Pérez-Guevara, F.; Kutralam-Muniasamy, G. Metro station free drinking water fountain- A potential "microplastics hotspot" for human consumption. *Environ. Pollut.* **2020**, *261*, 114227. [CrossRef]
94. Johnson, A.C.; Ball, H.; Cross, R.; Horton, A.A.; Jürgens, M.D.; Read, D.S.; Vollertsen, J.; Svendsen, C. Identification and Quantification of Microplastics in Potable Water and Their Sources within Water Treatment Works in England and Wales. *Environ. Sci. Technol.* **2020**, *54*, 12326–12334. [CrossRef] [PubMed]
95. Kuptsov, A.; Zhizhin, G. *Fourier-Raman and Fourier-IR Spectra of Polymers*; Fizmatlit: Moscow, Russia, 2001; 657p. Available online: https://www.researchgate.net/publication/262416516_Kupcov_AH_Zizin_GN_Fure-KR_i_Fure-IK_spektry_polimerov_m_Fizmatlit_2001_657_s/references (accessed on 1 May 2024).
96. Pittroff, M.; Mueller, Y.K.; Witzig, C.S.; Scheurer, M.; Storck, F.R.; Zumbuelte, N. Microplastic analysis in drinking water based on fractionated filtration sampling and Raman microspectroscopy. *Environ. Sci. Pollut. Res.* **2021**, *28*, 59439–59451. [CrossRef] [PubMed]
97. Zhou, G.; Wu, Q.; Wei, X.-F.; Chen, C.; Ma, J.; Crittenden, J.C.; Liu, B. Tracing microplastics in rural drinking water in Chongqing, China: Their presence and pathways from source to tap. *J. Hazard. Mater.* **2023**, *459*, 132206. [CrossRef] [PubMed]

98. Ramaremisa, G.; Tutu, H.; Saad, D. Detection and characterisation of microplastics in tap water from Gauteng, South Africa. *Chemosphere* **2024**, 141903. [CrossRef]
99. Strand, J.; Feld, L.; Murphy, F.; Mackevica, A.; Hartmann, N.B. *Analysis of Microplastic Particles in Danish Drinking Water*; APA: Aarhus, Denmark, 2018.
100. Mukotaka, A.; Kataoka, T.; Nihei, Y. Rapid analytical method for characterization and quantification of microplastics in tap water using a Fourier-transform infrared microscope. *Sci. Total Environ.* **2021**, *790*, 148231. [CrossRef]

water

MDPI

Review

Microplastics in Groundwater: Pathways, Occurrence, and Monitoring Challenges

Elvira Colmenarejo Calero [1,*], Manca Kovač Viršek [1,2] and Nina Mali [1]

[1] Geological Survey of Slovenia, Dimičeva 14, 1000 Ljubljana, Slovenia; manca.kovacvirsek@geo-zs.si or manca.virsek@izvrs.si (M.K.V.); nina.mali@geo-zs.si (N.M.)
[2] Institute for Water of the Republic of Slovenia, Einspielerjeva ulica 6, 1000 Ljubljana, Slovenia
* Correspondence: elvira.colmenarejo@geo-zs.si

Abstract: Microplastics (MPs), defined as plastic particles measuring less than 5 mm, are considered an emerging pollutant. Their presence in the water cycle and their interaction with ecological processes pose a significant environmental threat. As groundwater (GW) represents the primary source of drinking water, monitoring MPs in GW and investigating their potential sources and pathways is of urgent importance. This article offers a comprehensive overview of the primary contamination pathways of MPs from surface water, seawater, and soil into the GW. Moreover, it presents an examination of the occurrence of MPs in GW and identifies the challenges associated with their monitoring in GW. This study also discusses the difficulties associated with comparing research results related to MPs in GW, as well as indicating the need for implementing standardised techniques for their sampling and detection. On the basis of our experience and the literature review, we highlight the importance of understanding the specific hydrogeological and hydrogeographic conditions, collecting representative samples, using sampling devices with comparable specifications and comparable laboratory techniques for MP identification, and preventing contamination at all stages of the monitoring process. This review offers valuable insights and practical guidelines on how to improve the reliability and comparability of results between studies monitoring MPs in GW.

Keywords: microplastics; groundwater; sampling; monitoring; aquifer; borehole

Citation: Colmenarejo Calero, E.; Kovač Viršek, M.; Mali, N. Microplastics in Groundwater: Pathways, Occurrence, and Monitoring Challenges. *Water* **2024**, *16*, 1228. https://doi.org/10.3390/w16091228

Academic Editors: Grigorios L. Kyriakopoulos, Antonis A. Zorpas, María Rocío Rodríguez Barroso and Vassilis J. Inglezakis

Received: 15 March 2024
Revised: 21 April 2024
Accepted: 22 April 2024
Published: 25 April 2024

1. Introduction

In recent decades, the use of plastics has increased to such an extent that they have become indispensable to society [1,2]. In 2022, the global production of plastics reached 400.3 million tonnes, with Europe contributing 14% to this total [3]. While only a small proportion of the plastics produced is recycled or incinerated, the majority is accumulated in natural environments or landfills [4]. MPs have attracted considerable attention as an emerging pollutant and have been recognised as a key contributor to environmental pollution [5–7].

MPs are water-insoluble, solid polymer particles characterised by a size of less than 5 mm. Although the lower limit has not yet been formally defined, particles under 1 μm in size are considered nanoparticles [8]. They differ in terms of their chemical composition, colour, shape, density, size, and other characteristics [9]. The most commonly produced polymer types, according to their chemical composition, are polypropylene (PP; 18.9%), low-density polyethylene (PE-LD; 14.1%), polyvinyl chloride (PVC; 12.7%), high-density polyethylene (PE-HD; 12.2%), polyethylene terephthalate (PET; 6.2%), polyurethane (PUR; 5.3%), and polystyrene (PS; 5.2%) [3]. While there is no standardised method for categorising particles based on their shape [10], some common shapes include fibres, filaments, spheres, fragments, beads, films, and pellets [10,11].

Based on their source, MPs can also be categorised into primary and secondary MPs. Primary MPs are tiny plastic particles released into the environment through the

erosion of products during their use [12,13], e.g., textile fibres and purposely manufactured particles such as microbeads used in cosmetics and medical products, as well as plastic pellets used for the production of plastics [14]. Secondary MPs are MPs resulting from fragmentation due to the weathering of larger plastics when these are released into the environment [13,15].

Although plastic particles smaller than 5 mm had been reported in earlier publications [16,17], the term "microplastics (MPs)" was coined by Thomson et al. [18]. At a workshop organised by NOAA in 2008 [19], the definition of MPs was expanded to include "plastic particles smaller than 5 mm", without specifying a lower size limit [20].

MPs are present in atmospheric, terrestrial, and aquatic environments [21–28] and thus also in the water cycle [29]. Due to their presence in the environment and the threat they pose to the ecosystem [30], MPs have received considerable attention as newly emerging pollutants [5,6,31,32].

In the environment, primary and secondary MP particles may enter GW via various pathways, e.g., via seawater, rivers, or lakes (GW interaction through the hyporheic zone), or by penetration through soil pores [29,33,34]. To gain a better understanding of these interactions, it is important to compare the presence of MPs in different aquifers under different environmental conditions [35].

GW represents the most important freshwater reservoir on our planet [36,37]. Despite numerous protective measures, the quality of GW sources has been threatened for decades by various contaminants and substandard pumping practices. Rapid population growth and urbanisation of rural areas have intensified MP contamination [38] and pressure on GW systems, leading to impaired water quality [39]. These factors carry far-reaching environmental, economic, and social implications [40].

On the environmental level, MPs in GW ecosystems can pose a threat to the stygofauna, which plays a crucial role in removing pathogenic organisms from water [41]. Although there is a lack of research on the effects of MPs on organisms in GW, studies have found that MPs can be harmful to aquatic creatures, as MPs can be ingested or accumulated in the organism's tissues, affecting their reproductivity, growth, and survival [42]. The impact of MPs varies depending on their characteristics, concentration levels, and duration of exposure [43]. Therefore, monitoring MPs in GW to assess the risk they may pose to the stygofauna is essential.

Due to the presence of MPs in GW, it is crucial to determine the vulnerability of GW to MPs pollution [33]. There is therefore an urgent need to improve our understanding of the mechanisms of MP transport.

This study aims to provide a comprehensive description of the pathway mechanisms for the transport of MPs into GW from rivers, lakes, and seawater, as well as from soil and the unsaturated zone. It presents the findings of previous research on the prevalence and differences of MPs found in GW. The main added value of this literature review is the discussion about the main challenges in monitoring MPs in GW, which is reinforced by our rich experience in this field.

2. Pathways of MPs Transport into the GW

2.1. Transport Mechanisms of MPs to GW from Rivers, Lakes, and Seawater

GW often interacts with surface water and seawater through recharge and discharge. These interactions represent potential pathways for the transport of MPs in GW systems.

MPs can enter rivers and lakes via different sources of pollution, e.g., effluents from wastewater treatment plants (WWTP); sewage sludge; atmospheric deposition; direct public disposal; and runoff from agricultural, recreational, industrial, and urban areas [44,45]. Due to their different origins, these polymers vary in composition, density, shape, and size [46,47]. The physical characteristics of the particles coupled with the hydrodynamic conditions of the open channel flow influence their transport parameters, as they may float or sink depending on these factors [48,49].

Due to their higher surface-to-volume ratio, small MP particles are often subject to an increase in density and size through different processes, such as aggregation with other MP particles, sediments, and organic matter, or the formation of a biofilm on their surface, which in turn affects their transport behaviour [48]. As the density of the particles increases, they tend to settle in the sediments due to the influence of gravity [48–52].

Scherer et al. [26] conducted a study on a river in the German river Elbe and found that the MP content in the sediment was significantly higher than in water. The average concentration of MPs in the sediment was 3,350,000 particles per cubic meter, i.e., 600,000 times higher than the average concentration of 5.57 particles per cubic meter detected in water. This difference is expected to be even greater in lakes due to the lower water flow velocities, which facilitate sedimentation processes [6].

MPs temporarily immobilised in the sediment can be remobilised by high-flow events [53] or by disaggregation of the biofilm formed on their surface. They may be taken up by benthic organisms or transported through the hyporheic zone (HZ) into the GW [54]. This process depends on the relationships between pore diameters and MPs' dimensions [55], particularly in the case of MPs of smaller diameters. Smaller size MPs can move through the pore space from the surface of the streambed into the subsurface layers [48,56]. In a field study conducted by Drummond et al. [48], 23% of MPs were reported to exhibit a hyporheic exchange ratio higher than the sedimentation ratio. This percentage increases to 42% for low-density MPs.

The HZ is an important interface between the stream and the shallow GW system [48]. Its significance lies in the fact that, depending on the situation, water from specific stream sections or the entire river can flow into or out of the local aquifer [57,58]. The transport of MPs through the HZ is conditioned by the properties of the particles, the hydrological and geochemical factors that condition the hyporheic exchange [48,56,57,59,60], including the shape of the streambed, local GW, material heterogeneities in the HZ, and turbulence in the stream [48]. Insight into these factors is crucial for understanding and addressing the problem of MP pollution.

Figure 1 displays the direction of water flow in a channel that either loses or gains water, along with the movement of MPs. When a stream constantly loses water, as shown in Figure 1(1.1,1.2), the water flows through the streambed sediments and reaches the aquifer. As illustrated in Figure 1(1.3), the flow of porewater causes MPs to move into the aquifer and eventually re-enter the water column through hyporheic flow paths [60]. On the other hand, when a stream constantly gains water (Figure 1(1.4)), it flows through the aquifer to the streambed.

Depending on the conditions of the channel, mobile pore-scale MPs in the HZ can be transported by advection from the HZ to the streambed or to the aquifer [56]. Similar mechanisms occur in lakebeds where MPs within sediments can be transported into the GW by continuously moving seeping water [29], which also reflects the bidirectional interaction between the lake and the GW.

Hydraulic gradients along the streambed and lakebed are the key drivers of hyporheic exchange [61,62]. Hydraulic conductivity, in turn, controls the flow rates of hyporheic exchange. If the material below and adjacent to the river channel is saturated (Figure 1(1.1)), the flow rates are controlled by saturated hydraulic conductivity. If the channel is perched above the underlying water table (Figure 1(1.2)), leakage is controlled by unsaturated hydraulic conductivity, which is much lower in value. Therefore, the transport of MPs under these conditions will be slower [57]. Hydraulic conductivity depends on various factors, including the materials present in the sediment [63]. For example, lakebeds retain sediments from the runoff, streamflow, and shoreline erosion. These sediments usually consist of organic matter, have a finer texture, and are less permeable; thus, they limit the rates and locations of the exchange [64]. Nevertheless, numerous studies have shown that there is still considerable interconnectivity between the two systems, even when sediments have low hydraulic conductivity [65–67].

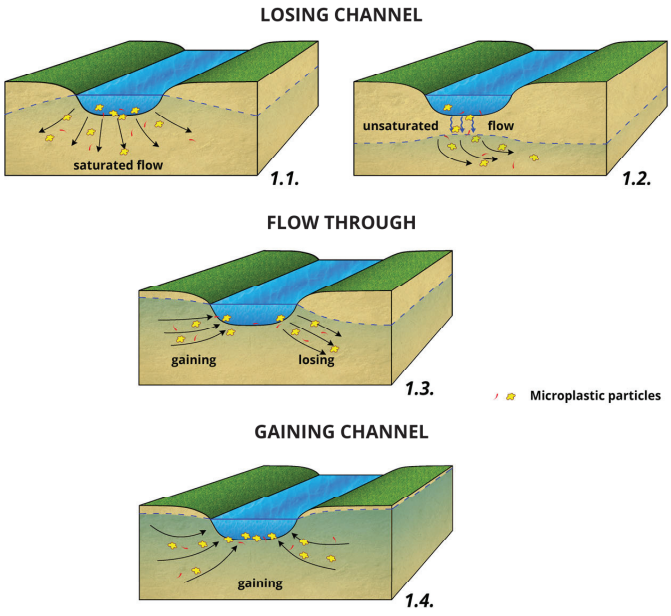

Figure 1. Pathways of MPs from the river channels: From the channel to the aquifer if (**1.1**) the material below and along the riverbed is saturated, or (**1.2**) the channel is above the lower GW level. (**1.3**) In and out of the channel to the aquifer. (**1.4**) From the aquifer to the channel. Adapted from Hoehn [57] and Singh and Bhagwat [29].

The spatial and temporal changes in water flow lead to corresponding changes in hydraulic gradients and hydraulic conductivity. This flow variability plays a crucial role in shaping fluvial hydrosystems by causing fluctuations in the water depth of the river channel laterally and along the longitudinal profile, thus creating or enhancing hydraulic gradients along the watercourse. This phenomenon is particularly pronounced in rivers with smaller drainage basins [61].

In general, the hydraulic conductivity in a river corridor decreases from the headwaters towards the lowlands. As a result, the exchange between surface and hyporheic waters is more pronounced in the headwaters of the river than in the lowlands. This trend can also be observed on a smaller scale, where the heads of the riffles act as infiltration zones while the tails of the riffles serve as discharge zones [26,56,61].

Fluctuations in seasonal flows have a considerable effect on hyporheic exchanges. In humid climates, the GW feeds the river during dry periods, while wet periods cause changes in flows from the surface to the hyporheic and floodplain water and vice versa due to river-level alterations. In arid climates, the vertical hydraulic gradient strengthens during the dry season, resulting in a shift from discharge to recharge in the direction of surface GW exchange [61].

In karstic limestone areas, fractures or conduits in the limestone regulate the main flow pathways through secondary porosity-dominated rocks, thereby intensifying interactions between the surface and the ground. During intense or prolonged rainfall, the recharge cannot drain fast enough, and the GW can rise above the ground level [68,69]. These dynamics can accelerate the migration of MPs from the surface into the GW [70].

While extensive research has been devoted to the transport of MPs through rivers and lakes, comparatively little attention has been paid to their transport through interactions between seawater and GW. However, the mechanisms involved in seawater–GW interactions are similar to those observed in river or lake–GW interactions.

The mixing of seawater with GW resources is a natural process known as seawater intrusion [29]. This phenomenon is primarily caused by prolonged changes in coastal GW levels, which can be triggered by many factors, including pumping, land use change, climate change, or sea level fluctuations [71]. Research has shown that seawater contaminants can enter GW through seawater intrusion [72,73]. The presence of MPs in seawater raises concern about the potential infiltration of MPs into GW through the mechanisms of seawater intrusion [29]. The high demand for fresh water in coastal areas has led to intensive GW extraction, resulting in elevated hydraulic gradients that drive seawater towards the inland area [29] and may increase the infiltration of MPs in GW.

Li et al. [74] investigated the impact of GW–seawater displacement on the transport behaviours of marine plastic particles through experiments on flow chamber systems and packed column systems. Their results confirm that plastic particles can enter the aquifer via seawater intrusion. Particles that attach to porous media during this process are more easily moved by GW–seawater displacement, especially when there is low ionic strength as the colloids attached to porous media tend to detach [75]. As a result, these particles can return into the seawater.

2.2. Transport of MPs through the Soil and the Unsaturated Zone to GW

MPs can enter the soil through poorly managed landfills, agricultural activities, the use of untreated wastewater for irrigation, flooding, bioturbation, atmospheric deposition, and illegal dumping of waste [76–78]. Agricultural activities can have a significant impact on the presence of MPs in the environment. For example, once in the soil, plastic mulches used in modern agricultural systems can be degraded to MPs and transported into the GW [54,77,79].

Larger plastic particles in soils can undergo degradation due to UV radiation, physical abrasion, and biological processes, thus transforming into MPs [80,81]. The generated MPs and MPs of primary origin can undergo fragmentation and phototransformation due to UV radiation and physical forces. In combination with aging, these processes can lead to the production of smaller MPs [82–84] with rougher surfaces, oxygen-containing functional groups, and more specific surface areas. These properties improve their capacity to adsorb pollutants from the environment, e.g., heavy metals and pesticides [85–88].

Due to their small size and large surface area, soil organisms such as earthworms, collembolans, and mites can transport MPs from the soil surface into deeper layers by various mechanisms such as pushing, ingestion, egestion, and adhesion to their exterior [89–91]. Invertebrates can also indirectly influence the transport of MPs by creating macropores in the soil, which serve as conduits for the transport of MPs through the process of leaching [89]. In addition to their direct or indirect transport by soil organisms, MPs can also reach deeper soil layers via percolating water [92]. Conversely, the presence of plant roots in the soil tends to retain or lift MPs along the soil profile [93].

The unsaturated zone provides an important link between the land surface and the GW [93]. MPs that permeate the soil can cross the unsaturated zone and reach the GW [88]. The processes of transport and binding of MPs to the substrate depend on particle properties, soil properties, and environmental factors [39,87,92]. Recent column experiments (Table 1) have investigated the impact of different environmental factors or particle properties on the transport or retention behaviour of MPs [87,94–100].

Particle properties such as surface hydrophobicity, density (which depends on the chemical composition or plastic material), shape, and size can influence the transport of MPs through porous media [98]. Smaller and regular shapes have better mobility than larger and irregular particles. While particle size and shape are the main determinants of MP transport, particles with higher hydrophobicity and density exhibit greater migration [98,100,101].

Table 1. Column experiments under unsaturated conditions investigating MP transport influenced by different experimental factors and MP properties.

Reference	Factor	Column Characteristics (cm)	Polymer	Shape	Media Material	Max Infiltration Depth (cm)	dMPs/dMedia	Rainfall Intensity
Waldschläger and Schüt-trumpf. [96]	/	19.40 × 30.00	CoPA; PA; PE; PET; PP; PS; PVC; SBR	Fibre; fragment; pellet; pellet cubic; sphere	Glass sphere	0.00–30.00	0.05–3.33	4600 mL/min
Ranjan et al. [97]	Wet-dry cycles	9.00 × 33.00	PE; PET; PP	Fragment	Sand	6.00–>30.00	<0.02–1.40	2.5–7–7.5 mm/h
Gao et al. [98]	Wet-dry cycles and presence of DOM	4.00 × 25.00	PA; PE; PET; PP	Pellet	Sand	0.00–13.50	0.017–2.14	/
Zhang et al. [99]	Artificial rainfall	39.00 × 9.00 × 29.00	PET; PE	Particle; fibre: film	0.03% MO; 0.77% clay; 17.27% silt; 81.73% sand	7.00	/	15.00 mm/day
		39.00 × 9.00 × 29.00	PET; PE	Particle; fibre: film	0.03% MO; 0.77% clay; 17.27% silt; 81.73% sand	6.00	/	25.00 mm/day
	Natural rainfall	39.00 × 9.00 × 29.00	PET; PE	Particle; fibre: film	0.03% MO; 0.77% clay; 17.27% silt; 81.73% sand	5.00	/	3.10 mm/day
O'Connor et al. [100]	/	25.00 × 4.00	PE	Spheres	Sand	7.500	0.05	83 mm/day
	/	25.00 × 4.00	PE; PP	Spheres	Sand	3.500	0.07–1.39	83 mm/day

Various media factors such as pH, humic acids, organic matter content, and electrolytes can lead to fluctuations in MP transport. However, as indicated by recent studies [102,103], these factors do not have a significant impact. Dong et al. [103] conducted a column experiment to investigate the effects of electrolyte concentration, pH, and humic acid on the transport of MPs. The study found that the mobility of PET MPs increased with decreasing electrolyte concentration, rising pH, and increasing humic acid concentration. The study also proved that the density and shape properties of PET MPs have a greater impact on their transport behaviour in porous media than the experimental chemical conditions.

Soil texture and MP size are both important factors that influence the movement of MPs through the soil. As MPs move through the soil, they travel through the spaces between the soil particles. If these spaces are smaller than the MPs, they can become trapped in the soil. The ratio between the size of the MP (dMP) and the size of the soil particles (dMedia) is a major factor influencing this process. Studies by Gao et al. [98], Ranjan et al. [97], and Waldschläger et al. [96] have shown that MPs tend to migrate deeper into the soil when the ratio of dMP/dMedia is less than 0.11. This ratio has been identified as the most important factor influencing the migration of MPs [98].

Soil layering may inhibit movement by enhancing MP deposition in the upper soil horizons, making MP transport in multilayer aquifers more complex [39]. Macropores, on the other hand, can promote preferential flow, increasing the likelihood of MPs reaching the GW [39,92,104]. To determine the size of MPs that can be transported through sediments, it is necessary to analyse the lithology and identify the pores and fissures in the soil [39].

In experiments with dry–wet cycles in a sandy soil column, O'Connor et al. [100], Ranjan et al. [97], and Gao et al. [98] found that weather patterns involving rainfall events followed by dry periods determined the depth of MP transport more than the volume of water flowing through the column.

Zhang et al. [99] conducted a leaching experiment to investigate the vertical migration of MPs under simulated and natural rainfall. Their experiment also showed that natural rainfall resulted in higher vertical migration compared to simulated rainfall. This emphasises the importance of conducting experiments under realistic environmental conditions to more accurately predict the distribution and fate of MPs in the natural environment.

3. Occurrence of MPs in GW

Early research on MPs focussed primarily on marine environments, with an emphasis on analytical approaches and reporting of data from monitoring campaigns [105]. The scope of studies focusing on freshwater environments remains limited. According to a recent review, 87% of the database of studies on MPs is associated with oceans, while only 13% refers to fresh water [106]. Few studies have been conducted on the detection of MPs in GW [2,25,33,59,107,108]. Table 2 summarises the results of the field studies selected for this review.

Currently, there is no standard procedure for the sampling and analysis of MPs in GW, and recent studies agree that there is an urgent need to standardise the sampling and analysis protocols [15,35,39,59]. Consequently, studies assessing the occurrence of MPs in GW are difficult to compare as they use different sampling and analytical approaches [15]. In addition, a multitude of factors can contribute to the variations in the number of MPs detected in GW across different studies, e.g., population density [109], climatology, hydrology, geology, land use of the study area [110], the presence of a particular source of contamination [111], and the distance between the surface and the aquifer [112].

Recent studies have identified various MP contamination sources in GW, including septic tanks, landfills, sewage treatment plants, and agricultural areas [109,113–115]. A significant correlation has been found between MP abundance in GW and the total concentration of antibiotics, proving that sewage treatment plants are a source of contamination [109]. Several studies have attempted to determine the impact of landfills on the occurrence of MPs in GW. They show that municipal and informal landfills, including abandoned municipal landfills, are sources of MPs that can contaminate GW. Informal landfills, which are built without proper construction and operated with a lack of environmental protection measures, are a cause for concern due to their insufficient protection and poor management [115]. Wan et al. [115] found a concentration of 11 to 17 MP/L of MPs in GW around an informal landfill, while Leideu et al. [113] investigated the presence of MPs in GW around a former municipal landfill located in an alluvial aquifer and found even wider concentration range of 0.71 to 106 MP/L. In addition, a study from Chennai, South India, reported higher levels of MPs in GW samples in the vicinity of municipal solid waste landfills, ranging from 2 to 80 MP/L [114]. MPs originating from industrial wastewater can also be an important vector for the transfer of heavy metals [2].

The capacity of karst systems to transport MPs into GW has been demonstrated in three studies from different parts of the world. The highest concentrations were reported from Italian cave water, where 12 to 54 MPs/L was measured, with no difference between tourist and non-tourist areas [116]. In a ground-breaking study from the USA, Panno et al. [25] discovered the presence of MPs in karst aquifers with an average of 7 ± 4.3 MP fibres per litre. Private septic tanks appear to be the source of MPs that can enter GW through sinkholes in the investigated area. In a remote area of China without a specific source of pollution, a low MP concentration was reported (ranging between 0 and 4 MP particles/L) [117].

Even fewer studies have been conducted on the occurrence of MPs in alluvial aquifers, namely, only two. However, recent studies by Esfandiari et al. [110] and Samandra et al. [108] have shed some light on this topic. In Iran, they found MPs in the range of 0.1 to 1.3 MP/L [110]. In contrast, much higher concentrations were detected in Australia, averaging 38 ± 8 MP/L in an unconfined alluvial aquifer [108].

Sample volume in the studies included in this review [2,25,108–110,113–115] ranged from one to twenty litres. When measuring MPs, a small sample volume can lead to an overestimation [118]. A minimum sample size of five hundred litres is necessary to ensure reliable results [15,119,120]. However, only two studies, both from Korea, sampled this many litres or more directly from GW [112,121]. These studies were conducted in a fractured rock aquifer and showed differences in the number of MPs detected. The first study reported MP concentrations between 0.02 and 3.48 MP/L. Their chemical composition was similar to the results of previous studies conducted on soils from the same area,

suggesting that the MPs present in the soils are leaching into the GW [121]. The second study detected fewer MP particles, with results ranging from 0.006 to 0.193 MP/L [112]. The highest MP concentrations were detected at the lowest altitude points, suggesting that the distance between the surface and the aquifer may be a factor in the entry of MPs into the GW [112].

The most frequently reported types of MPs in GW were PP and PE, which are also the most extensively manufactured types of plastics on the global scale [3] and the most highly prevalent types in marine environments [122], rivers [123], lakes [124], freshwater sediments [125], and soils [126,127]. The large amount of PP and PE particles in these environments poses a significant risk to the environment partly due to their potential to adsorb metals even at low concentrations [2]. To mitigate their negative impact, it is therefore crucial to understand the transport parameters of these particles.

The correct classification of MP particles in GW is important as the MP shapes detected can provide information about their source [128]. For example, MP fibres originate from the degradation of synthetic textiles and represent the predominant shape of MPs in wastewater treatment plant (WWTP) effluents [129]. Their presence may therefore indicate contamination from a WWTP effluent. On the other hand, fragment shapes are typical for weathering degradation of MPs, and their presence in GW may indicate contamination from a soil source [121]. Understanding the transport parameters of MP fibres and fragments is crucial for predicting GW contamination by MPs, as these are their most prevalent forms in GW [130].

It is crucial to ensure reliable and comparable results across different types of aquifers. Therefore, analysing the presence of MPs in different types of aquifers must be conducted using the same methodology for their sampling, quantification, and identification.

Table 2. Summary of the results of the analysed field studies investigating the presence of MPs in GW.

			Sampling Procedure				Analytical Method			Results			
Reference	**Country**	**Landfill/Aquifer Type**	**Well Cleaning before Sampling** *	**Pump**	**Filter**	**Sampled Volume (L)**	**Sample Treatment**	**MPs Detection and Quantification**	**Quality Control**	**MP Concentration (MP/L)**	**Main MP Type**	**Main, MP Shape**	**Size of Detected MPs (µm)**
Kim et al. [112]	Korea	Fractured rock mass and basal aquifer	5 min	Peristaltic pumps	Stainless steel—20 µm	500	50 mL of 30% H_2O_2 24 h—20 µm steel mesh filter—density separation 40 mL solution $Li_2O_{13}W_4{}^{-24}$ and ultra-pure water—metal filter	µ-FT-IR	Field blanks	0.006–0.192	PP, PE, PET	Fragments and fibres	20–50
Cha et al. [121]	Korea	Weathered and/or fractured rock aquifers	Yes	Peristaltic pump	Stainless steel—100 and 20 µm	300–500	30% H_2O_2 solution for 24 h—20 µm stainless-steel filter—40 mL solution of a Li_2WO_4—20 µm stainless-steel filter—dried at room temp	µ-FT-IR—Imaging microscope	Lab blanks/field blanks	0.02–3.48	PP, PE	Fragments	50–100
Panno et al. [25]	USA	Cracked and open karst aquifer	/	/	0.45 µm	2	Dried at 75 °C for 24 h	Dissecting microscope—py-GCMS	Lab blanks	Average 7.00 St. dev. 4.30	PE	Fibres	<1500
Shu et al. [117]	Southwest China	Karts aquifer	Yes	Extruded outlet device	0.45 µm	1	30% H_2O_2 at 65 °C and 100 rmp for 12 h—250 mL saturated NaCl 2 min—dried at 50 °C for 24 h	Stereoscopic microscope—RAMAN—FT-IR	Lab blanks	0.00–4.00	PS, PP, PET	Fibres	3–20
Balestra et al. [116]	Italy	Cave waters	/	/	Silver—0.8 µm	1	Dried for 2 h at 40 °C—2 mL of 15% H_2O_2 30 min—dried for 2 h at 50 °C	UV flashlight under a microscope—Infrared (IR) spectroscopy	/	12.00–54.00	PE, PVA	Fibres	100–990
Selvam et al. [2]	South India	Costal aquifer	/	12 V Teflon pump	Stainless steel—50 µm	20	30% H_2O_2 and Fe (II) solution—microline filter paper—diluted with deionised water	Stereoscopic microscope—µ-FT-IR—Atomic Force Microscopy	Field blanks	0.00–4.30	PA, PE	Fibres	120–2500
Shi et al. [109]	North China	Drinking water source	Yes	/	Polycarbonate—5 µm	1	30 mL of 30% H_2O_2 24 h at 40 °C	Optical microscope—µ-FT-IR	Lab blanks	4.00 -72.00	PA, PE, PP, PVC, PS	Fragments	<50

Table 2. *Cont.*

			Sampling Procedure				Analytical Method			Results			
Reference	**Country**	**Landfill/Aquifer Type**	**Well Cleaning before Sampling** *	**Pump**	**Filter**	**Sampled Volume (L)**	**Sample Treatment**	**MPs Detection and Quantification**	**Quality Control**	**MP Concentration (MP/L)**	**Main MP Type**	**Main, MP Shape**	**Size of Detected MPs (µm)**
Samandra et al. [108]	Australia	Alluvial unconfined aquifer	Yes	Bailer	Polycarbonate—15 µm	1	40 mL of 30% H_2O_2 for 12–24 h at 60 °C—density separation with 35 mL of a saturated $CaCl_2$ solution	LDIR	Field blanks, method blanks. Positive control: Lab control	Average 38.00 ± 8.00	PE, PP, PS, PVC	Fragments	18–491
Esfandiari et al. [110]	Southwest Iran	Alluvial aquifer	30 min	/	Paper—2 µm	20	250 mL of 30% H_2O_2 1 day—filter paper 2 µm—dried at room temp	Binocular microscope—RAMAN -SEM	Lab blanks, Positive control: Lab control	0.10–1.30	PS, PE, PET	Fibres	< 500
Ledieu et al. [113]	France	Landfill—alluvial groundwater	Yes	Supernova 21 pump	Metal—10 µm	8.8–10.2	Alumina filter 0.1 µm—100 mL of 30 wt% H_2O_2 48 to 72 h—ultrasonic bath—densimetric separation with NaI solution—JAMSS unit 24 h	µFTIR	Lab blanks, field blanks	0.71–106.70	PE, PP	/	32–2758
Manikanda et al. [114]	South India	Landfill	/	/	/	1	/	Dissecting microscope—SEM—ATR-FTIR	/	2.00–80.00	Nylon, PP, PS	Pellets, foam, fragments, fibres	/
Wan et al. [115]	South China	Landfill	/	/	Stainless steel 150, 75, 45 and 25 µm	4	40 ml 0.05 M Fe (II) solution and 40 ml 30% H_2O_2—0.45 mm filter membrane—density separation	LDIR Chemical Imaging System	Blanks in the entire procedure	11.00–17.00	PE, PP, PET	Fibres	20–150

Notes: * When yes, it was mentioned that the water discharged before sampling was at least three times the volume of water in the borehole or/and the physico-chemical water parameters were stable.

4. Challenges in GW MP Research

On the basis of our experience and analysis of the research articles listed in Table 2, we identified several challenges associated with the monitoring of MPs in GW. The main challenge is that there is currently no internationally agreed upon sampling method and MP detection procedure [131]. The challenges associated with sampling methods and sample analysis associated with quality assurance are described below.

4.1. Challenges Associated with Sampling Methods

Researchers conducting research on MPs in GW face several challenges in the field of GW sampling. Firstly, they need to select a suitable sampling site and then the correct sampling technique to ensure high-quality samples and comparable results. The important aspects of both are described below.

a Sampling point selection

Improper selection of the sampling point is a major concern, as it can lead to incorrect or questionable results. The sampling point must be defined on the horizontal and vertical scale (depth). To determine the optimal sampling location, we need to know the precise hydrogeographic conditions of the sampling site and the positions of the available boreholes or pumping stations.

In this context, Viaroli et al. [39] note the lack of information on the hydrogeographic properties of sampling points in many studies on MPs in GW. In order to conduct reliable research, sampling from a borehole requires consideration and inclusion of the following hydrogeographic features of the sampling sites: (a) data on the lithological profile of the sampling site, (b) data on the protection and maintenance of the sampling site, (c) data on the quality status of the sampling site, and (d) data on the borehole design (drilling method, borehole material, borehole depth and height, quantitative borehole capacity). For a correct interpretation of the pathways and origins of the detected MPs, it is also important to understand which GW (aquifer) enters the borehole [119].

There appears to be a lack of specific guidelines in the literature for determining the optimal depth for sampling in a well or below the GW table. In some articles, the depth at which sampling was performed is not mentioned [109,110,113]. Selvam et al. [2] state that sampling was carried out at a depth of 2–5 m. Samandra et al. [108] report that sampling was performed at the mid-point of the screened interval of the boreholes. Although further studies are required on the appropriateness of the sampling depth, it is expected that sampling should be carried out at the level of the screened section of the borehole. Sampling a few metres below the water table may result in sampling stagnant water from the well rather than from the aquifer itself, depending on the hydrogeological conditions of the aquifer and the pumping force.

When selecting the sampling points, the maintenance of the sampling boreholes must also be taken into account. Inadequate maintenance of the sampling points can lead to the presence of plastics and consequently MPs at the sampling points, which should be avoided. Boreholes that are open to the atmosphere and constructed with plastic materials should be avoided as they can be a source of contamination. However, some of the articles analysed report sampling in open or plastic-lined boreholes [108,110].

When sampling from a spring, it is possible to take samples either directly from the spring or from a pumping system. Sampling from a spring can lead to problems with air pollution, so it is important to sample as close to the spring as possible. If this is not possible, the distance at which sampling was performed should be indicated. On the other hand, sampling from a pumping system can lead to MP contamination due to the potential use of plastics in the construction of the facilities. Nevertheless, it is beneficial to compare the data from both sampling points in order to validate the reliability of the results.

b. Sampling procedure

Sampling with the pumping system

When sampling from a well, obtaining the necessary amount of sample water requires the use of pumping systems consisting of the pump and the filtration system which allow for the filtration of large amounts of water at the sampling point. Typically, the pumping systems also contain plastic parts that can potentially contaminate the sample. Of the articles analysed, only one indicates the use of Teflon-made pumps [2], while some [112,113,121] mention the type of pump used without specifying its material composition. All components of the pumping system need to be specified with regard to their material structure using FTIR analysis. The spectra of materials used must be included in the library, and the particles from the samples must be compared with them.

Furthermore, larger MP particles can be damaged and even fragmented as they pass through the pump, especially when pumps with impellers are used. This may lead to an overestimation of the presence of MPs. Therefore, it is important that researchers first test the pump for the potential fragmentation of plastic particles.

Design of the field filtration system

Designing a filtration system to sample large quantities of samples in the field can be challenging, as this requires a closed filtering system to prevent contamination from the atmosphere. Direct contact between plastic materials and samples should be avoided. This can complicate the construction of the system due to the limited options for suitable materials. Although some plastics have the desired properties such as elasticity or impermeability, they can be difficult to replace and are associated with higher costs. For example, silicone hoses and stainless-steel filter cartridges are favoured over plastic hoses and pipes, and hemp threads are a better alternative to plastic thread sealants.

Among the articles reporting on water sampling using pumping systems, two emphasise the use of silicone and stainless-steel materials [112,121] and mention the use of peristaltic pumps without providing any further information. Two other studies mention PVC components for the system [2,113].

It is also crucial to monitor the pressure in a closed system, as pressure-related problems can occur due to the strength of the pump and the likelihood of filter clogging. Such problems can result in damage to the system or filter, as well as loss of samples. It is, therefore, important to monitor this parameter closely by using a pressure gauge.

To control the amount of water collected, it is necessary to use a flow meter. Typically made of plastic, the flow meter can come into contact with the sampled water. This can be prevented by using it after the water has been filtered. This approach was used by Cha et al. [121].

Determination of the sample volume

To avoid underestimating the occurrence of MPs, the recommended minimum volume of GW sampled is 500 L [119]. The same volume has also been suggested for tap water [15]. Of all the studies examined, only two adhered to the recommended minimum sample volume of 500 L [112,121]. These studies detected a lower number of particles than other studies, which contradicts their initial assumptions. Both studies were conducted with similar sampling methods in the same type of aquifer. To establish whether the lower numbers observed are due to the type of aquifer or the sampling methods, further studies using the recommended minimum sample volume need to be conducted.

Collecting a minimum of 500 L of sample water poses a certain logistical challenge, especially when it comes to transporting the sample to a laboratory for filtration in a controlled, filtered air environment. It is therefore necessary to perform in situ filtration, despite the problems that this process may entail.

Selection of the proper filters

When analysing MPs in samples, studies report that the smallest size of MPs detected corresponded to the size limitation of the filter used [109–112,115]. This suggests that the filter used may have limited the detection of smaller MP particles during sampling. However, in most of the studies analysed, filter pore size did not appear to be a limiting factor, as the size of MPs detected was generally larger than the filter pore size. Nevertheless, it is worth noting that the detection limit of the analytical methods may influence the results.

For example, Kim et al. [112] mention the use of a filter size of 20 μm due to the 20 μm detection limit of the equipment for analysis. The use of a smaller pore size would therefore not help to extend the detection range of the MP particles but rather increase the risk of filter clogging. Sometimes also membranes >20 μm become rapidly clogged. To avoid clogging, the use of cascade filtering system is recommended.

In addition to the pore size, the material of the filters is also important. For the analysis of samples with an FTIR microscope or a Raman spectrometer, aluminium- or gold-coated polyester membranes and silicone membranes, which are suitable for analysis of particles in reflection mode, are particularly recommended. However, they have limitations in terms of pore size, which are generally too small and unable to filter large amount of water. Innovations and improvements are still needed in this area.

Purging before real sampling

The construction materials and work involved in building a well can lead to MP contamination. In addition, open-air wells can become contaminated with plastic waste or MPs due to atmospheric deposition. In order to accurately investigate the occurrence of MPs in GW through borehole sampling, it is necessary to purge the well before sampling begins. This involves pumping out two to three times the volume of the well or until the physicochemical parameters of water stabilise [132]. This standard procedure ensures that the sample is taken from the aquifer rather than the accumulated water in the well [108]. Moreover, sampling without purging yields information on the MPs present in the wells, which can provide useful information on the exposure to MPs for those using the water from that well for domestic purposes [131]. Some of the articles analysed in this study do not indicate whether purging was performed, while some of them mention purging for a specific duration, such as 5 or 30 min. Such a duration may or may not confirm the pumping of two to three times the well's volume or the stabilisation of physicochemical parameters. It may also result in inadequate purging. Therefore, it is important for studies to mention whether or not purging was performed prior to sampling as this would allow a more accurate interpretation of the results.

The importance of conducting blank sampling in the field and quality control

The difficulty of avoiding the use of plastics during in situ sampling reinforces the need to perform blank sampling for the pumping and filtration processes in the field. This is to ensure that the potential contact of the sample with plastic materials does not influence the results.

Replicating the properties of sampling and filtration in a field blank sample is a complex process as it is difficult to obtain a large volume of water that is free of MPs. One possible solution to this problem is to obtain a blank sample containing a smaller volume of water. For example, in a study conducted by Kim et al. [112], a blank sample of 20 L of water was collected, while the total volume of water sampled was 500 L. This approach helps to reduce friction and minimise the wear on the plastic materials in the system, although it may not exactly replicate the conditions of the target, which could potentially raise concerns about the accuracy of the results.

Given the complexity of obtaining a suitable and reliable field blank, one possible option to ensure that the materials in the sampling system do not interfere with the results is to perform an FTIR analysis of these materials. If there is a high degree of similarity between these materials and the MPs detected, as well as a match of other properties (e.g., colour and morphology), they should be excluded from the results.

4.2. Challenges Associated with Laboratory Analysis

Laboratory analysis of GW samples requires a lot of experience in the field of MP identification, as MPs are on average smaller and less colourful compared to MPs from surface water. Depending on the type of aquifer, samples may or may not need be pretreated before MP identification, which can be performed using FTIR or Raman spectroscopy. Both techniques require very experienced scientists for a good interpretation of the results.

a. Pretreatment of the samples

The process of preparing GW samples for MP analysis typically includes the following steps: filtration, digestion, and extraction. These steps, which are occasionally required to separate MPs from other particles such as sediment, organic matter, and minerals, can be challenging and may require labour-intensive sample preparation that carries the risk of contamination. Microplastics in marine environments have been extensively studied, and well-established analytical methods can be used as a reference for the pretreatment of groundwater samples if necessary. It is essential to mention that digestion steps, if necessary, should be performed before density separation, as MP particles can be entrapped in the organic material [133].

Most of the studies analysed used sample digestion to remove organic matter [2,106, 107,109,110,112,113,118], as this can interfere with the analysis and lead to an overestimation of MPs, or an inflated number of particles subjected to further analyses [134]. Although this technique has been shown to be effective for the analysis of MPs [135], any treatment of the sample may result in damage or loss of the sample or involve the risk of contamination. It is therefore advisable to avoid it as much as possible. Exposure to harsh chemicals and higher temperatures than 40 °C should be minimised [133]. As GW is not expected to contain large amounts of organic matter that could interfere with the analysis of MPs, digestion is therefore not recommended.

During density separation, in order to increase the recovery rate of microplastics, solutions with densities higher than 1.7 g/cm^3 are recommended [133]. For instance, sodium chloride (1.2 g/cm^3) is not recommended as its low density interferes with the polymer's recovery [133]. Zinc chloride ($ZnCl_2$), sodium polytungstate ($NaWO_4$), potassium carbonate (K_2CO_3), sodium bromide (NaBr), sodium iodide (NaI), and potassium formate (HCO_2K) are commonly used density solutions [133]. The density separation process typically involves shaking for 10 min and settling for 24 h [133]. During supernatant extraction, some microplastics may stick to the container wall, which can result in sample loss. To avoid this issue, it is recommended that the container walls be washed onto the filter [136].

Validation of the effectiveness of sample preparation methods is crucial and can be achieved with the use of positive controls. In addition, the possibility of sample contamination can be controlled by using blank samples during all steps of the analysis. Of all the articles selected for this review, only two mention the use of positive controls in the laboratory [108,110].

b. Limitations of methods for MP detection and quantification

Selecting the most appropriate sampling and detection method for all sizes of MP particles, which can range from 5 mm to 1 μm, is a major challenge. Chemical characterisation of plastics with spectroscopic methods is an extension to light microscopy. Spectroscopic techniques can help to exclude the doubtful particles of bigger sizes and include smaller particles, which are difficult to recognised on the basis of morphological features. Fourier-transform infrared (FTIR) spectroscopy or Raman spectroscopy produce the sample's molecular fingerprint represented with spectra. Spectroscopic data of each particle are compared with reference spectra in the library [137]. The ATR-FTIR method enables an easy determination of the chemical composition of particles larger than 0.5 mm, and the FTIR microscope enables the determination also of particles smaller than 0.5 mm. While all FTIR techniques can easily detect larger particles, they reach their limits when detecting smaller particles (<20 μm). As the particle size decreases, the reliability of the chemical analysis results also tends to decrease. Raman spectrometry is more suitable for smaller particles and also enables the detection of particles <20 μm. Among particle size limitations, there are a few more factors that can influence the detection of MPs with spectroscope techniques, e.g., additives, pigments, dyes, and fillers can overlay the signal of polymer, which can be seen as (i) a foreign band overlay, (ii) fluorescence, and (iii) absorbance [138].

The smaller the particles, the more difficult and time-consuming they are to detect, so complementary techniques are often required to facilitate this process. For example, in our laboratory, we work with an ATR-FTIR spectrometer for the identification of particles >0.5 mm, and with an FTIR microscope in reflection mode, we analyse particles <0.5 mm.

Shu et al. [117] used an FTIR microscope to identify larger MPs, as well as a microscope with a laser Raman spectrometer to detect smaller MPs. However, using more than one technique can lead to errors in counting the samples. To improve monitoring efficiency, the sampling and detection techniques should be suitable for similar sample sizes.

4.3. Challenges Associated with Quality Assurance

There is a risk of samples being contaminated with MPs during collection, transport, and processing, which can lead to inaccurate results. The accuracy of the results obtained depends on the sampling methods used and the precautions taken to avoid contamination [15,120]. Therefore, it is essential to follow the best practices recommended by experts to obtain reliable results. To minimise the potential for cross-contamination, it is advised to use glass or metal materials for sample preparation and avoid plastic [139]. Additionally, it is recommended to clean the materials with acid and filtered water or ultrapure water [139]. All personnel involved in the sampling should wear clean white cotton lab coats. To prevent air contamination, in situ filtration with a closed device is recommended, which eliminates any air contact with the sample. It is also suggested to use glass Petri dishes for the transport and storage of filters.

It is essential to take replicates while sampling to ensure precise and accurate results. Replicates are significant in comprehending the sampling's variability and enabling the collection of trustworthy data. Therefore, it is advised to use replicates while sampling also for sampling MPs in GW. To better distinguish the sources of variability affecting the sample, it is necessary to provide information on the exact process of collecting replicate samples. However, only three studies report the use of replicates in sampling. Selvam et al. [2] report the use of two replicates, while Shu et al. [117] used three replicates, as did Samandra et al. [108], who also report that they observed no consistent trend associated with the order of sample replicates.

The difficulty and time required to sample MPs can make performing replicates a challenging task. For this reason, a sampling system in which three simultaneous samples are collected with a single pump would be ideal to save sampling time and ensure the precision of research methods. In certain situations, conducting simultaneous replicates may not be possible due to certain limitations. For instance, the pumped flow may be low in some sampling locations, which may complicate sampling and, thus, make it difficult to carry out simultaneous duplicates. Under such circumstances, using sequential replicates can be a practical alternative to ensure an appropriate sampling.

5. Conclusions

MPs present in rivers, lakes, seas, and soils can enter GW via different pathways. The articles investigating the presence of MPs in GW show different results, both in terms of the quantity, shape, and size of the MPs detected, which could be the result of differences in the investigated areas (population density, climatology, geology, land use), as well as differences in the sampling methods used in the studies.

The articles included in this review agree that the lack of standardised techniques and procedures in the monitoring of MPs can lead to discrepancies between the results of individual studies. This poses a challenge when comparing results. Therefore, there is a need to develop a standardised methodology to enable a more accurate comparison of the occurrence of MPs in GW.

The complexity of monitoring MP particles is exacerbated by their diversity and the hydrogeological conditions of the sampling sites, as well as the associated risks of contamination. Based on the analysis of twelve research articles on the presence of MPs in GW and personal experiences, the following challenges or needs in sampling MPs in GW can be highlighted:

- An accurate interpretation of the origin and pathways of MPs found in a particular sampling area requires a thorough understanding and proper documentation of the hydrogeological and hydrogeographic conditions in the area.

- When collecting borehole samples, it is important to clean the borehole by pre-pumping a certain amount of water before the actual sampling. The appropriate sampling depth to obtain representative samples of the aquifer is also important.
- To ensure representativeness of the sample, a minimum of 500 L of sample water must be collected. This requires a more complex in situ sampling system, which makes the sampling process more challenging and time-consuming.
- During in situ filtration sampling, it is often difficult to avoid the use of plastics. The sampling system used must be carefully specified, and if plastic materials are present, they should be identified and accounted for in the final results.
- Sampling pumps can physically damage or fragment plastic particles, which can lead to an overestimation of the presence of MPs in the sample. To check for plastic particle fragmentation during operation, it is important to test the pump under conditions that reflect its actual power usage.
- Given that the presence of small particles in GW is expected, the filters and size limitations of detection methods may lead to underestimations of the presence of MPs in GW. To obtain accurate results, it is therefore necessary to develop more efficient filtration systems (e.g., cascade filtration) and improve the detection methods.
- In cases where the concentration of organic matter in the GW sample is low, it is recommended to omit the digestion step during sample preparation.
- To ensure accurate monitoring of MPs in GW, it is crucial to prevent contamination during all monitoring stages. This requires the blank sampling and implementation of quality control measures.

Last but not least, understanding the transport processes of MPs through the soil, the unsaturated zone and within aquifers is important to determine the sources of MPs in GW. These questions need to be clarified through experimental work, field measurements and mathematical modelling.

Research on the presence of MPs in the environment, including GW, is advancing rapidly. Our study focused on identifying the challenges with the ultimate aim of promptly developing standardised methods that will enable comparison of the results of different studies and form the basis for the implementation of measures, including legislative measures, to curb the entry of MPs into the environment.

Author Contributions: Conceptualisation, E.C.C., M.K.V. and N.M.; writing—original draft preparation, E.C.C.; writing—review and editing, M.K.V. and N.M.; supervision, M.K.V. and N.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Slovenian Research and Innovation Agency (ARIS) as part of the young researcher programme, specifically the Groundwater and Geochemistry research programme (P1-0020), as well as the research projects "Improved methods for determination of transport processes and Origin of microplastics in groundwater resources—(GWMicroPlast)" (J1-50030) and "Manca" (GeoZS RSF-O).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Egessa, R.; Nankabirwa, A.; Ocaya, H.; Pabire, W.G. Microplastic pollution in surface water of Lake Victoria. *Sci. Total Environ.* **2020**, *741*, 140201. [CrossRef] [PubMed]
2. Selvam, S.; Jesuraja, K.; Venkatramanan, S.; Roy, P.D.; Jeyanthi Kumari, V. Hazardous microplastic characteristics and its role as a vector of heavy metal in groundwater and surface water of coastal south India. *J. Hazard. Mater.* **2021**, *402*, 123786. [CrossRef]
3. Plastics Europe—The Facts 2023. 2023. Available online: https://plasticseurope.org/knowledge-hub/plastics-the-fast-facts-2023/ (accessed on 28 February 2024).

4. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci Adv.* **2017**, *3*, e1700782. [CrossRef] [PubMed]
5. Belzagui, F.; Gutiérrez-Bouzán, C.; Álvarez-Sánchez, A.; Vilaseca, M. When Size Matters—Textile Microfibers into the Environment. In Proceedings of the 2nd International Conference on Microplastic Pollution in the Mediterranean Sea, ICMPMS 2019, Capri, Italy, 15–18 September 2019; Springer: Cham, Switzerland, 2020. [CrossRef]
6. Campanale, C.; Losacco, D.; Triozzi, M.; Massarelli, C.; Uricchio, V.F. An Overall Perspective for the Study of Emerging Contaminants in Karst Aquifers. *Resources* **2022**, *11*, 105. [CrossRef]
7. Kumar, V.; Singh, E.; Singh, S.; Pandey, A.; Bhargava, P.C. Micro- and nanoplastics (MNPs) as emerging pollutant in ground water: Environmental impact, potential risks, limitations and way forward towards sustainable management. *Chem. Eng. J.* **2023**, *459*, 141568. [CrossRef]
8. Koelmans, A.A.; Besseling, E.; Shim, W.J. Nanoplastics in the Aquatic Environment. Critical Review. In *Marine Anthropogenic Litter*; Bergmann, M., Gutow, L., Klages, M., Eds.; Springer: Cham, Switzerland, 2015. [CrossRef]
9. Bergmann, M.; Gutow, L.; Klages, M. *Marine Anthropogenic Litter*; Springer: Berlin/Heidelberg, Germany, 2015.
10. Shim, W.J.; Hong, S.H.; Eo, S. Marine microplastics: Abundance, distribution, and composition. In *Microplastic Contamination in Aquatic Environments: An Emerging Matter of Environmental Urgency*; Elsevier: Amsterdam, The Netherlands, 2018; pp. 1–26. [CrossRef]
11. Hartmann, N.B.; Hüffer, T.; Thompson, R.C.; Hassellöv, M.; Verschoor, A.; Daugaard, A.E.; Rist, S.; Karlsson, T.; Brennholt, N.; Cole, M.; et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. *Environ. Sci. Technol.* **2019**, *53*, 1039–1047. [CrossRef]
12. Boucher, J.; Friot, D. *Primary Microplastics in the Oceans: A Global Evaluation of Sources*; IUCN: Gland, Switzerland, 2017; p. 43.
13. Napper, I.E.; Thompson, R.C. Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. *Mar. Pollut. Bull.* **2016**, *112*, 39–45. [CrossRef]
14. Pui, K.C.; Fok, L. Characterisation of plastic microbeads in facial scrubs and their estimated emissions in Mainland China. *Water Res.* **2017**, *122*, 53–61. [CrossRef]
15. Koelmans, A.A.; Mohamed Nor, N.H.; Hermsen, E.; Kooi, M.; Mintenig, S.M.; de France, J. Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. *Water Res.* **2019**, *155*, 410–422. [CrossRef]
16. Carpenter, E.J.; Anderson, S.J.; Harvey, G.R.; Miklas, H.P.; Peck, B.B. Polystyrene spherules in coastal waters. *Science* **1972**, *178*, 749–750. [CrossRef]
17. Carpenter, E.J.; Smith, K.L. Plastics on the Sargasso Sea surface. *Science* **1972**, *175*, 1240–1241. [CrossRef]
18. Thompson, R.C.; Olsen, Y.; Mitchell, R.P.; Davis, A.; Rowland, S.J.; John, A.W.G.; Mcgonigle, D.; Russell, A.E. Lost at Sea: Where Is All the Plastic? 2004. Available online: http://www.sciencemag.org/cgi/content/full/304/5672/838/ (accessed on 21 November 2023).
19. NOAA. Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris. 2009. Available online: http://www.MarineDebris.noaa.gov (accessed on 8 December 2023).
20. Frias, J.P.G.L.; Nash, R. Microplastics: Finding a consensus on the definition. *Mar. Pollut. Bull.* **2019**, *138*, 145–147. [CrossRef]
21. Alfaro-Núñez, A.; Astorga, D.; Cáceres-Farías, L.; Bastidas, L.; Soto Villegas, C.; Macay, K.; Christensen, J.H. Microplastic pollution in seawater and marine organisms across the Tropical Eastern Pacific and Galápagos. *Sci. Rep.* **2021**, *11*, 6424. [CrossRef]
22. Harms, I.K.; Diekötter, T.; Troegel, S.; Lenz, M. Amount, distribution and composition of large microplastics in typical agricultural soils in Northern Germany. *Sci. Total Environ.* **2021**, *758*, 143615. [CrossRef] [PubMed]
23. Lenaker, P.L.; Baldwin, A.K.; Corsi, S.R.; Mason, S.A.; Reneau, P.C.; Scott, J.W. Vertical Distribution of Microplastics in the Water Column and Surficial Sediment from the Milwaukee River Basin to Lake Michigan. *Environ. Sci. Technol.* **2019**, *53*, 12227–12237. [CrossRef]
24. Liao, Z.; Ji, X.; Ma, Y.; Lv, B.; Huang, W.; Zhu, X.; Fang, M.; Wang, Q.; Wang, X.; Dahlgren, R.; et al. Airborne microplastics in indoor and outdoor environments of a coastal city in Eastern China. *J. Hazard. Mater.* **2021**, *417*, 126007. [CrossRef]
25. Panno, S.V.; Kelly, W.R.; Scott, J.; Zheng, W.; McNeish, R.E.; Holm, N.; Hoellein, T.J.; Baranski, E.L. Microplastic Contamination in Karst Groundwater Systems. *Groundwater* **2019**, *57*, 189–196. [CrossRef] [PubMed]
26. Scherer, C.; Weber, A.; Stock, F.; Vurusic, S.; Egerci, H.; Kochleus, C.; Arendt, N.; Foeldi, C.; Dierkes, G.; Wagner, M.; et al. Comparative assessment of microplastics in water and sediment of a large European river. *Sci. Total Environ.* **2020**, *738*, 139866. [CrossRef]
27. Weber, C.J.; Opp, C. Spatial patterns of mesoplastics and coarse microplastics in floodplain soils as resulting from land use and fluvial processes. *Environ. Pollut.* **2020**, *267*, 115390. [CrossRef] [PubMed]
28. Wright, S.L.; Ulke, J.; Font, A.; Chan, K.L.A.; Kelly, F.J. Atmospheric microplastic deposition in an urban environment and an evaluation of transport. *Environ. Int.* **2020**, *136*, 105411. [CrossRef]
29. Singh, S.; Bhagwat, A. Microplastics: A potential threat to groundwater resources. *Groundw. Sustain. Dev.* **2022**, *19*, 100852. [CrossRef]
30. Vivekanand, A.C.; Mohapatra, S.; Tyagi, V.K. Microplastics in aquatic environment: Challenges and perspectives. *Chemosphere* **2021**, *282*, 131151. [CrossRef] [PubMed]

31. Geissen, V.; Mol, H.; Klumpp, E.; Umlauf, G.; Nadal, M.; van der Ploeg, M.; van de Zee, S.E.A.T.M.; Ritsema, C.J. Emerging pollutants in the environment: A challenge for water resource management. *Int. Soil Water Conserv. Res.* **2015**, *3*, 57–65. [CrossRef]
32. Kumar, R.; Sharma, P. Recent Developments in Extraction, Identification, and Quantification of Microplastics from Agricultural Soil and Groundwater. In *Fate and Transport of Subsurface Pollutants*; Springer: Berlin/Heidelberg, Germany, 2021; pp. 125–143. [CrossRef]
33. Re, V. Shedding light on the invisible: Addressing the potential for groundwater contamination by plastic microfibers. *Hydrogeol. J.* **2019**, *27*, 2719–2727. [CrossRef]
34. Kim, H.; Lee, J.Y. Emerging concerns about microplastic pollution on groundwater in South Korea. *Sustainability* **2020**, *12*, 5275. [CrossRef]
35. Chia, R.W.; Lee, J.Y.; Kim, H.; Jang, J. Microplastic pollution in soil and groundwater: A review. *Environ. Chem. Lett.* **2021**, *19*, 4211–4224. [CrossRef]
36. Kuri, G.H. Agua subterránea y soberanía interdependiente: El caso de los Sistemas Acuíferos Transfronterizos en la región binacional de Paso del Norte. *Norteamérica* **2017**, *12*, 113–145. [CrossRef]
37. Lapworth, D.J.; Boving, T.B.; Kreamer, D.K.; Kebede, S.; Smedley, P.L. Groundwater quality: Global threats, opportunities and realising the potential of groundwater. *Sci. Total Environ.* **2022**, *811*, 152471. [CrossRef] [PubMed]
38. Qiu, R.; Song, Y.; Zhang, X.; Xie, B.; He, D. Microplastics in Urban Environments: Sources, Pathways, and Distribution. In *Handbook of Environmental Chemistry*; Springer Science and Business Media Deutschland GmbH: Berlin/Heidelberg, Germany, 2020; Volume 95, pp. 41–61. [CrossRef]
39. Viaroli, S.; Lancia, M.; Re, V. Microplastics contamination of groundwater: Current evidence and future perspectives. A review. *Sci. Total Environ.* **2022**, *824*, 153851. [CrossRef]
40. Syafiuddin, A.; Boopathy, R.; Hadibarata, T. Challenges and Solutions for Sustainable Groundwater Usage: Pollution Control and Integrated Management. *Curr. Pollut. Rep.* **2020**, *6*, 310–327. [CrossRef]
41. Deng, L.; Krauss, S.; Feichtmayer, J.; Hofmann, R.; Arndt, H.; Griebler, C. Grazing of heterotrophic flagellates on viruses is driven by feeding behaviour. *Environ. Microbiol. Rep.* **2014**, *6*, 325–330. [CrossRef] [PubMed]
42. Koelmans, A.A.; Redondo-Hasselerharm, P.E.; Nor, N.H.M.; de Ruijter, V.N.; Mintenig, S.M.; Kooi, M. Risk assessment of microplastic particles. *Nat. Rev. Mater.* **2022**, *7*, 138–152. [CrossRef]
43. Ziajahromi, S.; Kumar, A.; Neale, P.A.; Leusch, F.D.L. Environmentally relevant concentrations of polyethylene microplastics negatively impact the survival, growth and emergence of sediment-dwelling invertebrates. *Environ. Pollut.* **2018**, *236*, 425–431. [CrossRef] [PubMed]
44. Dris, R.; Gasperi, J.; Saad, M.; Mirande, C.; Tassin, B. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? *Mar. Pollut. Bull.* **2016**, *104*, 290–293. [CrossRef] [PubMed]
45. Wagner, M.; Scherer, C.; Alvarez-Muñoz, D.; Brennholt, N.; Bourrain, X.; Buchinger, S.; Fries, E.; Grosbois, C.; Klasmeier, J.; Marti, T.; et al. Microplastics in freshwater ecosystems: What we know and what we need to know. *Environ. Sci. Eur.* **2014**, *26*, 12. [CrossRef] [PubMed]
46. Anderson, J.C.; Park, B.J.; Palace, V.P. Microplastics in aquatic environments: Implications for Canadian ecosystems. *Environ. Pollut.* **2016**, *218*, 269–280. [CrossRef] [PubMed]
47. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. Microplastics as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* **2011**, *62*, 2588–2597. [CrossRef] [PubMed]
48. Frei, S.; Piehl, S.; Gilfedder, B.S.; Löder, M.G.J.; Krutzke, J.; Wilhelm, L.; Laforsch, C. Occurence of microplastics in the hyporheic zone of rivers. *Sci. Rep.* **2019**, *9*, 15256. [CrossRef] [PubMed]
49. Kooi, M.; Besseling, E.; Kroeze, C.; van Wezel, A.P.; Koelmans, A.A. Modeling the fate and transport of plastic debris in freshwaters: Review and Guidance. In *Handbook of Environmental Chemistry*; Springer: Berlin/Heidelberg, Germany, 2018; Volume 58, pp. 125–152. [CrossRef]
50. Harrison, J.P.; Hoellein, T.J.; Sapp, M.; Tagg, A.S.; Ju-Nam, Y.; Ojeda, J.J. Microplastic-associated biofilms: A comparison of freshwater and marine environments. In *Handbook of Environmental Chemistry*; Springer: Berlin/Heidelberg, Germany, 2018; Volume 58, pp. 181–201. [CrossRef]
51. Kowalski, N.; Reichardt, A.M.; Waniek, J.J. Sinking rates of microplastics and potential implications of their alteration by physical, biological, and chemical factors. *Mar. Pollut. Bull.* **2016**, *109*, 310–319. [CrossRef]
52. Long, M.; Moriceau, B.; Gallinari, M.; Lambert, C.; Huvet, A.; Raffray, J.; Soudant, P. Interactions between microplastics and phytoplankton aggregates: Impact on their respective fates. *Mar. Chem.* **2015**, *175*, 39–46. [CrossRef]
53. Hurley, R.; Woodward, J.; Rothwell, J.J. Microplastic contamination of river beds significantly reduced by catchment-wide flooding. *Nat. Geosci.* **2018**, *11*, 251–257. [CrossRef]
54. Silva, C.J.M.; Machado, A.L.; Campos, D.; Rodrigues, A.C.M.; Patrício Silva, A.L.; Soares, A.M.V.M.; Pestana, J.L.T. Microplastics in freshwater sediments: Effects on benthic invertebrate communities and ecosystem functioning assessed in artificial streams. *Sci. Total Environ.* **2022**, *804*, 150118. [CrossRef] [PubMed]
55. Severini, E.; Ducci, L.; Sutti, A.; Robottom, S.; Sutti, S.; Celico, F. River–Groundwater Interaction and Recharge Effects on Microplastics Contamination of Groundwater in Confined Alluvial Aquifers. *Water* **2022**, *14*, 1913. [CrossRef]

56. Drummond, J.D.; Nel, H.A.; Packman, A.I.; Krause, S. Significance of Hyporheic Exchange for Predicting Microplastic Fate in Rivers. *Environ. Sci. Technol. Lett.* **2020**, *7*, 727–732. [CrossRef]
57. Hoehn, E. Exchange processes between rivers and ground waters—The hydrological and geochemical approach. In *Groundwater Ecology*; al Griebler, C., Ed.; European Commission Environment and Climate Programme: Brussels, Belgium, 2001; pp. 55–68.
58. Woessner, W.W. Hyporheic Zones. In *Methods in Stream Ecology*, 3rd ed.; Elsevier Inc.: Amsterdam, The Netherlands, 2017; Volume 1, pp. 129–157. [CrossRef]
59. Alimi, O.S.; Farner Budarz, J.; Hernandez, L.M.; Tufenkji, N. Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport. *Environ. Sci. Technol.* **2018**, *52*, 1704–1724. [CrossRef] [PubMed]
60. Drummond, J.D.; Schneidewind, U.; Li, A.; Hoellein, T.J.; Krause, S.; Packman, A.I. Microplastic accumulation in riverbed sediment via hyporheic exchange from headwaters to mainstems. *Sci. Adv.* **2022**, *8*, eabi9305. [CrossRef] [PubMed]
61. Breil, P.; Grimm, N.B.; Vervier, P. Surface Water-Groundwater Exchange Processes and Fluvial Ecosystem Function: An Analysis of Temporal and Spatial Scale Dependency. In *Hydroecology and Ecohydrology: Past, Present and Future*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2008; pp. 93–111. [CrossRef]
62. Xu, S.; Frey, S.K.; Erler, A.R.; Khader, O.; Berg, S.J.; Hwang, H.T.; Callaghan, M.V.; Davison, J.H.; Sudicky, E.A. Investigating groundwater-lake interactions in the Laurentian Great Lakes with a fully-integrated surface water-groundwater model. *J. Hydrol.* **2021**, *594*, 125911. [CrossRef]
63. Ren, X.W.; Santamarina, J.C. The hydraulic conductivity of sediments: A pore size perspective. *Eng. Geol.* **2018**, *233*, 48–54. [CrossRef]
64. Woessner, W.W. *Groundwater-Surface Water Exchange*; Groundwater Project: Guelph, ON, Canada, 2020; p. 158. ISBN 978-1-7770541-5-1.
65. Cieśliński, R.; Piekarz, J.; Zieliński, M. Groundwater supply of lakes: The case of Lake Raduńskie Górne (northern Poland, Kashubian Lake District). *Hydrol. Sci. J.* **2016**, *61*, 2427–2434. [CrossRef]
66. Engesgaard, P.; Solvang, I.S.; Steiness, M.; Kristensen, E.; Kragh, T.; Duque, C. Tracing the spatial distribution of whole-lake exchange of groundwater and lakewater in lowhydraulic gradient systems using δ18O and electrical conductivity and uncertain end-member mixing analysis. *Water* **2020**, *12*, 1608. [CrossRef]
67. Javadzadeh, H.; Ataie-Ashtiani, B.; Hosseini, S.M.; Simmons, C.T. Interaction of lake-groundwater levels using cross-correlation analysis: A case study of Lake Urmia Basin, Iran. *Sci. Total Environ.* **2020**, *729*, 138822. [CrossRef] [PubMed]
68. Basu, B.; Morrissey, P.; Gill, L.W. Application of Nonlinear Time Series and Machine. Learning Algorithms for Forecasting Groundwater Flooding in a Lowland Karst Area. *Water Resour. Res.* **2022**, *58*, e2021WR029576. [CrossRef]
69. Drew, D.P. Hydrogeology of lowland karst in Ireland. *Q. J. Eng. Geol. Hydrogeol.* **2008**, *41*, 61–72. [CrossRef]
70. Pradhan, B.; Chand, S.; Chand, S.; Rout, P.R.; Naik, S.K. Emerging groundwater contaminants: A comprehensive review on their health hazards and remediation technologies. *Groundw. Sustain. Dev.* **2023**, *20*, 100868. [CrossRef]
71. Werner, A.D.; Bakker, M.; Post, V.E.A.; Vandenbohede, A.; Lu, C.; Ataie-Ashtiani, B.; Simmons, C.T.; Barry, D.A. Seawater intrusion processes, investigation and management: Recent advances and future challenges. *Adv. Water Resour.* **2013**, *51*, 3–26. [CrossRef]
72. Jia, C.; Chen, Q.; Hao, D.; Lou, S.; An, M.; Li, Q.; Dong, F.; Yu, Y.; Cheng, W. Ground water copper levels in the seawater intrusion area and the possible physical and chemical dynamics. *Environ. Sci. Process. Impacts* **2021**, *23*, 335–343. [CrossRef] [PubMed]
73. Liu, S.; Liu, Y.; Wang, C.; Dang, X. The Distribution Characteristics and Human Health Risks of High-Fluorine Groundwater in Coastal Plain: A Case Study in Southern Laizhou Bay, China. *Front. Environ. Sci.* **2022**, *10*, 901637. [CrossRef]
74. Li, M.; Zhang, M.; Rong, H.; Zhang, X.; He, L.; Han, P.; Tong, M. Transport and deposition of plastic particles in porous media during seawater intrusion and groundwater-seawater displacement processes. *Sci. Total Environ.* **2021**, *781*, 146752. [CrossRef]
75. Johnson, W.P.; Ma, H.; Rasmuson, A.; VanNess, K.; Li, K.; Ron, C.; Erickson, B. Colloid Transport in Environmental Granular Porous Media. In *Encyclopedia of Water*; Wiley: Hoboken, NJ, USA, 2019; pp. 1–14. [CrossRef]
76. Allen, S.; Allen, D.; Phoenix, V.R.; le Roux, G.; Durántez Jiménez, P.; Simonneau, A.; Binet, S.; Galop, D. Atmospheric transport and deposition of microplastics in a remote mountain catchment. *Nat. Geosci.* **2019**, *12*, 339–344. [CrossRef]
77. Bläsing, M.; Amelung, W. Plastics in soil: Analytical methods and possible sources. *Sci. Total Environ.* **2018**, *612*, 422–435. [CrossRef]
78. Klein, M.; Fischer, E.K. Microplastic abundance in atmospheric deposition within the Metropolitan area of Hamburg, Germany. *Sci. Total Environ.* **2019**, *685*, 96–103. [CrossRef] [PubMed]
79. Steinmetz, Z.; Wollmann, C.; Schaefer, M.; Buchmann, C.; David, J.; Tröger, J.; Muñoz, K.; Frör, O.; Schaumann, G.E. Plastic mulching in agriculture. Trading short-term agronomic 18 benefits for long-term soil degradation? *Sci. Total Environ.* **2016**, *550*, 690–705. [CrossRef] [PubMed]
80. Barnes, D.K.A.; Galgani, F.; Thompson, R.C.; Barlaz, M. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 1985–1998. [CrossRef] [PubMed]
81. Cooper, D.A.; Corcoran, P.L. Effects of mechanical and chemical processes on the degradation of plastic beach debris on the island of Kauai, Hawaii. *Mar. Pollut. Bull.* **2010**, *60*, 650–654. [CrossRef] [PubMed]
82. Chae, Y.; An, Y.J. Current research trends on plastic pollution and ecological impacts on the soil ecosystem: A review. *Environ. Pollut.* **2018**, *240*, 387–395. [CrossRef] [PubMed]

83. Tian, L.; Jinjin, C.; Ji, R.; Ma, Y.; Yu, X. Microplastics in agricultural soils: Sources, effects, and their fate. *Curr. Opin. Environ. Sci. Health* **2022**, *25*, 100311. [CrossRef]
84. Zhu, K.; Jia, H.; Sun, Y.; Dai, Y.; Zhang, C.; Guo, X.; Wang, T.; Zhu, L. Long-term phototransformation of microplastics under simulated sunlight irradiation in aquatic environments: Roles of reactive oxygen species. *Water Res.* **2020**, *173*, 115564. [CrossRef] [PubMed]
85. Brennecke, D.; Duarte, B.; Paiva, F.; Caçador, I.; Canning-Clode, J. Microplastics as vector for heavy metal contamination from the marine environment. *Estuar. Coast. Shelf Sci.* **2016**, *178*, 189–195. [CrossRef]
86. Duan, J.; Bolan, N.; Li, Y.; Ding, S.; Atugoda, T.; Vithanage, M.; Sarkar, B.; Tsang, D.C.W.; Kirkham, M.B. Weathering of microplastics and interaction with other coexisting constituents in terrestrial and aquatic environments. *Water Res.* **2021**, *196*, 117011. [CrossRef]
87. Ren, Z.; Gui, X.; Xu, X.; Zhao, L.; Qiu, H.; Cao, X. Microplastics in the soil groundwater environment: Aging, migration, and co-transport of contaminants—A critical review. *J. Hazard. Mater.* **2021**, *419*, 126455. [CrossRef]
88. Zhang, W.; Ma, X.; Zhang, Z.; Wang, Y.; Wang, J.; Wang, J.; Ma, D. Persistent organic pollutants carried on plastic resin pellets from two beaches in China. *Mar. Pollut. Bull.* **2015**, *99*, 28–34. [CrossRef] [PubMed]
89. Maaß, S.; Daphi, D.; Lehmann, A.; Rillig, M.C. Transport of microplastics by two collembolan species. *Environ. Pollut.* **2017**, *225*, 456–459. [CrossRef] [PubMed]
90. Rillig, M.C.; Ziersch, L.; Hempel, S. Microplastic transport in soil by earthworms. *Sci. Rep.* **2017**, *7*, 1362. [CrossRef] [PubMed]
91. Yu, H.; Zhang, X.; Hu, J.; Peng, J.; Qu, J. Ecotoxicity of polystyrene microplastics to submerged carnivorous Utricularia vulgaris plants in freshwater ecosystems. *Environ. Pollut.* **2020**, *265*, 114830. [CrossRef] [PubMed]
92. Lwanga, E.H.; Beriot, N.; Corradini, F.; Silva, V.; Yang, X.; Baartman, J.; Rezaei, M.; van Schaik, L.; Riksen, M.; Geissen, V. Review of microplastic sources, transport pathways and correlations with other soil stressors: A journey from agricultural sites into the environment. *Chem. Biol. Technol. Agric.* **2022**, *9*, 20. [CrossRef]
93. Li, H.; Lu, X.; Wang, S.; Zheng, B.; Xu, Y. Vertical migration of microplastics along soil profile under different crop root systems. *Environ. Pollut.* **2021**, *278*, 116833. [CrossRef] [PubMed]
94. Okutan, H.M.; Sağir, Ç.; Fontaine, C.; Nauleau, B.; Kurtulus, B.; le Coustumer, P.; Razack, M. One-Dimensional Experimental Investigation of Polyethylene Microplastic Transport in a Homogeneous Saturated Medium. *Front. Environ. Sci.* **2022**, *10*, 885875. [CrossRef]
95. Qi, S.; Song, J.; Shentu, J.; Chen, Q.; Lin, K. Attachment and detachment of large microplastics in saturated porous media and its influencing factors. *Chemosphere* **2022**, *305*, 135322. [CrossRef]
96. Waldschläger, K.; Schüttrumpf, H. Infiltration Behavior of Microplastic Particles with Different Densities, Sizes, and Shapes-From Glass Spheres to Natural Sediments. *Environ. Sci. Technol.* **2020**, *54*, 9366–9373. [CrossRef]
97. Ranjan, V.P.; Joseph, A.; Sharma, H.B.; Goel, S. Preliminary investigation on effects of size, polymer type, and surface behaviour on the vertical mobility of microplastics in a porous media. *Sci. Total Environ.* **2023**, *864*, 161148. [CrossRef]
98. Gao, J.; Pan, S.; Li, P.; Wang, L.; Hou, R.; Wu, W.M.; Luo, J.; Hou, D. Vertical migration of microplastics in porous media: Multiple controlling factors under wet-dry cycling. *J. Hazard. Mater.* **2021**, *419*, 126413. [CrossRef] [PubMed]
99. Zhang, X.; Chen, Y.; Li, X.; Zhang, Y.; Gao, W.; Jiang, J.; Mo, A.; He, D. Size/shape-dependent migration of microplastics in agricultural soil under simulative and natural rainfall. *Sci. Total Environ.* **2022**, *815*, 152507. [CrossRef] [PubMed]
100. O'Connor, D.; Pan, S.; Shen, Z.; Song, Y.; Jin, Y.; Wu, W.M.; Hou, D. Microplastics undergo accelerated vertical migration in sand soil due to small size and wet-dry cycles. *Environ. Pollut.* **2019**, *249*, 527–534. [CrossRef] [PubMed]
101. Dong, Z.; Qiu, Y.; Zhang, W.; Yang, Z.; Wei, L. Size-dependent transport and retention of micron-sized plastic spheres in natural sand saturated with seawater. *Water Res.* **2018**, *143*, 518–526. [CrossRef]
102. Alimi, O.S.; Farner, J.M.; Tufenkji, N. Exposure of nanoplastics to freeze-thaw leads to aggregation and reduced transport in model groundwater environments. *Water Res.* **2021**, *189*, 116533. [CrossRef]
103. Dong, S.; Xia, J.; Sheng, L.; Wang, W.; Liu, H.; Gao, B. Transport characteristics of fragmental polyethylene glycol terephthalate (PET) microplastics in porous media under various chemical conditions. *Chemosphere* **2021**, *276*, 130214. [CrossRef]
104. Denovio, N.M.; Saiers, J.E.; Ryan, J.N. Colloid Movement in Unsaturated Porous Media: Recent Advances and Future Directions. 2004. Available online: http://www.vadosezonejournal.org (accessed on 29 February 2024).
105. Schmid, C.; Cozzarini, L.; Zambello, E. Microplastic's story. *Mar. Pollut. Bull.* **2021**, *162*, 111820. [CrossRef] [PubMed]
106. Blettler, M.C.M.; Abrial, E.; Khan, F.R.; Sivri, N.; Espinola, L.A. Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. *Water Res.* **2018**, *143*, 416–424. [CrossRef] [PubMed]
107. Huang, J.; Chen, H.; Zheng, Y.; Yang, Y.; Zhang, Y.; Gao, B. Microplastic pollution in soils and groundwater: Characteristics, analytical methods and impacts. *Chem. Eng. J.* **2021**, *425*, 131870. [CrossRef]
108. Samandra, S.; Johnston, J.M.; Jaeger, J.E.; Symons, B.; Xie, S.; Currell, M.; Ellis, A.V.; Clarke, B.O. Microplastic contamination of an unconfined groundwater aquifer in Victoria, Australia. *Sci. Total Environ.* **2022**, *802*, 149727. [CrossRef]
109. Shi, J.; Dong, Y.; Shi, Y.; Yin, T.; He, W.; An, T.; Tang, Y.; Hou, X.; Chong, S.; Chen, D.; et al. Groundwater antibiotics and microplastics in a drinking-water source area, northern China: Occurrence, spatial distribution, risk assessment, and correlation. *Environ. Res.* **2022**, *210*, 112855. [CrossRef] [PubMed]
110. Esfandiari, A.; Abbasi, S.; Peely, A.B.; Mowla, D.; Ghanbarian, M.A.; Oleszczuk, P.; Turner, A. Distribution and transport of microplastics in groundwater (Shiraz aquifer, southwest Iran). *Water Res.* **2022**, *220*, 118622. [CrossRef] [PubMed]

111. Dris, R.; Imhof, H.; Sanchez, W.; Gasperi, J.; Galgani, F.; Tassin, B.; Laforsch, C. Beyond the ocean: Contamination of freshwater ecosystems with (micro-)plastic particles. *Environ. Chem.* **2015**, *12*, 539–550. [CrossRef]
112. Kim, Y.-I.; Jeong, E.; Lee, J.-Y.; Chia, R.W.; Raza, M. Microplastic contamination in groundwater on a volcanic Jeju Island of Korea. *Environ. Res.* **2023**, *226*, 115682. [CrossRef] [PubMed]
113. Ledieu, L.; Phuong, N.-N.; Flahaut, B.; Radigois, P.; Papin, J.; le Guern, C.; Béchet, B.; Gasperi, J. May a Former Municipal Landfill Contaminate Groundwater in Microplastics? First Investigations from the "Prairie de Mauves Site" (Nantes, France). *Microplastics* **2023**, *2*, 93–106. [CrossRef]
114. Manikanda, K.B.; Natesan, U.; Vaikunth, R.; Praveen, K.; Ruthra, R.; Srinivasalu, S. Spatial distribution of microplastic concentration around landfill sites and its potential risk on groundwater. *Chemosphere* **2021**, *277*, 130263. [CrossRef] [PubMed]
115. Wan, Y.; Chen, X.; Liu, Q.; Hu, H.; Wu, C.; Xue, Q. Informal landfill contributes to the pollution of microplastics in the surrounding environment. *Environ. Pollut.* **2022**, *293*, 118586. [CrossRef] [PubMed]
116. Balestra, V.; Vigna, B.; de Costanzo, S.; Bellopede, R. Preliminary investigations of microplastic pollution in karst systems, from surface watercourses to cave waters. *J. Contam. Hydrol.* **2023**, *252*, 104117. [CrossRef]
117. Shu, X.; Xu, L.; Yang, M.; Qin, Z.; Zhang, Q.; Zhang, L. Spatial distribution characteristics and migration of microplastics in surface water, groundwater and sediment in karst areas: The case of Yulong River in Guilin, Southwest China. *Sci. Total Environ.* **2023**, *868*, 161578. [CrossRef]
118. Liu, K.; Zhang, F.; Song, Z.; Zong, C.; Wei, N.; Li, D. A novel method enabling the accurate quantification of microplastics in the water column of deep ocean. *Mar. Pollut. Bull.* **2019**, *146*, 462–465. [CrossRef]
119. Cha, J.; Lee, J.Y.; Chia, R.W. Comment on the paper "Microplastic contamination of an unconfined groundwater aquifer in Victoria, Australia". *Sci. Total Environ.* **2022**, *820*, 153121. [CrossRef] [PubMed]
120. Chia, R.W.; Lee, J.Y.; Jang, J.; Cha, J. Errors and recommended practices that should be identified to reduce suspected concentrations of microplastics in soil and groundwater: A review. *Environ. Technol. Innov.* **2022**, *28*, 102933. [CrossRef]
121. Cha, J.; Lee, J.Y.; Chia, R.W. Microplastics contamination and characteristics of agricultural groundwater in Haean Basin of Korea. *Sci. Total Environ.* **2023**, *864*, 161027. [CrossRef]
122. Nunes, B.Z.; Huang, Y.; Ribeiro, V.V.; Wu, S.; Holbech, H.; Moreira, L.B.; Xu, E.G.; Castro, I.B. Microplastic contamination in seawater across global marine protected areas boundaries. *Environ. Pollut.* **2023**, *316*, 120692. [CrossRef] [PubMed]
123. Matjašič, T.; Mori, N.; Hostnik, I.; Bajt, O.; Kovač Viršek, M. Microplastic pollution in small rivers along rural–urban gradients: Variations across catchments and between water column and sediments. *Sci. Total Environ.* **2023**, *858*, 160043. [CrossRef] [PubMed]
124. Dusaucy, J.; Gateuille, D.; Perrette, Y.; Naffrechoux, E. Microplastic pollution of worldwide lakes. *Environ. Pollut.* **2021**, *284*, 117075. [CrossRef] [PubMed]
125. Schwarz, A.E.; Ligthart, T.N.; Boukris, E.; van Harmelen, T. Sources, transport, and accumulation of different types of plastic litter in aquatic environments: A review study. *Mar. Pollut. Bull.* **2019**, *143*, 92–100. [CrossRef] [PubMed]
126. Fok, L.; Cheung, P.K.; Tang, G.; Li, W.C. Size distribution of stranded small plastic debris on the coast of Guangdong, South China. *Environ. Pollut.* **2017**, *220*, 407–412. [CrossRef] [PubMed]
127. Scheurer, M.; Bigalke, M. Microplastics in Swiss Floodplain Soils. *Environ. Sci. Technol.* **2018**, *52*, 3591–3598. [CrossRef]
128. Helm, P.A. Occurrence, sources, transport, and fate of microplastics in the great lakes–St. lawrence river basin. In *Handbook of Environmental Chemistry*; Springer Science and Business Media Deutschland GmbH: Berlin/Heidelberg, Germany, 2020; Volume 101, pp. 15–47. [CrossRef]
129. Falco, F.; di Pace, E.; Cocca, M.; Avella, M. The contribution of washing processes of synthetic clothes to microplastic pollution. *Sci. Rep.* **2019**, *9*, 6633. [CrossRef]
130. Bakaraki Turan, N.; Sari Erkan, H.; Onkal Engin, G. Microplastics in wastewater treatment plants: Occurrence, fate and identification. *Process Saf. Environ. Prot.* **2021**, *146*, 77–84. [CrossRef]
131. Lee, J.Y.; Cha, J.; Ha, K.; Viaroli, S. Microplastic pollution in groundwater: A systematic review. *Environ. Pollut. Bioavailab.* **2024**, *36*, 2299545. [CrossRef]
132. Wilde, F.D.; Radtke, D.B.; Gibs, J.; Iwatsubo, R.T. *National Field Manual for the Collection of Water-Quality Data, Book 4. Techniques of Water-Resources Investigations. Chapter A4. Collection of Water Samples*; U.S. Geological Survey: Reston, VA, USA, 1998.
133. Galgani, F.; Ruiz-Orejon, L.F.; Ronchi, F.; Tallec, K.; Fischer, E.; Matiddi, M.; Anastasopoulou, A.; Andresmaa, E.; Angiolillo, M.; Bakker, M.P.; et al. *Guidance on the Monitoring of Marine Litter in European Seas: An Update to Improve the Harmonised Monitoring of Marine Litter under the Marine Strategy Framework Directive*; JRC Technical Report No. 133594; Publications Office of the European Union: Luxembourg, 2023. [CrossRef]
134. Prata, J.C.; da Costa, J.P.; Girão, A.V.; Lopes, I.; Duarte, A.C.; Rocha-Santos, T. Identifying a quick and efficient method of removing organic matter without damaging microplastic samples. *Sci. Total Environ.* **2019**, *686*, 131–139. [CrossRef] [PubMed]
135. Masura, J.; Baker, J.E.; Foster, G.D.; Arthur, C.; Herring, C. Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommendations for Quantifying Synthetic Particles in Waters and Sediments. 2015. Available online: https://repository.library.noaa.gov/view/noaa/10296 (accessed on 17 April 2024).
136. Hidalgo-Ruz, V.; Gutow, L.; Thompson, R.C.; Thiel, M. Microplastics in the marine environment: A review of the methods used for identification and quantification. *Environ. Sci. Technol.* **2012**, *46*, 3060–3075. [CrossRef] [PubMed]

137. Löder, M.G.J.; Gerdts, G. Methodology Used for the Detection and Identification of Microplastics—A Critical Appraisal. In *Marine Anthropogenic Litter*; Bergmann, M., Gutow, L., Klages, M., Eds.; Springer: Cham, Switzerland, 2015. [CrossRef]
138. Lenz, R.; Enders, K.; Stedmon, C.A.; MacKenzie, D.M.A.; Nielsen, T.G. A critical assessment of visual identification of marine microplastic using Raman spectroscopy for analysis improvement. *Mar. Pollut. Bull.* **2015**, *100*, 82–91. [CrossRef]
139. Prata, J.C.; Reis, V.; da Costa, J.P.; Mouneyrac, C.; Duarte, A.C.; Rocha-Santos, T. Contamination issues as a challenge in quality control and quality assurance in microplastics analytics. *J. Hazard. Mater.* **2021**, *403*, 123660. [CrossRef]

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Spatial and Temporal Distribution Characteristics and Potential Sources of Microplastic Pollution in China's Freshwater Environments

Hualong He [1], Sulin Cai [1], Siyuan Chen [1], Qiang Li [1], Pengwei Wan [1], Rumeng Ye [1], Xiaoyi Zeng [1], Bei Yao [1], Yanli Ji [1], Tingting Cao [1], Yunchao Luo [3], Han Jiang [1], Run Liu [1], Qi Chen [1], You Fang [1], Lu Pang [1], Yunru Chen [1], Weihua He [1], Yueting Pan [1], Gaozhong Pu [4], Jiaqin Zeng [5] and Xingjun Tian [1,2,*]

1 School of Life Sciences, Nanjing University, Nanjing 210023, China; hehua@smail.nju.edu.cn (H.H.); mg1930001@smail.nju.edu.cn (S.C.); mg20300060@smail.nju.edu.cn (S.C.); waterliqiang@163.com (Q.L.); w18362983051@163.com (P.W.); yrm_666@163.com (R.Y.); xyzeng@connect.hku.hk (X.Z.); by-beiyao@hotmail.com (B.Y.); luckyjiyanli@163.com (Y.J.); caotting@zafu.edu.cn (T.C.); JH269962460@163.com (H.J.); recho24@126.com (R.L.); chen1455526947@163.com (Q.C.); youfang@smail.nju.edu.cn (Y.F.); panglugxs@163.com (L.P.); cherry960502@163.com (Y.C.); 18351870527@163.com (W.H.); njupyt@163.com (Y.P.)

2 Co-Innovation Center for Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, China

3 School of Life Sciences, Shanxi Normal University, Taiyuan 030031, China; luoyunchao@sxnu.edu.cn

4 Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, China; pukouchy@163.com

5 College of Resources and Environment, Tibet Agricultural & Animal Husbandry University, Linzhi 860000, China; zengjiaqin2009@163.com

* Correspondence: tianxj@nju.edu.cn

Citation: He, H.; Cai, S.; Chen, S.; Li, Q.; Wan, P.; Ye, R.; Zeng, X.; Yao, B.; Ji, Y.; Cao, T.; et al. Spatial and Temporal Distribution Characteristics and Potential Sources of Microplastic Pollution in China's Freshwater Environments. *Water* **2024**, *16*, 1270. https://doi.org/10.3390/w16091270

Academic Editors: Grigorios L. Kyriakopoulos, Antonis A. Zorpas, María Rocío Rodríguez Barroso and Vassilis J. Inglezakis

Received: 7 April 2024
Revised: 20 April 2024
Accepted: 22 April 2024
Published: 29 April 2024

Abstract: Microplastic pollution is a research hotspot around the world. This study investigated the characteristics of microplastic pollution in the freshwater environments of 21 major cities across China. Through indoor and outdoor experimental analysis, we have identified the spatial and temporal distribution characteristics of microplastic pollution in China's freshwater environments. Our findings indicate that the average concentration of microplastics in China's freshwater environments is 3502.6 n/m^3. The majority of these microplastics are fibrous (42.5%), predominantly smaller than 3 mm (28.1%), and mostly colored (64.7%). The primary chemical components of these microplastics are polyethylene (PE, 33.6%), polyvinyl chloride (PVC, 21.5%), polypropylene (PP, 16.8%), and polystyrene (PS, 15.6%). The abundance of microplastics in China's freshwater environments generally tends to increase from west to east and from south to north, with the lowest concentration found in Xining, Qinghai (1737.5 n/m^3), and the highest in Jiamusi, Heilongjiang (5650.0 n/m^3). The distribution characteristics of microplastics are directly related to land use types, primarily concentrated in areas of intense human activity, including agricultural, transport, and urban land. Seasonal changes affect the abundance of microplastics, peaking in summer, followed by spring and autumn, mainly due to variations in rainfall, showing a positive correlation.

Keywords: China's freshwater environment; microplastics pollution; spatial and temporal distribution characteristics; land use types; rainfall

1. Introduction

Globally, at least 300 million tons of plastic are produced annually [1], the majority of which enter the environment and remain for decades [2], posing severe risks to biological safety [3]. China, as a populous and major agricultural country [4], is also one of the largest plastic producers [5]. In 2018, China's plastic production reached 60.4215 million tons, accounting for 29% of the global total [6]. At the same time, China is one of the largest

consumers of plastic [7], generating massive amounts of plastic waste yearly [8]. The extensive use of plastic bags, fast food containers, plastic greenhouses, and agricultural film has led to a significant "white pollution" problem [9–12].

Plastics in the environment continuously degrade into microplastics (MPs) and nanoplastics (NPs) [13]. Microplastics are generally defined as plastics smaller than 5 mm [14]. The issue of microplastic pollution has been widely reported worldwide and has become a significant environmental problem, attracting public attention [15,16]. Due to their ubiquity, persistence, and potential ecological risks, microplastics have become a hotspot in new pollutant research [17,18]. MPs are difficult to degrade, can adsorb other pollutants, and accumulate in the food chain, thus posing substantial hazards [19–21]. Studies have shown that microplastics can alter the structure and function of ecosystems, ultimately affecting biodiversity [22–26].

Freshwater ecosystems include large bodies of water such as rivers and lakes, as well as smaller bodies like ditches and ponds [27–29]. These ecosystems, closely related to human life, boast high species diversity and provide numerous ecological services, making them highly susceptible to microplastic pollution [30,31]. Microplastics in trash, sludge, and wastewater can enter freshwater environments directly, while those in soil may be carried into water bodies by runoff [32–34]. Although current research on microplastic pollution mainly focuses on marine ecosystems, reports have identified terrestrial ecosystems as major sources of pollution [35,36]. Freshwater environments often have a more immediate physical proximity to human activities compared to marine environments, leading to a more direct impact on water quality and human health through recreational and consumption routes [37,38]. As freshwater environments are crucial pathways for microplastics to transfer from land to sea, research on microplastics in freshwater environments is growing and has yielded important insights. However, it remains less extensive compared to studies focused on marine environments [39,40].

The study of microplastic pollution in freshwater ecosystems focuses on water bodies, sediments, and biota, with factors affecting the distribution, accumulation, and migration of microplastics being key research topics [27,30,31]. It has been demonstrated that microplastics are widely distributed in China's freshwater environments [41]. An investigation in the Yangtze River Delta of China found the widespread presence of microplastics [42]. Even in the high-altitude area on the Tibetan Plateau, microplastics are also commonly present in freshwater [43]. However, due to the diversity of China's freshwater environments, the abundance of inhabiting species, varied geographical locations, and complex socioeconomic backgrounds, data are currently widely distributed and lack systematic integrity [44]. Moreover, China's rapid economic growth and the significant contribution of plastic waste highlight the urgent need for effective policies and actions to address this issue [45]. Hence, there is an urgent need for research on microplastic pollution in China's freshwater environments [46].

This study selects 21 major cities across China, primarily located in the Yangtze and Yellow River basins, encompassing most types of land use and representing China's main freshwater environments. By investigating and analyzing the characteristics of microplastic pollution in these cities' freshwater environments, this study aims to answer the following scientific questions: (1) What are the spatial and temporal distribution characteristics of microplastic pollution in China's freshwater environments? (2) What are the potential sources of microplastic pollution in China's freshwater environments? (3) What are the driving factors affecting the distribution of microplastic pollution in China's freshwater environments?

2. Materials and Methods

2.1. Sampling Locations

Our study systematically selected 21 cities across China, focusing on both urban and suburban areas. The sampling covered both large water bodies, such as rivers and lakes, and smaller ones like ditches and ponds, encompassing naturally occurring and man-made sites. These cities were strategically chosen to represent a broad spectrum of land use types,

including but not limited to agricultural, urban, and suburban areas. This diverse selection aims to capture the varying degrees of microplastic pollution influenced by different human activities across geographical locations.

Sampling was conducted four times in 2020, during April, June, August, and October, with three replicates at each site, resulting in a total of 252 water samples. We conducted our sampling during two distinct seasons: the dry season (spring and autumn) and the rainy season (summer). This approach allowed us to capture seasonal variations in microplastic abundance [47]. Detailed information and locations of the sampling sites are provided in Figure 1 and in Tables S1 and S2.

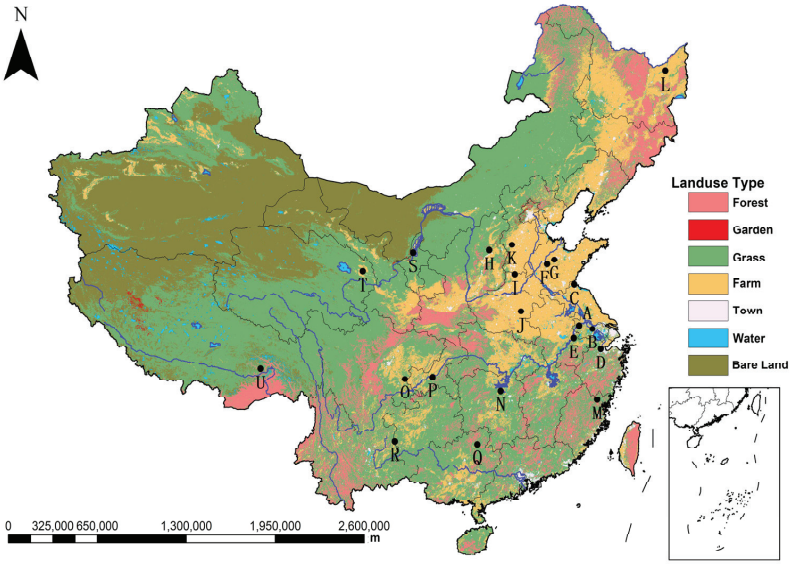

Figure 1. Spatial distribution of sampling locations across China. Each black dot represents a sampling site.

Winter was excluded from the seasonal sampling due to the freezing of rivers in northern China, which poses significant challenges for sampling. Additionally, the reduced biological and human activity during this season could potentially skew the representation of microplastic pollution levels.

2.2. Sampling Method

At each sampling site, GPS was used to determine the coordinates, and the surrounding environment and water body types were photographed for future reference. Surface water (5–10 cm deep) was collected randomly in 1000 mL glass bottles after rinsing the container with site water [48]. Each site had three replicates. The samples were then transported to the laboratory, stored at 2 °C in the dark, and sealed [49].

2.3. Extraction of Microplastics from Water Samples

Currently, there is a complete set of mature methodologies for the extraction and analysis of microplastics in water bodies [50–52]. Our experiment mainly referenced the work of Su and Hu [48,49], as shown in Figure S1. The extraction of microplastics from water samples involves three steps: (1) measure and record the volume of each site and its parallel water samples. (2) Using a vacuum pump (ME1, Vacuubrand, Wertheim, Germany) and a glass filter (XX1004700, Millipore, Boston, MA, USA), firstly filter the water sample onto a nylon membrane filter (NY2004700, Millipore, USA) with a diameter of 47 mm and a pore size of 20 μm, then rinse the substances on the filter membrane

into a 250 mL conical flask with 30% (v/v) hydrogen peroxide (H1009, Sigma-Aldrich, Saint Louis, MO, USA), and finally place it in a high-temperature shaker for digestion (80 rpm, 65 °C, not exceeding 72 h) until the organic matter is completely digested and the solution is transparent. (3) Filter the digested solution onto a nitrocellulose membrane filter (HAWP04700, Millipore, USA) with a diameter of 47 mm and a pore size of 0.45 μm, place it in a 6 cm diameter glass Petri dish, dry it in an oven, and seal and store it for subsequent analysis. Due to the high amounts of impurities, to ensure the accuracy of the experiment, each water sample needs to be filtered onto three membrane filters, resulting in a total of 756 nitrocellulose membrane filters.

2.4. Microscopic Examination and Statistical Analysis of Microplastic Samples

Microplastic samples were examined and photographed under a stereomicroscope (E100, Nikon, Tokyo, Japan) at 30–40× magnification, adjusting as needed for microplastic size. The ImageJ software (Version 1.8.0) was used for measuring and statistically analyzing the microplastic sizes. Photographs were used to classify microplastics by shape, size, color, and abundance.

2.5. Chemical Composition Identification of Microplastic Samples

In this experiment, micro-Fourier Transform Infrared Spectroscopy (μ-FTIR, Spectrum Two, PerkinElmer, Waltham, MA, USA) was employed to identify the chemical composition of microplastics, while a Field Emission Scanning Electron Microscope (FE-SEM, Mira 4, Tescan, Brno, Czech Republic) was used to photograph the surface structure of the microplastics. Initially, a small number of samples were selected for identification to gain experience, followed by the random selection of a large number of samples for formal identification.

The μ-FTIR identification process involved transferring samples to clean nitrocellulose membranes, placing them on the μ-FTIR sample platform, and using the OPUS software (Version 8.8) for analysis. The FE-SEM process included preparing the samples on conductive tape, coating them with gold to prevent charging, and photographing them at varying magnifications based on the sample type.

2.6. Data Acquisition on Population, Economy, Rainfall, and Land Use Types

The population, economic, and rainfall data used in this study were all sourced from statistical yearbooks published by various city statistics bureaus, while the data on land use types were derived from the results of China's third national land survey released by the Ministry of Natural Resources [53]. The third national land survey of China commenced in October 2017 and was completed in 2020. The survey comprehensively utilized satellite remote sensing images with a resolution better than 1 m to create base maps for the investigation. The survey, lasting three years, involved 219,000 survey personnel and compiled 295 million survey plot data points, thoroughly clarifying the status of land use in China. Therefore, the related data used in this study are reliable and credible.

2.7. Quality Assurance

The presence of procedural contamination or air blank contamination can impact the final results. Therefore, to minimize sample contamination during field sampling, the following measures were taken: First, before sampling, all containers and tools were cleaned with filtered water (distilled water filtered through a 47 mm diameter, 5 μm pore size filter membrane) and covered or wrapped in foil to prevent contamination. Second, gloves were worn during the sampling process. Third, samples were sealed and stored immediately after collection to avoid direct exposure to the atmosphere as much as possible.

In the laboratory analysis process, all solutions were filtered and prepared using a 47 mm diameter, 5 μm pore size polycarbonate filter membrane (TMTP04700, Millipore, USA). During the drying step, five open Petri dishes were used to estimate air blank contamination within the oven, and after being placed for 72 h, no microplastic blank

contamination was found in any of the Petri dishes, proving that the oven environment was clean. To reduce risk, samples were still slightly covered with aluminum foil during the drying process.

Additionally, we set up five blank controls to rigorously detect any system or experimental errors. These controls were tested alongside the experimental samples in identical conditions to ensure no contamination influenced our results. The analysis of these blank controls showed no detectable contamination, affirming the reliability of our experimental procedures and data.

2.8. Data Analysis

Data analysis and graphing were conducted using RStudio software (Version 4.3.2). The data were initially subjected to a normal distribution test (Shapiro–Wilk Test) and a homogeneity of variances test (Bartlett's Test). If the data were normally distributed, an ANOVA was used for intra-group variance analysis, followed by a Tukey Test for inter-group multiple comparisons. If the data did not follow a normal distribution, a non-parametric testing method (Kruskal–Wallis Test) was employed for intra-group variance analysis, with Dunn's Test and the Bonferroni correction method applied for inter-group multiple comparisons.

The map of vegetation in China was created using ArcGIS (Version 10.2). The map showing the distribution of sample abundance at sampling sites was generated using R extension packages (sf, ggspatial), with map vector data sourced from DataV.GeoAtlas [54]. The other figures were produced using RStudio software (Version 4.3.2) and the corresponding extension packages (tidyverse, etc.).

3. Results

3.1. Physical and Chemical Properties of Microplastic Samples

By analyzing 256 water samples through filtration and digestion, a total of 756 filter membranes were obtained. Microplastic samples on all filter membranes were observed, photographed, scanned, and identified, with results recorded (Figure 2). Under a stereomicroscope set at various magnifications, microplastics of different shapes were observed: fibers, films, fragments, and pellets. Field emission scanning electron microscopy (FE-SEM) revealed that the surfaces of these differently shaped microplastics were not smooth, with most being very rough. The chemical components of the extracted microplastic samples, identified using micro-Fourier transform infrared spectroscopy (μ-FTIR), were primarily polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), and polystyrene (PS).

Statistical analysis of all samples yielded distribution characteristics of microplastics in terms of shape, size, and color (Table 1 and Figure 3). Table 1 presents the analysis results of all samples. In terms of shape, fibers were the most common, accounting for 42.5% of microplastics, followed by films at 38.2%. Regarding size, microplastics smaller than 0.3 mm predominated, comprising 28.1% of the total, with those smaller than 0.5 mm making up 51.9%. In terms of color, transparent microplastics were most common at 35.3%, but colored samples accounted for 64.7%, mainly white and black, indicating that most microplastics were colored.

Table 1. Distribution characteristics of microplastics by shape, size, and color (%).

Shape	Percentage	Size (mm)	Percentage	Color	Percentage
Fiber	42.5	<0.3	28.1	Transparent	35.3
Film	38.2	0.3–0.5	23.7	White	20.8
Fragment	11.5	0.5–1	17.1	Black	13.5
Pellet	7.8	1–2	13.8	Blue	11.4
		2–3	10.9	Green	9.3
		3–5	6.4	Red	6.1
				Others	3.5

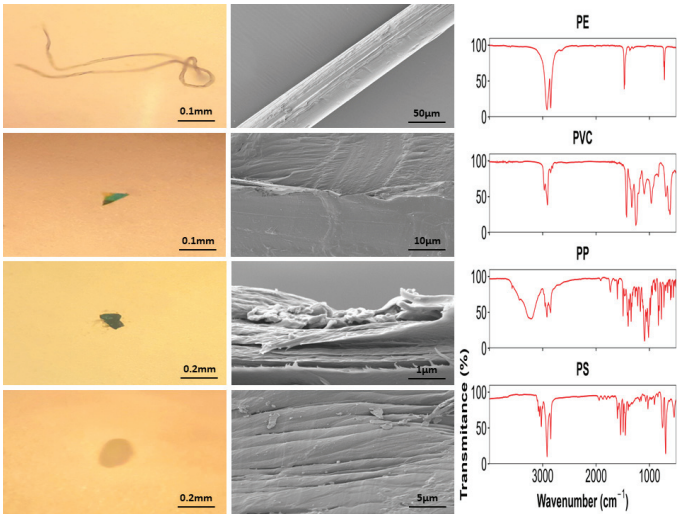

Figure 2. Stereomicroscope, SEM, and spectral images of microplastic samples.

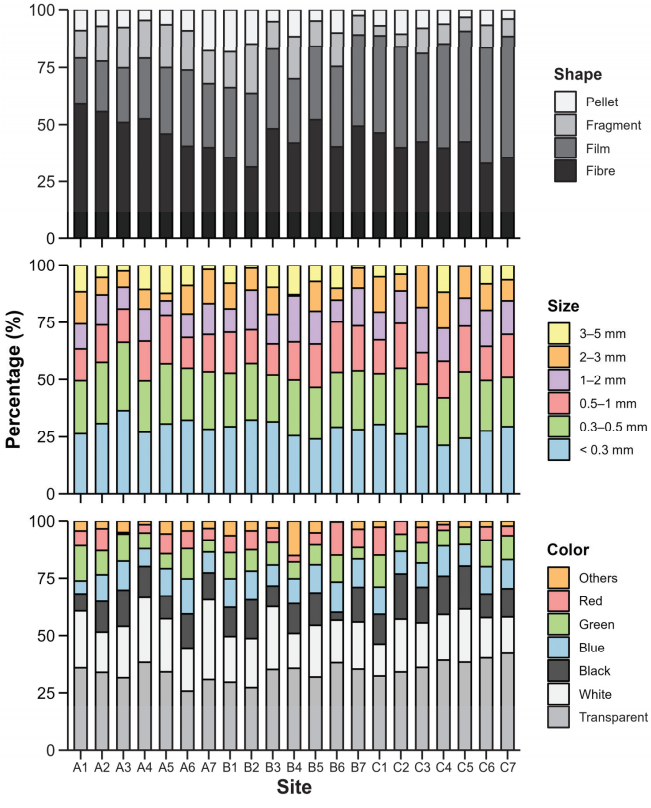

Figure 3. Distribution characteristics of microplastics by shape, size, and color. Group information details can be found in Table S3. All sampling sites were arranged in ascending order of microplastic abundance and divided into three groups.

The analysis of various sampling points (Figure 3) showed that in terms of shape, Group A had the highest proportion of fibers at 48.2%, surpassing the other groups. However, Group C had the highest proportion of films at 46.6%. In terms of size, Group A had the highest proportion of samples smaller than 0.5 mm at 55.4%, while Group C had a higher proportion of samples larger than 0.5 mm, indicating a larger average size. The distribution of colors among the three groups was relatively even, with no significant differences ($p > 0.05$).

Micro-Fourier transform infrared spectroscopy (μ-FTIR) was used for infrared spectroscopic analysis of all samples. The statistical analysis of these results provided the distribution characteristics of the chemical components of microplastics (Figure 4). It was found that the chemical components of the microplastic samples were mainly polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), and polystyrene (PS), with PE being the most prevalent at 33.6%, followed by PVC at 21.5%. Figure 4 also reveals that, among all analyzed samples, there were some non-plastic components, although they only constituted 7.5% of the materials. This indicates that the experimental process extracted not only plastic samples but also some non-plastic components. While there were minor errors, they were negligible and did not impact the experimental results.

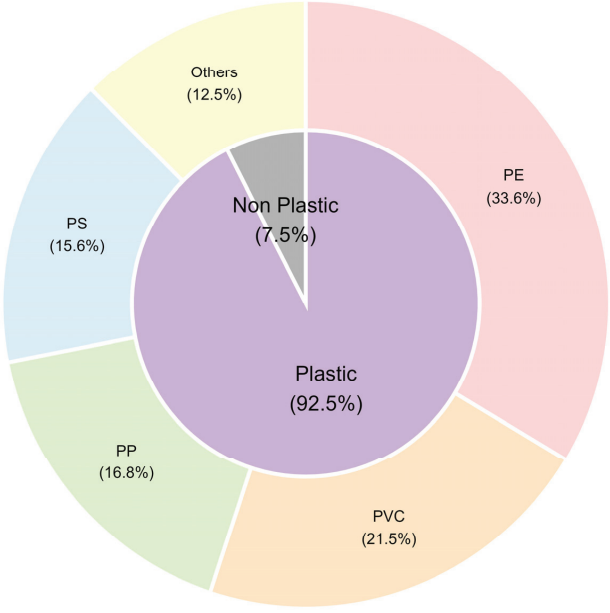

Figure 4. Distribution characteristics of microplastics by chemical composition. The external circular chart displays the percentage composition of different types of microplastics identified. The internal pie chart illustrates the proportion of non-plastic components detected in the samples.

3.2. Spatial Distribution Characteristics of Microplastic Abundance

Statistical analysis of all sampling results revealed the spatial distribution characteristics of microplastic abundance in China's freshwater environments, with an average abundance of 3502.6 n/m^3 (Figure 5). The lowest abundance was in Xining, Qinghai (1737.5 n/m^3), and the highest in Jiamusi, Heilongjiang (5650.0 n/m^3). Microplastics were primarily found in areas with frequent human activity, including economically developed and agriculturally intensive regions.

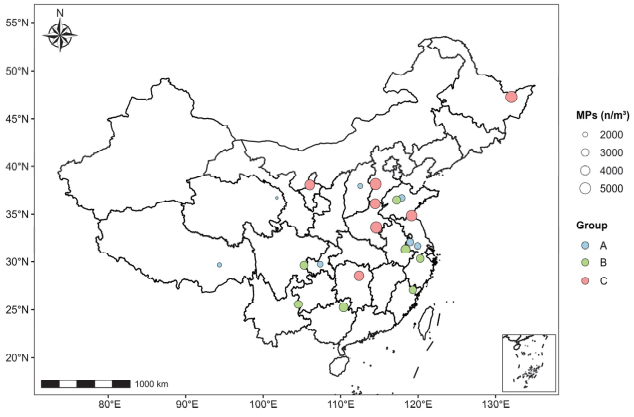

Figure 5. Spatial distribution characteristics of microplastic abundance. The points in the figure represent different sampling sites, with different colors indicating different groups. The varying sizes of the points denote different abundances; the larger the area, the higher the abundance.

We categorized all the sample sites into three groups according to the abundance of microplastics, ranging from lowest to highest (Table S3). Group A's sampling locations are predominantly situated in the eastern and western regions, exhibiting the lowest mean abundance of microplastics at merely 2228.9 n/m^3. The sampling sites of Group B are chiefly located in the southern region, demonstrating a relatively higher mean abundance of microplastics, calculated at 3346.7 n/m^3. Conversely, Group C's sampling locations are mainly concentrated in the central and northern regions, with the highest mean abundance of microplastics, recorded at 4932.3 n/m^3. Further analytical examination revealed that the differences between the groups are markedly significant ($p < 0.001$).

Subsequent regression analysis was conducted to assess the relationship between the abundance of microplastics and geographic coordinates (Figure 6). The analysis revealed that there is a general upward trend in microplastic abundance with increasing longitude, evidencing a notable positive correlation ($p < 0.05$). Similarly, an overall upward trend in microplastic abundance was observed with increasing latitude; however, the positive correlation in this case was less pronounced ($p > 0.05$). Thus, these findings allow us to deduce that the abundance of microplastics in China's freshwater environments exhibits a general increasing trend from west to east and from south to north.

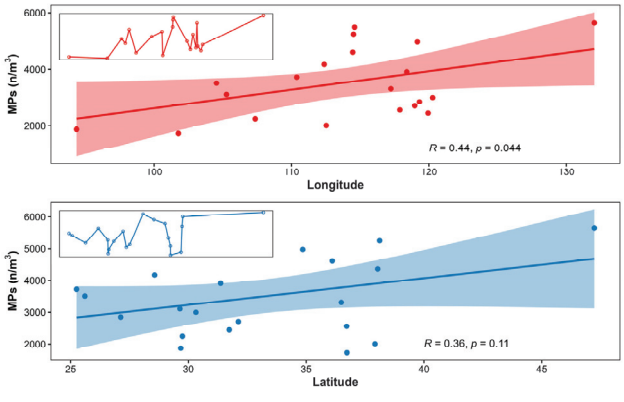

Figure 6. Geographic distribution characteristics of microplastic abundance. The major part of the figure is the regression analysis, with a small chart in the left corner showing the trend of microplastic abundance changes.

3.3. Temporal Distribution Characteristics of Microplastic Abundance

Statistical analyses of data from four sampling events elucidated the temporal distribution patterns of microplastic abundance in freshwater environments across China (Figure 7). Overall, the abundance of microplastics demonstrated an initial increase followed by a decrease within the period from April to October. Among these sampling points, the month of June recorded the peak abundance of microplastics, reaching 4776.2 n/m^3. This was succeeded by August, with an abundance of 3904.8 n/m^3, and subsequently October, showing 2771.4 n/m^3. The lowest abundance was observed in April, with a mere 2557.1 n/m^3.

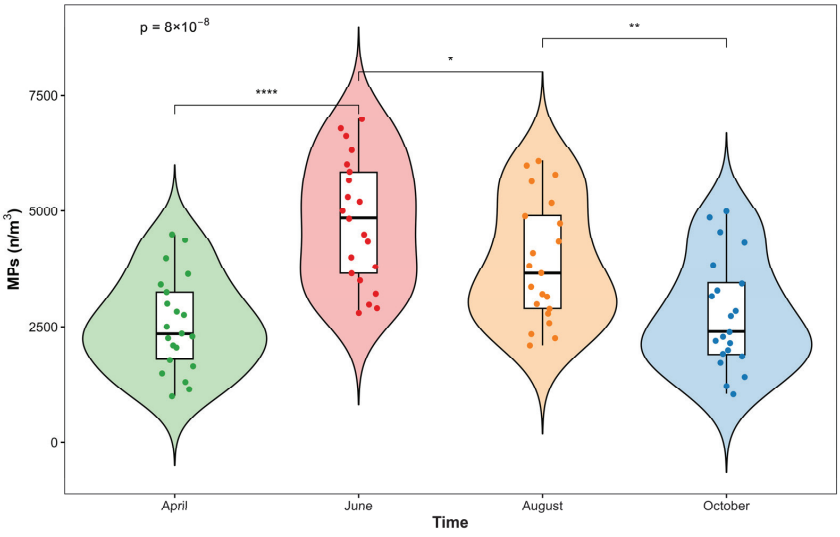

Figure 7. Temporal distribution characteristics of microplastic abundance. The *p*-value in the top left corner represents the difference among all groups. The number of asterisks * above the lines connecting groups indicates the size of the *p*-value; * for $p < 0.05$, ** for $p < 0.01$, **** for $p < 0.001$.

When comparing the microplastic abundances across the four sampling instances, the variations were found to be highly significant ($p < 0.001$). Specifically, the comparative analysis between adjacent sampling periods highlighted that the difference between April and June was the most pronounced, followed by a discernibly significant difference between August and October, while the gap between June and August was relatively minor.

In China, April typically corresponds to the spring season, and October to the autumn season, with both June and August falling within the summer period. Conducting an analysis based on these seasonal distinctions, the data reveal a seasonal distribution trend for microplastic abundance in China's freshwater environments: the highest abundance is recorded during the summer months, averaging 4340.5 n/m^3, followed by autumn with 2771.4 n/m^3, and the lowest in spring at 2557.1 n/m^3.

3.4. Relationship between Microplastic Distribution and Land Use Types

Figure 5 has already shown that microplastics are mainly distributed in areas with frequent human activities, including economically developed and agriculturally concentrated regions. However, the specific relationship between microplastic distribution and various types of land use, and the extent of this relationship, has yet to be determined. This section will further explore the relationship between the characteristics of microplastic distribution and land use types.

Using the R extension packages (linkET and vegan), a Mantel test was conducted to examine the relationship between the abundance of microplastics and types of land use (Figure 8). The Mantel test, a statistical method used to assess the correlation between

two distance matrices, helps us understand the relationship between the geographical distribution of microplastics and various land use types. This non-parametric test is particularly useful in ecological studies where data may not meet the assumptions required by more traditional parametric tests.

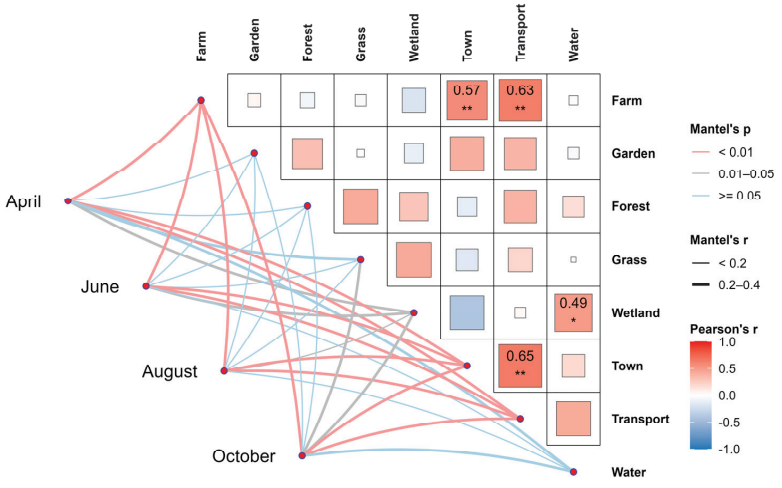

Figure 8. Mantel test. The lower left corner of the figure displays a correlation network diagram between microplastic abundance and land use types, with red lines indicating positive correlations and blue lines indicating negative correlations. The thicker the line, the stronger the correlation. The upper right corner shows a heatmap of correlations between land use types, with red indicating positive correlations and blue indicating negative correlations. The larger the square area, the larger the correlation coefficient. Asterisks indicate the level of significance of the correlation, * for $p < 0.05$, ** for $p < 0.01$.

The results showed a correlation between different types of land use. There was a relatively high correlation between urban and transport land (r = 0.65) and a considerable correlation between agricultural land and both urban (r = 0.57) and transport land (r = 0.63).

Examining the relationship between microplastic abundance and types of land use, with April sampling as an example, the highest correlation was found with agricultural land, followed by transport and then urban land. The correlation *p*-values were all less than 0.01, indicating a highly significant relationship. A weak correlation existed between microplastic abundance and wetlands, with a *p*-value less than 0.05 but greater than 0.01. No correlation was found between microplastic abundance and gardens, forests, grasslands, and water area lands, with all *p*-values greater than 0.05. Similar correlation patterns were observed in the analysis of samples from other months.

The Mantel test indicated that the distribution characteristics of microplastics are directly related to types of land use, mainly concentrated in areas with frequent human activities, including agricultural, transport, and urban land.

Further analysis through redundancy analysis (RDA) using R extension packages (ggpubr, ggrepel, and vegan) delved into the impact of land use types on microplastic distribution characteristics. The data were first subjected to Hellinger transformation for dimensionality reduction and normalization to enhance reliability.

Results (Figure 9) confirmed that agricultural, transport, and urban land have the most significant impact on microplastic abundance. This is in complete agreement with the Mantel test results, further proving that microplastic distribution characteristics are directly related to types of land use, primarily concentrating in areas of frequent human activity such as agricultural, transport, and urban land.

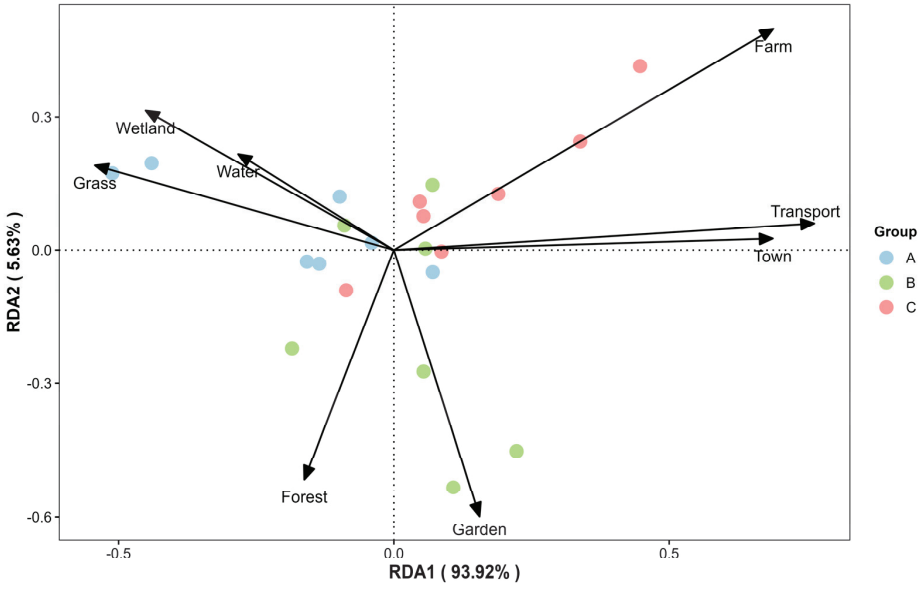

Figure 9. Redundancy analysis (RDA). The *x*-axis (RDA1) and *y*-axis (RDA2) represent the first and second principal components, respectively, explaining the highest proportions of variance in the data set. The points in the figure represent samples, with different colors indicating different groups, while arrows originating from the origin represent land use types. The length of an arrow indicates the strength of the impact of land use type on microplastic abundance, with longer arrows indicating a stronger influence of that land use type. The angle between an arrow and the axes represents the correlation between the land use type and the axes, with smaller angles indicating higher correlations. The vertical distance from a sample point to an arrow and its extension line indicates the strength of the impact of land use type on microplastic abundance; the closer a sample point is to an arrow, the stronger the influence of that land use type on microplastic abundance. If a sample point is in the same direction as an arrow, it indicates a positive correlation between microplastic abundance and that land use type. If a sample point is in the opposite direction of an arrow, it indicates a negative correlation between microplastic abundance and that land use type.

3.5. Relationship between Microplastic Distribution and Social and Natural Factors

Figure 7 has shown that the abundance of microplastics changes over time, with June showing higher abundance than other months. To understand why this variation occurs, a regression analysis was conducted between microplastic abundance and social and natural factors (Figure 10).

The results showed a negative correlation between microplastic abundance and regional GDP, indicating that economically developed areas do not necessarily have higher microplastic abundance (Figure 10A). A positive but not significant correlation ($p > 0.05$) was found between microplastic abundance and population size, suggesting that microplastic abundance tends to increase with population growth (Figure 10B).

A strong positive correlation (p-value close to 0.01) was found between microplastic abundance and regional area, indicating that microplastic abundance increases with the size of the area (Figure 10C). Correlation analysis between microplastic abundance at different sampling sites across various months and rainfall showed a very strong positive correlation ($p < 0.001$), identifying rainfall as the most critical factor affecting microplastic distribution (Figure 10D).

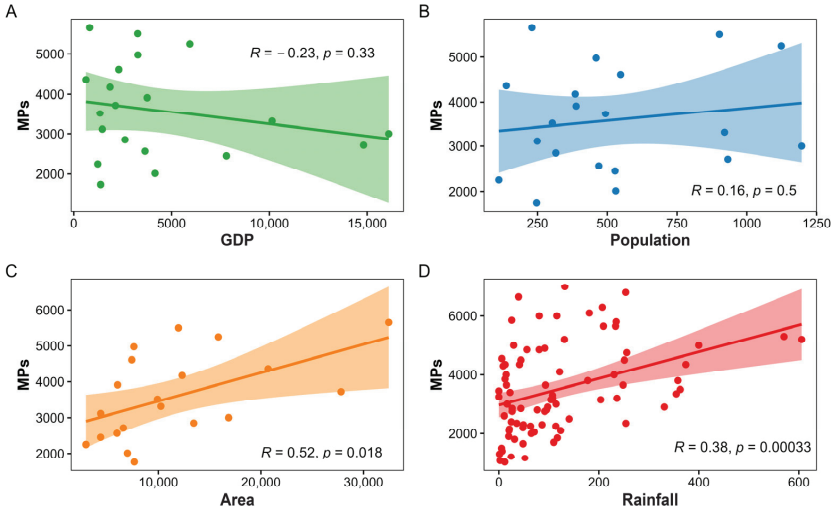

Figure 10. Regression analysis of microplastic abundance with social and natural factors. (**A**) Regression analysis of microplastic abundance with GDP. (**B**) Regression analysis of microplastic abundance with regional population. (**C**) Regression analysis of microplastic abundance with regional area. (**D**) Regression analysis of microplastic abundance with rainfall. (**A**–**C**) use the average values of four samplings. (**D**) uses all the values of four samplings, including microplastic abundance and rainfall.

4. Discussion

4.1. Spatial and Temporal Distribution Characteristics of Microplastic Pollution

This study discovered an overall trend of increasing microplastic abundance from west to east and from south to north in China's freshwater environments. Agricultural lands in China are mainly concentrated in the central, eastern, and northern regions [55], which is evident from the land type distribution shown in Figure 1. There is an inevitable correlation between the two. When categorizing all sampling points based on microplastic abundance from low to high, significant differences were observed between the groups ($p < 0.001$), with Group C sampling points showing the highest microplastic abundance, which also had the highest proportion of agricultural land. This precisely indicates that the possible source of microplastics in freshwater environments is the adjacent farmlands [56,57].

Overall, the abundance of microplastics increased first and then decreased from April to October, with the highest abundance recorded in June. This pattern corresponds with China's rainy season, which mainly occurs from June to August (summer). Although Group C did not experience the highest rainfall, the accumulation of microplastics during the dry season (spring) is expected to be higher than in the other groups.

In the dry season, microplastics accumulate on the soil surface or on roads covered with dust [58,59]. Kang et al. conducted a study in Goyang city, South Korea, and found that microplastic concentrations in road dust increased with the drying period, suggesting a significant accumulation of these pollutants on road surfaces in dry conditions [60]. Their research indicated that after a three-day drying period, the concentration of microplastics was notably higher, with a significant portion originating from vehicle tires and road materials.

During the rainy season, these microplastics are transported to freshwater environments with rainwater runoff [61–63]. Koutnik et al. conducted a global analysis on the distribution of microplastics in soil and freshwater environments, focusing on the factors affecting their concentration and the fundamental transport processes. Their findings indicate that microplastic concentrations in inland locations such as glaciers and urban stormwater were significantly higher than in rivers, suggesting the importance of rainwater runoff in microplastic transport [64].

Furthermore, regression analysis between regional population, area size, and microplastic abundance revealed a positive correlation, though not significant. However, there was no positive correlation between regional GDP and microplastic abundance, possibly because economically developed areas have a lower proportion of agricultural land and pay more attention to environmental protection, thereby limiting the use of plastic products [65–68].

4.2. Potential Sources of Microplastic Pollution

Both Mantel tests and redundancy analysis (RDA) indicate that the distribution characteristics of microplastics are directly related to land use types, predominantly concentrated in areas of frequent human activity, including agricultural, transport, and urban land.

Agricultural activities involve extensive use of plastic greenhouses and mulch films, mostly made of polyethylene (PE) or polyvinyl chloride (PVC) [69–71]. Zhang et al. found the agricultural plastic film usage in China in 2017 was 2,528,600 tons [72]. After agricultural film recycling and water erosion, the plastic debris amount was estimated as 465,016 tons. The water erosion process carried 4329 tons of plastic debris into the aquatic environment. Studies have shown that under the combined effects of sunlight and rainfall, these plastic films continuously break down into smaller pieces, forming microplastics that can migrate into deeper soil layers [73]. Additionally, the long-term application of sludge and organic fertilizers is another significant source of microplastic pollution in farmlands [74]. About 90% of microplastics in wastewater accumulate in sludge, which is often used as fertilizer after pretreatment [75]. However, conventional sludge pretreatment methods, such as anaerobic fermentation and heat drying, are ineffective in removing microplastics [76,77]. Thus, microplastics enter and accumulate in the soil through sludge used as fertilizer [78]. This explains why polyethylene and polyvinyl chloride are the main chemical components of microplastic samples in this study (Figure 4). Among all sampling points, Group C had the highest proportion of film-shaped microplastics and the largest average size, likely related to the high proportion of agricultural land in this group.

In transportation activities, microplastics primarily originate from two sources. The first source is road dust [79]. Su et al. found that the average abundance of microplastics in road dust collected from typical streets in Phillip Bay, Australia, and its upstream area during different precipitation seasons ranged from 20.6 to 529.3 items/kg [80]. Fibers (70.8%), individuals smaller than 1 mm (41.9%), and polymers like polyester and polypropylene (combined 26.3%) constituted the majority of microplastics. Monitoring road dust is an economical and effective method for preliminary screening of microplastic pollution levels from atmospheric or urban non-point source diffusion. Road dust has been proven to be an important site where microplastics enter the environment from non-point sources [60,79]. The second major source in transportation is tire and brake wear [81]. Evangeliou et al. conducted global simulations of the atmospheric transport of microplastic particles produced by road traffic, including tire wear particles (TWPs) and brake wear particles (BWPs) [82]. Their findings reveal a high transport efficiency of these particles to remote regions, suggesting a significant environmental impact far from their urban source areas. Recent research by Griffith University in Australia analyzed the quantity and type of tire wear particles (TWPs) in urban stormwater runoff [83]. As tires wear, they release particles of varying sizes, from visible rubber chunks to microplastics. Annually, 6.6 million tons of TWPs are released worldwide, becoming a significant source of microplastic pollution.

Urban areas, as major human settlements, continuously generate a vast amount of microplastic pollution [59]. This experiment showed that fibers are the predominant shape of microplastics in China's freshwater environments (42.5%). Similar findings have been reported in many studies, especially those investigating urban water bodies [84,85]. For instance, studies were conducted in the Ottawa River basin in Canada and the Rhine River basin in Germany, where fiber detection rates exceeded 60%, with some areas reaching up to 100% [86,87]. Additionally, Hu et al. investigated microplastic distribution in 25 small water bodies in the Yangtze River Delta urban agglomeration, including Shanghai and Zhe-

jiang [48]. The results showed that microplastics were universally present, with an average abundance of 0.5–21.5 items/liter, predominantly in fiber form, accounting for 87.8%. A significant source of high fiber content in freshwater environments is domestic scattered discharge, such as the washing of synthetic clothing [88,89]. Another significant source is sewage treatment plants, where a large amount of non-removed microplastics remaining in the effluent or sewage sludge enters the water and soil through direct discharge or sludge reuse [90]. Currently, most sewage treatment plants (STPs) have varying processes for removing fibrous microplastics and have not yet established a unified standard for effectively treating microplastics, which is a significant challenge in addressing urban microplastic pollution [91,92].

4.3. The Driving Role of Rainfall on the Distribution of Microplastics

In this study, regression analysis between the abundance of microplastics at all sampling points and the rainfall in different months shows a very strong correlation ($p < 0.001$). This indicates a positive correlation between the abundance of microplastics and the amount of rainfall. Moreover, the detection results at all sampling points vary with the seasons, showing significant differences in the abundance of microplastics across different months. Combined with the multi-variate statistical analysis of human activities and meteorological data, it was found that urbanization and precipitation significantly affect the abundance and distribution of microplastics. It can be assumed that there is a pattern where microplastics, originating from human activities including agricultural production, transportation, and daily life, first accumulate in the soil environment during the dry season, and then are washed away and transported by runoff during the rainy season, eventually entering freshwater environments.

Freshwater microplastics primarily come from land-based sources, which can be divided into point sources and non-point sources [93]. Point sources include sewage treatment plants, plastic manufacturing companies, etc., while non-point sources include farmlands, roads, residential buildings, and commercial areas [94]. The types and colors of microplastics in water environments change with different land pollution sources [40,95]. In the investigation of microplastics in Italy's Ofanto River, due to the impact of agricultural activities in the watershed, especially the use of plastic film, black film-shaped microplastics dominated [96]. This indirectly proves the driving role of rainfall on the distribution of microplastics. In this study, the color distribution ratio of the three groups of samples was relatively balanced, with no significant differences ($p > 0.05$). This may be due to different pollution sources near different sampling points, showing no clear pattern.

Apart from rainfall, wind is also an important driver affecting the distribution of microplastics [97]. This is because the density of microplastic particles is much lower than that of soil minerals like quartz, and they are less "sticky", making them less likely to be captured by moisture like soil minerals [98]. Therefore, microplastics are more easily carried away by the wind [99]. Sometimes, the wind may not be strong enough to lift dust, but it can still carry microplastics into the air [100]. A study showed that smaller plastic particles can travel farther in the atmosphere [101]. Microplastic particles of 10 μm or smaller tend to fall closer to their source, but many particles of 2.5 μm or smaller can be carried far from the source. We acknowledge recent findings by Xiao et al., which highlight the significant role of microplastic fiber shapes in their long-distance atmospheric transport [102]. These insights are particularly relevant to our discussion on the pathways and mechanisms of microplastic migration. In agricultural activities, wind can promote the degradation of plastic film into microplastics and facilitate the spread of microplastics in the atmosphere [103]. In transportation activities, two major sources of microplastics—road dust and tire wear—are also affected by wind in addition to being washed away by rainfall [104].

5. Conclusions

This study elucidated the spatial and temporal distribution characteristics and potential sources of microplastic pollution in China's freshwater environments. It was found that the abundance of microplastics generally increases from west to east and from south to north, with higher abundance observed during the rainy season (summer) compared to the dry season (spring and autumn). The main reason is that the distribution of microplastics is directly related to land use types, primarily originating from agricultural, transport, and urban land. The change in microplastics' abundance with the seasons is mainly driven by rainfall. However, the threshold of rainfall that triggers the migration of microplastics remains unclear, which is a direction for future research [105].

This study is the first to investigate the spatial and temporal distribution characteristics of microplastic pollution in China's freshwater environments on a national scale, enriching the data on microplastic pollution in China's freshwater environments and filling a research gap in this field. The findings of this study provide a solid scientific basis for the control and legislation of microplastics, thereby establishing reliable monitoring schemes and formulating effective measures to protect freshwater environments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16091270/s1, Figure S1: Work flow; Table S1: Detailed information of sampling sites; Table S2: Land use types of sampling sites (%); Table S3: Group information and microplastic abundance (n/m^3).

Author Contributions: Conceptualization, supervision and funding acquisition, X.T.; software—data analysis and visualization, H.H.; writing—original draft preparation, H.H.; writing—review and editing, X.T.; investigation, H.H., S.C. (Sulin Cai), S.C. (Siyuan Chen), Q.L., P.W., R.Y., X.Z., B.Y., Y.J., T.C., Y.L., H.J., R.L., Q.C., Y.F., L.P., Y.C., W.H., Y.P., G.P. and J.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Key Research and Development Program of China, grant number 2023YFC3905604; National Natural Science Foundation of China, grant number 31870598 and 31530007.

Data Availability Statement: Data set available on request from the authors.

Acknowledgments: The authors would like to extend sincere gratitude to everyone who provided assistance during the sampling process. Special thanks are due to the authors' families for invaluable contributions, without which this work would not have been possible.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Borrelle, S.B.; Ringma, J.; Law, K.L.; Monnahan, C.C.; Lebreton, L.; McGivern, A.; Murphy, E.; Jambeck, J.; Leonard, G.H.; Hilleary, M.A.; et al. Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. *Science* **2020**, *369*, 1515–1518. [CrossRef] [PubMed]
2. Brahney, J.; Hallerud, M.; Heim, E.; Hahnenberger, M.; Sukumaran, S. Plastic rain in protected areas of the United States. *Science* **2020**, *368*, 1257–1260. [CrossRef] [PubMed]
3. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci. Adv.* **2017**, *3*, e1700782. [CrossRef] [PubMed]
4. Brooks, A.L.; Wang, S.L.; Jambeck, J.R. The Chinese import ban and its impact on global plastic waste trade. *Sci. Adv.* **2018**, *4*, eaat0131. [CrossRef]
5. Chen, Y.D.; Cui, Z.J.; Cui, X.W.; Liu, W.; Wang, X.L.; Li, X.X.; Li, S.X. Life cycle assessment of end-of-life treatments of waste plastics in China. *Resour. Conserv. Recy.* **2019**, *146*, 348–357. [CrossRef]
6. Ma, Z.F.; Jiang, W.J.; Yang, S. China Plastics Industry (2018). *China Plast.* **2019**, *33*, 127–146. [CrossRef]
7. Wen, Z.G.; Xie, Y.L.; Chen, M.H.; Dinga, C.D. China's plastic import ban increases prospects of environmental impact mitigation of plastic waste trade flow worldwide. *Nat. Commun.* **2021**, *12*, 425. [CrossRef]
8. Zhang, K.; Shi, H.H.; Peng, J.P.; Wang, Y.H.; Xiong, X.; Wu, C.X.; Lam, P.K.S. Microplastic pollution in China's inland water systems: A review of findings, methods, characteristics, effects, and management. *Sci. Total Environ.* **2018**, *630*, 1641–1653. [CrossRef]

9. Green, D.S.; Boots, B.; Blockley, D.J.; Rocha, C.; Thompson, R. Impacts of Discarded Plastic Bags on Marine Assemblages and Ecosystem Functioning. *Environ. Sci. Technol.* **2015**, *49*, 5380–5389. [CrossRef]
10. Shen, M.C.; Song, B.; Zeng, G.M.; Zhang, Y.X.; Huang, W.; Wen, X.F.; Tang, W.W. Are biodegradable plastics a promising solution to solve the global plastic pollution? *Environ. Pollut.* **2020**, *263*, 114469. [CrossRef]
11. Xanthos, D.; Walker, T.R. International policies to reduce plastic marine pollution from single-use plastics (plastic bags and microbeads): A review. *Mar. Pollut. Bull.* **2017**, *118*, 17–26. [CrossRef]
12. Kim, M.H.; Song, C.M. Deep Neural Network Analysis on Uplift Resistance of Plastic Greenhouses for Sustainable Agriculture. *Sustainability* **2023**, *15*, 5632. [CrossRef]
13. Alimi, O.S.; Budarz, J.F.; Hernandez, L.M.; Tufenkji, N. Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport. *Environ. Sci. Technol.* **2018**, *52*, 1704–1724. [CrossRef] [PubMed]
14. de Souza Machado, A.A.; Kloas, W.; Zarfl, C.; Hempel, S.; Rillig, M.C. Microplastics as an emerging threat to terrestrial ecosystems. *Glob. Change Biol.* **2018**, *24*, 1405–1416. [CrossRef]
15. Rillig, M.C.; Bonkowski, M. Microplastic and soil protists: A call for research. *Environ. Pollut.* **2018**, *241*, 1128–1131. [CrossRef] [PubMed]
16. Thompson, R.C.; Moore, C.J.; vom Saal, F.S.; Swan, S.H. Plastics, the environment and human health: Current consensus and future trends. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 2153–2166. [CrossRef] [PubMed]
17. Rillig, M.C. Microplastic in Terrestrial Ecosystems and the Soil? *Environ. Sci. Technol.* **2012**, *46*, 6453–6454. [CrossRef] [PubMed]
18. Rillig, M.C. Microplastic Disguising As Soil Carbon Storage. *Environ. Sci. Technol.* **2018**, *52*, 6079–6080. [CrossRef]
19. Xu, Q.Y.; Xiang, J.Y.; Ko, J.H. Municipal plastic recycling at two areas in China and heavy metal leachability of plastic in municipal solid waste. *Environ. Pollut.* **2020**, *260*, 114074. [CrossRef]
20. Bouwmeester, H.; Hollman, P.C.H.; Peters, R.J.B. Potential Health Impact of Environmentally Released Micro- and Nanoplastics in the Human Food Production Chain: Experiences from Nanotoxicology. *Environ. Sci. Technol.* **2015**, *49*, 8932–8947. [CrossRef]
21. Ng, E.-L.; Huerta Lwanga, E.; Eldridge, S.M.; Johnston, P.; Hu, H.-W.; Geissen, V.; Chen, D. An overview of microplastic and nanoplastic pollution in agroecosystems. *Sci. Total Environ.* **2018**, *627*, 1377–1388. [CrossRef] [PubMed]
22. Steinmetz, Z.; Wollmann, C.; Schaefer, M.; Buchmann, C.; David, J.; Tröger, J.; Muñoz, K.; Frör, O.; Schaumann, G.E. Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? *Sci. Total Environ.* **2016**, *550*, 690–705. [CrossRef] [PubMed]
23. Boots, B.; Russell, C.W.; Green, D.S. Effects of Microplastics in Soil Ecosystems: Above and Below Ground. *Environ. Sci. Technol.* **2019**, *53*, 11496–11506. [CrossRef] [PubMed]
24. de Souza Machado, A.A.; Lau, C.W.; Kloas, W.; Bergmann, J.; Bachelier, J.B.; Faltin, E.; Becker, R.; Görlich, A.S.; Rillig, M.C. Microplastics Can Change Soil Properties and Affect Plant Performance. *Environ. Sci. Technol.* **2019**, *53*, 6044–6052. [CrossRef] [PubMed]
25. Sun, Y.; Xu, W.; Gu, Q.; Chen, Y.; Zhou, Q.; Zhang, L.; Gu, L.; Huang, Y.; Lyu, K.; Yang, Z. Small-Sized Microplastics Negatively Affect Rotifers: Changes in the Key Life-History Traits and Rotifer–Phaeocystis Population Dynamics. *Environ. Sci. Technol.* **2019**, *53*, 9241–9251. [CrossRef]
26. Yuan, W.; Zhou, Y.; Liu, X.; Wang, J. New Perspective on the Nanoplastics Disrupting the Reproduction of an Endangered Fern in Artificial Freshwater. *Environ. Sci. Technol.* **2019**, *53*, 12715–12724. [CrossRef] [PubMed]
27. Eerkes-Medrano, D.; Thompson, R.C.; Aldridge, D.C. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res.* **2015**, *75*, 63–82. [CrossRef] [PubMed]
28. Carpenter, S.R.; Stanley, E.H.; Vander Zanden, M.J. State of the World's Freshwater Ecosystems: Physical, Chemical, and Biological Changes. *Annu. Rev. Environ. Resour.* **2011**, *36*, 75–99. [CrossRef]
29. Wang, Z.Q.; Zhang, Y.L.; Kang, S.C.; Yang, L.; Shi, H.H.; Tripathee, L.; Gao, T.U. Research progresses of microplastic pollution in freshwater systems. *Sci. Total Environ.* **2021**, *795*, 148888. [CrossRef]
30. Ockenden, A.; Tremblay, L.A.; Dikareva, N.; Simon, K.S. Towards more ecologically relevant investigations of the impacts of microplastic pollution in freshwater ecosystems. *Sci. Total Environ.* **2021**, *792*, 148507. [CrossRef]
31. Foley, C.J.; Feiner, Z.S.; Malinich, T.D.; Höök, T.O. A meta-analysis of the effects of exposure to microplastics on fish and aquatic invertebrates. *Sci. Total Environ.* **2018**, *631–632*, 550–559. [CrossRef]
32. Corradini, F.; Meza, P.; Eguiluz, R.; Casado, F.; Huerta-Lwanga, E.; Geissen, V. Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. *Sci. Total Environ.* **2019**, *671*, 411–420. [CrossRef]
33. Eckert, E.M.; Di Cesare, A.; Kettner, M.T.; Arias-Andres, M.; Fontaneto, D.; Grossart, H.P.; Corno, G. Microplastics increase impact of treated wastewater on freshwater microbial community. *Environ. Pollut.* **2018**, *234*, 495–502. [CrossRef]
34. Leslie, H.A.; Brandsma, S.H.; van Velzen, M.J.M.; Vethaak, A.D. Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. *Environ. Int.* **2017**, *101*, 133–142. [CrossRef]
35. Baho, D.L.; Bundschuh, M.; Futter, M.N. Microplastics in terrestrial ecosystems: Moving beyond the state of the art to minimize the risk of ecological surprise. *Glob. Change Biol.* **2021**, *27*, 3969–3986. [CrossRef] [PubMed]
36. Rillig, M.C.; Lehmann, A. Microplastic in terrestrial ecosystems. *Science* **2020**, *368*, 1430–1431. [CrossRef] [PubMed]

37. Horton, A.A.; Walton, A.; Spurgeon, D.J.; Lahive, E.; Svendsen, C. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total Environ.* **2017**, *586*, 127–141. [CrossRef] [PubMed]
38. Xu, C.Y.; Zhang, B.B.; Gu, C.J.; Shen, C.S.; Yin, S.S.; Aamir, M.; Li, F. Are we underestimating the sources of microplastic pollution in terrestrial environment? *J. Hazard. Mater.* **2020**, *400*, 123228. [CrossRef] [PubMed]
39. Wang, J.; Bucci, K.; Helm, P.A.; Hoellein, T.; Hoffman, M.J.; Rooney, R.; Rochman, C.M. Runoff and discharge pathways of microplastics into freshwater ecosystems: A systematic review and meta-analysis. *Facets* **2022**, *7*, 1473–1492. [CrossRef]
40. Wang, W.F.; Ndungu, A.W.; Li, Z.; Wang, J. Microplastics pollution in inland freshwaters of China: A case study in urban surface waters of Wuhan, China. *Sci. Total Environ.* **2017**, *575*, 1369–1374. [CrossRef]
41. Talbot, R.; Chang, H.J. Microplastics in freshwater: A global review of factors affecting spatial and temporal variations*. *Environ. Pollut.* **2022**, *292*, 118393. [CrossRef] [PubMed]
42. Yu, F.; Pei, Y.Z.; Zhang, X.C.; Wu, X.G.; Zhang, G.B.; Ma, J. Occurrence and distribution characteristics of aged microplastics in the surface water, sediment, and crabs of the aquaculture pond in the Yangtze River Delta of China. *Sci. Total Environ.* **2023**, *871*, 162039. [CrossRef] [PubMed]
43. Feng, S.S.; Lu, H.W.; Yao, T.C.; Xue, Y.X.; Yin, C.; Tang, M. Spatial characteristics of microplastics in the high-altitude area on the Tibetan Plateau. *J. Hazard. Mater.* **2021**, *417*, 126034. [CrossRef] [PubMed]
44. Zhou, G.Y.; Wang, Q.G.; Zhang, J.; Li, Q.S.; Wang, Y.Q.; Wang, M.J.; Huang, X. Distribution and characteristics of microplastics in urban waters of seven cities in the Tuojiang River basin, China. *Environ. Res.* **2020**, *189*, 109893. [CrossRef]
45. Fu, Z.L.; Wang, J. Current practices and future perspectives of microplastic pollution in freshwater ecosystems in China. *Sci. Total Environ.* **2019**, *691*, 697–712. [CrossRef]
46. Xu, Y.Y.; Chan, F.K.S.; He, J.; Johnson, M.; Gibbins, C.; Kay, P.; Stanton, T.; Xu, Y.Y.; Li, G.; Feng, M.L.; et al. A critical review of microplastic pollution in urban freshwater environments and legislative progress in China: Recommendations and insights. *Crit. Rev. Environ. Sci. Technol.* **2021**, *51*, 2637–2680. [CrossRef]
47. Wang, G.L.; Lu, J.J.; Li, W.J.; Ning, J.Y.; Zhou, L.; Tong, Y.B.; Liu, Z.L.; Zhou, H.J.; Xiayihazi, N. Seasonal variation and risk assessment of microplastics in surface water of the Manas River Basin, China. *Ecotoxicol. Environ. Saf.* **2021**, *208*, 111477. [CrossRef]
48. Hu, L.; Chernick, M.; Hinton, D.E.; Shi, H. Microplastics in Small Waterbodies and Tadpoles from Yangtze River Delta, China. *Environ. Sci. Technol.* **2018**, *52*, 8885–8893. [CrossRef]
49. Su, L.; Xue, Y.; Li, L.; Yang, D.; Kolandhasamy, P.; Li, D.; Shi, H. Microplastics in Taihu Lake, China. *Environ. Pollut.* **2016**, *216*, 711–719. [CrossRef]
50. Fuller, S.; Gautam, A. A Procedure for Measuring Microplastics using Pressurized Fluid Extraction. *Environ. Sci. Technol.* **2016**, *50*, 5774–5780. [CrossRef]
51. Hurley, R.R.; Lusher, A.L.; Olsen, M.; Nizzetto, L. Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices. *Environ. Sci. Technol.* **2018**, *52*, 7409–7417. [CrossRef] [PubMed]
52. Zhang, S.L.; Yang, X.M.; Gertsen, H.; Peters, P.; Salánki, T.; Geissen, V. A simple method for the extraction and identification of light density microplastics from soil. *Sci. Total Environ.* **2018**, *616*, 1056–1065. [CrossRef] [PubMed]
53. Ministry of Natural Resources of China. China's Third National Land Survey. Available online: https://www.mnr.gov.cn/zt/td/dscqggtdc/ (accessed on 1 April 2024).
54. Aliyun. DataV.GeoAtlas. Available online: https://geo.datav.aliyun.com/areas_v3/bound/100000_full.json (accessed on 1 April 2024).
55. Frolking, S.; Qiu, J.J.; Boles, S.; Xiao, X.M.; Liu, J.Y.; Zhuang, Y.H.; Li, C.S.; Qin, X.G. Combining remote sensing and ground census data to develop new maps of the distribution of rice agriculture in China. *Glob. Biogeochem. Cycles* **2002**, *16*, 38-1–38-10. [CrossRef]
56. Liu, M.T.; Lu, S.B.; Song, Y.; Lei, L.L.; Hu, J.N.; Lv, W.W.; Zhou, W.Z.; Cao, C.J.; Shi, H.H.; Yang, X.F.; et al. Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai, China. *Environ. Pollut.* **2018**, *242*, 855–862. [CrossRef] [PubMed]
57. Zhang, M.J.; Zhao, Y.R.; Qin, X.; Jia, W.Q.; Chai, L.W.; Huang, M.K.; Huang, Y. Microplastics from mulching film is a distinct habitat for bacteria in farmland soil. *Sci. Total Environ.* **2019**, *688*, 470–478. [CrossRef]
58. O'Connor, D.; Pan, S.Z.; Shen, Z.T.; Song, Y.A.; Jin, Y.L.; Wu, W.M.; Hou, D.Y. Microplastics undergo accelerated vertical migration in sand soil due to small size and wet-dry cycles. *Environ. Pollut.* **2019**, *249*, 527–534. [CrossRef]
59. Xia, F.Y.; Liu, H.T.; Zhang, J.; Wang, D.Q. Migration characteristics of microplastics based on source-sink investigation in a typical urban wetland. *Water Res.* **2022**, *213*, 118154. [CrossRef]
60. Kang, H.; Park, S.; Lee, B.; Kim, I.; Kim, S. Concentration of Microplastics in Road Dust as a Function of the Drying Period-A Case Study in G City, Korea. *Sustainability* **2022**, *14*, 3006. [CrossRef]
61. McCormick, A.; Hoellein, T.J.; Mason, S.A.; Schluep, J.; Kelly, J.J. Microplastic is an Abundant and Distinct Microbial Habitat in an Urban River. *Environ. Sci. Technol.* **2014**, *48*, 11863–11871. [CrossRef] [PubMed]
62. Bian, P.Y.; Liu, Y.X.; Zhao, K.H.; Hu, Y.; Zhang, J.; Kang, L.; Shen, W.B. Spatial variability of microplastic pollution on surface of rivers in a mountain-plain transitional area: A case study in the Chin Ling-Wei River Plain, China. *Ecotoxicol. Environ. Saf.* **2022**, *232*, 113298. [CrossRef]

63. Chen, L.Y.; Yu, L.; Li, Y.J.; Han, B.J.; Zhang, J.D.; Tao, S.; Liu, W.X. Spatial Distributions, Compositional Profiles, Potential Sources, and Intfluencing Factors of Microplastics in Soils from Different Agricultural Farmlands in China: A National Perspective. *Environ. Sci. Technol.* **2022**, *56*, 16964–16974. [CrossRef] [PubMed]
64. Koutnik, V.S.; Leonard, J.; Alkidim, S.; DePrima, F.J.; Ravi, S.; Hoek, E.M.V.; Mohanty, S.K. Distribution of microplastics in soil and freshwater environments: Global analysis and framework for transport modeling. *Environ. Pollut.* **2021**, *274*, 116552. [CrossRef]
65. Yang, S.Y.; Zhou, M.; Chen, X.; Hu, L.P.; Xu, Y.F.; Fu, W.; Li, C. A comparative review of microplastics in lake systems from different countries and regions. *Chemosphere* **2022**, *286*, 131806. [CrossRef]
66. Kumar, R.; Verma, A.; Shome, A.; Sinha, R.; Sinha, S.; Jha, P.K.; Kumar, R.; Kumar, P.; Shubham; Das, S.; et al. Impacts of Plastic Pollution on Ecosystem Services, Sustainable Development Goals, and Need to Focus on Circular Economy and Policy Interventions. *Sustainability* **2021**, *13*, 9963. [CrossRef]
67. Chen, H.W.; Qin, Y.H.; Huang, H.; Xu, W.Y. A Regional Difference Analysis of Microplastic Pollution in Global Freshwater Bodies Based on a Regression Model. *Water* **2020**, *12*, 1889. [CrossRef]
68. Stoate, C.; Báldi, A.; Beja, P.; Boatman, N.D.; Herzon, I.; van Doorn, A.; de Snoo, G.R.; Rakosy, L.; Ramwell, C. Ecological impacts of early 21st century agricultural change in Europe—A review. *J. Environ. Manag.* **2009**, *91*, 22–46. [CrossRef]
69. Bai, X.Y.; Pan, K.W.; Shoaib, N.; Sun, X.M.; Wu, X.G.; Zhang, L. Status of phthalate esters pollution in facility agriculture across China: Spatial distribution, risk assessment, and remediation measures. *Sci. Total Environ.* **2024**, *908*, 168416. [CrossRef]
70. Jia, Z.F.; Wei, W.; Wang, Y.H.; Chang, Y.J.; Lei, R.; Che, Y.H. Occurrence characteristics and risk assessment of microplastics in agricultural soils in the loess hilly gully area of Yan' an, China. *Sci. Total Environ.* **2024**, *912*, 169627. [CrossRef]
71. Sintim, H.Y.; Flury, M. Is Biodegradable Plastic Mulch the Solution to Agriculture's Plastic Problem? *Environ. Sci. Technol.* **2017**, *51*, 1068–1069. [CrossRef]
72. Zhang, Q.Q.; Ma, Z.R.; Cai, Y.Y.; Li, H.R.; Ying, G.G. Agricultural Plastic Pollution in China: Generation of Plastic Debris and Emission of Phthalic Acid Esters from Agricultural Films. *Environ. Sci. Technol.* **2021**, *55*, 12459–12470. [CrossRef]
73. Qi, R.M.; Jones, D.L.; Li, Z.; Liu, Q.; Yan, C.R. Behavior of microplastics and plastic film residues in the soil environment: A critical review. *Sci. Total Environ.* **2020**, *703*, 134722. [CrossRef] [PubMed]
74. Weithmann, N.; Möller, J.N.; Löder, M.G.J.; Piehl, S.; Laforsch, C.; Freitag, R. Organic fertilizer as a vehicle for the entry of microplastic into the environment. *Sci. Adv.* **2018**, *4*, eaap8060. [CrossRef] [PubMed]
75. Zhang, L.S.; Xie, Y.S.; Liu, J.Y.; Zhong, S.; Qlan, Y.J.; Gao, P. An Overlooked Entry Pathway of Microplastics into Agricultural Soils from Application of Sludge-Based Fertilizers. *Environ. Sci. Technol.* **2020**, *54*, 4248–4255. [CrossRef]
76. Carrère, H.; Dumas, C.; Battimelli, A.; Batstone, D.J.; Delgenès, J.P.; Steyer, J.P.; Ferrer, I. Pretreatment methods to improve sludge anaerobic degradability: A review. *J. Hazard. Mater.* **2010**, *183*, 1–15. [CrossRef] [PubMed]
77. Wei, W.; Zhang, Y.T.; Huang, Q.S.; Ni, B.J. Polyethylene terephthalate microplastics affect hydrogen production from alkaline anaerobic fermentation of waste activated sludge through altering viability and activity of anaerobic microorganisms. *Water Res.* **2019**, *163*, 114881. [CrossRef] [PubMed]
78. Koutnik, V.S.; Alkidim, S.; Leonard, J.; DePrima, F.; Cao, S.Q.; Hoek, E.M.V.; Mohanty, S.K. Unaccounted Microplastics in Wastewater Sludge: Where Do They Go? *ACS Est. Water* **2021**, *1*, 1086–1097. [CrossRef]
79. Kitahara, K.I.; Nakata, H. Plastic additives as tracers of microplastic sources in Japanese road dusts. *Sci. Total Environ.* **2020**, *736*, 114881. [CrossRef] [PubMed]
80. Su, L.; Nan, B.; Craig, N.J.; Pettigrove, V. Temporal and spatial variations of microplastics in roadside dust from rural and urban Victoria, Australia: Implications for diffuse pollution. *Chemosphere* **2020**, *252*, 126567. [CrossRef] [PubMed]
81. Gossmann, I.; Halbach, M.; Scholz-Böttcher, B.M. Car and truck tire wear particles in complex environmental samples—A quantitative comparison with "traditional" microplastic polymer mass loads. *Sci. Total Environ.* **2021**, *773*, 145667. [CrossRef]
82. Evangeliou, N.; Grythe, H.; Klimont, Z.; Heyes, C.; Eckhardt, S.; Lopez-Aparicio, S.; Stohl, A. Atmospheric transport is a major pathway of microplastics to remote regions. *Nat. Commun.* **2020**, *11*, 3381. [CrossRef]
83. Rauert, C.; Charlton, N.; Okoffo, E.D.; Stanton, R.S.; Agua, A.R.; Pirrung, M.C.; Thomas, K.V. Concentrations of Tire Additive Chemicals and Tire Road Wear Particles in an Australian Urban Tributary. *Environ. Sci. Technol.* **2022**, *56*, 2421–2431. [CrossRef]
84. Li, H.X.; Ma, L.S.; Lin, L.; Ni, Z.X.; Xu, X.R.; Shi, H.H.; Yan, Y.; Zheng, G.M.; Rittschof, D. Microplastics in oysters &ITSaccostrea cucullata&IT along the Pearl River Estuary, China. *Environ. Pollut.* **2018**, *236*, 619–625. [CrossRef] [PubMed]
85. Lin, L.; Zuo, L.Z.; Peng, J.P.; Cai, L.Q.; Fok, L.; Yan, Y.; Li, H.X.; Xu, X.R. Occurrence and distribution of microplastics in an urban river: A case study in the Pearl River along Guangzhou City, China. *Sci. Total Environ.* **2018**, *644*, 375–381. [CrossRef] [PubMed]
86. Ballent, A.; Corcoran, P.L.; Madden, O.; Helm, P.A.; Longstaffe, F.J. Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. *Mar. Pollut. Bull.* **2016**, *110*, 383–395. [CrossRef]
87. Mani, T.; Hauk, A.; Walter, U.; Burkhardt-Holm, P. Microplastics profile along the Rhine River. *Sci. Rep.* **2015**, *5*, 17988. [CrossRef]
88. Napper, I.E.; Thompson, R.C. Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. *Mar. Pollut. Bull.* **2016**, *112*, 39–45. [CrossRef]
89. Napper, I.E.; Barrett, A.C.; Thompson, R.C. The efficiency of devices intended to reduce microfibre release during clothes washing. *Sci. Total Environ.* **2020**, *738*, 140412. [CrossRef]
90. Ben-David, E.A.; Habibi, M.; Haddad, E.; Hasanin, M.; Angel, D.L.; Booth, A.M.; Sabbah, I. Microplastic distributions in a domestic wastewater treatment plant: Removal efficiency, seasonal variation and influence of sampling technique. *Sci. Total Environ.* **2021**, *752*, 141880. [CrossRef] [PubMed]

91. Yaseen, A.; Assad, I.; Sofi, M.S.; Hashmi, M.Z.; Bhat, S.U. A global review of microplastics in wastewater treatment plants: Understanding their occurrence, fate and impact. *Environ. Res.* **2022**, *212*, 113258. [CrossRef]
92. Sun, J.; Dai, X.H.; Wang, Q.L.; van Loosdrecht, M.C.M.; Ni, B.J. Microplastics in wastewater treatment plants: Detection, occurrence and removal. *Water Res.* **2019**, *152*, 21–37. [CrossRef]
93. Li, J.Y.; Liu, H.H.; Chen, J.P. Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water Res.* **2018**, *137*, 362–374. [CrossRef] [PubMed]
94. Simmerman, C.B.; Wasik, J.K.C. The effect of urban point source contamination on microplastic levels in water and organisms in a cold-water stream. *Limnol. Ocean. Lett.* **2020**, *5*, 137–146. [CrossRef]
95. Wen, X.F.; Du, C.Y.; Xu, P.; Zeng, G.M.; Huang, D.L.; Yin, L.S.; Yin, Q.D.; Hu, L.; Wan, J.; Zhang, J.F.; et al. Microplastic pollution in surface sediments of urban water areas in Changsha, China: Abundance, composition, surface textures. *Mar. Pollut. Bull.* **2018**, *136*, 414–423. [CrossRef] [PubMed]
96. Campanale, C.; Stock, F.; Massarelli, C.; Kochleus, C.; Bagnuolo, G.; Reifferscheid, G.; Uricchio, V.F. Microplastics and their possible sources: The example of Ofanto river in southeast Italy. *Environ. Pollut.* **2020**, *258*, 113284. [CrossRef] [PubMed]
97. Kannankai, M.P.; Devipriya, S.P. Atmospheric microplastic deposition in a coastal city of India: The influence of a landfill source on monsoon winds. *Sci. Total Environ.* **2024**, *908*, 168235. [CrossRef] [PubMed]
98. Jin, Z.K.; Ren, Y.P.; Tao, W.; Chen, Z.Y.; Xu, G.H. Study on the release of microplastic particles with different particle sizes in sediments caused by wave-induced liquefaction. *Sci. Total Environ.* **2024**, *911*, 168788. [CrossRef] [PubMed]
99. Rezaei, M.; Riksen, M.J.P.M.; Sirjani, E.; Sameni, A.; Geissen, V. Wind erosion as a driver for transport of light density microplastics. *Sci. Total Environ.* **2019**, *669*, 273–281. [CrossRef] [PubMed]
100. Bullard, J.E.; Ockelford, A.; O'Brien, P.; Neuman, C.M. Preferential transport of microplastics by wind. *Atmos Environ.* **2021**, *245*, 118038. [CrossRef]
101. Zhang, Y.L.; Gao, T.G.; Kang, S.C.; Sillanpää, M. Importance of atmospheric transport for microplastics deposited in remote areas. *Environ. Pollut.* **2019**, *254*, 112953. [CrossRef]
102. Xiao, S.L.; Cui, Y.F.; Brahney, J.; Mahowald, N.M.; Li, Q. Long-distance atmospheric transport of microplastic fibres influenced by their shapes. *Nat. Geosci.* **2023**, *16*, 863–870. [CrossRef]
103. Wang, T.; Zou, X.Q.; Li, B.J.; Yao, Y.L.; Li, J.S.; Hui, H.J.; Yu, W.W.; Wang, C.L. Microplastics in a wind farm area: A case study at the Rudong Offshore Wind Farm, Yellow Sea, China. *Mar. Pollut. Bull.* **2018**, *128*, 466–474. [CrossRef] [PubMed]
104. Huang, Y.M.; He, T.; Yan, M.T.; Yang, L.; Gong, H.; Wang, W.J.; Qing, X.; Wang, J. Atmospheric transport and deposition of microplastics in a subtropical urban environment. *J. Hazard. Mater.* **2021**, *416*, 126168. [CrossRef] [PubMed]
105. Zhang, X.T.; Chen, Y.X.; Li, X.Y.; Zhang, Y.L.; Gao, W.; Jiang, J.; Mo, A.Y.; He, D.F. Size/shape-dependent migration of microplastics in agricultural soil under simulative and natural rainfall. *Sci. Total Environ.* **2022**, *815*, 152507. [CrossRef]

water

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Monitoring of Microplastics in Water and Sediment Samples of Lakes and Rivers of the Akmola Region (Kazakhstan)

Natalya S. Salikova [1], Javier Rodrigo-Ilarri [2,*], Lyudmila A. Makeyeva [1], María-Elena Rodrigo-Clavero [2], Zhulduz O. Tleuova [1] and Anar D. Makhmutova [1]

[1] Department of Ecology, Life Safety and Environmental Protection, Abay Myrzakhmetov Kokshetau University, Kokshetau 020000, Kazakhstan; natsal66@mail.ru (N.S.S.); ludmila_mak_72@mail.ru (L.A.M.); lady.zhulduz@bk.ru (Z.O.T.); anar_mahmutova@mail.ru (A.D.M.)

[2] Instituto de Ingeniería del Agua y del Medio Ambiente (IIAMA), Universitat Politècnica de València, 46022 Valencia, Spain; marodcla@upv.es

* Correspondence: jrodrigo@upv.es

Abstract: This paper provides a detailed description of the findings and methodology related to the monitoring of microplastics in three lakes and one river of the Akmola Region in Kazakhstan. The concentration of microplastic particles and the analysis of water and sediment quality of the Yesil River and Kopa, Zerendinskoye, and Borovoe lakes have been analyzed. A total of 64 water samples were collected across the spring, summer, and autumn seasons, with subsequent analysis revealing a seasonal increase in microplastic concentrations. The average microplastic content ranged from 1.2×10^{-1} particles/dm^3 in spring to 4.5×10^{-1} particles/dm^3 in autumn. Lakes exhibited higher concentrations compared to the Yesil River. Correlation analysis highlighted a connection between microplastic content and turbidity, particularly notable during the spring season. Analysis of sediments revealed a decrease in microplastic concentrations from the coastal zone toward open waters sediments. Microplastic fibers were predominant in sediments (69.6%), followed by fragments (19.1%), films (7.4%), and granules (3.9%). Larger particles (>500 μm) were found in beach sediments, constituting an average of 40.5% of the total plastics found. This study contributes valuable insights into the spatial and temporal distribution of microplastics, emphasizing the need for ongoing monitoring and management strategies to address this environmental concern.

Keywords: microplastics; Akmola region; Yesil river; sediments; water quality indicators

Citation: Salikova, N.S.; Rodrigo-Ilarri, J.; Makeyeva, L.A.; Rodrigo-Clavero, M.-E.; Tleuova, Z.O.; Makhmutova, A.D. Monitoring of Microplastics in Water and Sediment Samples of Lakes and Rivers of the Akmola Region (Kazakhstan). *Water* **2024**, *16*, 1051. https://doi.org/10.3390/w16071051

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

Received: 11 March 2024
Revised: 31 March 2024
Accepted: 3 April 2024
Published: 5 April 2024

1. Introduction

Improved water supply, sanitation, and better water resource management can boost economic growth and aid in poverty reduction. Access to clean and safe water is crucial for maintaining a healthy society [1]. However, the increasing presence of suspended organic and inorganic substances, along with anthropogenic macro and micro ions in water bodies, hampers natural self-purification processes and harms aquatic life. Elevated levels of these substances in watercourses can lead to severe diseases and health issues in humans [2–4].

This study builds upon extensive scientific research conducted in Northern Kazakhstan, specifically in the North Kazakhstan and Akmola provinces. Previous investigations focused on protecting and efficiently utilizing water resources, as well as evaluating water quality [5–7]. Both natural and human-induced factors affecting hydrochemical indicators in Northern Kazakhstan's natural waters were explored. Long-term studies have shown that anthropogenic activities significantly impact hydrochemical indicators in surface waters, contributing to their deterioration [8–11].

The challenges surrounding the management and processing of solid domestic waste persist as critical and unresolved issues in contemporary Kazakhstan. Recent studies have examined various aspects, including the sorting and utilization of solid domestic

waste [12–14], the recycling of plastic waste [15,16], and the impact of plastic bottle landfills and dumps on environmental components [17–19].

Plastics are widely manufactured and utilized for various purposes, ranging from medical and technological applications to packaging and wrapping, owing to their unique properties such as low density, thermal and electrical conductivity, corrosion resistance, and cost-effectiveness. However, despite its widespread use, plastic has evolved into a global menace for the natural environment [20], human health, and living organisms. The convenience of plastic has led to approximately 40% of it being single-use with a brief lifespan of just a few minutes but a decomposition period in the environment that can extend to several hundred years [21]. According to [22], "2500 million tons of plastic, equivalent to 30% of all plastics ever produced, are currently in use. Between 1950 and 2015, the cumulative generation of primary and secondary (recycled) plastic waste was 6300 million tons. Of this, about 800 million tons (12%) of plastic were incinerated, and 600 million tons (9%) were recycled, with only 10% of it being recycled more than once".

Due to their low density, which is similar to that of water, plastic microparticles tend to float on the water's surface. The y are easily transported by surface runoff from coastal areas, landfills, and unauthorized dumps into rivers and lakes. This situation is particularly severe in regions with high tourism potential and a poor culture of plastic waste disposal, leading to the littering of coastal zones with plastic debris. Inadequate infrastructure and a lack of environmental awareness among the population and tourists in the Akmola region and Kazakhstan as a whole contribute to the widespread pollution of coastal areas, which then extends to rivers and lakes. The problem is exacerbated by numerous unauthorized landfills and the discharge of household wastewater into lakes, increasing the risk of plastic pollution in water bodies. According to reports from the Ministry of Ecology, Geology, and Natural Resources of the Republic of Kazakhstan and Forbes Kazakhstan, satellite images taken in 2021 identified over 600 unauthorized solid waste disposal sites in the Akmola region, with the regional center of Kokshetau accounting for 23.5% (147 dumps). The region lacks proper facilities for sorting and processing solid domestic waste, resulting in a recycling rate of no more than 3% [23].

The absence of effective plastic recycling solutions results in the gradual degradation of plastic waste in the environment due to solar radiation, mechanical forces, and biological processes, forming particles of various sizes, including macro-, micro-, and nano-sized, known as microplastics (MP) [19]. While there are ongoing discussions regarding the definition and classification of microplastics, as well as the lower size limit, it is widely accepted that they encompass plastic particles smaller than 5 mm [24–27]. The se small plastic particles pose significant environmental hazards and can enter the bodies of animals and humans through water and food intake [28–30]. Studies suggest that Americans consume an estimated 13% of their daily diet in the form of microplastic particles. Additionally, previous research has examined drinking water as a potential source of microplastic ingestion. A study conducted by Newcastle University for WWF in 2019 found that, on average, individuals may ingest up to 5 g of plastic per week through food consumption [31].

In 2017, it was reported that microplastic (MP) particles can accumulate in the liver, kidney, and intestine of mammals, with the rate of tissue accumulation and distribution influenced by the particle size. Exposure to microplastics has been observed to disrupt energy and lipid metabolism, as well as induce oxidative stress [32]. In 2019, a study highlighted the inadvertent ingestion of microplastics by humans from various sources. The authors underscored the need for further research into the extent of microplastic consumption and its potential impact on human health [33]. Additionally, the presence of microplastics has been detected in all parts of the placenta, including maternal, fetal, and amniochorionic membranes. This suggests that microplastics carry substances that act as endocrine disruptors, potentially leading to long-term health effects [34].

The danger of microplastic (MP) intake into the bodies of animals and humans is also linked to its ability to absorb and hinder the sedimentation of organic pollutants and heavy metals, leading to increased turbidity and coloration of natural water.

Pollutants absorbed in this manner can subsequently enter the bodies of aquatic organisms and humans, elevating the risk of toxic effects [35–37]. The significance of international research on microplastics as an environmental pollutant is evident from the substantial increase in scholarly works over the past decade. Due to the relatively limited study of this issue, research topics are diverse, encompassing analyses of extent, behavior, risks to human health and specific species, and the impacts of microplastic pollution in freshwater systems [38]. Other areas of research include the standardization of monitoring methods in marine regions to facilitate comparison and assessment of microplastic pollution over time [39], as well as the historical development of marine anthropogenic litter [40]. Analysis of scientific literature available in open sources indicates a lack of research on microplastics in Kazakhstan's environment, with existing publications often lacking in-depth analyses of the problem. For instance, scientists at the Institute of Hydrobiology and Ecology of the Republic of Kazakhstan conducted an analysis of macro- and microplastics based on monitoring results from the Caspian Sea. The ir study concludes that further research is necessary to qualitatively and quantitatively assess the extent of plastic pollution and its impact on living organisms and the ecosystem of the sea [41].

One of the few studies conducted in Russia, a neighboring country to Kazakhstan, was published in 2021, reporting for the first time on microplastics in inland lakes of South Siberia. This study investigated particle sizes, their concentrations in lakes, surface morphology, and the elemental composition of plastics using spectroscopic methods. Additionally, factors influencing the presence of microplastics in lakes were identified. The paper emphasizes the necessity of increasing attention to waste management practices [42]. The quantity and distribution of microplastics in natural waters can be influenced by various environmental factors, including salinity, water depth, pH, and temperature [43]. However, further research with additional factors and experimental data are required to establish their impact on the prevalence of microplastics in natural waters [44]. Given the lack of comprehensive studies on microplastics in natural waters of Kazakhstan, it is pertinent to monitor microplastics in rivers and lakes, seek correlations between microplastic content, water depth, and quality, and analyze microplastic content in contact media such as coastal and bottom sediments.

In this work, the behavior of microplastics in the natural water objects of the Akmola region (Kazakhstan) is analyzed, giving recommendations on the sustainable and safe use of natural waters in Kazakhstan. This research helps to find a solution to the issue of the safety of natural waters in the region while contributing to the study of the problem of pollution of natural waters by microplastics.

2. Description of the Study Area

Microplastic monitoring was conducted within the Yesil River basin, the sole watercourse in the Akmola region of Kazakhstan, running from southeast to northwest and further north towards the North Kazakhstan region [45]. The Akmola region encompasses a 1027 km-long section of the river, covering approximately 20,000 km^2 [46]. The formation of a stable ice cover typically occurs by the end of November, with ice formation potentially beginning as early as October. The river experiences significant spring flooding, which typically subsides by the end of May in the upper reaches and by the end of June in the northern reaches [47]. Based on these characteristics, sampling was scheduled during the spring period (at the end of the spring flood) in May and during the autumn period (before ice cover formation) in September. Summer precipitation has minimal impact on the river's water regime, and summer floods are uncommon [48]. During the sampling period in the summer season of 2023, no rainfall was observed.

The Yesil River within the city of Astana, the capital of Kazakhstan, is a popular destination for both local residents and visitors. The water quality of the Yesil River in Astana is influenced by various factors in the sampling area, including industrial enterprises such as the "GazMashApparat" plant, overflow dams, river tributaries (such as the Akbulak river mouth), Triathlon Park, and city embankments frequented by the population.

Additionally, within the Akmola region, lakes Kopa, Zerendinskoye, and Borovoe were examined for microplastic content. The se lakes are all heavily visited by the population, with Zerendinskoye and Borovoe Lakes being particularly popular during the summer months. Lake Kopa, located within the city of Kokshetau, sees visitors throughout the year. The locations of these three lakes are indicated in Figure 1.

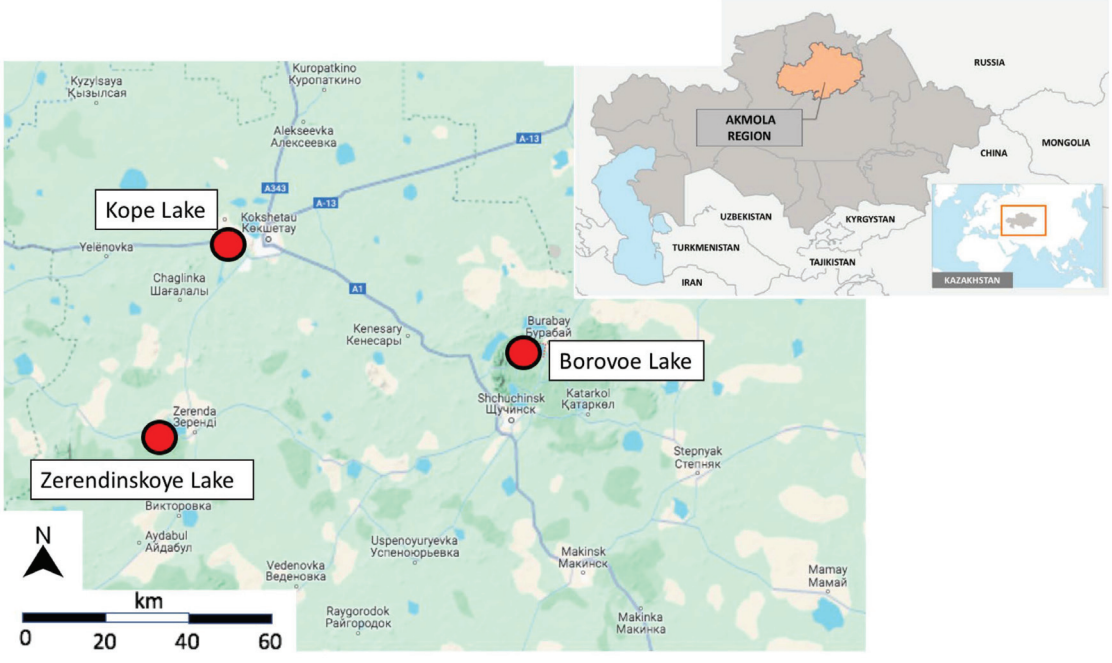

Figure 1. Location of Kopa, Zerendinskoye and Borovoe lakes.

Lake Kopa is situated near the base of the Kokshetau Upland, in the northwestern part of Kokshetau City. It spans an average area of 14 km^2 with depths ranging from 2.0 to 3.0 m. The total catchment area is 3860 km^2, primarily consisting of the lake's tributaries: the Chaglinka River from the southwest and the Kylshakty River from the southeast, with only a small portion (80 km^2) directly contributing to the lake [49]. The lake remains perennial and does not dry up. The water quality of Lake Kopa may be influenced by various factors including public groundwater wells, the Urker Palace restaurant, the Shagalaly River which flows into the lake carrying pollution from Krasny Yar village, as well as the presence of railway tracks and the city beach. Sampling sediment was challenging due to dense reed and cattail thickets, with an average width of 300 m, and a viscous lake bottom covered with a layer of clayey, loamy silt ranging from 0.5 to 2.8 m thick.

Lake Borovoe, located in the Shchuchinsky district of the Akmola region, is a drainless lake situated at the eastern base of Mount Kokshe. Covering an area of 10.5 km^2 (with dimensions of 4.5 km in length and 3.9 km in width), it boasts an average depth ranging from 4.5 to 7.0 m [50,51]. Lake Borovoe serves as a resort destination, frequented not only by local residents but also by tourists from distant regions of Kazakhstan and abroad. The water quality of Lake Borovoe may be affected by various tourist attractions, including the boat station, Baitas Hotel, S. Seifullin Secondary School, the ring transport road, the mouth of the Sarybulak Brook, and the Aynakol entertainment complex.

Lake Zerendinskoye, located in northern Kazakhstan within the Yesil River basin, is approximately 50 km away from Kokshetau. It measures 5.3 × 3.5 km in size, with an area of 9.61 km^2 and a coastline spanning 19.4 km. The lake boasts an average depth of

4.2 m and features a flat sandy bottom, occasionally adorned with pebbles and scattered boulders. Sandy beaches adorn the shores, and the water of the lake is fresh and transparent. While Lake Zerendinskoye is not a popular tourist destination, certain areas along its shoreline may pose a risk to water quality. The se include the M. Gabdulin secondary educational school, a rural hospital, the Zeren Nur recreation center, a fishery enterprise, the Vostochnaya recreation center, and a public recreation area near the Balkadisha monument.

3. Materials and Methods

3.1. Location of the Sampling Points

The selection of natural water sampling points was carried out in collaboration with specialists from the Astana and Kokshetau branches of the Republican State Enterprise "KazHydromet". During the selection process, consideration was given to potential sources of pollution and the key characteristics of the rivers and lakes under investigation. Detailed information regarding the sampling points, as well as the main characteristics of the water bodies, can be found in Tables 1–4 and Figures 2–5.

Table 1. Sampling points at Kopa Lake.

Sampling Point	GPS Coordinates	Description
1	53.286948, 69.354102	Public groundwater wells
2	53.297121, 69.336914	Urker Palace Restaurant
3	53.313779, 69.322168	Mouth of the Shagalaly River (Krasny Yar village)
4	53.327312, 69.328756	Railway tracks (Krasnoye village)
5	53.319875, 69.364748	Kokshetau meteorological station "Kazhidromet" for Akmola region
6	53.300309, 69.379781	City beach
7	53.312930, 69.351676	Center of the lake
8	53.305157, 69.357763	Center of the lake

Table 2. Sampling points at Lake Zerendinskoye.

Sampling Point	GPS Coordinates	Description
1	52.914085, 69.141020	M. Gabdulin Secondary School
2	52.906375, 69.126523	Rural hospital
3	52.922582, 69.083956	Zeren Noor Recreation Centre
4	52.940660, 69.113021	Fishery enterprise
5	52.940180, 69.125558	Vostochnaya recreation center
6	52.935238, 69.136085	Public recreation site, Balkadisha Monument
7	52.928451, 69.121305	Center of the lake
8	52.920951, 69.127372	Center of the lake

Table 3. Sampling points at Lake Borovoe.

Sampling Point	GPS Coordinates	Description
1	53.089900, 70.254179	Boat station
2	53.087245, 70.268173	Hotel Baitas
3	53.084186, 70.299261	S. Seifullin Secondary School
4	53.076950, 70.302128	Ring Road
5	53.067756, 70.301272	Mouth of Sarybulak Creek
6	53.061712, 70.296819	Aynakol Entertainment Complex
7	53.078176, 70.278866	Center of the lake
8	53.071580, 70.280757	Center of the lake

Table 4. Sampling points on the Yesil River (within Astana city limits).

Sampling Point	GPS Coordinates	Description
1	51.159911, 71.398368	GasMashApparat plant area
2	51.156111, 71.407128	Overflow dam
3	51.160420, 71.422823	City embankment, area of Kenesary monument location
4	51.149496, 71.437259	Mouth of Akbulak River
5	51.132982, 71.447133	Triotlon Park
6	51.124678, 71.453330	City embankment, the area of the residence of the President of the Republic of Kazakhstan Akorda
7	51.152855, 71.427425	Riverbank
8	51.159903, 71.417597	Riverbank

Figure 2. Map-scheme of sampling points location at Kopa Lake.

Figure 3. Map-scheme of sampling points location at Lake Zerendinskoye.

Figure 4. Map-scheme of sampling points location at Lake Borovoe.

Figure 5. Map-scheme of sampling points on the Yesil River within the city of Astana.

For sediment sampling, a total of 30 sampling points were identified, and their coordinates were meticulously recorded. The sampling points were categorized as DO (denoting bottom sediments), SW (representing the water's edge), and ZZ (indicating the splash zone). Detailed information regarding these sampling points, along with their respective coordinates, can be found in Tables 5–8.

Table 5. Sediment sampling points at Kopa Lake.

Sample Number	GPS Coordinates	Location
1	53.300309, 69.379781	DO
2		UW
3		ZZ
4	53.297121, 69.336914	DO
5		UW
6		ZZ

Table 6. Sediment sampling points at Lake Zerendinskoye.

Sample Number	GPS Coordinates	Location
7	52.940180, 69.125558	DO
8		UW
9		ZZ
10	52.935238, 69.136085	DO
11		UW
12		ZZ
13	52.914085, 69.141020	DO
14		UW
15		ZZ

Table 7. Sediment sampling points at Lake Borovoe.

Sample Number	GPS Coordinates	Location
16	53.076950, 70.302128	DO
17		UW
18		ZZ
19	53.067756, 70.301272	DO
20		UW
21		ZZ
22	53.061712, 70.296819	DO
23		UW
24		ZZ

Table 8. Sediment sampling points on the Yesil River.

Sample Number	GPS Coordinates	Location
25	51.156111, 71.407128	DO
26		UW
27		ZZ
28	51.101215, 71.514357	DO
29		UW
30		ZZ

3.2. Water Sampling Equipment and Sampling Methodology

The following equipment was utilized for water sampling to analyze physico-chemical parameters and for the extraction of microplastics and sediment sampling:

- GR-91 rod dredger with a bucket volume of 300 cm^3;
- Ruttner bathometer with a volume of 5 dm^3;
- Metal bucket with a volume of 10 dm^3;
- Metal scoops and spatulas;

- Glass jars;
- Glass Petri dishes;
- Metal tweezers;
- Aluminum foil;
- Sefar polyamide mesh with a mesh size of 100 μm.

For sampling surface water sources for microplastic analysis, a metal bucket with a volume of 10 dm^3 was used. Water was collected by scooping from the surface layer at a depth ranging from 5 to 20 cm. Filtering was conducted until a total volume of 100 L (equivalent to 10 buckets of 10 dm^3) of water was filtered.

Water was filtered from a depth of 1.5 m using a bathometer with a volume of 5 dm^3, with a total volume filtered from this depth amounting to 50 dm^3. The study refrained from using manta trawl nets due to concerns regarding the potential for cross-contamination and microplastic transfer between samples [52]. To mitigate this risk, a new filter was employed for each sample. Despite the widespread global practice of using meshes ranging from 300 to 390 μm for monitoring microplastics in surface waters [53–55], the study opted for the smallest mesh size available in Kazakhstan, which was 100 μm. Volumetric sampling, followed by filtration similar to the approach outlined in [56], was employed in the study. At each new sampling point, the sampler, bucket, and filter device were thoroughly rinsed with distilled water. Following filtration, the filters were transferred to glass Petri dishes, with each filter placed in a separate dish labeled with the sample number and location.

Recent estimates indicate that 70% to 90% of aquatic microplastic particles accumulate in sediment profiles [57]. To validate the hypothesis of microplastic input into water bodies from the coastal zone and to gain a more comprehensive understanding of microplastic distribution, the content of microplastics in bottom sediments of open waters and coastal zone sediments was assessed at three distinct locations [58]:

(i) Wave splash zone;
(ii) Water edge;
(iii) Bottom sediment zone at depths ranging from approximately 1.23 to 1.53 m (the maximum feasible depth for manual sediment sampling, determined by the geological structure of the bottom).

In each zone, samples were collected at five evenly distributed points along the entire 10-m length of the site. This method resulted in a total of five individual samples for each zone, covering bottom sediments, the water's edge, and the splash zone. The boundaries of the sampling zones were delineated using a measuring tape. Cut-off and sediment samples were gathered with a metal scoop into glass jars, while bottom sediments were obtained using a hand dredge. Accessible sites were chosen for sediment sampling to ensure the collection of all three intended sediment groups: sediments, the water's edge, and the wave splash zone. Sampling was avoided in areas where shoreline access was impractical, such as locations with overgrown reeds or areas where the natural shoreline had been altered into a stone embankment.

Bottom sediments were sampled to a depth of 5.0 ± 0.5 cm and packed in glass containers with metal lids. Each sample was carefully labeled and transported to the laboratory for further analysis. Upon arrival, the samples were dried at 30 °C and subsequently stored in glass containers in a refrigerator at 4 °C until analysis. Beach sediments (referenced in Table 9) were sampled from an area located on the same transect (perpendicular) as the sampled sediments. This area was positioned at a far distance of 5 m from the water's edge line, extending 5 m from the perpendicular line in each direction. The defined area was marked with colored tape, with a total area of 10×5 m.

Table 9. Beach sediment sampling points.

Sample No.	Place of Selection	GPS Coordinates
1	Kopa Lake	53.300309, 69.379781
2	Kopa Lake	53.297121, 69.336914
3	Zerendinskoye Lake	52.940180, 69.125558
4	Zerendinskoye Lake	52.929259,69.142834
5	Zerendniskoye Lake	52.914085, 69.141020
6	Borovoe Lake	53.076950, 70.302128
7	Borovoe Lake	53.067756, 70.301272
8	Borovoe Lake	53.061712, 70.296819
9	Yesil River	51.101215, 71.514357

3.3. Water Quality Standards

All water samples collected during the spring, summer, and autumn seasons were subjected to analysis for 10 physical and chemical indicators as per the regulations outlined in the Order of the Minister of Health of the Republic of Kazakhstan dated 24 November 2022, No. KR DSM-138, titled "On approval of hygienic standards of safety indicators for household and cultural and domestic water use" [59]. The methods utilized for analyzing the physico-chemical parameters of water reservoirs are detailed in Table 10.

Table 10. List of the main measured indicators of water quality and methods for their determination.

Indicator	Method of Measurement	Detection Limit	Standards and References
Color degree	Photometric	±1 degree	Interstate standard 31868-2012. Water. Methods for determining color [60]
Turbidity	Photometric	±0.01 mg/dm^3	Interstate standard 3351-74. Drinking water. Method for determination of odor, taste, color, and turbidity [61]
pH	Potentiometric	±0.01 unit	RK ISO standard 4316-2019. Surfactants. Determination of pH of aqueous solutions. Potentiometric method [62]
Oxidizability (COD)	Titrimetric	±0.1 mg/dm^3	State mandatory standard 26449.1-85. Stationary distillation and desalination plants. Methods for chemical analysis of salt waters [63]
Ammonium ions	Photometric	±0.01 mg/dm^3	Interstate standard 33045-2014. Water. Methods for determining nitrogen-containing substances [64]
Hardness (concentration of calcium and magnesium ions)	Titrimetric	±0.01 mg/dm^3	Interstate standard 31954-20120. Drinking water. Methods of hardness determination [65]
Mineralization (dry residue)	Gravimetric	±1 mg/dm^3	State mandatory standard 18164-72. Drinking water. Method for determination of total solids content [66]
Sulfates	Titrimetric	±0.1 mg/dm^3	Interstate standard 4389-72. Methods for determination of sulfate content [67]
Total iron	Photometric	±0.01 mg/dm^3	Interstate standard 4011-72. Drinking water. Methods for determination of total iron [68]
Carbonates	Titrimetric	±1 mg/dm^3	State mandatory standard 26449.1-85. Stationary distillation and desalination plants. Methods for chemical analysis of salt waters [63]

3.4. Methodology to Extract and Analyze Microplastics from Water and Sediment Samples

To extract and analyze microplastics from water and sediment samples, the following equipment was used:

- Microscope: DTX 500 LCD Levenhuk with photo and video registration;
- Analytical electronic scales: AX-200 Shimadzu (measurement accuracy 0.0001 g);
- Stainless steel sieves with mesh sizes: 3, 2, 1, 0.3, 0.175 mm;
- Electric dry-air thermostat: TS-1/80 SPU (maximum deviation of the average temperature not more than ±1 °C, maximum deviation of the temperature at any point ±0.4 °C);
- Water bath: "Ekros" model 4310;
- Ultrasonic bath: UZV-4.0 "Sapphire" with a digital thermostat (temperature range from 15 to 70 °C, ultrasound frequency 35 kHz, timer from 1 to 99 min);
- Filters for quantitative analysis: Whatman No.2;
- Laboratory centrifuge: Opn-3.01 "Dastan" centrifuge with rotation speeds of 1000, 1500, and 3000 rpm;
- Set of areometers;
- 5.75 M ZnCl solution.

Based on the analysis of 100 sources [69], and their adaptation to laboratory conditions, we developed a working protocol for extracting and separating microplastic particles from organic substances and foreign impurities in both aqueous and solid media. The quality of the research was ensured by implementing the "negative" control method to prevent cross-contamination of samples with plastic. The efficiency of microplastic extraction was evaluated using the "positive" control method [70,71]. Measures were taken, as suggested by [72,73], to prevent cross-contamination of samples with microplastics. To prevent contamination from the air, most of the work was conducted in a fume cupboard, and the analyzed filters were consistently stored in glassware, specifically Petri dishes with a closed lid [74]. Throughout all experiments, synthetic materials were avoided, and only cotton lab coats and metal instruments were used [75]. Before each session, all instruments were washed with distilled water, and surfaces were cleaned with ethyl alcohol, followed by wiping with distilled water.

A "negative" control was implemented by checking materials (Petri dishes, polyamide filters, flasks, beakers, bottles, and tables) for the presence of microplastics. For this control, polyamide filters pre-wrapped in foil and aged in muffle ovens at a temperature of 350 °C were utilized. Additionally, weekly checks of clean filters in labeled Petri dishes with an open lid were conducted.

Positive controls were implemented to prevent the loss of microplastic particles at different stages of extraction from the sample [76–78], involving the addition of UV fluorescent microplastic particles of various fractions. In the laboratory, filters used for the filtration of natural waters without visually noticeable organic contamination were dried either in a desiccator at room temperature or in a desiccator at 35 °C, within closed Petri dishes. Subsequently, these filters were examined under a microscope with a magnification range of 100–500. For contaminated filters, the particles were washed off the surface with distilled water into a 250 mL laboratory glass beaker or conical flask, followed by treatment through saline extraction and peroxide oxidation.

During the extraction and determination of microplastic concentration from sediments, we followed established procedures:

1. Drying the sample at 30 °C for a minimum of 24 h.
2. Weighing the dried sample.
3. Density separation by placing a 100-g portion of the sample in a saturated saline solution (5.75 M ZnCl solution). The volume of the solution was three times that of the sample, with an exposure time of 5–8 h and three repetitions [79–83].
4. The resulting supernatant was filtered through a 100–150 mm glass funnel using a filter for quantitative analysis (Whatman #42). The filters were replaced when clogged.
5. The filter containing retained plastic and organic matter particles was washed from the saline solution with distilled water.
6. Microplastic particles, along with organic matter from all filters related to the analysis of one sample, were washed into a glass beaker with distilled water.

7. The oxidation of organic impurities was carried out with a ratio of hydrogen peroxide to Fe(II) salt—1:1. The oxidation time was 30 min, adding 25 cm^3 of 30% hydrogen peroxide and 25 cm^3 of Fe(II) catalyst solution to a glass beaker with 150 cm^3, containing solids extracted through density separation. The beaker was placed in a water bath, with the thermostat at 50 °C, with periodic stirring for 30 min [84–86].
8. An additional portion of hydrogen peroxide was added to the beaker if undissolved organic matter was visually observed. The beaker was then covered with aluminum foil and left for a period of 8–12 h.

The density separation procedure was repeated using separation funnels, followed by filtration through a filter for quantitative analysis. The resulting filter, containing particulate matter, was placed in a Petri dish, covered with a lid, and dried at room temperature for 24 h or in a desiccator at a temperature not exceeding 35 °C [87,88]. The dried filters underwent microscopic examination, where each filter was carefully placed on a slide. The microscope was systematically navigated from edge to edge, and plastic particles were identified, categorized by type (fibers or non-fibrous materials like angular and hard fragments, flexible and thin films, or rounded and hard granules), and noted along with their respective sizes.

Qualitative analysis of microplastic particles larger than 1 mm was conducted through Fourier-transform infrared spectroscopy (FTIR) analysis. Infrared (IR) spectra were acquired using the Shimadzu IR-Prestige 21 instrument (Japan) within the wavelength range of 400–4000 cm^{-1}. The analysis was performed on the broken total internal reflection DuraSampl IR II with single reflection (prism material diamond on ZnSe substrate) from Smiths (USA). The IR spectra were matched against library databases such as IRs Polymer2, Polymer, and T-Polymer. For particles smaller than 1 mm found on filters after water filtration, they were considered plastics if they exhibited a shiny surface, bright color, sharp geometric shapes, and did not break under pressure from metal tweezers [89–91]. Depending on the amount of water filtered through the filter, the concentration of particles per 1 dm^3 was calculated. The content of microplastics in sediments was determined based on the number of particles per 1 kg of an absolutely dry sample.

Expeditions were conducted for water sampling from surface sources, organized by seasons: spring (from 19 May 2023 to 31 May 2023), summer (from 4 August 2023 to 11 August 2023), and autumn (from 14 September 2023 to 20 September 2023). The sampling locations included Kopa Lake, Zerendinskoye Lake, Borovoe Lake, and Yesil River within Astana city limits. The sampling occurred during the morning hours, specifically from 6:00 to 9:00 a.m., at each location. Water samples were obtained for the analysis of physical and chemical parameters from 8 sampling points: surface samples were collected by scooping with a metal bucket, and samples from a depth of 1.5 m were taken using a bathometer. Each sample, totaling 16 for each site in every season, was collected in 2-L containers.

For the microplastics analysis, water filtration was conducted from the surface and a depth of 1.5 m using a filtration device with polyamide filters. This process was carried out at a total of 16 filtration points for each river or lake in every season:

- With a bathometer from a depth of 1.5 m—the total volume of 50 dm^3 from each sampling point;
- From the surface with a metal bucket—volume 100 dm^3 from each point.

4. Results and Discussion

4.1. Analysis of MP Content in Water Samples

A total of 64 water samples were collected during the spring, summer, and autumn periods for the analysis of physical and chemical parameters. Water filtration was carried out at 64 points in each season. All collected samples were transported to the university laboratory for further study. When calculating microplastic concentrations, it was considered that the volume of filtered water from the surface was 100 dm^3, and from a depth of 1.5 m, it was 50 dm^3. The results of the physico-chemical analysis of water and the microplastic content in the studied lakes during spring, summer, and autumn are presented

in Tables S1–S12 (Supplementary Materials). Table 11 displays the microplastic content found in sediments, and Figure 6 showcases some examples of fibers discovered in the water samples.

Table 11. Microplastic content in sediments (particles/kg).

Sediment Type	Kopa Lake	Zerendinskoye Lake	Borovoe Lake	Yesil River
Open water bottom sediments	113.48 48.32	76.51 66.16 134.45	68.62 77.15 47.41	88.11 76.10
Sediments of the water's edge zone	118.92 74.39	129.23 99.50 95.08	93.95 76.09 83.60	103.59 150.38
Splash zone deposits	212.52 147.45	152.28 105.19 147.30	84.98 85.33 99.09	121.10 120.53
Beach sediments	184.09 179.96	192.46 161.97 204.42	169.36 189.00 183.98	179.73

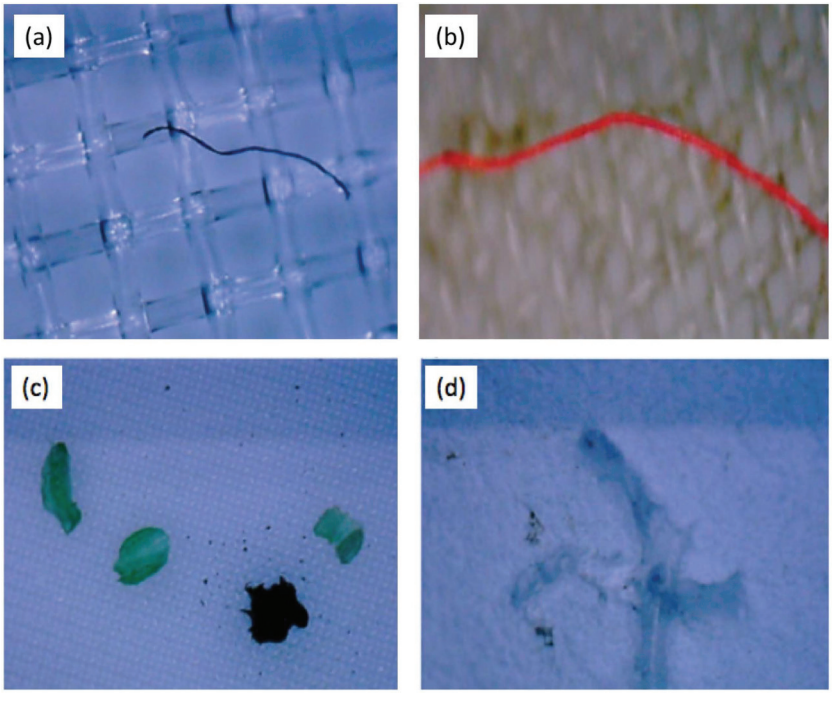

Figure 6. Samples of fibers (**a**,**b**), fragments (**c**), and films (**d**) of microplastic found in natural waters of Akmola region.

Tables 12–14 show the microplastic content found for the samples taken in Spring, Summer, and Autumn. Table 15 shows the microplastic concentration found in sediments.

Table 12. Microplastic content in surface water bodies of Akmola region (spring period).

Water Body	Fibers	Fragments	Films	Concentration (Particles/dm^3)	Average MP Concentration (Particles/dm^3)	Mean MP Concentration (Particles/dm^3) at Surface/Depth 1.5 m
Kopa Lake	120	15		1.0×10^{-2}–6.2×10^{-1}	1.4×10^{-1}	5.5×10^{-2}/2.3×10^{-1}
Zerendinskoye Lake	151	6		2.0×10^{-2}–4.4×10^{-1}	1.5×10^{-1}	9.9×10^{-2}/2.0×10^{-1}
Borovoe Lake	111	20	2	1.0×10^{-2}–4.8×10^{-1}	1.3×10^{-1}	5.3×10^{-2}/2.3×10^{-1}
Yesil River	40	30		0–4.0×10^{-1}	7.7×10^{-2}	2.5×10^{-2}/1.4×10^{-1}
Total in spring period	422	71	2	0–6.2×10^{-1}	1.2×10^{-1}	5.8×10^{-2}/2.0×10^{-1}

Table 13. Microplastic content in surface water bodies of Akmola region (summer period).

Water Body	Fibers	Fragments	Films	Concentration (Particles/dm^3)	Average MP Concentration (Particles/dm^3)	Mean MP Concentration (Particles/dm^3) at Surface/Depth 1.5 m
Kopa Lake	239	1	11	6.0×10^{-2}–4.6×10^{-1}	2.4×10^{-1}	1.5×10^{-1}/3.4×10^{-1}
Zerendinskoye Lake	440		35	1.6×10^{-1}–1.8	4.5×10^{-1}	2.3×10^{-1}/6.6×10^{-1}
Borovoe Lake	145		8	6.0×10^{-2}–2.4×10^{-1}	2.7×10^{-1}	1.0×10^{-1} /1.7×10^{-1}
Yesil River	170			6.0×10^{-2}–2.4×10^{-1}	1.5×10^{-1}	1.3×10^{-1}/1.8×10^{-1}
Total in summer period	994	1	54	6.0×10^{-2}–1.8	4.5×10^{-1}	1.5×10^{-1}/3.4×10^{-1}

Table 14. Microplastic content in surface water bodies of Akmola region (autumn period).

Water Body	Fibers	Fragments	Films	Concentration (Particles/dm^3)	Average MP Concentration (Particles/dm^3)	Mean MP Concentration (Particles/dm^3) at Surface/Depth 1.5 m
Kopa Lake	190	3		2.0×10^{-2}–1.22	1.9×10^{-1}	5.6×10^{-2}/3.3×10^{-1}
Zerendinskoye Lake	111	0		3.0×10^{-2}–3.2×10^{-1}	1.1×10^{-1}	5.6×10^{-2}/1.6×10^{-1}
Borovoe Lake	182	1		5.0×10^{-3}–4.8×10^{-1}	1.7×10^{-1}	6.7×10^{-2}/2.8×10^{-1}
Yesil River	113	1		1.0×10^{-3}–2.0×10^{-1}	1.1×10^{-1}	4.8×10^{-2}/1.7×10^{-1}
Total in autumn period	596	5		1.0×10^{-2}–1.22	1.5×10^{-1}	5.7×10^{-2}/2.4×10^{-1}

Table 15. Microplastic concentration in sediments (particles/kg).

Sampling Area	Kopa Lake	Zerendinskoye Lake	Borovoe Lake	Yesil River
Open water bottom	80.90	92.37	64.39	82.11
Water's edge zone	96.66	107.94	84.55	126.99
Splash zone	179.99	134.92	89.80	120.82
Beach	182.03	186.28	180.78	179.73

The average microplastic content in the studied samples increases from the spring to the autumn season: 1.2×10^{-1} particles/dm^3 in the spring period, 1.5×10^{-1} in summer, and 4.5×10^{-1} in the autumn sampling period. Microplastic concentrations in the lakes' water showed consistent levels, averaging 2.1×10^{-1} particles per dm^3 of filtered water, surpassing the content in the Yesil River (average for three seasons—1.1×10^{-1} particles/dm^3). The observed microplastic content in the natural waters of the Akmola region aligns with published data in scientific literature, ranging from 1×10^{-3} to 10 particles per dm^3 [92].

In the studied water samples, microplastics primarily manifest as fibers, constituting 93.8% of the total number of microplastic particles. Fragments follow at 3.6%, and films at 2.6%. This distribution aligns with published data, where the prevalence of fibers in surface waters ranges from 62% to 98%, fragments rank second with proportions of 2–18%, and films take the third spot with proportions ranging from 0% to 14% [93].

Despite using Whatman No. 42 filters with a pore size of 8 μm in sample processing, the lower limit for the detected microplastic particles is estimated to be 100 μm, based on

the mesh size of the polyamide mesh used for water filtration. The optical particle sorting method used in filter analysis may have led to the underestimation of transparent particles. Fragment sizes ranged from 250 to 100 μm in the longest dimension, while fiber sizes along the length varied from 100 to 500 μm. The detected fibers had a diameter significantly smaller than the mesh size of the polyamide mesh (100 μm), suggesting that only those fibers caught or entangled in the mesh were detected. Consequently, it is plausible that the actual fiber content in the studied river and lakes of the Akmola region might be higher than detected.

4.2. Correlation Dependencies

For most samples, MP content showed a correlation with water turbidity and sampling depth (surface and 1.5 m depth). The average MP concentration at a depth of 5–20 cm from the surface was 8.8×10^{-2} particles/dm^3, while at a depth of 1.5 m from the surface, it was 2.6×10^{-1} particles/dm^3. Despite turbidity, no correlation was found between microplastic content and other water quality parameters. Similar results were observed for microplastic content in plankton [94]. However, a noticeable correlation between microplastic content and turbidity was identified, particularly during the spring period.

Figures 7–9 present the results obtained when comparing microplastic concentration in water samples with turbidity. Conclusions diverge when analyzing results from water samples taken at the lake shoreline (sample points one to six in every lake) compared to those observed for water samples taken at inner waters in the lakes (sample points seven and eight). Figures 7–9 have been plotted using the same scale on the X-axis (Turbidity ranging from 0 to 40 mg/dm^3) and Y-axis (microplastic concentration ranging from 0 to 1.4 particles/dm^3) to facilitate easy comparison and draw conclusions from these comparisons. Results also exhibit a significant dependence on the season, as explained below.

Figure 7 displays the turbidity–microplastic concentration correlograms for the spring, summer, and autumn seasons for the three lakes. Figure 7a presents the results for spring at points one to six, revealing highly significant correlations in all three cases. In each instance, the linear correlation coefficients R^2 exceed 0.71 for Kopa Lake, with some values surpassing 0.80 ($R^2 = 0.805$ for Zerendinskoye Lake and $R^2 = 0.87$ for Borovoe Lake).

Figure 7b illustrates the values obtained for spring at sampling points within the three lakes (points 7 and 8). Correlations remain high for Kopa Lake ($R^2 = 0.63$) and Zerendinskoye Lake ($R^2 = 0.67$), while slightly lower for Borovoe Lake ($R^2 = 0.46$). In all cases, MP concentrations are below 0.65 particles/dm^3, and turbidity values are below 35 mg/dm^3.

The graphs illustrating the correlations obtained for the summer season are depicted in Figure 7c,d. For points located on the lakeshores, the highest values are once again observed in Kopa Lake ($R^2 = 0.85$). However, in this case, MP concentrations increase significantly, with values exceeding 1.2 particles/dm^3. The values obtained for Zerendinskoye Lake are very similar to those obtained for the spring season, both in terms of the correlation coefficient ($R^2 = 0.74$) and turbidity and MP concentration values. In the case of Borovoe Lake ($R^2 = 0.73$), turbidity values recorded in summer are significantly lower than those recorded in spring (below 8 mg/dm^3), while MP concentration values remain in the same order as before, albeit slightly lower (below 0.4 particles/dm^3).

During the summer season, results for sampling points within the three lakes vary significantly compared to those observed in the spring season. No significant correlations have been detected in any of them, turbidity values are below 7.0 mg/dm^3, and MP concentration is very low, below 0.3 particles/dm^3, in all three lakes.

The correlations observed in summer, and especially in spring, do not replicate in the autumn season, as depicted in Figure 7e,f. Figure 7e displays the values obtained for points one to six in autumn for the three lakes. In none of them is the existence of any correlation observed ($R^2 < 0.13$), while turbidity values remain very low (below 7 mg/dm^3) with MP concentration values below 0.5 particles/dm^3, except for Borovoe Lake, where concentrations reach around 0.8 mg/dm^3.

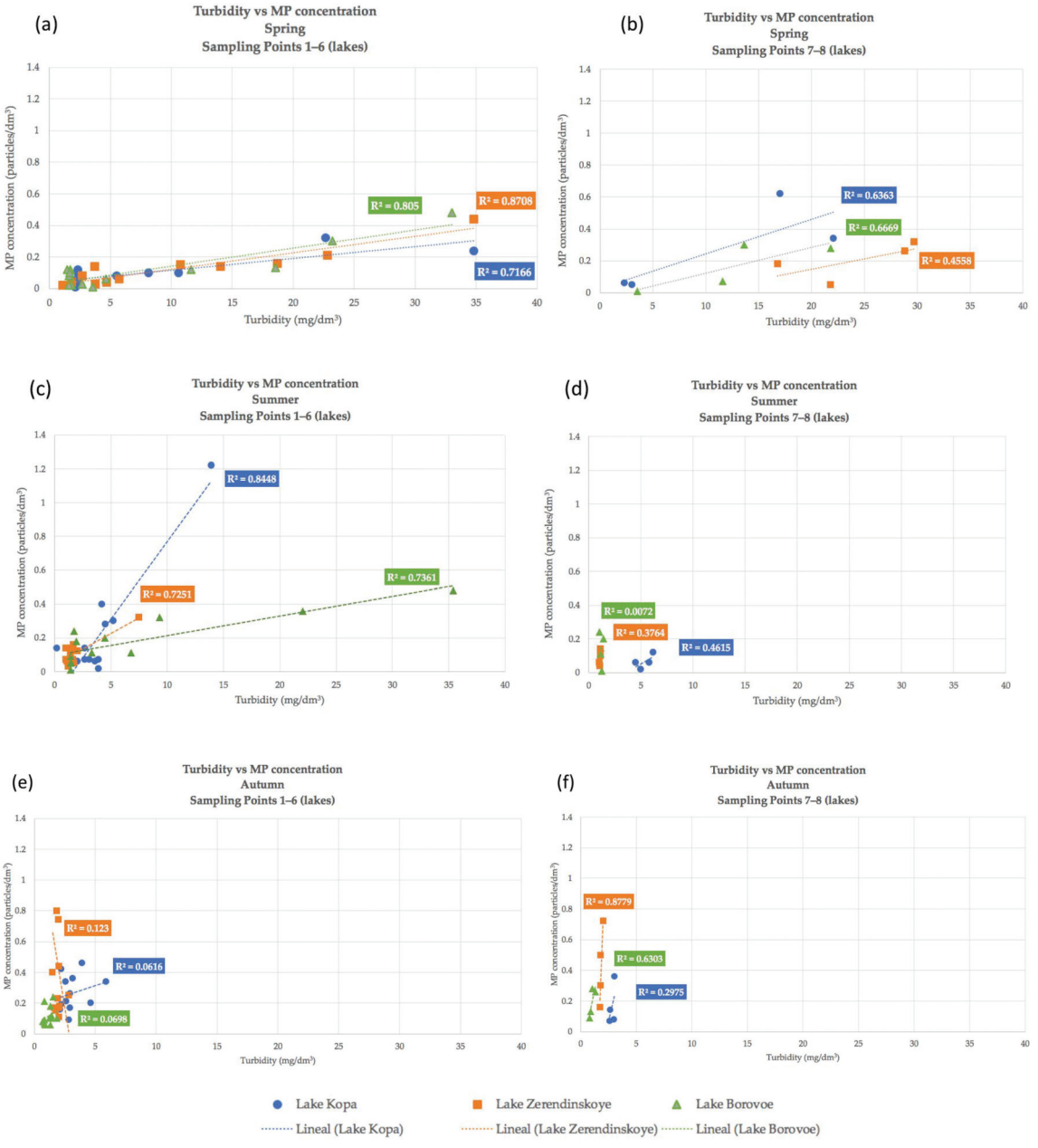

Figure 7. Turbidity vs microplastic concentration in waters for the Spring, Summer, and Autumn seasons. (**a**) Sampling points 1–6 in Spring; (**b**) Sampling points 7–8 in Spring; (**c**) Sampling points 1–6 in Summer; (**d**) Sampling points 7–8 in Summer; (**e**) Sampling points 1–6 in Autumn; (**f**) Sampling points 7–8 in Autumn.

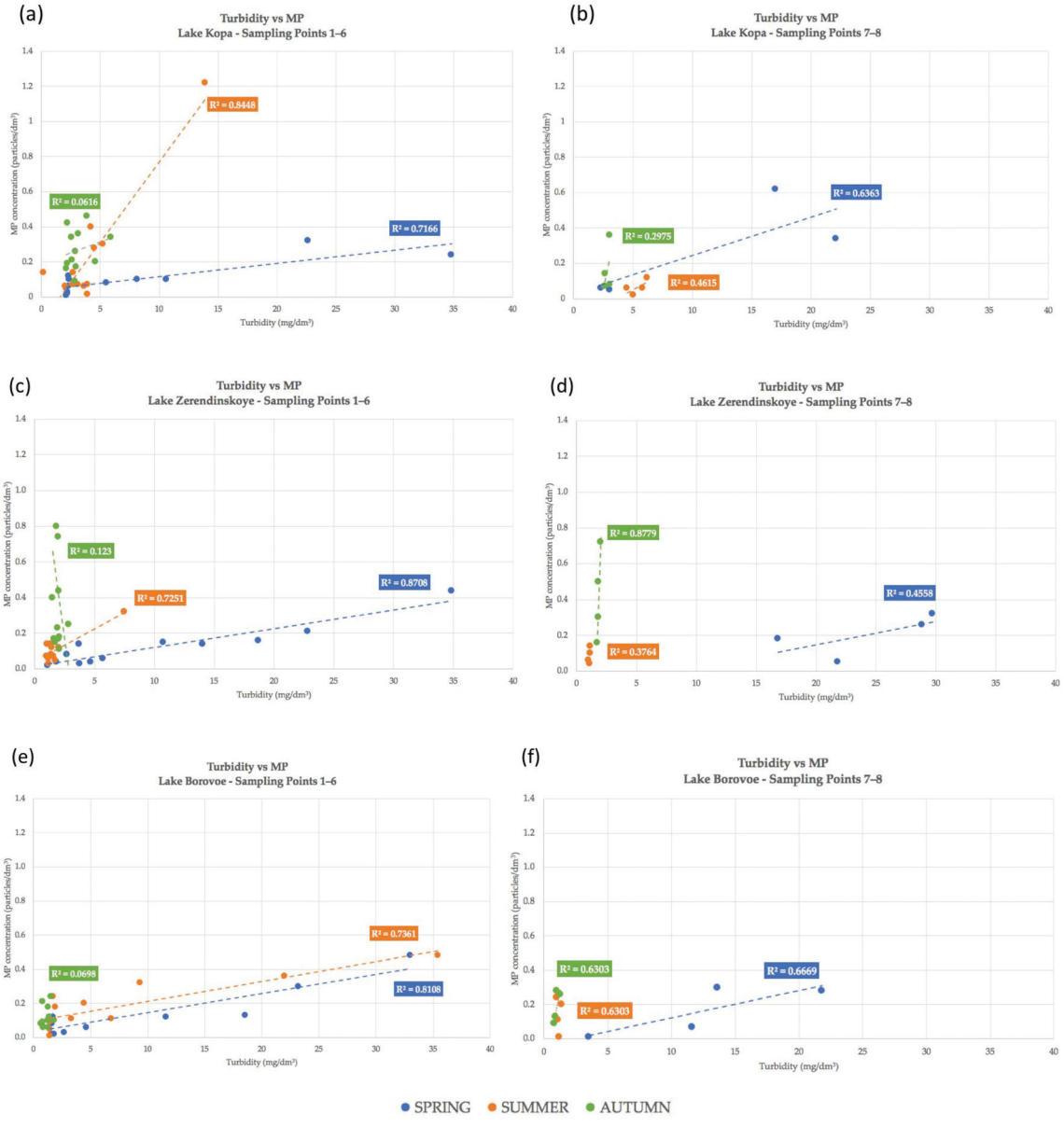

Figure 8. Turbidity vs microplastic concentration in waters: (**a**) Kopa Lake samples 1–6; (**b**) Kopa Lake samples 7–8; (**c**) Zerendinskoye Lake samples 1–6; (**d**) Zerendinskoye Lake samples 7–8; (**e**) Borovoe Lake samples 1–6; and (**f**) Borovoe Lake samples 7–8.

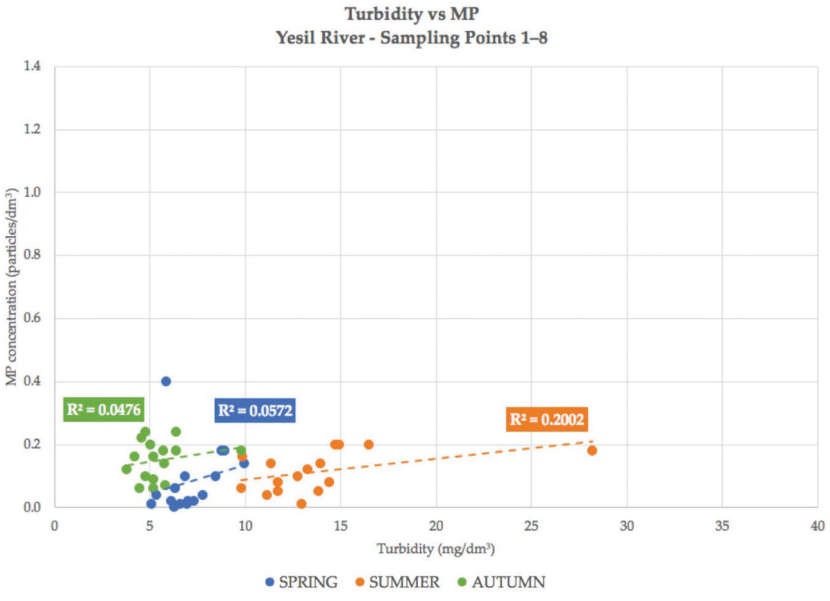

Figure 9. Turbidity vs. microplastic concentration in the Yesil River waters.

For the autumn season, values observed within the lakes (points seven and eight) are very similar to the corresponding values obtained for points located on the shores. In this case, turbidity values do not exceed 5 mg/dm^3 in any of the analyzed cases, and MP concentration values are below 0.4 particles/dm^3 for Kopa Lake and Zerendinskoye Lake, while for Borovoe Lake, they are slightly higher, exceeding 0.7 particles/dm^3. For sampling points seven and eight, correlation coefficient values exceeding 0.60 have been found for Zerendinskoye Lake (R^2 = 0.63) and Borovoe Lake (R^2 = 0.88).

Figure 8 shows the relationship between turbidity and microplastic concentration in waters for three lakes during the spring, summer, and autumn seasons. Figure 8 has been generated to observe the temporal evolution of turbidity and MP concentration more clearly in each lake independently.

Figure 8a,b illustrate the results obtained for Kopa Lake. For sampling points located on the shores of Kopa Lake, the results depicted in Figure 8a highlight strong correlations between turbidity and MP concentration in Kopa Lake during the spring (R^2 = 0.72) and summer (R^2 = 0.85) seasons. The highest turbidity values were observed in spring, reaching values close to 35 mg/dm^3. The highest MP concentration values in Kopa Lake were recorded in the summer season, with concentrations exceeding 1.2 particles/dm^3. However, during the autumn season, these correlations disappear, while turbidity and MP concentration values decrease significantly. During the autumn season, turbidity values in Kopa Lake are below 7 mg/dm^3, and MP concentration does not exceed 0.5 particles/dm^3.

Figure 8b displays the results obtained for sampling points within Kopa Lake (points seven and eight). The high correlations observed for the spring and summer seasons at points one and six are now only preserved for the spring season (R^2 = 0.63), with a drastic decrease in the correlation coefficient in the summer (R^2 = 0.46) and autumn (R^2 = 0.30) seasons. The highest turbidity values are still observed in spring, although they decrease to approximately 22 mg/dm^3. The highest MP concentrations are also observed in spring, reaching slightly above 0.6 particles/dm^3. It is noteworthy that compared to the values observed at points 1–6 in summer, MP concentrations in summer at the interior sampling points in Kopa Lake only have values of 0.2 particles/dm^3.

The results obtained for Zerendinskoye Lake are presented in Figure 8c,d. For sampling points located on the shores of Zerendinskoye Lake, the results depicted in Figure 8c

highlight a very strong correlation between turbidity and MP concentration during the spring (R^2 = 0.87). However, the correlation decreases in the summer (R^2 = 0.72) and autumn (R^2 = 0.12) seasons. The highest turbidity values were observed in spring, reaching values close to 35 mg/dm^3. Surprisingly, the highest MP concentration values in Zerendinskoye Lake were recorded in the autumn season, with concentrations exceeding 0.8 particles/dm^3. However, during the autumn season, turbidity values are lower than 3 mg/dm^3, and the correlations found for spring and summer disappear. During the spring season, turbidity values in Zerendinskoye Lake are the highest (just below 35 mg/dm^3), and observed MP concentrations are just over 0.4 particles/ dm^3.

Similarly, the results obtained for Borovoe Lake are shown in Figure 8e,f. As with Kopa Lake, the highest correlations were obtained for the spring (R^2 = 0.81) and summer (R^2 = 0.73) seasons. The maximum turbidity values are very similar in these cases (35 mg/dm^3 for summer and 33 mg/dm^3 for spring), and similar MP concentrations are observed, with maximum values around 0.5 particles/dm^3. Similar to what was found in Kopa Lake and Zerendinskoye Lake, the highest MP concentrations were found in the summer season, although their values are very similar to those observed in spring. As mentioned for Kopa Lake and Zerendinskoye Lake, the correlations found during spring and summer disappear in the autumn season (R^2 = 0.07), where turbidity values decrease drastically to below 3 mg/dm^3, and MP concentrations are below 0.25 particles/dm^3.

The conclusions already presented for Kopa Lake and Zerendinskoye Lake are valid for the sampling points located within Borovoe Lake. In this case, the regression coefficient value in spring for sampling points seven and eight reaches the value R^2 = 0.67, a slightly higher value than that recorded for Kopa Lake (R^2 = 0.63) but lower than that recorded for Zerendinskoye Lake (R^2 = 0.87).

Figure 9 displays the results obtained for the Yesil River. A discernible correlation between turbidity and MP concentration in the Yesil River water has been identified only during the summer season, with a very low correlation coefficient (R^2 = 0.20). The maximum values of turbidity are observed in summer (28 mg/dm^3). However, the highest values for MP concentration are found in the spring season (0.4 particles/dm^3).

A discernible pattern emerged from the analysis, indicating that samples taken from a depth of 1.5 m exhibit a higher microplastic content compared to water samples taken and filtered from the surface of the reservoir at the same point.

4.3. Analysis of MP Content in Sediment Samples

The results of sediment analysis (including bottom sediments, water edge, splash zone, and beach sediments located perpendicularly to each other) revealed a decrease in microplastic concentrations from the coastal zone towards the sediments in open waters. The average concentrations of microplastics were as follows: 79.63 particles/kg in bottom sediments, 102.47 particles/kg in the water edge zone, and 127.58 particles/kg in the splash zone. The beach sediment zone exhibited the highest content of microplastics, with an average of 179.73 particles/kg. In terms of particle shape, microplastic fibers predominated in sediments (69.6%), followed by fragments (19.1%), with films and granules accounting for 11.3%.

Published data suggest that predominant particles may include fibers (up to 77%) [95] and fragments (up to 73%) [96]. However, in most studies, fibers are identified as the predominant microplastic particles [97]. The size of microplastic fragments and films in water ranged from 250–100 μm in the largest dimension, while the size of fibers along the length ranged from 100 to 500 μm, consistent with published data [98]. Particles larger than 500 μm were found in beach sediments, constituting an average of 40.5%.

4.4. Analysis of FTIR Results

The type of microplastic was determined by comparing the IR spectra of the samples with those in libraries (IRs Polymer2, Polymer, T-Polymer, T-Organic) and by analyzing absorption bands caused by stretching and bending vibrations of groups characteristic of

certain types of polymers [99]. Based on the results of FTIR analysis, 95% of the samples were identified as particles of polymer origin (see Figure 10). The IR spectra of 3% of the samples were identified as belonging to the natural polymer cellulose, while unidentified structures accounted for 2% of microplastics (likely due to the degradation of the structures in the natural environment).

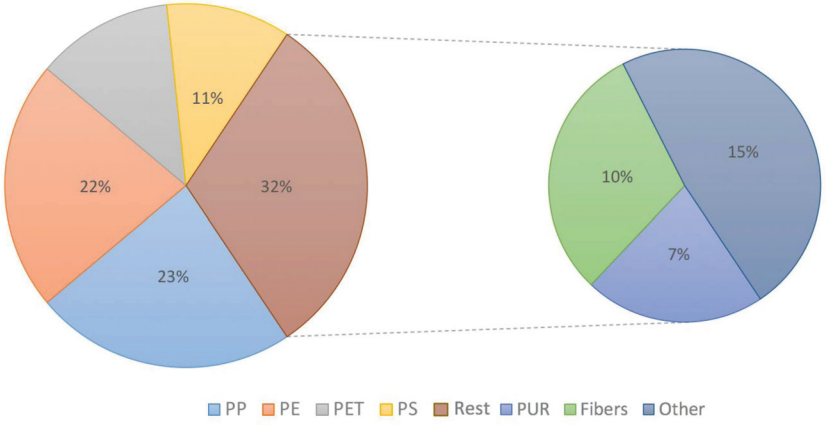

Figure 10. FTIR analysis. Qualitative composition of microplastics.

The total amount of identified microplastics is predominantly comprised of particles of polypropylene (23.3%), polyethylene (22.2%), polyethylene terephthalate (12.2%), and polystyrene (11.1%). Synthetic fibers (such as polyacrylic, polyamide, and polyester fibers) account for 9.5% of all identified microplastic particles. Similar results, indicating the predominance of microparticles of polypropylene and polyethylene in natural water bodies, are explained by their resistance to degradation and the challenges posed to decomposition by microbial biota [100]. Consequently, the inadequate management of plastic waste in Kazakhstan contributes to the presence of microplastics in all natural water bodies and their sediments in the Akmola region [19,101].

Based on the identified qualitative composition of microplastics, we can infer that the primary sources in water bodies and sediments of the Akmola region in Kazakhstan are likely household plastic waste, particularly disposable tableware made from polypropylene, polyethylene terephthalate, and polystyrene, along with packaging materials predominantly composed of polypropylene, which are widely utilized in Kazakhstan [102,103]. Another significant source of microplastics entering natural water bodies could be domestic wastewater, which carries rinsing water from washing activities, containing synthetic fibers such as polyacrylic, polyester, and polyamide fibers [44,104]. Additionally, storm drains from areas such as car repair shops and roadways may contribute to microplastic pollution, as they can carry rubber waste, including polyurethanes [105].

5. Conclusions

This paper presents, for the first time in Kazakhstan, the results of a comprehensive sampling campaign assessing microplastic (MP) content in three lakes and one river, alongside various water quality indicators. The investigation into MP concentrations revealed distinct seasonal and locational patterns. Strong correlations between turbidity and MP concentration were observed in Kopa Lake, Zerendinskoye Lake, and Borovoe Lake during spring and summer, with peak values in these seasons. However, these correlations diminished in autumn, with consistently higher MP concentrations during summer. In the Yesil River, a discernible correlation between turbidity and MP concentration emerged only in summer, with a low correlation coefficient.

The analysis extended to sediment samples, exploring different zones including bottom sediments, water edge, splash zone, and beach sediments. Results indicated a decreasing trend in microplastic concentrations from the coastal zone towards the sediments in open waters. The beach sediment zone exhibited the highest MP content, with microplastic fibers being the predominant particle type. Particle size analysis revealed variations, with microplastic fragments and films in water ranging from 250–100 μm, while fibers ranged from 100 to 500 μm. Larger particles exceeding 500 μm were notably found in beach sediments, constituting an average of 40.5%.

Overall, the findings highlight the complexity of MP distribution, influenced by seasonal variations, river or lake characteristics, and sediment composition, providing valuable insights for understanding and managing microplastic pollution in aquatic environments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16071051/s1, Tables S1–S12: Results of the studies of water quality at Lakes Kopa, Zerendinskoye, and Borovoe and at Yesil River. The Excel file with the details of the correlation analysis is also provided.

Author Contributions: Conceptualization, N.S.S. and J.R.-I.; methodology, N.S.S.; software, J.R.-I. and M.-E.R.-C.; validation, N.S.S.; formal analysis, N.S.S. and J.R.-I.; investigation, N.S.S. and J.R.-I.; resources, N.S.S. and J.R.-I.; data curation, N.S.S., L.A.M., Z.O.T. and A.D.M.; writing—original draft preparation, N.S.S. and J.R.-I.; writing—review and editing, J.R.-I. and M.-E.R.-C.; visualization, J.R.-I. and M.-E.R.-C.; supervision, N.S.S. and J.R.-I.; project administration, N.S.S.; funding acquisition, N.S.S. and J.R.-I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan through the research project entitled "Health Risk Modelling Based on the Identification of Microplastics in Water Systems and the Reasoning About Actions to Manage the Water Resources Quality" (Grant No. AP14869081).

Data Availability Statement: Data is contained within the article or Supplementary Materials.

Acknowledgments: The authors thank Nurbol Appazov, Director of the Institute of Chemical Research and Technology at Korkyt Ata Kyzylorda University, for his leadership in the quantitative analysis of microplastics using FTIR analysis.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Drinking-Water. Available online: https://www.who.int/news-room/fact-sheets/detail/drinking-water (accessed on 5 March 2024).
2. Levallois, P.; Villanueva, C.M. Drinking Water Quality and Human Health: An Editorial. *Int. J. Environ. Res. Public Health* **2019**, *16*, 631. [CrossRef] [PubMed]
3. Chang, K.Y.; Wu, I.-W.; Huang, B.-R.; Juang, J.-G.; Wu, J.-C.; Chang, S.-W.; Chang, C.C. Associations between Water Quality Measures and Chronic Kidney Disease Prevalence in Taiwan. *Int. J. Environ. Res. Public Health* **2018**, *15*, 2726. [CrossRef] [PubMed]
4. Meride, Y.; Ayenew, B. Drinking water quality assessment and its effects on residents health in Wondo genet campus, Ethiopia. *Environ. Syst. Res.* **2016**, *5*, 1. [CrossRef]
5. Salikova, N.S.; Rodrigo-Ilarri, J.; Alimova, K.K.; Rodrigo-Clavero, M.-E. Analysis of the Water Quality of the Ishim River within the Akmola Region (Kazakhstan) Using Hydrochemical Indicators. *Water* **2021**, *13*, 1243. [CrossRef]
6. Salikova, N.S.; Seidakhmetova, G.I.; Zhaparova, S.B.; Bayazitova, Z.E.; Kapbasova, G.A. Assessment of the conformity of the quality of decentralised sources of water supply to the sanitary and hygienic requirements of household and drinking purposes. *Bull. KazNITU* **2020**, *6*, 109–113. (In Russian)
7. Salikova, N.S.; Uralbaeva, A.A. Monitoring of hydrochemical indicators of surface water bodies of the Akmola region. *Actual Probl. Humanit. Nat. Sci.* **2016**, *2*, 185–188. (In Russian)
8. Salikova, N.S.; Likhodumova, I.N. Influence of natural and anthropogenic factors on the surface waters quality in the North Kazakhstan region. *Water Chem. Ecol.* **2015**, *5*, 9–14. (In Russian)
9. Salikova, N.S.; Uralbaeva, A.A. On a comprehensive assessment and factors in the formation of the hydrochemical composition of surface waters in the Akmola region. *Bull. D. Serikbaev EKSTU. Serikbaev EKSTU. Ser. Earth Sci.* **2016**, *1*, 22–28. (In Russian)
10. Salikova, N.S. *The Quality of Natural Waters in Northern Kazakhstan: A Monograph*; IP "Salem": Almaty, Kazakhstan, 2019.
11. Mukatova, A.A.; Tleuova, Z.O.; Bayazitova, Z.E. The surface waters quality in the Republic of Kazakhstan. *Sci. Real.* **2021**, *1*, 84–85. (In Russian)

12. Mukhamadeyeva, R.M.; Bayazitova, Z.E.; Elyubaev, S.Z.; Makeyeva, L.A.; Nurmaganbetov, Z.O. Development of Integrated Technology of Collection and Recycling of Plastic in Small Towns. *J. Environ. Manag. Tour.* **2018**, *9*, 1366–1376. [CrossRef]
13. Bayazitova, Z.E.; Zhaparova, S.B.; Mukhamadeyeva, R.M.; Tleuova, Z.O.; Makeeva, L.A.; Yelyubayev, S.Z. Studying the ways to reduce the harmful effects of plastic bottles in municipal solid waste on the environment in small towns. *J. Environ. Manag. Tour.* **2016**, *7*, 538–547.
14. Bayazitova, Z.; Zhaparova, S.; Tleuova, Z.; Makeeva, L.; Kapbasova, G.; Timeeva, M.; Makhmutova, A. *Development of an Integrated Technology for the Collection, Sorting and Processing of Plastic Bottles in Small Towns of the Akmola Region. Monograph*; Alla Prima: Almaty, Kazakhstan, 2017. (In Russian)
15. Zhaparova, S.B.; Salikova, N.S.; Bayazitova, Z.E.; Bekbergenova, Z.B. Method of Obtaining Polymer-Sand Paving Slabs. Utility Model Patent RK No. 6808, 14 January 2022.
16. Zhaparova, S.B.; Bayazitova, Z.E.; Salikova, N.S.; Tleuova, Z.O.; Bekpergenova, Z.B. Development of an effective technology for processing household plastic waste for the development of small business. *Bull. KazNITU* **2020**, *1*, 109–113. (In Russian)
17. Makeeva, L.A.; Tleuova, Z.O.; Zhabaeva, M.U.; Amrina, S.K. Ecological assessment of the impact of municipal solid waste landfills on the state of atmospheric air. *Bull. Shakarim Semipalat. State Univ.* **2017**, *1*, 155–160. (In Russian)
18. Makeeva, L.A.; Tleuova, Z.O.; Bayazitova, Z.E.; Temirbekov, N.G.; Kazytay, A. Influence of accumulation of plastic bottles on the ecological state of soils (on the example of the Schuchinsk landfill). *Bull. Shakarim Semipalat. State Univ.* **2017**, *1*, 151–155. (In Russian)
19. Salikova, N.S.; Rodrigo-Ilarri, J.; Rodrigo-Clavero, M.-E.; Urazbayeva, S.E.; Askarova, A.Z.; Magzhanov, K.M. Environmental Assessment of Microplastic Pollution Induced by Solid Waste Landfills in the Akmola Region (North Kazakhstan). *Water* **2023**, *15*, 2889. [CrossRef]
20. Jambeck, R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K.L. Plastic waste inputs from land into the ocean. *Science* **1979**, *347*, 768–771. [CrossRef] [PubMed]
21. U.S. National Park Service. *Decomposition Times of Marine Debris.* Available online: https://ourworldindata.org/grapher/decomposition-rates-marine-debris (accessed on 4 April 2024).
22. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci. Adv.* **2017**, *3*, e1700782. [CrossRef]
23. Government of the Republic of Kazakhstan. Landfills in Akmola. Available online: https://www.gov.kz/memleket/entities/ecogeo/press/news/details/244813?lang=ru (accessed on 22 February 2024).
24. Hartmann, N.B.; Hüffer, T.; Thompson, R.C.; Hassellöv, M.; Verschoor, A.; Daugaard, A.E.; Rist, S.; Karlsson, T.; Brennholt, N.; Cole, M.; et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. *Environ. Sci. Technol.* **2019**, *53*, 1039–1047. [CrossRef]
25. Frias, J.P.G.L.; Nash, R. Microplastics: Finding a consensus on the definition. *Mar. Pollut. Bull.* **2019**, *138*, 145–147. [CrossRef]
26. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. Microplastics as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* **2011**, *62*, 2588–2597. [CrossRef]
27. Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection. Sources, Fate and Effects of Microplastics in the Marine Environment (Part 2). 2016. Available online: https://unesdoc.unesco.org/ark:/48223/pf0000247517 (accessed on 20 February 2024).
28. Andrady, A.L. *Plastics and Environmental Sustainability*; John Wiley & Sons: Hoboken, NJ, USA, 2015. [CrossRef]
29. Wright, S.L.; Thompson, R.C.; Galloway, T.S. The physical impacts of microplastics on marine organisms: A review. *Environ. Pollut.* **2013**, *178*, 483–492. [CrossRef] [PubMed]
30. Cox, K.D.; Covernton, G.A.; Davies, H.L.; Dower, J.F.; Juanes, F.; Dudas, S.E. Human Consumption of Microplastics. *Environ. Sci. Technol.* **2019**, *53*, 7068–7074. [CrossRef] [PubMed]
31. De Wit, W.; Bigaud, N. *No Plastic in Nature: Assessing Plastic Ingestion from Nature to People: An Analysis for WWF*; WWF: Gland, Switzerland, 2019.
32. Deng, Y.; Zhang, Y.; Lemos, B.; Ren, H. Tissue accumulation of microplastics in mice and biomarker responses suggest widespread health risks of exposure. *Sci. Rep.* **2017**, *7*, srep46687. [CrossRef]
33. Schwabl, P.; Köppel, S.; Königshofer, P.; Bucsics, T.; Trauner, M.; Reiberger, T.; Liebmann, B. Detection of Various Microplastics in Human Stool. *Ann. Intern. Med.* **2019**, *171*, 453–457. [CrossRef] [PubMed]
34. Ragusa, A.; Svelato, A.; Santacroce, C.; Catalano, P.; Notarstefano, V.; Carnevali, O.; Papa, F.; Rongioletti, M.C.A.; Baiocco, F.; Draghi, S.; et al. Plasticenta: First evidence of microplastics in human placenta. *Environ. Int.* **2021**, *146*, 106274. [CrossRef] [PubMed]
35. Torres, F.G.; Dioses-Salinas, D.C.; Pizarro-Ortega, C.I.; De-la-Torre, G.E. Sorption of chemical contaminants on degradable and non-degradable microplastics: Recent progress and research trends. *Sci. Total Environ.* **2021**, *757*, 143875. [CrossRef]
36. Fu, L.; Li, J.; Wang, G.; Luan, Y.; Dai, W. Adsorption behavior of organic pollutants on microplastics. *Ecotoxicol. Environ. Saf.* **2021**, *217*, 112207. [CrossRef] [PubMed]
37. Yang, H.; Dong, H.; Huang, Y.; Chen, G.; Wang, J. Interactions of microplastics and main pollutants and environmental behavior in soils. *Sci. Total Environ.* **2022**, *821*, 153511. [CrossRef] [PubMed]
38. Mahon, A.M.; Officer, R.; Nash, R.; O'Connor, I. *Scope, Behaviour, Risks and Impacts of Microplastic Pollution in Irish Freshwater Systems*; Environmental Protection Agency by Marine and Freshwater Research Centre, Galway-Mayo Institute of Technology: Galway, Ireland, 2017.
39. Maes, T.; Van der Meulen, M.D.; Devriese, L.I.; Leslie, H.A.; Huvet, A.; Frère, L.; Robbens, J.; Vethaak, A.D. Microplastics baseline surveys at the water surface and in sediments of the North-East Atlantic. *Front. Mar. Sci.* **2017**, *4*, 257485. [CrossRef]

40. Bergman, M.; Gutow, L.; Klages, M. *Marine Anthropogenic Litter*; Springer: Berlin, Germany, 2015.
41. Baimukanov, M.T.; Baimukanova, Z.M. On plastic pollution and its potential impact on the biodiversity of the Caspian Sea. *Sci. News Kazakhstan* **2021**, *2*, 174–179. (In Russian)
42. Malygina, N.; Mitrofanova, E.; Kuryatnikova, N.; Biryukov, R.; Zolotov, D.; Pershin, D.; Chernykh, D. Microplastic Pollution in the Surface Waters from Plain and Mountainous Lakes in Siberia, Russia. *Water* **2021**, *13*, 2287. [CrossRef]
43. Wakkaf, T.; El Zrelli, R.; Kedzierski, M.; Balti, R.; Shaiek, M.; Mansour, L.; Tlig-Zouari, S.; Bruzaud, S.; Rabaoui, L. Characterization of microplastics in the surface waters of an urban lagoon (Bizerte lagoon, Southern Mediterranean Sea): Composition, density, distribution, and influence of environmental factors. *Mar. Pollut. Bull.* **2020**, *160*, 111625. [CrossRef]
44. Kye, H.; Kim, J.; Ju, S.; Lee, J.; Lim, C.; Yoon, Y. Microplastics in water systems: A review of their impacts on the environment and their potential hazards. *Heliyon* **2023**, *9*, e14359. [CrossRef]
45. Kazbekov, A.K.; Kabiev, E.K. *Hydrogeological and Engineering-Geological Studies in Northern Kazakhstan*; Polygraphy: Kokshetau, Kazakhstan, 1997.
46. Klushev, S.L.; Arefieva, V.A.; Zatenatskaya, T.I.; Isachenko, N.P.; Pachikina, Y.I.; Rubinshtein, L.I.; Feldman, M.I. Brief information about natural conditions and study of the Kokchetav region. In *Natural Zoning of Northern Kazakhstan*; AN SSSR: Moscow-Leningrad, Russia, 1960.
47. Berkaliev, Z.T. *Hydrological Regime of the Rivers of Central, Northern and Western Kazakhstan*; AN SSSR: Moscow-Leningrad, Russia, 1959.
48. Dolatova, L.A. Features of the hydrochemical composition of the middle reaches of the Ishim River and lakes of its basin. *World Sci. Cult. Educ.* **2011**, *1*, 347–351.
49. Muravlev, G.G. *Small Lakes of Kazakhstan*; Gidrometeoizdat: Leningrad, Russia, 1973.
50. Filonets, P.P. *Lakes of Northern, Western and Eastern Kazakhstan: A Reference Book*; Gidrometeoizdat: Leningrad, Russia, 1974.
51. Davitaya, F.F. (Ed.) *Agroclimatic and Water Resources of Areas of Development of Virgin and Fallow Lands*; Gidrometeoizdat: Leningrad, Russia, 1955.
52. Doyle, M.J.; Watson, W.; Bowlin, N.M.; Sheavly, S.B. Plastic particles in coastal pelagic ecosystems of the Northeast Pacific ocean. *Mar. Environ. Res.* **2011**, *71*, 41–52. [CrossRef] [PubMed]
53. Gago, J.; Galgani, F.; Maes, T.; Thompson, R.C. Microplastics in seawater: Recommendations from the marine strategy framework directive implementation process. *Front. Mar. Sci.* **2016**, *3*, 214280. [CrossRef]
54. Park, H.; Park, B. Review of Microplastic Distribution, Toxicity, Analysis Methods, and Removal Technologies. *Water* **2021**, *13*, 2736. [CrossRef]
55. Lindeque, P.K.; Cole, M.; Coppock, R.L.; Lewis, C.N.; Miller, R.Z.; Watts, A.J.; Wilson-McNeal, A.; Wright, S.L.; Galloway, T.S. Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size. *Environ. Poll.* **2020**, *265*, 114721. [CrossRef]
56. Dubaish, F.; Liebezeit, G. Suspended microplastics and black carbon particles in the Jade system, southern North Sea. *Water Air Soil Poll.* **2013**, *224*, 1352. [CrossRef]
57. Uddin, S.; Fowler, S.W.; Uddin, M.F.; Behbehani, M.; Naji, A. A review of microplastic distribution in sediment profiles. *Mar. Pollut. Bull.* **2021**, *163*, 111973. [CrossRef]
58. Darabi, M.; Majeed, H.; Diehl, A.; Norton, J.; Zhang, Y. A Review of Microplastics in Aquatic Sediments: Occurrence, Fate, Transport, and Ecological Impact. *Curr. Pollut. Rep.* **2021**, *7*, 40–53. [CrossRef]
59. Minister of Health of the Republic of Kazakhstan, On Approval of Hygienic Norms of Safety Indicators of Household Drinking and Cultural and Domestic Water Use. 2022. Available online: https://adilet.zan.kz/rus/docs/V2200030713 (accessed on 5 March 2024).
60. Interstate Standard 31868-2012. Water. Methods for Determining Color. Interstate Council for Standardization, Metrology and Certification: Moscow, Russia, 2019; p. 12. Available online: https://docs.cntd.ru/document/1200097407 (accessed on 31 March 2024).
61. Interstate Standard 3351-74. Drinking Water. Method for Determinination of Odour, Taste, Color and Turbidity. Publishing House of Standards: Moscow, Russia, 2003; p. 8. Available online: https://meganorm.ru/Index2/1/4294850/4294850608.htm (accessed on 31 March 2024).
62. ISO Standard 4316-2019. Surfactants. Determination of pH of Aqueous Solutions. Potentiometric Method. Technical Committee ISO/TC91:1977. International Organization for Standardization: Geneva, Switzerland, 1977. Available online: https://online.zakon.kz/Document/?doc_id=33823182&pos=30;-10#pos=30;-10 (accessed on 31 March 2024).
63. State Mandatory Standard 26449. 1-85. Stationary Distillation and Desalination Plants. Methods for Chemical Analysis of Salt Waters. USSR State Committee on Standards: Moscow, Russia, 1985; p. 101. Available online: https://files.stroyinf.ru/Index2/1/4294850/4294850335.htm (accessed on 31 March 2024).
64. Interstate Standard 33045-2014. Water. Methods for Determining Nitrogen-Containing Substances. Interstate Council for Standardization, Metrology and Certification: Moscow, Russia, 2014; p. 24. Available online: https://docs.cntd.ru/document/1200115428 (accessed on 31 March 2024).
65. Interstate Standard 31954-20120. Drinking Water. Methods of Hardness Determination. Interstate Council for Standardization, Metrology and Certification: Moscow, Russia, 2018; p. 18. Available online: https://docs.cntd.ru/document/1200097815 (accessed on 31 March 2024).
66. State Mandatory Standard 18164-72. Drinking Water. Method for Determination of Total Solids Content. USSR State Committee on Standards: Moscow, Russia, 1972; p. 4. Available online: https://docs.cntd.ru/document/1200012556 (accessed on 31 March 2024).

67. Interstate Standard 4389-72. Methods for Determination of Sulphate Content. Publishing House of Standards: Moscow, Russia, 1972; p. 9. Available online: https://files.stroyinf.ru/Data2/1/4294850/4294850724.htm (accessed on 31 March 2024).
68. Interstate Standard 4011-72. Drinking Water. Methods for Determination of Total Iron. Publishing House of Standards: Moscow, Russia, 1972; p. 8. Available online: https://docs.cntd.ru/document/1200008210 (accessed on 31 March 2024).
69. Salikova, N.S.; Makeeva, L.A.; Urazbayeva, S.E.; Askarova, A.J.; Magzhanov, K.M. Method of Extraction and Purification of Microplastic from Natural Media. Copyright Certificate for the Work of Science No. 39146 Issued on 21 September 2023, Kazakh National Institute of Intellectual Property (Kazpatent). Available online: https://copyright.kazpatent.kz/?!.iD=PLEk. (accessed on 4 April 2024).
70. Cable, R.N.; Beletsky, D.; Beletsky, R.; Wigginton, K.; Locke, B.W.; Duhaime, M.B. Distribution and modeled transport of plastic pollution in the Great Lakes, the world's largest freshwater resource. *Front. Environ. Sci.* **2017**, *5*, 246530. [CrossRef]
71. Ziajahromi, S.; Neale, P.A.; Rintoul, L.; Leusch, F.D.L. Wastewater treatment plants as a pathway for microplastics: Development of a new approach to sample wastewater-based microplastics. *Water Res.* **2017**, *112*, 93–99. [CrossRef]
72. Allen, S.; Allen, D.; Phoenix, V.R.; Le Roux, G.; Jiménez, P.D.; Simonneau, A.; Binet, S.; Galop, D. Atmospheric transport and deposition of microplastics in a remote mountain catchment. *Nat. Geosci.* **2019**, *12*, 339–344. [CrossRef]
73. Wesch, C.; Bredimus, K.; Paulus, M.; Klein, R. Towards the suitable monitoring of ingestion of microplastics by marine biota: A review. *Environ. Pollut.* **2016**, *218*, 1200–1208. [CrossRef] [PubMed]
74. Hermsen, E.; Pompe, R.; Besseling, E.; Koelmans, A.A. Detection of low numbers of microplastics in North Sea fish using strict quality assurance criteria. *Mar. Pollut. Bull.* **2017**, *122*, 253–258. [CrossRef] [PubMed]
75. Vandermeersch, G.; Van Cauwenberghe, L.; Janssen, C.R.; Marques, A.; Granby, K.; Fait, G.; Kotterman, M.J.; Diogène, J.; Bekaert, K.; Robbens, J.; et al. A critical view on microplastic quantification in aquatic organisms. *Environ. Res.* **2015**, *143*, 46–55. [CrossRef] [PubMed]
76. Hermsen, E.; Mintenig, S.M.; Besseling, E.; Koelmans, A.A. Quality Criteria for the Analysis of Microplastic in Biota Samples: A Critical Review. *Environ. Sci. Technol.* **2018**, *52*, 10230–10240. [CrossRef] [PubMed]
77. Gao, W.; Deng, X.J.; Zhang, J.; Qi, L.; Zhao, X.Q.; Zhang, P.Y. Assessment of quality control measures in the monitoring of microplastic: A critical review. *Environ. Pollut. Bioavailab.* **2023**, *35*, 2203349. [CrossRef]
78. Praveena, S.M.; Laohaprapanon, S. Quality assessment for methodological aspects of microplastics analysis in bottled water—A critical review. *Food Control.* **2021**, *130*, 108285. [CrossRef]
79. Monteiro, S.S.; Rocha-Santos, T.; Prata, J.C.; Duarte, A.C.; Girão, A.V.; Lopes, P.; Cristovão, T.; da Costa, J.P. A straightforward method for microplastic extraction from organic-rich freshwater samples. *Sci. Total Environ.* **2022**, *815*, 152941. [CrossRef] [PubMed]
80. Fries, E.; Dekiff, J.H.; Willmeyer, J.; Nuelle, M.T.; Ebert, M.; Remy, D. Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. *Environ. Sci. Process. Impacts* **2013**, *15*, 1949–1956. [CrossRef] [PubMed]
81. Imhof, H.K.; Ivleva, N.P.; Schmid, J.; Niessner, R.; Laforsch, C. Contamination of beach sediments of a subalpine lake with microplastic particles. *Curr. Biol.* **2013**, *23*, R867–R868. [CrossRef] [PubMed]
82. Joint Research Centre and Institute for Environment and Sustainability, Guidance on Monitoring of Marine Litter in European Seas—Publications Office of the EU. 2013. Available online: https://op.europa.eu/en/publication-detail/-/publication/76da424f-8144-45c6-9c5b-78c6a5f69c5d/language-en (accessed on 5 March 2024).
83. Hidalgo-Ruz, V.; Gutow, L.; Thompson, R.C.; Thiel, M. Microplastics in the marine environment: A review of the methods used for identification and quantification. *Environ. Sci. Technol.* **2012**, *46*, 3060–3075. [CrossRef]
84. Oni, B.A.; Sanni, S.E. Occurrence of Microplastics in Borehole Drinking Water and Sediments in Lagos, Nigeria. *Environ. Toxicol. Chem.* **2022**, *41*, 1721–1731. [CrossRef]
85. Lavoy, M.; Crossman, J. A novel method for organic matter removal from samples containing microplastics. *Environ. Pollut.* **2021**, *286*, 117357. [CrossRef]
86. Al-Azzawi, M.S.M.; Kefer, S.; Weißer, J.; Reichel, J.; Schwaller, C.; Glas, K.; Knoop, O.; Drewes, J.E. Validation of Sample Preparation Methods for Microplastic Analysis in Wastewater Matrices—Reproducibility and Standardization. *Water* **2020**, *12*, 2445. [CrossRef]
87. Hernandez, L.M.; Grant, J.; Fard, P.S.; Farner, J.M.; Tufenkji, N. Analysis of ultraviolet and thermal degradations of four common microplastics and evidence of nanoparticle release. *J. Hazard. Mater. Lett.* **2023**, *4*, 100078. [CrossRef]
88. Munno, K.; Helm, P.A.; Jackson, D.A.; Rochman, C.; Sims, A. Impacts of temperature and selected chemical digestion methods on microplastic particles. *Environ. Toxicol. Chem.* **2018**, *37*, 91–98. [CrossRef]
89. Corradini, F.; Meza, P.; Eguiluz, R.; Casado, F.; Huerta-Lwanga, E.; Geissen, V. Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. *Sci. Total Environ.* **2019**, *671*, 411–420. [CrossRef]
90. Lusher, A.L.; McHugh, M.; Thompson, R.C. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Mar. Pollut. Bull.* **2013**, *67*, 94–99. [CrossRef]
91. Mariano, S.; Tacconi, S.; Fidaleo, M.; Rossi, M.; Dini, L. Micro and Nanoplastics Identification: Classic Methods and Innovative Detection Techniques. *Front. Toxicol.* **2021**, *3*, 636640. [CrossRef] [PubMed]
92. Koelmans, A.A.; Nor, N.H.M.; Hermsen, E.; Kooi, M.; Mintenig, S.M.; De France, J. Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. *Water Res.* **2019**, *155*, 410–422. [CrossRef] [PubMed]

93. Li, Y.; Zhang, Y.; Chen, G.; Xu, K.; Gong, H.; Huang, K.; Yan, M.; Wang, J. Microplastics in Surface Waters and Sediments from Guangdong Coastal Areas, South China. *Sustainability* **2021**, *13*, 2691. [CrossRef]
94. Pazos, R.S.; Bauer, D.E.; Gómez, N. Microplastics integrating the coastal planktonic community in the inner zone of the Río de la Plata estuary (South America). *Environ. Pollut.* **2018**, *243*, 134–142. [CrossRef] [PubMed]
95. Petroody, S.S.A.; Hashemi, S.H. Wastewater treatment plants as a pathway for the release of microplastics into the environment: Investigation of sludge and treated effluent of Sari wastewater treatment plant. *Amirkabir J. Civ. Eng.* **2021**, *53*, 3751–3762. [CrossRef]
96. Bakir, A.; Doran, D.; Silburn, B.; Russell, J.; Archer-Rand, S.; Barry, J.; Nicolaus, E.M. A spatial and temporal assessment of microplastics in seafloor sediments: A case study for the UK. *Front. Mar. Sci.* **2023**, *9*, 1093815. [CrossRef]
97. Ghanbari, N.; Fataei, E.; Naji, A.; Imani, A.A.; Nasehi, F. Microplastic pollution in sediments in the urban section of the Qara Su River, Iran. *Appl. Water Sci.* **2022**, *12*, 192. [CrossRef]
98. Almasi, A.; Mahmoudi, M.; Mohammadi, M.; Dargahi, A.; Biglari, H. Optimizing biological treatment of petroleum industry wastewater in a facultative stabilization pond for simultaneous removal of carbon and phenol. *Toxin Rev.* **2021**, *40*, 189–197. [CrossRef]
99. Kuptsov, K.; Zhizhin, G. *Fourier-Raman and Fourier-IR Spectra of Polymers*; Fizmatlit: Moscow, Russia, 2011; p. 657. Available online: https://www.researchgate.net/publication/262416516_Kupcov_AH_Zizin_GN_Fure-KR_i_Fure-IK_spektry_polimerov_m_Fizmatlit_2001_657_s/references (accessed on 4 April 2024).
100. Li, Y.; Chen, G.; Xu, K.; Huang, K.; Wang, J. Microplastics Environmental Effect and Risk Assessment on the Aquaculture Systems from South China. *Int. J. Environ. Res. Public Health* **2021**, *18*, 1869. [CrossRef]
101. Golwala, H.; Zhang, X.; Iskander, S.M.; Smith, A.L. Solid waste: An overlooked source of microplastics to the environment. *Sci. Total Environ.* **2021**, *769*, 144581. [CrossRef] [PubMed]
102. Chappell, B.; Pramanik, A.; Basak, A.; Sarker, P.; Prakash, C.; Debnath, S.; Shankar, S. Processing household plastics for recycling—A review. *Clean. Mater.* **2022**, *6*, 100158. [CrossRef]
103. Ajaj, R.; Abu Jadayil, W.; Anver, H.; Aqil, E. A Revision for the Different Reuses of Polyethylene Terephthalate (PET) Water Bottles. *Sustainability* **2022**, *14*, 4583. [CrossRef]
104. Carr, S.A.; Liu, J.; Tesoro, A.G. Transport and fate of microplastic particles in wastewater treatment plants. *Water Res.* **2016**, *91*, 174–182. [CrossRef]
105. Ory, N.C.; Lehmann, A.; Javidpour, J.; Stöhr, R.; Walls, G.L.; Clemmesen, C. Factors Influencing the Spatial and Temporal Distribution of Microplastics at the Sea Surface—A Year-Long Monitoring Case Study from the Urban Kiel Fjord, Southwest Baltic Sea. *Sci. Total Environ.* **2020**, *736*, 139493. [CrossRef]

microplastics

MDPI

Article

Comparison of Methodologies for Microplastic Isolation through Multicriteria Analysis (AHP)

Valentina Phinikettou, Iliana Papamichael, Irene Voukkali and Antonis A. Zorpas *

Laboratory of Chemical Engineering and Engineering Sustainability, Faculty of Pure and Applied Sciences, Open University of Cyprus, Giannou Kranidioti 89, Nicosia 2231, Cyprus; v.phini@gmail.com (V.P.); iliana.papamichael@gmail.com (I.P.); voukkei@yahoo.gr (I.V.)
* Correspondence: antoniszorpas@yahoo.com or antonis.zorpas@ouc.ac.cy

Abstract: Environmental pollution caused by microplastics has evolved into a global concern; however, limited knowledge exists about microplastics in soils due to the absence of standardized extraction methods. This research aimed to develop an inexpensive, rapid method with user-friendly and environmentally sustainable outcomes for microplastics retrieval. Three salt solutions (Sodium Chloride, Magnesium Sulfate, Sodium Hexametaphosphate) and an oil solution (canola oil) underwent evaluation for microplastics extraction through the flotation process due to the density and oleophilic properties of plastics. Four widely used plastic types, obtained through fragmentation using a grinding mill from clean new plastic containers or membranes, were subjected to analysis. The experimental procedures for microplastics retrieval varied among the evaluated solutions. Through a comprehensive multicriteria analysis, the saturated Sodium Chloride solution emerged as the optimal scenario for microplastics extraction, followed closely by the canola oil scenario. The recovery method utilizing Sodium Chloride demonstrated economic feasibility, safety, and reliability. This study provides valuable insights into an effective and sustainable approach for mitigating microplastic pollution in soil, offering a promising avenue for future environmental conservation efforts.

Keywords: microplastics; extraction; soil; multicriteria analysis; AHP; conservation

Citation: Phinikettou, V.; Papamichael, I.; Voukkali, I.; Zorpas, A.A. Comparison of Methodologies for Microplastic Isolation through Multicriteria Analysis (AHP). *Microplastics* **2024**, *3*, 184–204. https://doi.org/10.3390/microplastics3010011

Academic Editors: Grigorios L. Kyriakopoulos and Nicolas Kalogerakis

Received: 25 January 2024
Revised: 1 March 2024
Accepted: 7 March 2024
Published: 10 March 2024

1. Introduction

The conceptualization of microplastics (MPs) has undergone significant evolution since their initial description as microscopic particles in the 20 μm diameter range by Thompson et al. in 2004 [1]. The latest literature defines MPs as synthetic organic polymers [2] with an upper size limit of 5 mm as shown in Figure 1 [3–6]. Since 2010, the European Union (EU) has witnessed a significant rise in total plastics production, reaching nearly 700 million metric tons [7]. Despite remaining a key player in the plastics industry, the annual production in the EU has seen a decline in recent years. In 2020, production hit its lowest point since 2009, standing at 55 million metric tons, but showed a modest recovery in 2021, reaching 57.2 million metric tons. According to Statista [8], this annual global plastic production decrease in 2020 was attributed to the impact of the health crisis. In Europe, the decline in production reached 5.1% with the rate of decline in France reaching 11%. The automotive industry, facing disruptions in production, witnessed the highest plastic consumption decline, with a rate of 18.1% in the EU (28% in France) compared to the previous year [9,10].

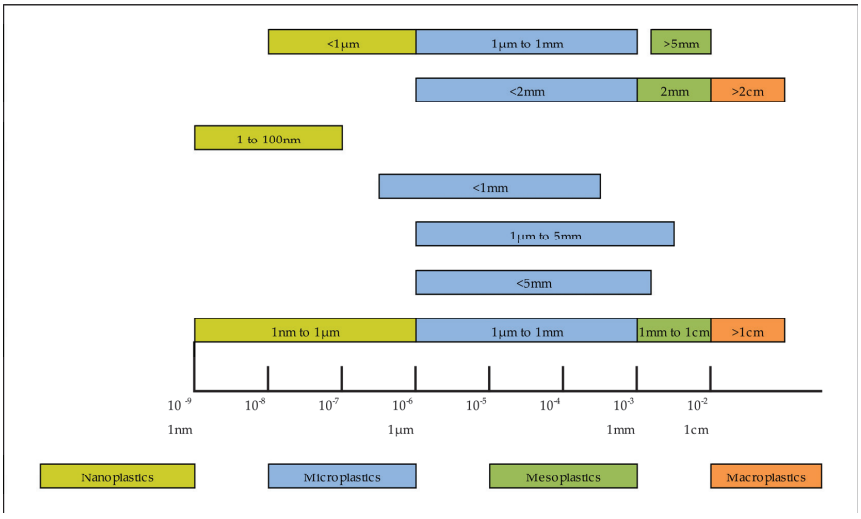

Figure 1. Comparison of plastic sizes according to the references [4,5,11–15].

Despite the commencement of plastic industrialization in the 1950s [16], research on the environmental implications of plastic production and usage emerged decades later [17], initially concentrating on marine plastic deposition [18]. While investigations into plastic waste in aquatic ecosystems have been prominent, research on terrestrial ecosystems lags, with studies often focusing on the transport of plastics in surface water flows [19]. Scientific approaches commonly involve measuring plastic litter to infer its life cycle [20]. Some studies emphasize point sources like wastewater treatment plants and the transport mechanisms of MPs through hydrographic networks and air masses [21,22]. Despite advancements, research on detecting and analyzing MPs in soil remains limited, even though a significant 79% of plastic produced from 1950 to 2015 ended up in landfills or was released into the environment without control.

1.1. Environmental Impacts and Sources of MPs

In the year 2022, with the global population nearing 8 billion people [23], the demand for annual plastic production surged to 450 million tons, equivalent to the collective weight of the entire human population on Earth. Projections indicate that by 2050 the quantity of plastics in the ocean is expected to surpass the population of fish [24]. The escalation in the prevalence of MPs in the oceans, a concerning global phenomenon, was underscored by the United Nations in 2017 [25] when a minimum of 51 trillion MP particles present in marine environments was estimated. The presence of plastic waste in terrestrial and aquatic environment constitutes a major threat to biodiversity and human existence. According to Chatziparaskeva et al. [26], 80% of plastic waste ends up in marine environments after being discarded on land [27]. Geomorphology, surface runoff, and air masses are the most important reasons for the transport and deposition of plastics in the aquatic environment [26,28].

The adverse impacts on marine pollution stem from various anthropogenic activities, including port facilities [29], coastal landfills [30], dumping sites along coastlines, and litter accumulation from aqua tourism and recreational activities [31], shipping, and fishing operations [26] (Figure 2). Additionally, the discharge of both treated and untreated sewage is identified as a significant pathway for MPs entering the sea [32]. In recent decades, due to the increase in tourism which constitutes a large portion of the global gross domestic product (GDP) share (9.2% in 2022, while by 2033 it is expected to reach 11.6%) [33], every year the arrivals of tourists around the world exceed one billion, with the largest percentage of visitors being in the coastal areas of the Mediterranean. Notably,

the Mediterranean experiences a 40% surge in marine litter during the summer, attributed to the influx of approximately 200 million tourists annually. In addition, tourism, the many recreational activities and insufficient recycling have the effect of dumping plastic in the terrestrial environment and by extension in the sea [26,30]. With an economic lens, financial impact of MPs accumulation in marine environments is staggering, with estimates suggesting a cost of 2.5 trillion USD worldwide. Specifically, the Mediterranean Sea bears a substantial burden, receiving between 150,000 and 500,000 tons of microplastics and 70,000 to 130,000 tons of MPs annually [34]. This environmental challenge imposes significant financial consequences on various sectors, including tourism, fisheries, and maritime activities, with associated costs for businesses in the Mediterranean totaling around 641 million euros [26,35].

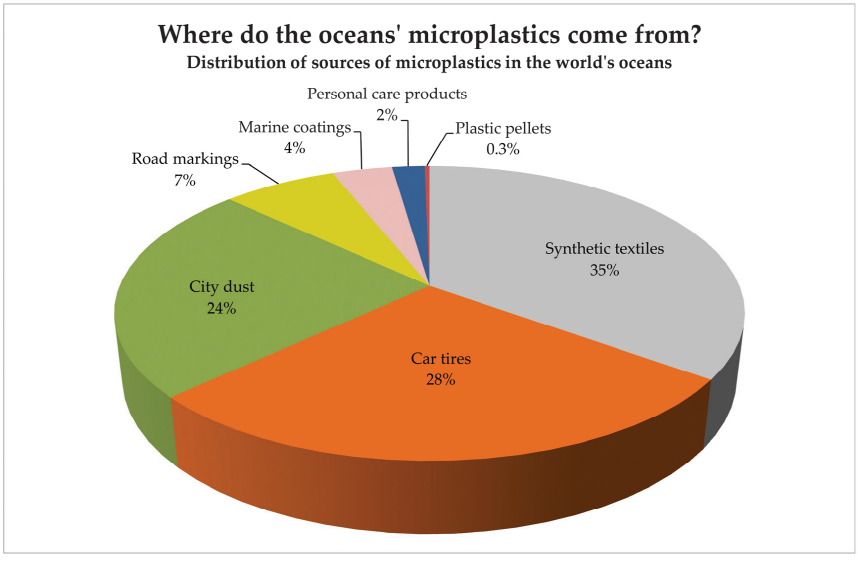

Figure 2. Distribution of sources of MPs in the world's oceans figure created by the authors; data from [9].

The widespread use of plastic in aquaculture and its destruction by weather conditions, accidents, and even by marine organisms results in the breakdown of plastic into MPs, their release, and transport in the marine environment [36,37]. Regarding marine life, MPs are responsible for 90% of damage to marine wildlife since 700 marine species (17% of which are endangered) face severe threats due to plastic accumulation in marine environments [26,38]. Since benthic environments constitute primary feeding ecosystems for marine organisms, the presence of MPs disrupts various feeding mechanism such as phytoplankton, lobsters, fish, corals, and others [39]. On a surface level, according to Serwandi Dharmadasa et al. [28] it is predicted that 99% of sea birds and turtles will have ingested plastic by 2050.

While plastic pollution in oceans has garnered significant attention, the presence of MPs in soil has been a relatively understudied but critical concern. Research indicates that plastic accumulation on land can be 4–23 times higher than that in the oceans [40,41], underscoring the substantial impact of plastic waste on terrestrial ecosystems. Despite these findings, human understanding of soil pollution by MPs is still limited. The global surge in plastic waste is a growing environmental challenge, with approximately 79% [42] of this waste accumulating in landfills and other terrestrial compartments, including agroecosystems [43–46]. This emphasizes the pressing need to investigate and comprehend the extent of MP pollution in soil, as it plays a crucial role in agricultural and ecological systems [47].

1.2. EU Legislation and Initiatives on Plastic Waste

The European Commission (EC) is dedicated to combating MP pollution, as outlined in both the European Green Deal and the recently introduced Circular Economy Action Plan [26,48,49]. Within the framework of the Zero Pollution Action Plan, the Commission has established a goal to achieve a 30% reduction in MP pollution by the year 2030. To achieve this goal, the European Parliament has undertaken a series of strategic moves with several key initiatives, to address plastic pollution within the EU.

The Packaging and Packaging Waste Directive (PPWD–Directive 94/62/EC) [50], adopted in 1994, established measures aimed at promoting sustainable and eco-friendly packaging solutions, while minimizing the generation of packaging waste and encouraging the reuse, recycling, and other methods of recovering packaging waste. In 2024, the Packaging and Packaging Waste Directive will see the implementation of new regulations, encompassing the introduction of producer responsibility schemes for all packaging. The directive also sets ambitious recycling targets, mandating the recycling of at least 65% of all packaging by weight by 2025 and a further increase to 70% by 2030 [51].

The Marine Strategy Framework Directive (MSFD) 2008/56/EC [52] was embedded in 2008, with the aim of preserving clean, healthy, productive, and resilient marine ecosystems, all the while promoting a more sustainable utilization of marine resources. Additional measures have been proposed to tackle marine litter and 'ghost fishing'; proposals include the involvement of all involved parties to retrieve as much discarded gear as possible, integrating it into waste and recycling processes. Furthermore, producers of plastic fishing gear will bear the expenses of waste collection, transportation, treatment, and awareness-raising efforts. The call was accompanied by a push for increased investment in research and innovation to develop environmentally friendly fishing gear, thus contributing to cleaner oceans [53].

Fishermen are bound by the regulations outlined in Council Regulation (EC) No 1224/2009, and within this framework they are required to either retrieve lost fishing gear or report such losses. Fishermen should actively work to recover any fishing gear that has been lost. This could include nets, lines, or other equipment used in their fishing operations. If retrieval is not possible, fishermen are obligated to report the loss of gear. This involves informing relevant authorities about the incident, providing details such as the location and circumstances of the loss [54].

The Waste Framework Directive (EU 2018/851) [55] was launched on May 2018, with the approval of new rules designed to enhance the recycling rate of plastic waste. The directive included the obligation of Member States to set up separate collection for at least paper, metal, plastic, and glass waste. This legislative measure aligns with a broader commitment to circular economy principles, emphasizing the importance of reusing and recycling materials [26,48,49]. At the same time, single-use plastic products refer to items made entirely or partially from plastic and are usually designed to be used only once or for a brief period before being discarded. Recognizing that the 10 most frequently discovered single-use plastic items on European beaches, along with fishing gear, make up 70% of all marine litter in the EU, the European Parliament has taken strict actions by supporting the ban of single-use plastics [56]. The EU Single-Use Plastics Directive (SUPD) [57] has set specific targets, including achieving a 77% separate collection rate for plastic bottles by 2025 and incorporating 25% recycled plastic in PET beverage bottles from 2025, with a further increase to 30% in all plastic beverage bottles by 2030. Various measures such as awareness campaigns, design requirements (like connecting caps to bottles), and labeling regulations that inform consumers about plastic content and proper disposal, were implemented to achieve these goals. Additionally, fisheries, representing a substantial 27% of marine litter, became a specific focus for targeted measures [26,58].

The next step was taken on October 2023 and emphasized preventing plastic pellet losses to reduce MP pollution. This proposal saw the advocacy of measures to minimize the release of MPs from diverse sources such as textiles, tires, paints, and even cigarettes, acknowledging the complex pathways through which these particles enter the environment.

The overarching goal is to improve the understanding of pellet losses in the supply chain, focusing on improving accuracy in loss estimates, raise awareness among stakeholders, and ensure the effective mitigation of impacts on Small and Medium Enterprises (SMEs) involved in the pellet supply chain [59].

The overarching theme tying these initiatives together is the ongoing integration of circular economy principles. Emphasized in the European Green Deal [48], the EU Biodiversity Strategy [60], and the Farm to Fork strategy [61], there is a concerted effort to accelerate the development of a circular economy culture across all sectors of the economy [62]. Strengthening ocean protection is identified as a crucial component of these overarching strategies. In essence, these initiatives collectively represent a comprehensive and dynamic approach by the European Parliament to tackle plastic pollution head-on and foster a sustainable, circular economy within the EU [63].

In 2015, the United Nations (UN) introduced the 17 Sustainable Development Goals (SDGs), accompanied by a set of targets and indicators [64]. Monitoring the accumulation of MPs is a crucial step in implementing strategies to mitigate MP pollution. SDG 12, which focuses on ensuring sustainable consumption and production patterns, plays a pivotal role in addressing MP pollution. Specifically, Target 12.5 encourages the reduction of plastic pollution through measures such as prevention, reduction, reuse, and recycling of plastic. To track the progress in meeting this target, the national rate and quantity of recycling are taken into account [64,65].

MPs pose a significant threat to marine life and terrestrial ecosystems, as they can be ingested by marine and terrestrial organisms, leading to various ecological imbalances. Diminishing the release of MPs alleviates the pressure on aquatic and soil ecosystems, where these pollutants pose a significant threat. Linking MP accumulation to SDG 14 'Life Below Water' and SD 15 'Life on Land' highlights the need to protect and restore aquatic and land ecosystems [65]. Runoff from water sources can transport MPs to soil and water bodies potentially impacting land-dwelling and aquatic organisms and ecosystems [26]. Sustainable Development Goal 3 (SDG 3) focuses on "Good Health and Well-being" and aims to ensure healthy lives and promote well-being for all at all ages. The SDG 3 primarily addresses health-related issues and MP accumulates in the food chain impacting human health. Therefore, addressing MP pollution requires a holistic approach that considers its impact on both aquatic and terrestrial environments, as well as its implications for human well-being [64].

In the pursuit of efficient MP extraction from soils, density separation remains a popular method, although it has traditionally been applied to water and sediments. Recent advancements have enhanced its applicability to soil matrices [66]. Plastic particles, interacting with charged ions, allow the utilization of various salt solutions [67]. However, the choice of solute becomes crucial, with the optimal density range defined at 1.6–1.8 g cm^{-3} [68]. Among the solutes, saturated Sodium Chloride solution (1.2 g cm^{-3}) emerges as a safe and environmentally friendly option [68], effectively removing low-density MP types but proving unsuitable for high-density plastics like polyvinyl chloride (PVC) and polyethylene terephthalate (PET) [69]. Calcium Chloride ($CaCl_2$) (1.5 g cm^{-3}) encounters challenges with organic matter agglomeration [69], while Zinc Chloride ($ZnCl_2$), known for its toxicity and alteration potential, raises health and structural concerns [70]. Sodium Iodide (NaI), although efficient, faces limitations due to its cost [71]. In addition to conventional density separation, oil separation gains attention by capitalizing on the oleophilic properties of MPs [66,72]. Studies reveal that the application of oils, such as castor oil and olive oil, yields higher recovery rates compared to density separation using only salt solutions [72–74].

The aim of this research is the comparison of four methodologies through multicriteria analysis. During the research, three salt solutions and an oil solution were examined for the extraction of MPs through the process of floatation. Specifically, canola oil, Sodium Chloride (NaCl) solution, Magnesium Sulfate ($MgSO_4$) solution, and Sodium Hexametaphosphate ($Na_6[(PO_3)_6]$) solution were evaluated in relation to four types of widely used plastics:

polyethylene (PE), polystyrene (PS), low density polyethylene (LDPE), and PET in standard sand soil (ISO standard sand, EN 196-1). The process involves extraction through floatation and sedimentation, followed by filtration. The authors of this research endeavor to identify the most effective technique for extracting MPs from soil. The objective is to develop a method applicable to actual soil samples, such as those from crops or coastal areas, enabling the detection, quantitative assessment, and qualitative analysis of MPs.

2. Materials and Methods

In the comparison of the four methods, a crucial aspect is the choice of a control sample that serves as a benchmark for evaluation. The control sample selected for this purpose is CEN Standard sand, adhering to the ISO standard for EN 196-1. This particular sand is recognized as a standard natural siliceous sand, and its selection as a control sample is justified for the following reasons [75]:

- Purity: CEN Standard sand is known for its high purity. Its composition is representative of a well-defined natural siliceous sand without additional contaminants, ensuring that the control sample is free from external influences that could affect the comparison.
- Isometric Rounded Grains: The sand grains in CEN Standard sand exhibit isometric rounded shapes. This characteristic contributes to the homogeneity of the control sample, providing a consistent and well-defined structure for evaluation across different methodologies.
- Certification (EN 196-1): CEN Standard sand is certified according to the EN 196-1 standard. This certification establishes a common denominator for the evaluation of the three methodologies under consideration. It ensures that the control sample adheres to specific quality and performance standards, enhancing the reliability and consistency of the comparison. By utilizing CEN Standard sand as the control sample, the study aims to establish a baseline that facilitates a meaningful and standardized comparison of the four methods. This approach ensures that any variations observed in the results can be attributed to the methodologies themselves rather than differences in the characteristics of the control sample. Table 1 showcases the grading size of CEN Standard Sand used to compare the four solutions.

Table 1. Grading Size CEN Standard Sand of EN 196-1 [75] ISO 679:2009 [76].

Square Mesh Size (mm)	Cumulative Retained (%)
0.08	99 ± 1
0.16	87 ± 5
0.50	67 ± 5
1.00	33 ± 5
1.60	7 ± 5
2.00	0

In this research, four distinct types of polymers, namely PE, PS, LDPE, and PET, were employed for analysis. The primary polymers were manufactured by the Cypriot company Lordos United Group. The process of obtaining these polymer samples involved shredding and utilizing a Kenwood grinding mill, with the source materials being clean new plastic containers or films. It is noteworthy that the resulting plastic samples were intentionally limited to sizes less than 5 mm; the size distribution of MPs was determined by the dry-sieving method [77]. The choice of these specific polymers is significant, as they represent commonly used plastics with diverse applications across various industries. Each polymer type is usually associated with a plethora of common applications. PE is commonly employed for packaging materials [78], plastic bags [79], bottles [80], and plastic toys [81]. PS has common application in food packaging [81], disposable cutlery [82], and other uses. LDPE is commonly used for containers [83], dispensing and squeeze bottles [84], tubing [85], automotive parts [86], molded laboratory equipment [87], etc. Lastly, PET has

a vast reach of applications, including, but not limited to, bottles [88], food packaging [89], textile fibers [90], and other uses.

The intentional reduction of the plastic samples to sizes less than 4 mm aligns with the focus on MPs, which are defined as particles with dimensions typically smaller than 5 mm. This size range is crucial for understanding the behavior and impact of MPs in various environmental contexts, emphasizing the relevance of the research in addressing contemporary challenges associated with plastic pollution.

The densities of common polymers, as indicated in Table 2, exhibit a range from 0.9 g/mL to 1.6 g/mL [91]. However, it is important to note that the remaining particles present in the samples tend to settle out due to their higher density. For instance, typical densities for quartz sand particles are around 2.65 g/mL [92]. The challenge arises when dealing with high-density polymers such as PVC and PET, which necessitate the use of high-density solutions like Sodium Iodide (NaI) and Zinc Chloride ($ZnCl_2$) for effective separation. While these solutions are suitable for the separation of high-density polymers, they pose significant concerns regarding safety for both human use and the environment due to their toxic nature [92–95]. A cutting-edge approach in the field involves harnessing the oleophilic (oil-attracting) properties of MPs and utilizing oils for density separation, representing a novel perspective [66,72]. Recent studies highlight that this innovative method, using oils, can achieve higher recovery rates of MPs compared to traditional density separation methods employing salt solutions [66,73,74,96]. The research conducted by Mani et al. [96] in 2019 demonstrated an impressive recovery rate of 99 $\pm$ 4% for MPs using castor oil. This underscores the effectiveness of the oleophilic approach in achieving high recovery rates. The separation process proposed by Scopetani et al. [73] achieved substantial recovery rates, ranging from 90% to 97%, for six different types of polymers. This further validates the efficacy of utilizing oils for MP separation.

Table 2. Density of examine polymers.

Polymer	Density (g/cm^{-3})
PE	0.95~0.97
PS	0.95~1.06
LDPE	0.89~0.93
PET	1.37~1.41

Sodium Chloride with a density of 1.2 g/mL is a solution that has been widely used by researchers to extract MPs, a method that is an economical and safe solution [69,97–99]. The application of oils to extract MPs is a new method that combines the low density of oil and the oleophilic properties of plastics. Canola oil with a density of 0.92 g/mL is an oil that has been used by researchers and shows excellent results [66,73,74,96]. The choice of the Magnesium Sulfate solution was made due to its high density (1.32 g/mL) and due to the availability of the salt in the laboratory for conducting the laboratory tests. The choice of the Sodium Hexametaphosphate Solution was made due to its low density (1.18 g/mL) and due to the availability of the salt in the laboratory for conducting the laboratory tests. This salt finds a variety of applications in industry, while in geological research laboratories it is used to break up grains of silt and clay to classify and evaluate soil [100].

2.1. Spiking and Recovery Test

The experimental MPs recovery process differs between the three salts (Sodium Chloride, Magnesium Sulfate, Sodium Hexametaphosphate) and the oil (canola oil) and is analyzed below. It is noted that the experimental procedure was repeated four times per solvent, once per type of polymer examined.

2.1.1. Solutions of Sodium Chloride, Magnesium Sulfate, Sodium Hexametaphosphate

A sample of CEN Standard Sand with a constant mass of 100 g was placed in a glass bowl and 5 g of the examined polymer were added as they were obtained from

their shredding in a grinding mill. The sample was mixed with a metal spatula. The sample was carefully transferred to a glass volumetric cylinder, then 500 mL of Sodium Chloride solution was added; the volumetric cylinder was sealed with a suitable stopper and manually stirred for 30 s (stirring is carried out by 180-degreerotations of the volumetric cylinder), then the stopper was removed and washed with a few ml of deionized water (about 5 mL) over the roller to remove any granules and MPs. To ensure the completeness of the transfer, the volumetric cylinder was weighed both before and after the MPs were placed, confirming that the entire required mass was transferred. The sample was left undisturbed for 6 h at a laboratory temperature of 25 °C. After 6 h, the elements that floated were removed by overflow and placed on filter paper to dry (Figure 3). The process was repeated for the three types of salt solvents and for the four polymers (Figure 4).

Figure 3. Steps of the experimental procedure.

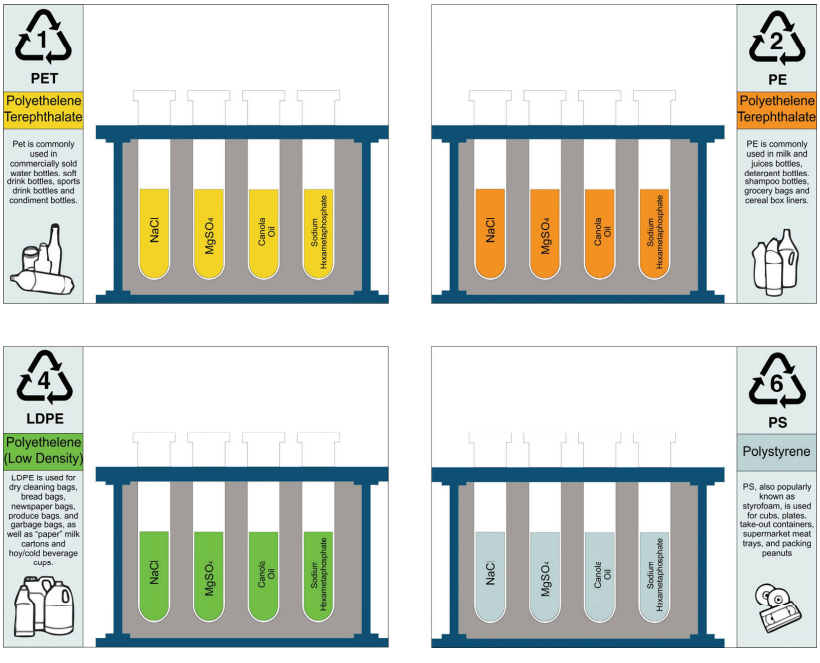

Figure 4. Execution phase comparison of four solutions on four types of polymers.

2.1.2. Canola Oil

A sample of CEN Standard Sand of constant mass of 100 g was placed in a glass bowl and 5 g of the examined polymer were added as they were obtained from their fragmentation in a grinding mill. The sample was mixed with a metal spatula. The sample was carefully transferred to a glass volumetric cylinder, then 200 mL of deionized water and 100 mL of canola oil were added; the volumetric cylinder was sealed with a suitable stopper and manually stirred for 30 s (stirring is carried out by 180-degree rotations of the volumetric cylinder), then the stopper was removed and washed with a few ml of deionized water (about 5 mL) over the cylinder, in order to detach any granules and MPs. After the end of stirring, another 200 mL of deionized water was added. The sample was left undisturbed for 6 h at a laboratory temperature of 25 °C. At the end of the 6 h, the elements that floated were removed by overflow, rinsed with deionized water, and placed on filter paper to dry. The polymers identified per sample were placed in clear plastic air-tight plastic bags of specific mass and then weighed on a calibrated balance to three decimal places. The initial amount of plastics added to the sand sample was then compared to the mass of recovered plastics.

2.2. Protocol Validation

Ensuring the integrity of experimental samples and controls is crucial in research, especially when dealing with MPs [101]. In our study, several measures were implemented to prevent possible contamination during sample processing and experimental controls:

1. Use of Glass and Metal Equipment: Glass and metal equipment was exclusively utilized during sample processing and for experimental controls. These materials are chosen for their inert nature, minimizing the risk of introducing foreign elements or contaminants into the samples;
2. Selection of Plastic Grinding Mill: While glass and metal equipment were predominantly used, the grinding mill employed for shredding the polymers was made of plastic. This decision likely considered the mechanical properties needed for effective polymer shredding. It is noteworthy that this equipment choice was a conscious decision and did not compromise the integrity of the experiment;
3. Cleaning and Rinsing Protocols: All equipment used, whether glass, metal, or the plastic grinding mill, underwent thorough cleaning, rinsing with distilled water, and subsequent drying in an oven. This meticulous cleaning process aims to eliminate any residual contaminants that might affect the experimental outcomes;
4. Clothing and Sealing Measures: Cotton clothes were used during experimental tests to prevent contamination of samples with plastic fibers. This precaution is essential, as airborne MPs or fibers from clothing could inadvertently contaminate the samples. Additionally, proper sealing of samples during idle times provided further safeguards against external influences.

These measures collectively demonstrate a commitment to maintaining the purity and reliability of the experimental setup. The attention to detail in the choice of materials, cleaning procedures, and handling protocols contributes to the robustness of the study and the accuracy of the obtained results [102].

2.3. Multi-Criteria Decision Analysis for MPs Extraction Method

In this phase of the study, a Multi-Criteria Decision Analysis (MCDA) is employed to evaluate alternative scenarios for selecting the optimal solvent for extracting micro and nanoplastics from soil samples [103]. This decision-making methodology, which is used in business research, becomes crucial when dealing with complex decision-making problems that require a multidimensional approach [104,105]. The complexity and importance of decision-making problems necessitate a multidimensional analysis rather than one-sided and one-dimensional approaches. MCDA is employed to prioritize alternative scenarios by resolving conflicting parameters [106].

The MCDA involves the calculation of the frequency of occurrence of management methodologies in the ranking positions during sensitivity analysis. The application of MCDA in this research aims to provide a reliable and scientifically documented approach to compare and select the optimal MPs extraction method. By considering multiple criteria and conducting a multifaceted analysis, the study seeks to derive an optimal solution that balances technical, economic, and environmental considerations [106–109]. In order to apply the MCDA and determine the optimal MPs extraction method, the following approach was initially applied:

- Characteristics of four solvents are collected and evaluated;
- Experimental controls are conducted to evaluate solvents;
- Scenarios are identified, and evaluation criteria per scenario are determined;
- Calculations of the frequency of occurrence of the management methodologies in the ranking positions during the sensitivity analysis.

Numerous MCDA methods have been developed in recent years to address multi-dimensional problems with conflicting parameters [106,109–112]. Widely used methods include ELECTRE II, AHP, PROMETHEE, Gray Relational Analysis, and Regime. These methods vary in the way criteria are defined, expressed, and applied [107–109,113–115]. The selection of the solvent for MPs extraction is a critical factor in the research due to the abundance of alternative solvents, each with its own advantages and disadvantages (technical, economic, environmental, etc.).

2.4. Analytical Hierarchy Process (AHP)

AHP, developed by Saaty [116], is applied in this study to evaluate restoration methodologies. AHP structures a problem in a hierarchical way, descending from a goal to criteria, sub-criteria, and alternatives in successive levels. Firstly, the complexity of the problem is decomposed into decision elements to make it more comprehensible. AHP in particular employs a binary comparison of user-selected criteria and prioritizes them using a standard scaling system. The relative weight of each element is determined through a pairwise assessment of the proposed criteria, establishing preferences and prioritizing alternative scenarios based on the assigned weighting factors [117,118].

The weighting factors assigned to each criterion play a crucial role in determining the overall preference for each scenario. Direct coefficients may be applied for a small number of criteria, while indirect coefficients involve ranking the criteria in order of importance. The AHP structure of the evaluation problem is visually represented in Figure 5, showcasing the interconnected relationships between criteria and sub-criteria. This structured approach aids in systematically assessing and prioritizing the alternative scenarios. The criteria for evaluating the alternative scenarios encompass economic, environmental, and technical aspects, each contributing to the overall effectiveness of the extraction method (Figure 6).

The complexity of polymers and their varying properties across different soils necessitates the development of a versatile and efficient extraction methodology. The application of an analytical prioritization method aims to identify a cost-effective, rapid, and reliable technique that is user and environmentally friendly. The alternative scenarios considered for evaluation are Sodium Chloride solution, Magnesium Sulfate solution, canola oil, and Sodium Hexametaphosphate solution. Two sustainability pillars, economic and environmental, are selected as primary criteria, with additional consideration given to technical aspects such as the simplicity of application and user safety. The third pillar, social sustainability, is regarded as of minor importance in this assessment. Additionally, the criterion of validity is incorporated into the evaluation framework. The criteria are further categorized as follows:

1. Economic Criteria: Solvent Cost—Assessing the economic feasibility of each scenario, considering the cost of the solvent involved;
2. Environmental Criteria: Safety—Evaluating the environmental impact and safety considerations associated with each scenario;

3. Technical Criteria: Simplicity of Method—Gauging the simplicity and ease of application of the extraction method. User Security—Considering the safety aspects for the individuals involved in the extraction process. Validity—Assessing the scientific validity and reliability of the extraction results.

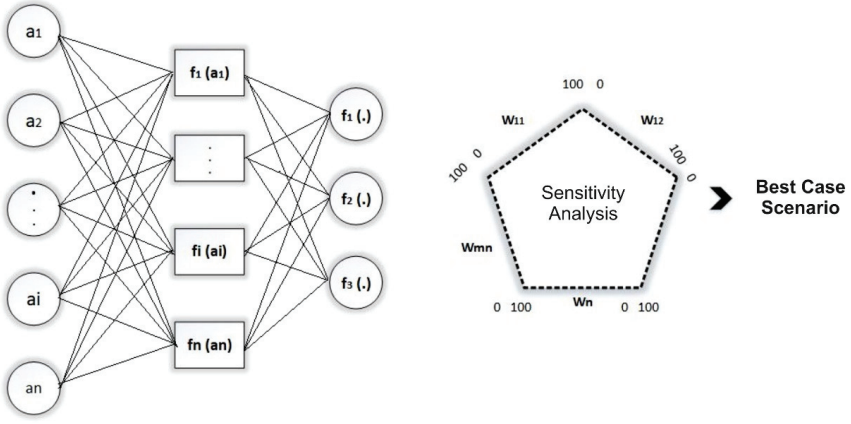

Figure 5. Diagram of the MCDA process showing the relationships between the performance scenarios, the criteria, and the weighting coefficients.

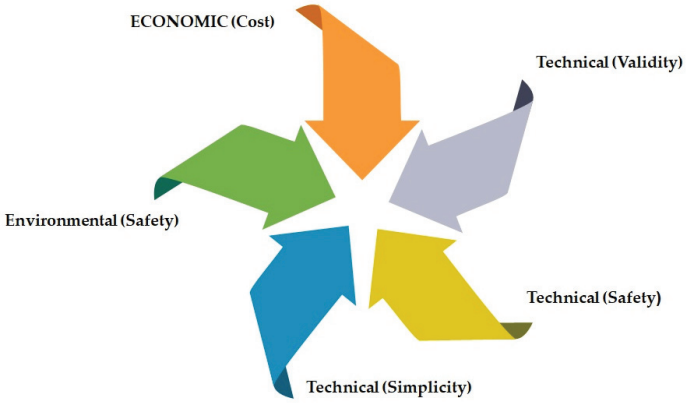

Figure 6. Combination of SWOT and AHP analysis.

The scoring of criteria is guided by Table 3, which employs a fundamental scale developed by Saaty [116] in 1990. This scale allows for a consistent and standardized assessment of the criteria, contributing to an objective and comprehensive evaluation. In summary, the methodology employs a MCDA, considering economic, environmental, and technical factors to prioritize alternative scenarios for MPs extraction. The robustness of the AHP ensures a systematic and informed decision-making process in the selection of the optimal extraction method [119].

AHP method was implemented using the online version of Topsis Software https://onlineoutput.com/ (accessed 8 March 2024). The analysis was conducted in four successive stages:

1. Defining Alternative Scenarios: The alternative scenarios were defined based on the extraction methods under consideration;
2. Defining Criteria: Criteria for evaluation were defined, encompassing economic, environmental, and technical aspects;

3. Rating of Criteria and Sub-criteria through Weighting Factors: Weighting factors were assigned to criteria and sub-criteria to reflect their relative importance in the decision-making process. The ratings were performed by the user to establish the hierarchy of preferences;
4. Presentation of Results: The software analyzed the defined alternative scenarios, criteria, and weighting factors to present the results of the AHP analysis. This stage was an outcome of the software's processing of the user-defined inputs.

Table 3. Fundamental scale of Saaty [116].

Value	Definition	Interpretation
1	Equal Preference	Both criteria/scenarios contribute equally to the goal
3	Moderate Strong Preference	One criterion/scenario is slightly more important than the other
5	Strong Preference	One criterion/scenario is significantly more important than the other
7	Very Strong Preference	One criterion/scenario is very important compared to the other
9	Extremely Strong Preference	One criterion/scenario is extremely more important than the other
2, 4, 6, 8	Intermediate Preference Values	Used to express intermediate preferences

To address the problem at hand, the software was applied with equal grading (weighting) of criteria. This approach aimed to ensure a fair and unbiased evaluation of the alternative scenarios. The systematic use of AHP, facilitated by the Topsis Software [120,121], provided a structured and data-driven approach to prioritize and select the most suitable MPs extraction method.

3. Results and Discussion

The various methods implemented for the recovery of MPs demonstrated different levels of recovery accuracy depending on the type of plastic. Knowing the initial mass of plastics introduced into the solution and the mass recovered allows us to calculate the recovery rates. The results of the four solvents in relation to the four polymers evaluated are given in Table 4.

Table 4. Recovery rates of plastic particles by the oil and density separation method using a mixture of canola oil and three types of salt solutions.

	Average Recovery Performance (%)			
Type pf Polymer	Sodium Chloride Solution	Magnesium Sulfate Solution	Canola Oil	Sodium Hexametaphosphate
PE	91	94	97	62
PS	98	99	99	98
LDPE	86	87	94	78
PET	42	63	75	12

Most highlighted is the recovery method using canola oil which showed the highest accuracy rates compared to the three salt solutions. More precisely, the MPs recovery achieved a rate of 97% in PE, 99% in PS, 94% in LDPE, and 75% in PET. The Magnesium Sulfate solution demonstrated the same rate of efficiency in recovering PS and achieved high rates in the other three categories of plastics as well. Therefore, Magnesium Sulfate yields favorable results, especially for high-density polymers like PET. Contrastingly, the Sodium Hexametaphosphate solution exhibited relatively low efficiency for PE and PET, but remarkably achieved a 98% recovery rate for PS. This can be attributed to the low-density of PS compared to the salt solutions, which causes PS to easily float. The recovery method using a saturated Sodium Chloride solution is suitable for recovery of low-density polymers, while for high-density plastics canola oil Oil is a more valid method (Figures 7 and 8).

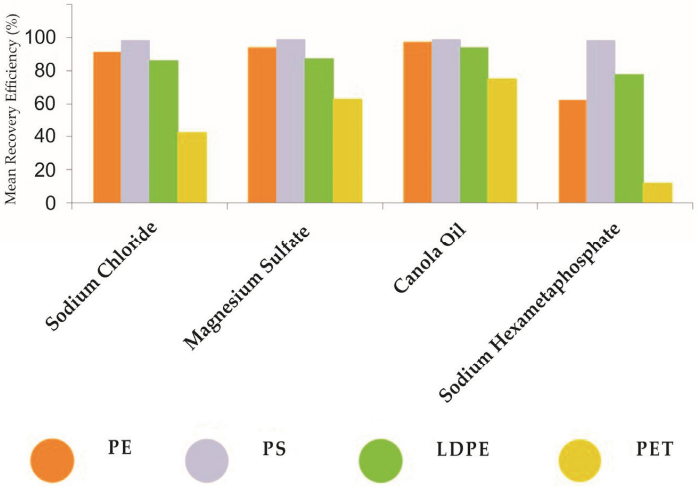

Figure 7. Efficiency of Solution for each type of MP.

Figure 8. Efficiency of Solution for each type of MP.

The oleophilic properties of the MPs resulted in 'trapping' of the MPs in the oil column after separation due to agitation [71,72]. Due to its high density, the Magnesium Sulfate solution shows high turbidity, so the detection and extraction of MPs is particularly difficult although the results are quite high even in high density polymers (PET). The evaluation of the results of the experimental procedure and their comparison with previous related research on the recovery methods with saturated Sodium Chloride solution and canola oil

provide validation for the efficacy of these two recovery methods. The study aligns with previous research [71,122] enhancing the credibility and reliability of the recovery methods applied in this research.

Particularly, Kononov et al. [71] explored the development of a cost-effective and straightforward technique for extracting MPs from soil by utilizing canola oil and employing the density separation process with Sodium Chloride. Through their research, numerous oils were evaluated for the extraction of MPs including canola, castor, olive, rice, and turpentine oils for the extraction of LDPE, PP, and PVC. Canola, silicone, rice, and turpentine oils exhibited recoveries of 95% and above; however, turpentine oil was not recommended for further applications, due to its ability to dissolve weakly structured plastics. On the other hand, castor oil, even though highly efficient [96], demonstrated a lower recovery rate along with olive oil and silicon oil. In summary, the research revealed that the best option for recovery amongst the oils employed was in fact canola oil [71]. The attraction between MPs and canola oil might be linked to the lipophilicity of the hydrocarbon chains and the oil molecules [74]. According to Kim et al. [123], the correlation between the interfacial tension of oil and the oil–water emulsions during agitation is the minimization of the stability of the oil layer and buoyancy of oil-absorbed MPs due to the development of the oil–water emulsion.

AHP Results

Based on the scenarios and criteria described for the problem under consideration, Table 5 was structured. This table describes the performance of each alternative scenario with respect to the evaluation criteria, a means of determining the degree of preference of the criteria in obtaining the optimal solution. The scoring of this table was based on a numerical scale of 1 to 9, whereby the best option is number 9 and the worst option is number 1. Sensitivity analysis was carried out by varying the weight of these evaluation criteria and showed that small changes in their scores affect the final decision to determine the optimal solution.

Table 5. Criteria evaluation ranking using the AHP method.

	Criteria				
	Economic Cost	**Environmental Safety**	**Technical**		
			Simplicity	**Users' Safety**	**Validity**
Sodium Chloride	9	8	8	8	7
Magnesium Sulfate	5	5	8	6	8
Canola Oil	7	4	8	7	9
Sodium Hexametaphosphate	4	7	8	8	4

The application of AHP through the TOPSIS software showed that based on the evaluation criteria the Sodium Chloride solvent scenario is presented as the best scenario, followed by canola oil (Figure 9). The Sodium Chloride recovery method is an economical, safe, and valid method for MPs recovery [71,97]. Although the MPs recovery method using oil shows the best results, with the existing laboratory equipment the use of the Sodium Chloride solution is a more user-friendly method to use. Regarding Sodium Hexametaphosphate it is deemed impractical due to the increased cost and difficulties in supplying the solution compared to the other three options and the significant low validity.

On the other hand, canola oil received a low score in environmental performance as the disposal of the solution requires a specific recycling procedure, which may not be available in certain areas. The oleophilic properties of MPs contribute to their effective separation in the oil column after agitation [71]. Post-procedure, cleaning the laboratory equipment used poses challenges, and oil residues persist on MPs. However, it attains a very high validity score as the results are highly comparable with those of other researchers [71,74]. Magnesium Sulfate, aside from the high purchase cost, exhibits significant turbidity resulting from the solution's high density, which complicates the detection and extraction of MPs.

Moreover, the production of Magnesium Sulfate solution demands a substantial amount of time for the salt to dissolve in the distilled water.

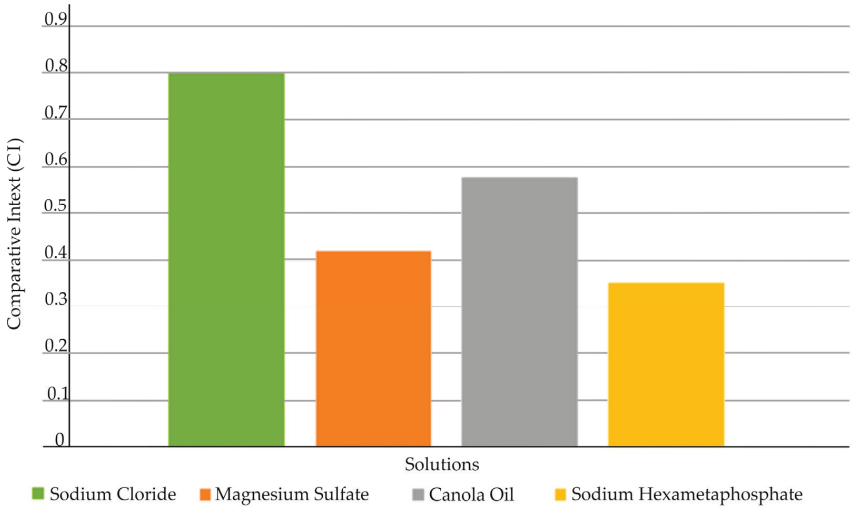

Figure 9. Multicriteria Results of the Solutions Effectiveness (CI—Comparative Intext).

Lastly, the AHP analysis assigns the highest score to Sodium Chloride, commonly known as table salt, also due to its extreme affordability and easy supply. It poses no significant threats to the environment or the user and the processing is relatively straightforward [66,73,74,96]. However, the necessity for recycling, reusing, and reclaiming hazardous waste applies to both costly and environmentally harmful density separation solutions [124]. Various salts have been investigated regarding their recyclability including NaI, NaBr, and others. The reuse of NaI [125] demonstrates its capability to be recycled up to ten times through rinsing and evaporation stages, maintaining a cost-effective approach (3.7 EUR/kg) without chemical contamination or significant loss thus deeming it applicable due to its environmental friendliness and the potentiality for multiple recycling cycles. At the same time, NaBr can be successfully recycled five times during the extraction of MPs from soil, achieving a recovery rate of over 90% [126].

These findings collectively emphasize the promising nature of the oleophilic approach, providing a valuable alternative to conventional methods. The oleophilic properties of plastics were initially considered a crucial characteristic for developing an effective method of separating MPs from soil, deviating from conventional density separation techniques and offering a hypothetical solution to the challenges posed by the density differences of plastics [97]. The use of oils not only enhances recovery rates but also introduces a potential avenue for safer and more environmentally friendly MPs separation techniques. As this research area continues to evolve, it holds promise for contributing to more sustainable practices in the field of MPs analysis and remediation [92,95]. The dilemma is to strike a balance between achieving efficient separation based on polymer densities and ensuring the safety of the separation methods employed. The toxicity associated with certain high-density solutions underscores the need for exploring alternative, safer methods or finding ways to mitigate the environmental impact of the chosen separation techniques. This is particularly crucial for applications involving MPs, where environmental safety and minimal ecological impact are paramount considerations [93,94].

4. Conclusions

The application of the AHP and the subsequent analysis through software have yielded a clear and decisive result in favor of the Sodium Chloride solvent scenario as the optimal

method for low-density MPs recovery. This conclusion is based on a comprehensive evaluation of various criteria, including economic viability, safety, and overall validity of the extraction method. The AHP analysis, considering economic and safety factors along with technical validity, has positioned the Sodium Chloride solvent scenario as the most favorable for MPs recovery. The Sodium Chloride recovery method is highlighted as an economically feasible option, aligning with sustainable practices and contributing to efficient waste management. The AHP evaluation takes into account the safety aspects of the recovery method, emphasizing the importance of a solvent that is not only effective but also safe for users and the environment. The Sodium Chloride scenario is recognized for its scientific validity in MPs recovery, further reinforcing its status as a reliable and efficient method. The endorsement of the Sodium Chloride solvent scenario suggests its potential for practical implementation in MPs extraction from soil samples. The findings contribute to the development of environmentally friendly practices, aligning with the goals of sustainable waste management and environmental protection. While the study provides a robust foundation, ongoing research is encouraged to explore additional solvents and refine methodologies for continuous improvement in MPs extraction techniques. Communicating the results to relevant stakeholders, policymakers, and the public is crucial for promoting the adoption of effective and sustainable MPs recovery practices. In conclusion, the research, employing the AHP methodology, has successfully identified the Sodium Chloride recovery method as the optimal scenario for MPs extraction. This outcome not only advances scientific knowledge in the field but also contributes practical solutions to address the growing concern of MP pollution in soil environments. Further research is required to explore the utilization of a broader range of oils and salt solutions with higher density for MP extraction. The application of distilled water as a solvent for MP cleaning post-filtration is deemed problematic in the context of oil solvents. Therefore, it is advisable to explore alternative filtration solutions or adsorbents to facilitate the more seamless and efficient removal of oils.

Author Contributions: Conceptualization, V.P., I.V. and A.A.Z.; methodology, V.P. and A.A.Z.; software, V.P. and A.A.Z.; validation, V.P., I.V. and A.A.Z.; formal analysis, V.P., I.P., I.V. and A.A.Z.; investigation, V.P. and A.A.Z.; resources, I.V. and A.A.Z.; data curation, V.P., I.V. and A.A.Z.; writing—original draft preparation, V.P., I.P. and A.A.Z.; writing—review and editing, V.P., I.P. and A.A.Z.; visualization, V.P., I.P., I.V. and A.A.Z.; supervision, I.V. and A.A.Z.; project administration, A.A.Z.; funding acquisition, A.A.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data will be released upon reasonable request.

Acknowledgments: The authors would like to acknowledge the Laboratory of Chemical Engineering and Engineering Sustainability of the Open University of Cyprus for supporting this research.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Thompson, R.C.; Olsen, Y.; Mitchell, R.P.; Davis, A.; Rowland, S.J.; John, A.W.G.; McGonigle, D.; Russell, A.E. Lost at Sea: Where Is All the Plastic? *Science* **2004**, *304*, 838. [CrossRef]
2. Zhang, Q.; Song, M.; Xu, Y.; Wang, W.; Wang, Z.; Zhang, L. Bio-based polyesters: Recent progress and future prospects. *Prog. Polym. Sci.* **2021**, *120*, 101430. [CrossRef]
3. Lusher, A.L.; McHugh, M.; Thompson, R.C. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Mar. Pollut. Bull.* **2013**, *67*, 94–99. [CrossRef]
4. Desforges, J.-P.W.; Galbraith, M.; Dangerfield, N.; Ross, P.S. Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. *Mar. Pollut. Bull.* **2014**, *79*, 94–99. [CrossRef]

5. Rocha-Santos, T.; Duarte, A.C. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. *TrAC Trends Anal. Chem.* **2015**, *65*, 47–53. [CrossRef]
6. Liu, J.; Liang, J.; Ding, J.; Zhang, G.; Zeng, X.; Yang, Q.; Zhu, B.; Gao, W. Microfiber pollution: An ongoing major environmental issue related to the sustainable development of textile and clothing industry. *Environ. Dev. Sustain.* **2021**, *23*, 11240–11256. [CrossRef]
7. Statista Plastics Industry in Europe—Statistics & Facts. Available online: https://www.statista.com/topics/8641/plastics-industry-in-europe/#topicOverview (accessed on 23 January 2024).
8. Statista Annual Production of Plastics Worldwide from 1950 to 2021. Available online: https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/ (accessed on 22 January 2024).
9. Plastics Europe Plastics—The Facts. 2021. Available online: https://plasticseurope.org/wp-content/uploads/2021/12/Plastics-the-Facts-2021-web-final.pdf (accessed on 22 January 2024).
10. Eionet Portal ETC/CE Report 2023/2 The Fate of EU Plastic Waste. Available online: https://www.eionet.europa.eu/etcs/etc-ce/products/etc-ce-report-2023-2-the-fate-of-eu-plastic-waste (accessed on 22 January 2024).
11. Browne, M.A.; Galloway, T.; Thompson, R. Microplastic—An emerging contaminant of potential concern? *Integr. Environ. Assess. Manag.* **2007**, *3*, 559–561. [CrossRef] [PubMed]
12. Ryan, P.G.; Moore, C.J.; van Franeker, J.A.; Moloney, C.L. Monitoring the abundance of plastic debris in the marine environment. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 1999–2012. [CrossRef] [PubMed]
13. Official Journal of the European Union Commission Recommendation of 18 October 2011 on the Definition of Nanomaterial. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011H0696 (accessed on 22 January 2024).
14. Claessens, M.; Van Cauwenberghe, L.; Vandegehuchte, M.B.; Janssen, C.R. New techniques for the detection of microplastics in sediments and field collected organisms. *Mar. Pollut. Bull.* **2013**, *70*, 227–233. [CrossRef] [PubMed]
15. Hartmann, N.B.; Hüffer, T.; Thompson, R.C.; Hassellöv, M.; Verschoor, A.; Daugaard, A.E.; Rist, S.; Karlsson, T.; Brennholt, N.; Cole, M.; et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. *Environ. Sci. Technol.* **2019**, *53*, 1039–1047. [CrossRef] [PubMed]
16. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci. Adv.* **2017**, *3*, e1700782. [CrossRef]
17. Hoellein, T.J.; Rochman, C.M. The "plastic cycle": A watershed-scale model of plastic pools and fluxes. *Front. Ecol. Environ.* **2021**, *19*, 176–183. [CrossRef]
18. Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K.L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347*, 768–771. [CrossRef] [PubMed]
19. Horton, A.A.; Dixon, S.J. Microplastics: An introduction to environmental transport processes. *WIREs Water* **2018**, *5*, e1268. [CrossRef]
20. Kiessling, T.; Knickmeier, K.; Kruse, K.; Brennecke, D.; Nauendorf, A.; Thiel, M. Plastic Pirates sample litter at rivers in Germany—Riverside litter and litter sources estimated by schoolchildren. *Environ. Pollut.* **2019**, *245*, 545–557. [CrossRef] [PubMed]
21. Windsor, F.M.; Durance, I.; Horton, A.A.; Thompson, R.C.; Tyler, C.R.; Ormerod, S.J. A catchment-scale perspective of plastic pollution. *Glob. Chang. Biol.* **2019**, *25*, 1207–1221. [CrossRef]
22. Hoellein, T.J.; Shogren, A.J.; Tank, J.L.; Risteca, P.; Kelly, J.J. Microplastic deposition velocity in streams follows patterns for naturally occurring allochthonous particles. *Sci. Rep.* **2019**, *9*, 3740. [CrossRef] [PubMed]
23. Goodwine, K. The Ethics of Single-Use Plastics. Student Research. Honor Scholar Thesis, DePauw University, Greencastle, IN, USA, 2019. 105. Available online: https://scholarship.depauw.edu/cgi/viewcontent.cgi?article=1104&context=studentresearch (accessed on 24 January 2024).
24. Barcelo, D.; Pico, Y. Case studies of macro- and microplastics pollution in coastal waters and rivers: Is there a solution with new removal technologies and policy actions? *Case Stud. Chem. Environ. Eng.* **2020**, *2*, 100019. [CrossRef]
25. United Nations. 'Turn the Tide on Plastic' Urges UN, as Microplastics in the Seas Now Outnumber Stars in Our Galaxy. Available online: https://news.un.org/en/story/2017/02/552052-turn-tide-plastic-urges-un-microplastics-seas-now-outnumber-stars-our-galaxy (accessed on 22 January 2024).
26. Chatziparaskeva, G.; Papamichael, I.; Zorpas, A.A. Microplastics in the coastal environment of Mediterranean and the impact on sustainability level. *Sustain. Chem. Pharm.* **2022**, *29*, 100768. [CrossRef]
27. European Environment Agency (EEA). From Source to Sea—The Untold Story of Marine Litter. Available online: https://www.eea.europa.eu/publications/european-marine-litter-assessment#:~:text=Plastic%20packaging%20and%20small%20plas-tic,waste%20management%20capacity%20is%20limited (accessed on 22 January 2024).
28. Sevwandi Dharmadasa, W.L.S.; Andrady, A.L.; Kumara, P.B.T.P.; Maes, T.; Gangabadage, C.S. Microplastic pollution in Marine Protected Areas of Southern Sri Lanka. *Mar. Pollut. Bull.* **2021**, *168*, 112462. [CrossRef] [PubMed]
29. Cutroneo, L.; Cincinelli, A.; Chelazzi, D.; Fortunati, A.; Reboa, A.; Spadoni, S.; Vena, E.; Capello, M. Baseline characterisation of microlitter in the sediment of torrents and the sea bottom in the Gulf of Tigullio (NW Italy). *Reg. Stud. Mar. Sci.* **2020**, *35*, 101119. [CrossRef]
30. Expósito, N.; Rovira, J.; Sierra, J.; Folch, J.; Schuhmacher, M. Microplastics levels, size, morphology and composition in marine water, sediments and sand beaches. Case study of Tarragona coast (western Mediterranean). *Sci. Total Environ.* **2021**, *786*, 147453. [CrossRef] [PubMed]

31. Nachite, D.; Maziane, F.; Anfuso, G.; Williams, A.T. Spatial and temporal variations of litter at the Mediterranean beaches of Morocco mainly due to beach users. *Ocean Coast. Manag.* **2019**, *179*, 104846. [CrossRef]
32. Alfaro-Núñez, A.; Astorga, D.; Cáceres-Farías, L.; Bastidas, L.; Soto Villegas, C.; Macay, K.; Christensen, J.H. Microplastic pollution in seawater and marine organisms across the Tropical Eastern Pacific and Galápagos. *Sci. Rep.* **2021**, *11*, 6424. [CrossRef] [PubMed]
33. Statista Share of Travel and Tourism's Total Contribution to GDP Worldwide in 2019 and 2022, with a Forecast for 2023 and 2033. Available online: https://www.statista.com/statistics/1099933/travel-and-tourism-share-of-gdp/ (accessed on 22 January 2024).
34. Zorpas, A.A.; Navarro-Pedreño, J.; Panagiotakis, I.; Dermatas, D. Steps forward to adopt a circular economy strategy by the tourism industry. *Waste Manag. Res.* **2021**, *39*, 889–891. [CrossRef] [PubMed]
35. DeWit, W.; Burns, E.T.; Guinchard, J.C.; Ahmed, N. *Plastics: The Costs to Society, the Environment and the Economy*; World Wildlife Fund (WWF): Gland, Switzerland, 2021.
36. Davidson, T.M. Boring crustaceans damage polystyrene floats under docks polluting marine waters with microplastic. *Mar. Pollut. Bull.* **2012**, *64*, 1821–1828. [CrossRef] [PubMed]
37. Rapp, J.; Herrera, A.; Martinez, I.; Raymond, E.; Santana, Á.; Gómez, M. Study of plastic pollution and its potential sources on Gran Canaria Island beaches (Canary Islands, Spain). *Mar. Pollut. Bull.* **2020**, *153*, 110967. [CrossRef] [PubMed]
38. Alessi, E.; Di Carlo, G.; Campogianni, S.; Tangerine, B.; Pietrobelli, E. *Out of the Plastic Trap: Saving the Mediterranean from Plastic Pollution*; World Wildlife Fund (WWF): Gland, Switzerland, 2018.
39. Sharma, S.; Sharma, V.; Chatterjee, S. Microplastics in the Mediterranean Sea: Sources, Pollution Intensity, Sea Health, and Regulatory Policies. *Front. Mar. Sci.* **2021**, *8*, 634934. [CrossRef]
40. Horton, A.A.; Walton, A.; Spurgeon, D.J.; Lahive, E.; Svendsen, C. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total Environ.* **2017**, *586*, 127–141. [CrossRef] [PubMed]
41. De Souza Machado, A.A.; Kloas, W.; Zarfl, C.; Hempel, S.; Rillig, M.C. Microplastics as an emerging threat to terrestrial ecosystems. *Glob. Chang. Biol.* **2018**, *24*, 1405–1416. [CrossRef]
42. González-Fernández, D.; Cózar, A.; Hanke, G.; Viejo, J.; Morales-Caselles, C.; Bakiu, R.; Barceló, D.; Bessa, F.; Bruge, A.; Cabrera, M.; et al. Floating macrolitter leaked from Europe into the ocean. *Nat. Sustain.* **2021**, *4*, 474–483. [CrossRef]
43. Igalavithana, A.D.; Mahagamage, M.G.Y.L.; Gajanayake, P.; Abeynayaka, A.; Gamaralalage, P.J.; Ohgaki, M.; Takenaka, M.; Fukai, T.; Itsubo, N. Microplastics and Potentially Toxic Elements: Potential Human Exposure Pathways through Agricultural Lands and Policy Based Countermeasures. *Microplastics* **2022**, *1*, 102–120. [CrossRef]
44. Nizzetto, L.; Futter, M.; Langaas, S. Are Agricultural Soils Dumps for Microplastics of Urban Origin? *Environ. Sci. Technol.* **2016**, *50*, 10777–10779. [CrossRef] [PubMed]
45. Hossain, M.N.; Rahman, M.M.; Afrin, S.; Akbor, M.A.; Siddique, M.A.B.; Malafaia, G. Identification and quantification of microplastics in agricultural farmland soil and textile sludge in Bangladesh. *Sci. Total Environ.* **2023**, *858*, 160118. [CrossRef] [PubMed]
46. Loizia, P.; Voukkali, I.; Chatziparaskeva, G.; Navarro-Pedreño, J.; Zorpas, A.A. Measuring the Level of Environmental Performance on Coastal Environment before and during the COVID-19 Pandemic: A Case Study from Cyprus. *Sustainability* **2021**, *13*, 2485. [CrossRef]
47. Okoffo, E.D.; Donner, E.; McGrath, S.P.; Tscharke, B.J.; O'Brien, J.W.; O'Brien, S.; Ribeiro, F.; Burrows, S.D.; Toapanta, T.; Rauert, C.; et al. Plastics in biosolids from 1950 to 2016: A function of global plastic production and consumption. *Water Res.* **2021**, *201*, 117367. [CrossRef] [PubMed]
48. European Commission. Resolution of the European Committee of the Regions—The Green Deal in Partnership with Local and Regional Authorities. In Proceedings of the Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions The European Green Deal, Brussels, Belgium. 2019; p. 24. Available online: https://eur-lex.europa.eu/resource.html?uri=cellar:b828d165-1c22-11ea-8c1f-01aa75ed71a1.0002.02/DOC_1&format=PDF (accessed on 8 March 2024).
49. European Commission. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions Closing the Loop—An EU Action Plan for the Circular Economy. In Proceedings of the Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions Closing the Loop—An EU Action Plan for the Circular Economy, Brussels, Belgium. 2015. Available online: https://eur-lex.europa.eu/resource.html?uri=cellar:9903b325-6388-11ea-b735-01aa75ed71a1.0017.02/DOC_1&format=PDF (accessed on 8 March 2024).
50. European Parliament and Council Directive 94/62/EC on Packaging and Packaging Waste (OJ L 365, 31.12.1994, pp. 10–23). Available online: https://eur-lex.europa.eu/legal-content/EL/LSU/?uri=celex:31994L0062 (accessed on 11 March 2022).
51. The Guardian Polystyrene to Be Phased out Next Year under Australia's Plastic Waste Plan. Available online: https://www.theguardian.com/australia-news/2021/mar/04/polystyrene-to-be-phased-out-next-year-under-australias-plastic-waste-plan (accessed on 10 January 2024).
52. Official Journal of the European Union Directive 2008/56/EC of the European Parliament and of the Council. Marine Environmental Policy (Marine Strategy Framework Directive). Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008L0056&from=EN (accessed on 23 February 2022).

53. European Commission. New Proposal Will Tackle Marine Litter and "Ghost Fishing". Available online: https://ec.europa.eu/newsroom/mare/items/628060/en (accessed on 10 January 2024).
54. Official Journal of the European Union Council Regulation (EC) No 1224/2009. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009R1224 (accessed on 22 January 2024).
55. The European Parliament and the Council of the European Union Directive (EU) 2018/851 of the European Parliament and of the Council of 30 May 2018 amending Directive 2008/98/EC on Waste. L 150. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018L0851 (accessed on 27 January 2024).
56. European Commission. Single-Use Plastics: New EU Rules to Reduce Marine Litter. Available online: https://ec.europa.eu/commission/presscorner/detail/en/IP_18_3927 (accessed on 23 January 2024).
57. Official Journal of the European Union Commission Guidelines on Single-Use Plastic Products in Accordance with Directive (EU) 2019/904 of the European Parliament and of the Council on the Reduction of the Impact of Certain Plastic Products on the Environment. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52021XC0607(03)&from=EN (accessed on 11 March 2022).
58. Jacobs, C.; Soulliere, K.; Sawyer-Beaulieu, S.; Sabzwari, A.; Tam, E. Challenges to the Circular Economy: Recovering Wastes from Simple versus Complex Products. *Sustainability* **2022**, *14*, 2576. [CrossRef]
59. Council of the European Union Commission Staff Working Document Impact Assessment Report: Combatting Microplastic Pollution in the European Union. Available online: https://op.europa.eu/en/publication-detail/-/publication/f56a1d32-6c36-11ee-9220-01aa75ed71a1/language-en (accessed on 10 January 2024).
60. Montanarella, L.; Panagos, P. The relevance of sustainable soil management within the European Green Deal. *Land Use Policy* **2021**, *100*, 104950. [CrossRef]
61. European Commission Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions: A Farm to Fork Strategy for a Fair, Healthy and Environmentally-Friendly Food System. Available online: https://eur-lex.europa.eu/resource.html?uri=cellar:ea0f9f73-9ab2-11ea-9d2d-01aa75ed71a1.0001.02/DOC_1&format=PDF (accessed on 25 October 2023).
62. Modibbo, U.M.; D'Adamo, I.; Morone, P.; Ali, I. *The Implementation Challenges to Circular Economy Via-Sectoral Exploration BT—Computational Modelling in Industry 4.0: A Sustainable Resource Management Perspective*; Ali, I., Chatterjee, P., Shaikh, A.A., Gupta, N., AlArjani, A., Eds.; Springer: Singapore, 2022; pp. 11–21, ISBN 978-981-16-7723-6.
63. D'Adamo, I.; Mazzanti, M.; Morone, P.; Rosa, P. Assessing the relation between waste management policies and circular economy goals. *Waste Manag.* **2022**, *154*, 27–35. [CrossRef]
64. United Nations. Transforming our World: The 2030 Agenda for Sustainable Development. Available online: https://www.un.org/ga/search/view_doc.asp?symbol=A/RES/70/1&Lang=E (accessed on 30 March 2022).
65. Walker, T.R. (Micro)plastics and the UN Sustainable Development Goals. *Curr. Opin. Green Sustain. Chem.* **2021**, *30*, 100497. [CrossRef]
66. Nabi, I.; Bacha, A.-U.-R.; Zhang, L. A review on microplastics separation techniques from environmental media. *J. Clean. Prod.* **2022**, *337*, 130458. [CrossRef]
67. Felsing, S.; Kochleus, C.; Buchinger, S.; Brennholt, N.; Stock, F.; Reifferscheid, G. A new approach in separating microplastics from environmental samples based on their electrostatic behavior. *Environ. Pollut.* **2018**, *234*, 20–28. [CrossRef]
68. Van Cauwenberghe, L.; Devriese, L.; Galgani, F.; Robbens, J.; Janssen, C.R. Microplastics in sediments: A review of techniques, occurrence and effects. *Mar. Environ. Res.* **2015**, *111*, 5–17. [CrossRef]
69. Liu, M.; Lu, S.; Song, Y.; Lei, L.; Hu, J.; Lv, W.; Zhou, W.; Cao, C.; Shi, H.; Yang, X.; et al. Microplastic and mesoplastic pollution in farmland soils in suburbs of Shanghai, China. *Environ. Pollut.* **2018**, *242*, 855–862. [CrossRef]
70. He, D.; Luo, Y.; Lu, S.; Liu, M.; Song, Y.; Lei, L. Microplastics in soils: Analytical methods, pollution characteristics and ecological risks. *TrAC Trends Anal. Chem.* **2018**, *109*, 163–172. [CrossRef]
71. Kononov, A.; Hishida, M.; Suzuki, K.; Harada, N. Microplastic Extraction from Agricultural Soils Using Canola Oil and Unsaturated Sodium Chloride Solution and Evaluation by Incineration Method. *Soil Syst.* **2022**, *6*, 54. [CrossRef]
72. Crichton, E.M.; Noël, M.; Gies, E.A.; Ross, P.S. A novel, density-independent and FTIR-compatible approach for the rapid extraction of microplastics from aquatic sediments. *Anal. Methods* **2017**, *9*, 1419–1428. [CrossRef]
73. Scopetani, C.; Chelazzi, D.; Mikola, J.; Leiniö, V.; Heikkinen, R.; Cincinelli, A.; Pellinen, J. Olive oil-based method for the extraction, quantification and identification of microplastics in soil and compost samples. *Sci. Total Environ.* **2020**, *733*, 139338. [CrossRef] [PubMed]
74. Lechthaler, S.; Hildebrandt, L.; Stauch, G.; Schüttrumpf, H. Canola oil extraction in conjunction with a plastic free separation unit optimises microplastics monitoring in water and sediment. *Anal. Methods* **2020**, *12*, 5128–5139. [CrossRef]
75. *BS EN 196-1:2016*; Methods of Testing Cement Determination of Strength. European Standards: Brussels, Belgium. 2016. Available online: https://www.en-standard.eu/bs-en-196-1-2016-methods-of-testing-cement-determination-of-strength/ (accessed on 22 January 2024).
76. *ISO 679:2009*; Cement Test Methods Determination of Strength. ISO: Geneva, Switzerland. 2009. Available online: https://www.iso.org/standard/45568.html (accessed on 10 January 2024).
77. Mushtak, F.; Prakash, J.; Katoch, S.S. Microplastics in complex soil matrix: Recovery, identification and removal using micro nano techniques. *Micro Nano Eng.* **2024**, *22*, 100237. [CrossRef]

78. Xu, D.; Huang, Q.; Yang, L.; Chen, Y.; Lu, Z.; Liu, H.; Han, P.; Guo, L.; Wang, C.; Liu, C. Experimental design of composite films with thermal management and electromagnetic shielding properties based on polyethylene glycol and MXene. *Carbon* **2023**, *202*, 1–12. [CrossRef]
79. Abdelhameed, M.; Elbeh, M.; Baban, N.S.; Pereira, L.; Matula, J.; Song, Y.-A.; Ramadi, K.B. High-yield, one-pot upcycling of polyethylene and polypropylene waste into blue-emissive carbon dots. *Green Chem.* **2023**, *25*, 1925–1937. [CrossRef]
80. Onwucha, C.N.; Ehi-Eromosele, C.O.; Ajayi, S.O.; Schaefer, M.; Indris, S.; Ehrenberg, H. Uncatalyzed Neutral Hydrolysis of Waste PET Bottles into Pure Terephthalic Acid. *Ind. Eng. Chem. Res.* **2023**, *62*, 6378–6385. [CrossRef]
81. Jang, M.; Lee, M.; Yang, H.; Lee, H.; Park, S.B.; Jeon, H.; Hwang, S.Y.; Kim, H.J.; Oh, D.X.; Park, J. Method to analyze phthalate esters from soft toys dissolving into water mimicking infant playing. *Chemosphere* **2023**, *330*, 138695. [CrossRef] [PubMed]
82. Gupta, A.; Singh, G.; Ghosh, P.; Arora, K.; Sharma, S. Development of biodegradable tableware from novel combination of paddy straw and pine needles: A potential alternative against plastic cutlery. *J. Environ. Chem. Eng.* **2023**, *11*, 111310. [CrossRef]
83. Abbasi, T.; Jaafarzadeh Haghighi Fard, N.; Madadizadeh, F.; Eslami, H.; Ebrahimi, A.A. Environmental Impact Assessment of Low-Density Polyethylene and Polyethylene Terephthalate Containers Using a Life Cycle Assessment Technique. *J. Polym. Environ.* **2023**, *31*, 3493–3508. [CrossRef]
84. Rabot, C.; Chen, Y.; Bijlani, S.; Chiang, Y.-M.; Oakley, C.E.; Oakley, B.R.; Williams, T.J.; Wang, C.C.C. Conversion of Polyethylenes into Fungal Secondary Metabolites. *Angew. Chem. Int. Ed.* **2023**, *62*, e202214609. [CrossRef] [PubMed]
85. Nyquist, T.; Warren, K. A Study on Residual Stresses on Autofrettaged LDPE Tubing Including the Bauschinger Effect and Strain Aging. In Proceedings of the ASME 2021 Pressure Vessels & Piping Conference, Virtual, 13–15 July 2021.
86. Sadiku, R.; Ibrahim, D.; Agboola, O.; Owonubi, S.J.; Fasiku, V.O.; Kupolati, W.K.; Jamiru, T.; Eze, A.A.; Adekomaya, O.S.; Varaprasad, K.; et al. 15—Automotive components composed of polyolefins. In *Polyolefin Fibres*, 2nd ed.; The Textile Institute Book Series; Ugbolue, S.C.O.; Woodhead Publishing: Cambridge, UK, 2017; pp. 449–496, ISBN 978-0-08-101132-4.
87. Shahid, S.; Andreasson, E.; Petersson, V.; Gukhool, W.; Kang, Y.; Kao-Walter, S. Simplified Characterization of Anisotropic Yield Criteria for an Injection-Molded Polymer Material. *Polymers* **2023**, *15*, 4520. [CrossRef]
88. Niccolucci, V.; Botto, S.; Rugani, B.; Nicolardi, V.; Bastianoni, S.; Gaggi, C. The real water consumption behind drinking water: The case of Italy. *J. Environ. Manage.* **2011**, *92*, 2611–2618. [CrossRef] [PubMed]
89. Fabrizio, L.; Arrigo, R.; Scrivani, M.T.; Monti, M.; Fina, A. Upcycling of PET from recycled food packaging trays via vitrimers chemistry. *Polymer* **2023**, *266*, 125618. [CrossRef]
90. Palacios-Mateo, C.; van der Meer, Y.; Seide, G. Analysis of the polyester clothing value chain to identify key intervention points for sustainability. *Environ. Sci. Eur.* **2021**, *33*, 2. [CrossRef] [PubMed]
91. Liebezeit, G.; Dubaish, F. Microplastics in Beaches of the East Frisian Islands Spiekeroog and Kachelotplate. *Bull. Environ. Contam. Toxicol.* **2012**, *89*, 213–217. [CrossRef] [PubMed]
92. Mathalon, A.; Hill, P. Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Mar. Pollut. Bull.* **2014**, *81*, 69–79. [CrossRef] [PubMed]
93. Hidalgo-Ruz, V.; Gutow, L.; Thompson, R.C.; Thiel, M. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. *Environ. Sci. Technol.* **2012**, *46*, 3060–3075. [CrossRef] [PubMed]
94. Imhof, H.K.; Schmid, J.; Niessner, R.; Ivleva, N.P.; Laforsch, C. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. *Limnol. Oceanogr. Methods* **2012**, *10*, 524–537. [CrossRef]
95. Nuelle, M.-T.; Dekiff, J.H.; Remy, D.; Fries, E. A new analytical approach for monitoring microplastics in marine sediments. *Environ. Pollut.* **2014**, *184*, 161–169. [CrossRef] [PubMed]
96. Mani, T.; Frehland, S.; Kalberer, A.; Burkhardt-Holm, P. Using castor oil to separate microplastics from four different environmental matrices. *Anal. Methods* **2019**, *11*, 1788–1794. [CrossRef]
97. Scheurer, M.; Bigalke, M. Microplastics in Swiss Floodplain Soils. *Environ. Sci. Technol.* **2018**, *52*, 3591–3598. [CrossRef]
98. Quinn, B.; Murphy, F.; Ewins, C. Validation of density separation for the rapid recovery of microplastics from sediment. *Anal. Methods* **2017**, *9*, 1491–1498. [CrossRef]
99. Radford, F.; Zapata-Restrepo, L.M.; Horton, A.A.; Hudson, M.D.; Shaw, P.J.; Williams, I.D. Developing a systematic method for extraction of microplastics in soils. *Anal. Methods* **2021**, *13*, 1695–1705. [CrossRef]
100. Bedaiwy, M.N.A. A simplified approach for determining the hydrometer's dynamic settling depth in particle-size analysis. *CATENA* **2012**, *97*, 95–103. [CrossRef]
101. Bessa, F.; Frias, J.; Kögel, T.; Lusher, A.; Andrade, J.; Antunes, J.; Sobral, P.; Pagter, E.; Nash, R.; O'Connor, I.; et al. *Harmonized Protocol for Monitoring Microplastics in Biota*; JPI-Oceans BASEMAN Project: Brussels, Belgium, 2019.
102. Shanmugam, S.D.; Praveena, S.M.; Sarkar, B. Quality assessment of research studies on microplastics in soils: A methodological perspective. *Chemosphere* **2022**, *296*, 134026. [CrossRef]
103. Ravichandran, R.; Ayyavoo, R.; Rajangam, L.; Madasamy, N.; Murugaiyan, B.; Shanmugam, S. Identification of groundwater potential zone using analytical hierarchical process (AHP) and multi-criteria decision analysis (MCDA) for Bhavani river basin, Tamil Nadu, southern India. *Groundw. Sustain. Dev.* **2022**, *18*, 100806. [CrossRef]
104. Benedetti, L.; De Baets, B.; Nopens, I.; Vanrolleghem, P.A. Multi-criteria analysis of wastewater treatment plant design and control scenarios under uncertainty. *Environ. Model. Softw.* **2010**, *25*, 616–621. [CrossRef]

105. Appolloni, A.; D'Adamo, I.; Gastaldi, M.; Santibanez-Gonzalez, E.D.R.; Settembre-Blundo, D. Growing e-waste management risk awareness points towards new recycling scenarios: The view of the Big Four's youngest consultants. *Environ. Technol. Innov.* **2021**, *23*, 101716. [CrossRef]
106. Papamichael, I.; Voukkali, I.; Loizia, P.; Pappas, G.; Zorpas, A.A. Existing tools used in the framework of environmental performance. *Sustain. Chem. Pharm.* **2023**, *32*, 101026. [CrossRef]
107. Gilliams, S.; Raymaekers, D.; Muys, B.; Orshoven, J. Van Comparing multiple criteria decision methods to extend a geographical information system on afforestation. *Comput. Electron. Agric.* **2005**, *49*, 142–158. [CrossRef]
108. Behzadian, M.; Kazemzadeh, R.B.; Albadvi, A.; Aghdasi, M. PROMETHEE: A comprehensive literature review on methodologies and applications. *Eur. J. Oper. Res.* **2010**, *200*, 198–215. [CrossRef]
109. Anane, M.; Bouziri, L.; Limam, A.; Jellali, S. Ranking suitable sites for irrigation with reclaimed water in the Nabeul-Hammamet region (Tunisia) using GIS and AHP-multicriteria decision analysis. *Resour. Conserv. Recycl.* **2012**, *65*, 36–46. [CrossRef]
110. D'Adamo, I.; Gastaldi, M.; Imbriani, C.; Morone, P. Assessing regional performance for the Sustainable Development Goals in Italy. *Sci. Rep.* **2021**, *11*, 24117. [CrossRef] [PubMed]
111. Adem Esmail, B.; Geneletti, D. Multi-criteria decision analysis for nature conservation: A review of 20 years of applications. *Methods Ecol. Evol.* **2018**, *9*, 42–53. [CrossRef]
112. Tsangas, M.; Zorpas, A. Sustainability analysis of Cyprus hydrocarbons sector by a PESTEL—Swot indicators AHP based evaluation. In Proceedings of the 3rd EWaS International Conference, Lefkada Island, Greece, 27–30 June 2018.
113. Papapostolou, A.; Karakosta, C.; Kourti, K.A.; Doukas, H.; Psarras, J. Supporting Europe's energy policy towards a decarbonised energy system: A comparative assessment. *Sustainability* **2019**, *11*, 4010. [CrossRef]
114. Stanković, J.J.; Janković-Milić, V.; Marjanović, I.; Janjić, J. An integrated approach of PCA and PROMETHEE in spatial assessment of circular economy indicators. *Waste Manag.* **2021**, *128*, 154–166. [CrossRef] [PubMed]
115. Kumar, P.; Singh, R.K.; Kumar, V. Managing supply chains for sustainable operations in the era of industry 4.0 and circular economy: Analysis of barriers. *Resour. Conserv. Recycl.* **2021**, *164*, 105215. [CrossRef]
116. Saaty, R.W. The analytic hierarchy process—What it is and how it is used. *Math. Model.* **1987**, *9*, 161–176. [CrossRef]
117. Tsangas, M.; Jeguirim, M.; Limousy, L.; Zorpas, A. The Application of Analytical Hierarchy Process in Combination with PESTEL-SWOT Analysis to Assess the Hydrocarbons Sector in Cyprus. *Energies* **2019**, *12*, 791. [CrossRef]
118. Zorpas, A.A. Strategy development in the framework of waste management. *Sci. Total Environ.* **2020**, *716*, 137088. [CrossRef] [PubMed]
119. Vardopoulos, I.; Tsilika, E.; Sarantakou, E.; Zorpas, A.; Salvati, L.; Tsartas, P. An integrated SWOT-PESTLE-AHP model assessing sustainability in adaptive reuse projects. *Appl. Sci.* **2021**, *11*, 7134. [CrossRef]
120. Tutak, M.; Brodny, J.; Siwiec, D.; Ulewicz, R.; Bindzár, P. Studying the Level of Sustainable Energy Development of the European Union Countries and Their Similarity Based on the Economic and Demographic Potential. *Energies* **2020**, *13*, 6643. [CrossRef]
121. Yarahmadi, R.; Moridi, H.; Farshad, A.A.; Taheri, F. Weighing and Prioritizing the Eight Principles of Integrated Health, Safety, Environment and Energy Management in Industries Covered by the Ministry of Industry, Mining and Trade. *Salāmat-I Kār-I Īrān* **2020**, *17*, 1–10.
122. Phuong, N.N.; Poirier, L.; Pham, Q.T.; Lagarde, F.; Zalouk-Vergnoux, A. Factors influencing the microplastic contamination of bivalves from the French Atlantic coast: Location, season and/or mode of life? *Mar. Pollut. Bull.* **2018**, *129*, 664–674. [CrossRef] [PubMed]
123. Kim, J.; Lee, Y.-J.; Park, J.-W.; Jung, S.M. Repeatable separation of microplastics integrating mineral oil extraction and a PDMS-Ni foam adsorbent in real soil. *Chem. Eng. J.* **2022**, *429*, 132517. [CrossRef]
124. Rani, M.; Ducoli, S.; Depero, L.E.; Prica, M.; Tubić, A.; Ademovic, Z.; Morrison, L.; Federici, S. A Complete Guide to Extraction Methods of Microplastics from Complex Environmental Matrices. *Molecules* **2023**, *28*, 5710. [CrossRef] [PubMed]
125. Kedzierski, M.; Le Tilly, V.; César, G.; Sire, O.; Bruzaud, S. Efficient microplastics extraction from sand. A cost effective methodology based on sodium iodide recycling. *Mar. Pollut. Bull.* **2017**, *115*, 120–129. [CrossRef] [PubMed]
126. Liu, M.; Song, Y.; Lu, S.; Qiu, R.; Hu, J.; Li, X.; Bigalke, M.; Shi, H.; He, D. A method for extracting soil microplastics through circulation of sodium bromide solutions. *Sci. Total Environ.* **2019**, *691*, 341–347. [CrossRef] [PubMed]

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Review

Interaction between Microplastics and Pathogens in Subsurface System: What We Know So Far

Hongyu Zhao [1,2], Xiaotao Hong [1,2], Juanfen Chai [1,2], Bo Wan [1,2], Kaichao Zhao [1,2], Cuihong Han [1,2], Wenjing Zhang [1,2,*] and Huan Huan [3,*]

[1] Key Laboratory of Groundwater Resources and Environment (Jilin University), Ministry of Education, Changchun 130021, China
[2] College of New Energy and Environment, Jilin University, Changchun 130021, China
[3] Technical Centre for Soil, Agriculture and Rural Ecology and Environment, Ministry of Ecology and Environment, Beijing 100012, China
* Correspondence: zhangwenjing80@hotmail.com (W.Z.); hhuan0825@163.com (H.H.)

Abstract: Microplastics (MPs) are abundant in soil and the subsurface environment. They can co-transport with pathogens or act as vectors for pathogens, potentially causing severe ecological harm. The interaction of MPs with pathogens is an important topic. To describe the origins and features of MPs in the subsurface environment, we evaluated relevant studies conducted in the laboratory and field groundwater habitats. We explore the interactions between pathogens and microplastics from three perspectives including the respective physicochemical properties of microplastics and pathogens, external environmental factors, and the binding between microplastics and pathogens. The effects of some interaction mechanisms and environmental factors on their co-transport are discussed. The key factors affecting their interaction are the particle size, specific surface area, shape and functional groups of MPs, the zeta potential and auxiliary metabolic genes of pathogens, and the hydrophobicity of both. Environmental factors indirectly affect MPs and the interaction and co-transport process of pathogens by changing their surface properties. These findings advance our knowledge of the ecological behavior of MPs–pathogens and the associated potential health hazards.

Keywords: microplastic–pathogen interactions; environmental factor; co-migration; health risk

Citation: Zhao, H.; Hong, X.; Chai, J.; Wan, B.; Zhao, K.; Han, C.; Zhang, W.; Huan, H. Interaction between Microplastics and Pathogens in Subsurface System: What We Know So Far. *Water* **2024**, *16*, 499. https://doi.org/10.3390/w16030499

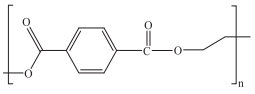
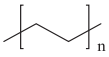
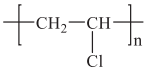
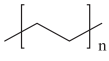
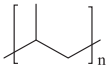
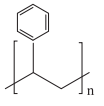
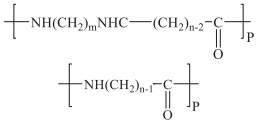
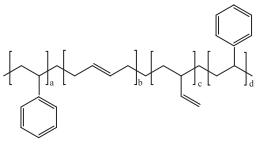
Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

Received: 4 January 2024
Revised: 26 January 2024
Accepted: 29 January 2024
Published: 4 February 2024

1. Introduction

Thompson developed the term "microplastics" (MPs) in 2004 to refer to the tiny plastics found in the ocean [1]. The concept was then used to describe tiny plastic trash fragments found in the environment as a result of consumer and industrial waste disposal and decomposition. Recent studies and government reports put the particle size of MPs between 5 mm and 1 μm, with nanoplastics having particle sizes less than 1 μm [2–5]. Currently, there are two classification methods for MPs: primary and secondary [6,7]. Primary MPs are plastic fragments or fibers that are less than or equal to 5 mm in size before entering the environment [8]. These include microfibers from clothes and plastic fragments from cosmetics and industrial manufacturing [9]. Secondary MPs comprise a range of plastic fragments with an initial size greater than 5 mm. These MPs can be reduced in size over time by a variety of biological, physical, and chemical weathering processes [4,10]. Nanoplastics have been less studied and are generally considered to be generated during the degradation, manufacturing, and use of plastics [11–13]. There is a public misconception that MPs in the environment are a single contaminant rather than a mixture of multiple plastic particles [14,15]. In actuality, the environment contains more than seven different kinds of MPs (Table 1) [16].

Table 1. Types of plastic polymers commonly found in the environment.

Type	Molecular Structure	Classification	Brief
Polyethylene terephthalate (PET)	 $((C_{10}H_8O_4)n)$		Polyethylene terephthalate, often abbreviated as PET, is the fourth most produced polymer in the world and is commonly used in producing synthetic fibers, food, and liquid containers.
High-density polyethylene	 $((C_2H_4)n)$		HDPE, or high-density polyethylene, is recognized for its high strength-to-density ratio and is often used to produce plastic bottles and corrosion-resistant products.
Polyvinyl chloride (PVC)	 $((C_2H_3Cl)n)$		Polyvinyl chloride, often abbreviated as PVC, is the third largest synthetic polymer produced in the world. Rigid PVC is commonly used in profile applications such as doors and windows, while flexible PVC is used for insulating cables, rainwear, and inflatable products.
Low-density polyethylene	 $((C_2H_4)n)$		Low-density polyethylene is one of the world's most widely produced plastics with low tensile strength and high elasticity. Its most common use is in plastic bags and films.
Polypropylene(PP)	 $((C_3H_6)n)$		Polypropylene is a chemically resistant material and is the second most produced polymer after polyethylene. It is used in a wide range of applications including medical, packaging, and industrial.
Polystyrene (PS)	 $((C_8H_8)n)$		Polystyrene is one of the most frequently used polymers, with an annual production capacity of millions of tons. Its primary applications include protective packaging, disposable dinnerware, and model construction kits.
Nylon			Nylon, also known as polyamide, was the first synthetic fiber in the world to have extraordinarily high abrasion resistance. It works in several applications including fabrics and wear-resistant components.
Styrene block copolymers			SBCs are a thermoplastic elastomer family. It has qualities comparable to natural rubber and offers high elongation, processability, and environmental stability, making it an important raw material for toys, furniture, medical, and automotive parts.

Even though there have been recent restrictions on the use of plastic in some countries, its use in everyday life inevitably poses potential risks to the ecosystem [17,18]. The government of Brazil has promoted economic growth in the Amazon Basin, leading to a

dramatic increase in the region's population. Increasing human activity has accelerated the industrialization of the Amazon Basin. Although the Amazon Basin covers 4.7 percent of the world's land area and has only 0.4 percent of the global population, it is thought to generate 10 percent of the plastic waste in the world's oceans [19]. The concentration of MPs in water bodies in southern India's coastal regions can reach 19.9 items/L, with the most common types being polyamide (PA), polypropylene (PP), polyethylene (PE), and polyvinyl chloride (PVC) [20]. The average abundance of MPs in the Pearl River's urban portion and estuary is 19.86 items/L and 8.902 items/L, respectively, with the primary types being PA and cellophane [21]. MPs have also been found in the sedimentary aquifer of the Bacchus Swamp in Australia, with an average concentration of 38 ± 8 items/L [22]. PE, PP, polystyrene (PS), and PVC were the most common. More than 90% of the fish sampled in the Nandu River carried MPs, with an average concentration of 0.6–5.8 [23]. MPs have also been discovered in mussels, raptors, and even drinking water [24–26]. According to Cox et al. [27], the number of MPs consumed per adult every year ranges between 74,000 and 121,000, which is the equivalent of 52 bank cards. The majority of MPs in an organism collect in the gut. MP accumulation and toxicity in the fish gut have been studied, and it appears that MP accumulation in the fish gut causes a range of toxic consequences such as intestinal mucosal damage and inflammation, and may also contribute to the dysbiosis of gut microbes [28]. Yong et al. [29] evaluated the relevant findings in a mouse model, where long-term MPs ingestion may result in intestinal liver lesions and other metabolic issues such as decreased energy metabolism.

Multiple outbreaks of viral and bacterial infections such as H1N1, COVID-19, pulmonary tuberculosis, and cholera in the past few decades have aroused serious concerns regarding the movement of dangerous germs. There is evidence that MPs can affect how harmful germs are transported while acting as carriers [30]. Nevertheless, it is unclear whether pathogens adsorbing on MPs influence the health of organisms when they consume such MPs. According to research on an e-waste disposal site in Guangdong, MPs form a new biological niche in the soil environment, and bacteria are well-suited to dwell on the surface of MPs [31]. More than 20 species were found colonizing the surface of MPs in a well-urbanized river in Chicago, USA, with average cell densities ranging from 0.037 to 0.063 cells μm^{-2} [32]. It has been shown that floating microplastic contaminants can facilitate the spread of pathogens over long distances to pristine locations far from land-based sources of contamination, potentially mediating the spread of pathogens in the marine environment, with important implications for wildlife and human health. Studies have shown that floating microplastic contaminants can facilitate the long-range dispersal of pathogens to pristine locations far from land-based sources of contamination, potentially mediating the spread of pathogens in the marine environment, with significant impacts on wildlife and human health [33]. Harmful microorganisms and gut-associated pathogens in wastewater colonize the surface of microplastics upon entering the aquatic environment [34]. Therefore, the ingestion of microplastics may pose a threat to aquatic organisms not only because of their inherent toxicity, but also because of their potential to act as vectors for disease transmission. Fabra et al. [35] studied the uptake, accumulation, and physiological responses of oysters to virgin and *E. coli*-coated MPs and discovered that oysters exhibited a greater uptake of *E. coli*-coated MPs. Although oysters retain less than 0.5% of total MPs and bioaccumulate minimally over short periods, germs on their surfaces may be transferred along the food chain to higher trophic levels by eating MPs. This could endanger both the ecosystem and human health. Microplastics can act as collectors of pathogens and transporters in the trophic chain.

Due to the diversity of MPs and pathogens, it is of great significance to study the interaction mechanisms between MPs and pathogens in the environment. Several research studies have attempted to investigate the potential interaction mechanisms between pathogenic microorganisms and MPs in the marine environment. For example, Khalid et al. [30] analyzed the possibility of marine MPs as vectors of pathogenic microorganisms and Jiang et al. [36] used high-throughput sequencing to examine the dominating bacterial

populations on the surface of MPs in the intertidal zone of the Yangtze River estuary in China. Between 4.8 million and 12.7 million tons of plastic waste are discharged from land into the oceans each year. In total, 98 percent of primary MPs come from land-based activities and only 2 percent from marine activities. Soil has become the greatest repository of MPs, with an abundance of 4–23 times that of the ocean [37]. However, due to the concealment and complexity of hydrogeological conditions, there are significant differences in the interaction mechanism between MPs and pathogens in aquifer media compared to other environmental systems. There has been no extensive research on the processes of interaction between pathogens and MPs in the subsurface environment.

A total of 212 keywords (filtered by keyword frequency ≥ 5) were screened in the study of microplastic–pathogen interactions, with the most common keywords being "microplastics", "pathogens", "wastewater", "biofilm", "biofilm formation", "marine-environment", and "temperature", with a total of five clusters, as shown in Figure 1. The larger clusters are blue, green and red, which represent the various elements of the microplastics research field. The blue color indicates microplastics research in wastewater; green indicates microplastic species and pollution studies. Red represents research on pathogen colonization on microplastic surfaces, etc.

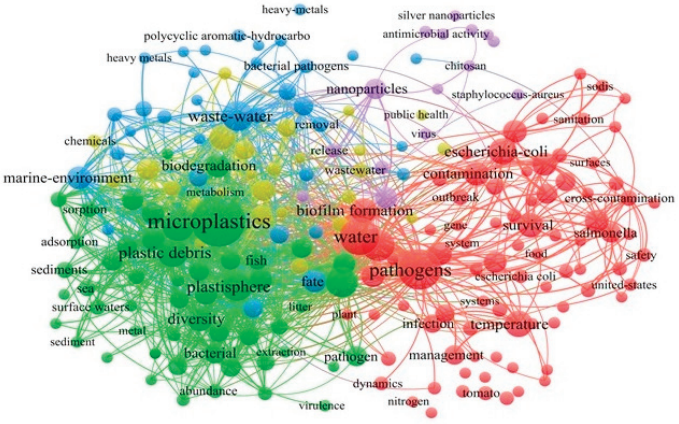


Figure 1. Keyword co-occurrence network visualization was generated by VOSviewer. Each color represents a topic cluster, where the font size and density (background color) of the keyword indicate the total link strength (TLS). A greater font indicates greater TLS, and the closer the distance between keywords, the higher the relevance of these studied topics.

The goals of this review were to (1) Summarize the sources and features of MPs in groundwater; (2) Discuss the possible interaction mechanisms between MPs and pathogens in aquifer media; (3) Explore the impact of environmental conditions on interaction mechanisms.

2. Sources and Features of Groundwater Microplastics

The sources of microplastics in the subsurface environment are complex (Figure 2). Because groundwater receives recharge from atmospheric precipitation, MPs on the soil surface may enter the soil pore space through leaching and eventually enter the groundwater system [38]. Soil not only stores the most MPs, but is also a potential pathway for MPs to enter the groundwater system [39,40]. Understanding the properties of MP distribution in soil is crucial to conducting an accurate assessment of MPs in groundwater. Trash, sewage sludge, and plastic film are the main three sources of plastics in global topsoil [16]. In the United States, 24.3 million tons of plastic were dumped in landfills in 2017 [41]. MPs from decomposition enter the soil with landfill leachate and may be harmful to the groundwater environment. The analytical results of landfill leachate from various places in China show that PE and PP are the most dominant MPs [7,42,43]. Plastic packaging,

sludge, and medical supplies are its primary sources [44,45]. The predominant size of these fragmented or fibrous MPs is 20–80 μm, with larger sizes significantly decreasing with soil depth [42,46]. Additionally, agricultural films made of PE and PVC as well as sludge wastewater discharged from sewage disposal plants are the primary sources of MPs in agricultural soils [47,48]. The analysis of 384 soil samples by Huang et al. [47] revealed a substantial linear association between the number of MPs in soil and the use of plastic films. Sewage sludge released from wastewater disposal plants is frequently high in organic matter and is commonly utilized for agricultural irrigation [49]. However, due to the limits of wastewater filtering technology, around 1.59% of MPs remain in already treated wastewater [50]. These MPs include microfibers from clothing, personal skin care products, and fertilizers. The abundance of MPs in sewage varies greatly depending on the local population density and lifestyle [51]. In eastern Spain, the abundance of MPs in agricultural land, which is irrigated by effluent from sewage treatment plants in various regions, can vary by more than threefold. Atmospheric deposition is the main source of MPs in natural terrestrial environments such as forests [52,53].

Figure 2. Sources and entry pathways of microplastics in groundwater.

Another significant source for recharging groundwater is river water. Isotopic evidence suggests that riverbank groundwater is recharged by rivers [54,55]. Past studies have confirmed that rivers are not only sinks that trap microplastics, but also media for the transport of microplastics from terrestrial to aquatic environments [56]. Microplastics in rivers can enter the groundwater environment through the lateral recharge of rivers to groundwater. Therefore, it is critical to comprehend the sources and distribution of MPs in rivers. Studies of MPs in rivers have shown that the type and concentration of MPs are closely related to population density and land use type [21,56–59]. Generally speaking, the river reaches in agriculturally developed areas have more MPs in the form of films, with the highest percentage of PE [59]. This is due to the widespread usage of plastic mulch films made from PE in agricultural farming. The highest abundance of MPs was found in urban rivers. This can be explained by the greater MP discharge rates per capita and population density [58]. More than 80% of these MPs are less than 0.5 mm in diameter, with the most common form being fibers with fragments [21,57,59]. Nylon fibers (polyamide, PA) from clothing and plastic particles (polyethylene, PP) from cosmetics are commonly found in waterways via sewage drainage systems [8,9]. Furthermore, plastic dust (styrene block copolymers, SBCs) from tire wear can be carried to water bodies by rainfall and runoff [60]. The abundance of MPs is expected to be higher in the downstream section of the river due to the transportation of MPs by the river [61]. However, studies by Yan et al. [21] and He et al. [56] yielded different results. It is not possible to move all MPs from upstream to

downstream. Changes in hydrodynamic circumstances, where some MPs are deposited on the river bottom, may reduce MP accumulation in estuaries [62]. Furthermore, industrial MPs such as PET are common in the downstream area, which is related to industrial wastewater discharges in the area [39].

3. Interactions between Pathogens and Microplastics

Micro- and nanoplastics can be used as carriers to carry pathogens for long-distance migration [53,63], and Fabra et al. [35] defined this adsorption–desorption behavior in terms of a "Trojan horse". Thus, the ability of MPs to carry pathogens for migration in porous surfaces is primarily determined by the strength of their pathogen adsorption capability. Furthermore, MPs may cause the long distance transmission of pathogenic microorganisms in aquifers by impeding their contact with the porous media or by competing for deposition sites of pathogenic microorganisms [5].

Most of the time, electrostatic repulsion occurs between pathogens and MPs because they both have net negatively charged surfaces. However, the repulsion is overcome due to the complexity of pathogen flagella, proteins, and surface charges, and the hydrophobicity of the cell surface [64]. A large number of studies have shown that microplastics are a vector that can be colonized by various algae and microorganisms. Laboratory investigations have shown that as the soil depth increases, the variety of bacteria on the surface of MPs diminishes [65]. The pathogen characteristics also influence their co-transport mechanisms with MPs. In this section, we will address the mechanism of interaction between microplastics and pathogens in aquifer media from their perspectives, respectively.

3.1. Effects from the Physical Properties of Microplastics

A large specific surface area and small particle size are key factors in the adsorption and transport of MPs in aquifer media. Li et al. [59] found that the adsorption capacity of Legionella at 50 μm MPs was 1–2 orders of magnitude higher than that at 3000 μm MPs. The increase in bacterial adsorption on MPs with decreasing particle size can be explained by a greater specific surface area. Therefore, for MPs to be able to travel vertically in the soil, a particle size smaller than the soil pore space is necessary [66]. Liu et al. [65] discovered that 0.5 mm MPs recovered in soil were less than 1 mm. However, adsorption is no longer the primary interaction mechanism when the particle size of MPs is comparable to that of pathogens. When the particle size of MPs is sufficiently tiny, MPs will compete with *E. coli* for adsorption sites in the soil, reducing *E. coli* adsorption in the soil and facilitating the transfer of *E. coli* in the aquifer medium through electrostatic repulsion [5].

Similarly, microplastic species is one of the key factors influencing the relationship between microplastic–pathogen interactions. Fibers were shown to be the most common type of MPs in different places in China, accounting for 86.37%, 59.4%, and 49% of marine, river, and soil MPs, respectively [67–69]. Microfibers have a higher potential for interaction with pathogens than other types of MPs. When compared to microspheres, microfibers had a better ability to adsorb pathogens on their surface [33]. The primary explanation was that increased surface roughness and heterogeneity promoted the microfibers' ability to attach to pathogens. Some earlier studies [70,71] suggested that nanoscale roughness on colloidal surfaces tends to minimize the repulsive interaction energy barriers between colloids and enhances the aggregation of MPs with bacteria, even in adverse conditions. However, the scanning electron microscopy (SEM) and atomic force microscopy (AFM) of various polymers [72] revealed that PVC had stronger cell adhesion than PP, even though the surface roughness of PVC (13.78 ± 0.65 nm) was somewhat lower than that of PP (14.07 ± 1 nm). Therefore, surface roughness is not a significant element affecting pathogen adsorption. One study reported the same conclusion that surface roughness did little to affect the adsorption of *S. sanguinis* on titanium surfaces [73]. Cohen and Radian [74] first described the alterations that microfibers undergo when migrating across aquifer media. As a result of friction with the coarse soil particles, microfibers break apart and release smaller,

more mobile pieces during flow, which may enable pathogens adsorbed on surfaces to move farther, increasing the environmental and ecological health risks.

Different shapes of microplastics have also been detected in organisms [75]. However, the comparison of bioaccumulation and the toxicity of microplastics with different shapes is still largely unknown. It has been shown that shape-dependent effects should not be ignored when conducting health risk assessments of microplastics. In comparison, non-spherical microplastics had more severe effects on the gut microbiota. Some specific species of gut bacteria are very sensitive to microplastic exposure. For example, microplastics induced a significant increase in the abundance of *Proteobacteria*. Microbial diversity studies continue to demonstrate the important role of *Proteobacteria* in gut inflammation. The increased *Proteobacteria* may produce more bacterial products such as lipopolysaccharide (LPS), which trigger inflammation, disrupt the intestinal mucosal barrier, and increase intestinal permeability. Microplastic fibers also reduce the relative abundance of *Pseudomonas* and *Aeromonas*, which can secrete signals to promote the proliferation and renewal of intestinal epithelial cells [76], compared to microplastic beads and microplastic debris [77]. Decreases in *Pseudomonas* and *Aeromonas* may further inhibit the regeneration of intestinal epithelial cells and reduce the coverage of cup cells [78]. In addition, fibrous microplastics lead to a decrease in the abundance of *actinobacteria*, which may weaken the function of the intestinal barrier and increase its susceptibility to immune stimulation, as *actinobacteria* play a key role in the synthesis of secondary metabolites that can be used as invasive antibiotics for invasive pathogens. In addition, *Gordonia* abundance was significantly increased in the gut of fish treated with microplastic fibers. *Gordonia* has a strong ability to degrade plastic-related compounds such as plasticizers [79], polypropylene [80], and phthalates [81]. These findings suggest that a high accumulation of microplastic fibers in the gut leads to specific changes in the gut microbiota associated with plastic exposure, and that gut flora dysbiosis would be a potential new mechanism by which microplastics cause or exacerbate gut toxicity in fish [28].

3.2. Effects from Chemical Characteristics of Microplastics

In addition to their physical characteristics, the chemical properties of MPs such as hydrophobicity and surface functional groups influence their adsorption behavior toward pathogens. Microplastics, as exogenous particles with a hydrophobic surface, are highly likely to provide new substrates for heterotrophic microbial activities, making their surface microbial communities significantly different from those of the surrounding environment and other organic residues [82]. As a result, a richer bacterial community exists on the surface of hydrophobic MPs compared to hydrophilic MPs [83,84]. Thanks to the hydrophobicity of MPs, the interaction affinity of SARS-CoV-2 with MPs in water is more than 10 times higher than that in air [85]. The impact of MP surface functional groups on the adsorption of pathogens is mostly observed in viruses. Liu et al. [86] found that compared to MP-NH2 (average zeta potential before adsorption -7.85 mV, virus adsorption rate $51.4 \pm 12.5\%$) and MP with no groups (average zeta potential before adsorption -16.06 mV, virus adsorption rate $83.6 \pm 0.8\%$), MP-COOH (average zeta potential before adsorption -23.72 mV, virus adsorption rate $94.3 \pm 0.8\%$) could adsorb more viruses. The absolute zeta potential values of MPs were favorably linked with the viral adsorption rate under various functional group alteration settings. As a result, while studying the interaction process between pathogenic microbes and MPs, hydrophobicity and surface functional groups must be considered.

3.3. Effects from Characteristics of Pathogens

3.3.1. Hydrophobicity

Hydrophobic interactions occur due to the mutual repulsion of hydrophobic nonpolar groups with water, and this action draws the hydrophobic groups closer together. Therefore, hydrophobicity is crucial in the early adsorption of germs to hydrophobic surfaces. The non-specific adsorption of germs on abiotic surfaces is favored by strong hydrophobic-

ity [87], while hydrophilic viruses and bacteria exhibit greater migratory potential in aquifer media [88,89]. In a study of the colonization of microplastic surfaces by different bacteria in the presence of Tween-80, it was found that highly hydrophobic strains colonized the microplastic surfaces with significantly higher biomass [90]. When there are enough carbon sources in the environment, bacteria with high cell surface hydrophobicity are better able to colonize. Interfacial tension may also be used to describe how hydrophobicity affects the growth of biofilms on MP surfaces. The decrease in interfacial tension is directly related to the hydrophobicity of bacterial cells [64]. During growth, some bacteria create surfactants, which might result in the replacement of certain proteins, lowering the surface tension and changing viscous moduli [91–93]. Therefore, bacterial adsorption is made easier by the development of protein networks with lower surface tension, particularly on the surfaces of MPs with low interfacial tension.

3.3.2. Surface Charge

In the DLVO theory, the positive and negative valves of the interaction energy (E_{int}) respectively indicate intermolecular interactions of mutual repulsion or attraction [94]. The E_{int} values of MPs containing SARS-CoV-2 RNA fragments were negative across the whole temperature range of water, which means that the two may assemble into a stable complex [85]. Further research revealed that the E_{int} values derived from the electrostatic energy between the two are often more similar to the E_{int} values derived from the total energy/potential energy than those derived from the van der Waals energy. This suggests that the primary mode of interaction between MPs and viruses is electrostatic contact. Electrostatic interaction is the primary adsorption method used by PS-MPs to capture 98.6% of the viral dose [86]. Experimental research by Dika et al. [95] demonstrated the role of electrostatic interactions in viral adsorption. The fact that viruses are normally negatively charged means that the electrostatic interaction force between viruses and positively charged MPs must grow in proportion to the overall negative charge carried by phages. When MPs have a negative charge, the higher the phage bulk charge density, the stronger the electrostatic repulsion and the lower the phage adsorption capability. Unlike the definition of relevant parameters about viruses, bacteria typically use the zeta potential or isoelectric point to assess the electrostatic interactions with non-biological surfaces. Positively charged MPs and negatively charged bacteria typically associate to create heterogeneous aggregates (with negative total zeta potential), which facilitates the movement of MPs across aquifer media [71]. However, when both bacteria and MPs are negatively charged, the zeta potentials of the different types of plastics are comparable, and electrostatic interaction is of little importance in the adhesion of bacteria to plastic surfaces [72].

3.3.3. Specific Properties

Pathogen adsorption levels on the surface of MPs varied substantially due to changes in the pathogen properties. *Proteobacteria* and *Actinobacteria* made up the majority of the bacterial communities that predominated on the surface of MPs in soil, together comprising more than 65% of the community [65]. On the one hand, both exhibited the highest relative abundance in the agricultural soil bacterial community, which favors their adsorption on plastics [96]. On the other hand, certain bacteria from these two phyla have unique genes for auxiliary metabolism that enable them to use additives and polymer resins as carbon sources and energy to promote their development [97,98]. *I. sakaiensis* may release two enzymes that break down PET and PET degradation pathways, respectively [99]. *P. aeruginosa* and *Achromobacter* sp. can be involved in the degradation of PVC in the presence of epoxidized *Mesua ferrea* L. seed oil [100]. *Enterobacter* sp., *Bacillus* sp., and *Pseudomonas* sp. are considered to be involved in the degradation of PS [101,102]. In addition, surface-associated proteins of *Streptomycetaceae* can reduce the surface tension to very small levels within minutes, which facilitates their adhesion to various surfaces [103].

Phage presence in biofilms is determined by the makeup of the biofilm matrix [104]. Consequently, phage diversity is strongly influenced by the variety of bacterial populations. Biofilms are known to be selective for phage enrichment, with *Caudovirales* being the most prevalent [98]. In addition, phages such as *Podoviridae* and *Autographiviridae* were more likely to be abundant in MP biofilms compared to stone. *Podoviridae* and *Autographiviridae* have a limited host range including *Enterobacteriaceae*, *Pseudomonas*, *Bacillus*, and *Streptococcus*, which include the majority of MP-degrading bacteria. Furthermore, the pathogenicity surface charge distribution may be heterogeneous. For example, the phage tail structure is positively charged, but the entire phage is negatively charged [105]. This heterogeneous surface charge can have a substantial impact on how it interacts with MPs.

We plotted all the factors that could affect the interaction between microplastics and pathogens in Figure 3. In addition to the nature of the pathogens and microplastics themselves, external environmental factors are also important in influencing the interaction between them.

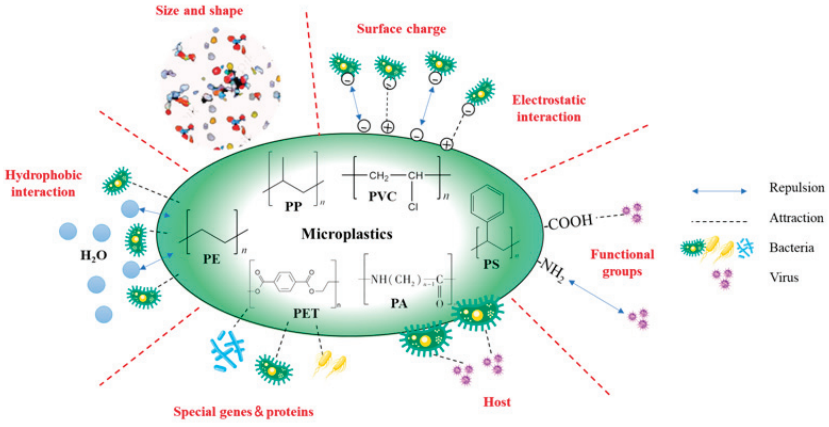


Figure 3. Mechanisms of interaction between MPs and pathogens in the subsurface environment.

4. Effects of Environmental Factors on the Interactions between Microplastics and Pathogens

4.1. Soil Physicochemical Properties

Soil physicochemical parameters have a significant impact on the structure and variety of the bacterial community, which in turn impacts the adsorption of pathogens by MPs [106–108]. Chai et al. [31] discovered a high percentage of common species on multiple MPs from the same plot of soil, showing that different MPs had highly similar core microbiota. Furthermore, when the physicochemical characteristics of soils from various plots were varied, the organization of bacterial communities populating the surface of the same MPs differed significantly. For example, heavy metal ions such as Cu, Pb, and Zn are found in high quantities in the soil of e-waste disposal sites, as are polymers such as PP and PC, which are commonly used in electronic devices [109]. Through surface complexation, electrostatic interactions, and hydrogen bonds, MPs adsorb heavy metals and serve as carriers for transfer in aquifer media [110–112]. Likewise, several heavy metals are utilized as additives in the manufacture of plastics [113]. Correspondingly, *Anaerobicbacteria*, represented by *Desulfovibrio*, made up the majority of the bacterial community composition on the surface of MPs in the target soil. This is because *Desulfovibrio* may thrive in severe oligotrophic settings and precipitate certain heavy metals via hydrogen sulfide synthesis [114]. Long-term MP persistence in the soil will alter the soil physicochemical features including a drop in soil organic matter and total nitrogen [115]. This would reduce the number of actinomycetes adsorbed on soil particles and the surface of MPs while increasing the prevalence of *Proteus*, *Bacillus*, and *Sphingomonas* [31,65]. *Sphingomonas* are

more suited to the surface environment of MPs and have a higher adsorption capability than *actinomycetes* [116]. Therefore, the physicochemical characteristics of the soil can either increase or decrease the MP adsorption capability for certain diseases.

4.2. Weathering

On the one hand, weathering has a direct impact on MP adsorption by changing their surface characteristics through physical fragmentation and photo-oxidation. Yuan et al. [117] demonstrated that the adsorption capability of aged MPs for *E. coli*, plasmid, and phage harboring antibiotic resistance genes was 6.6, 5.2, and 8.3 times greater than that of virgin MPs. Plastic aging is often characterized by polymer chain breaking and the formation of surface cracks and pores, which eventually fragment into micro- and nanoparticles. This means that weathered MPs have a larger specific surface area, microporous area, surface roughness, and attraction for antibiotic resistance gene (ARG) vectors. Long-term UV irradiation initiates the photolytic destruction of plastics, resulting in many microscopic pieces that serve as colonization sites for certain functional bacteria [90]. However, Lu et al. [86] showed that prolonged UV irradiation significantly decreased the absolute zeta potential of carboxyl group-modified MPs, which reduced their virus adsorption capacity, but increased the absolute zeta potential of amino group-modified MPs, which facilitated their virus adsorption capacity. On the other hand, weathering can have an indirect effect on the adsorption ability of MPs for pathogens. Aged PP-MPs can absorb more antibiotics because of a more developed pore structure [118,119]. This will encourage pathogens with ARG to bind to the surface of MPs. Weathering therefore directly influences the surface characteristics of MPs, which subsequently directly or indirectly affects the pathogen adsorption behavior.

4.3. Biofilm

Biofilms, a community of microorganisms accumulated in the matrix of self-developed extracellular polymeric substances (EPS), are present ubiquitously in both natural aquatic environments and engineering systems. Some studies have shown that the biofilms clinging to sand surfaces enhance the surface roughness of aquifer media and reduce flow channels, which can impede pathogen–MP co-transport [120]. Low crystallinity and high hardness matrix surfaces typically exhibit higher pathogenic microbial diversity [121]. However, McGivney et al. [122] discovered that biofilm-mediated weathering increases the crystallinity of PE and decreases the hardness of PP. This may lead to the selective adsorption of microorganisms by MPs and impact the diversity of adsorbed pathogens. However, it has also been demonstrated that the surface hydrophobicity and crystallinity of MPs are reduced by biofilm adherence [65]. Compared to the uncertainty of bacterial adsorption by biofilm-coated MPs, biofilms play a positive role in virus adsorption by MPs. Some viruses can interact with and bind to lipopolysaccharides (LPS) and peptidoglycans (PG) in biofilms [123]. This may provide novel locations for virus adsorption while also improving virus stability and transmission [124]. Biofilms can be crucial in the adsorption of MPs since they have colonized the majority of the surfaces of MPs in the water bodies.

In addition to microorganisms that can colonize the surface of microplastics, nanomaterials (NMs) can also be adsorbed onto the surface of microplastics. In 2013, Fries et al. classified MPs from sediment samples collected from Norderney and found TiO_2 nanoparticles on their surfaces, confirming that MPs can act as carriers of NMs [125,126]. Existing studies have reported that the oral administration of Ag nanoparticles decreases the *Firmicutes/Bacteroidetes* values and microbial community density in the gut of mice [127]. In addition, Sprague-Dawley rats orally exposed to Ag nanoparticles showed an increase in the proportion of Gram-negative bacteria and a decrease in the abundance of *Firmicutes* and *Lactobacillus* [128]. Zhao et al. found that TiO_2 nanoparticles further interfered with the diversity, composition, and KEGG pathways of the gut microbiota and led to inflammatory damage in the colon of mice with metabolic syndrome.

Antibiotics are widely used in the pharmaceutical aquaculture industry, and the overuse of antibiotics has led to antibiotics entering the water column. Despite the low concentration of antibiotics in this fraction, they can promote the development of antibiotic resistance in natural bacteria [129]. Natural bacteria may then transfer resistance to other bacteria including human pathogens [130]. At the same time, antibiotics selectively adsorb onto aged microplastics, favoring opportunistic pathogen colonization.

4.4. Ionic Strength

Ionic strength changes the surface charge of MPs and pathogens, influencing their interaction mechanism and movement pathway. He et al. [5] evaluated the migration of *E. coli* with MPs in aquifer media under various circumstances and discovered that MPs impact pathogen migration via diverse pathways at different ionic strengths. Because of the great mobility of *E. coli* and the poor deposition rates of MPs at low ionic strengths, the presence of MPs (<2 μm) had no influence on *E. coli* movement in aquifer media. Ionic strength changes impact the charge balance of colloids, influencing their stability. This may cause MPs to aggregate and depose, affecting their migration behavior in groundwater. The zeta potential of MPs and bacterial surfaces drops dramatically when the solution ionic strength increases, which is due to a large number of ions in the solution compressing the electrical double layer of the colloidal particles [131]. As a result, MPs and pathogens are more likely to overcome energy barriers and adsorb onto the media [132,133]. However, He et al. [5] demonstrated that MPs continue to facilitate *E. coli* transport at high ionic strengths. In a similar manner to *E. coli*, MPs with a size smaller than 2 μm exhibit increased deposition rates with higher ionic strength. This leads to competition between MPs and bacteria for deposition sites, ultimately resulting in a decrease in bacterial deposition in high ionic strength solutions. In contrast, nanoplastics maintain high mobility under high ionic strength. The suspended nanoplastics reduce the potential for *E. coli* attachment through repulsive effects, eventually increasing bacterial transportation in aquifer media.

4.5. pH

pH determines the fate and transport of pathogens and MPs in the subsurface environment by altering surface charge and adsorption–desorption processes. The transport of MPs in aquifer media is significantly affected by pH [132]. Slightly alkaline solutions facilitate the transport of MPs in aquifer media. At higher pH, MPs tend to carry more negative charges along with the aquifer media particles. As a result, MP colloids find it challenging to hit and adhere to the media surface because of the higher electrostatic repulsion and potential energy barrier between the two. In addition, the hydrodynamic size of the MPs shrinks with increasing pH, which aggravates Brown motion. Pathogens exhibit similar migration patterns. Decreasing pH increases the pathogen attachment to aquifer media and colloids, resulting in increased pathogen retention in the subsurface environment [82]. It is worth noting that pathogens can group due to weak electrostatic interaction and deposit in aquifer media when the pH of the solution is near the isoelectric point of the pathogens [134]. Therefore, when the solution pH is slightly acidic, the electrostatic repulsion between MPs and pathogens is minimized, and the adsorption capacity of MPs on pathogens is enhanced. A larger pH range may result in different surface charges for pathogens and MPs, thereby affecting their form of interaction. In contrast, if the pH range in the subsurface environment is modest, the changes in surface charges for both may be meaningless, and pH may not be the primary factor influencing their interaction.

4.6. Temperature

Temperature affects the interaction of MPs with pathogens mainly by altering the physicochemical characteristics, physiological properties, and adsorption thermodynamics. Many researchers have found that temperature affects the pathogen adhesion to solid surfaces and migration behavior in the subsurface environment [135,136]. Based on the results of rank correlation analysis [137], temperature is a major factor determining the

colonization of MPs by dominant pathogens. There are studies that illustrate the temporal and successional dynamics of biofilms and clearly show that an increase in temperature plays a role in the formation of plastic-specific microbial communities [138]. Cappello and Guglielmino [139] investigated *P. aeruginosa* adherence to polystyrene at 15 °C, 30 °C, and 47 °C and discovered that bacterial adhesion increased with temperature rise. The surrounding temperature affects the bacterial surface charge, hydrophobicity, and outer membrane components (e.g., lipopolysaccharides and flagella), which explains the variance in pathogen adhesion to MP surfaces [140,141]. Higher temperatures often boost pathogen attachment and limit their ability to move in groundwater [82]. Nevertheless, MP migratory behavior increases the uncertainty of the mentioned processes. According to the releasing behavior of various plastics in aqueous settings, higher temperatures stimulate plastic breaking, which in turn promotes MP migration [142,143]. Therefore, the co-transport behavior of MPs and pathogens in groundwater has to be researched further.

The influence of environmental factors on the interaction and synergistic transport between MPs and pathogens is extremely complex. Weathering processes such as physical fragmentation, photo-oxidation, and UV irradiation affect interactions due to changes in the morphological structure and surface characteristics of MPs. Ionic strength, pH, and temperature alter the surface charge of MPs and pathogens, thereby affecting their adsorption and transport capacities. Soil physicochemical characteristics and biofilms alter the composition of pathogen communities (Figure 4).

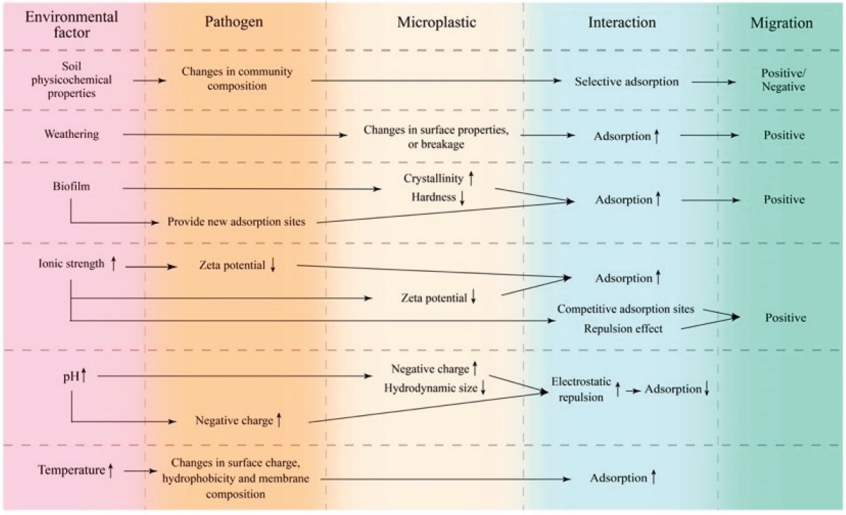


Figure 4. The effects of environmental factors on the interaction and co-migration between MPs and organic compounds.

5. Effects of Combined Exposure to MPs and Pathogens on Organisms

Microbial colonization boosts MP absorption by marine species. Filter feeders consume colonized MPs 10 times more than virgin MPs, and sea urchins exhibit a similar tendency [35,144]. These marine invertebrates seek for and absorb particulate matter through chemical sensing, and they prefer aged MPs over virgin particles [145]. Biofilm colonization may have changed the characteristics of MPs, making them more appealing. Environmental MPs may increase pathogen virulence. Disposable plastic tubes have been shown to promote significant expression of the central virulence-associated protein (VapA) of *Rhodococcus equi* at lower temperatures than standard glass tubes [146]. The surface nature of the plastic can help it activate transcriptional control of VirR and VirS proteins, thereby boosting the mRNA levels of VapA by 70-fold. The combined activity of MPs and their adsorbed pathogens may affect the organism's immune system. Sea urchins exposed

to colonized MPs had a substantial decrease in coelomocytes but an increase in vibratile cells and red/white amoebocyte ratio [144]. In addition, the exposed individuals' digestive systems were shown to have increased levels of total antioxidant activity and catalase. It has been reported that colonized MPs have a greater toxic effect on mussels than single MPs. *V. parahaemolyticus*-attached MPs affect hemocyte function in mussels, causing apoptosis and inhibiting antioxidant enzyme activity in the gills [147]. The combined impacts of pathogens and MPs may harm not just animals but even humans. In cancer patients with indwelling central venous catheters, *Rhodococcus equi* produces biofilms on the surface of the catheters made of polyurethane, which can cause bacteremia [148]. Although marine species have an exceptional ability to consume MPs, MPs may not be transferred up the food chain to higher levels after ingestion and excretion. Furthermore, it should be further researched to determine the risks that MPs or colonized MPs have to human health.

6. Conclusions

The damage that MPs cause to the environment has started to draw attention since the idea of them was first proposed. People have also started to pay attention to the transmission of microbes, particularly pathogens, in the natural environment out of concern for their health. Both are transported through the environment similarly to colloids, and their interactions are quite complicated. Groundwater, a critical supply of drinking water, is under threat from industrial and agricultural activities as well as the vertical migration of tiny particle pollutants from landfills, which may increase the degree of pollution and ecological risk from MPs and pathogens. This study focused on the major processes of MP–pathogen interactions as well as the sources and characteristics of MPs in the subsurface environment. The primary elements influencing their interactions include particle size, specific surface area, shape, hydrophobicity, and surface functional groups of MPs as well as pathogen hydrophobicity, zeta potential, and physiological features. The surface characteristics, crystallinity, and surface charge of MPs as well as changes in pathogens and the environment (e.g., soil physicochemical parameters and ionic strength) all have an impact on interactions. Furthermore, we investigated the co-transport of pathogens and MPs in aquifer media. The cohabitation of the two in subsurface environments may mutually enhance or prevent their migration, depending on the interactions. Future research should concentrate on (i) the synergistic transport behavior of pathogens and MPs in aquifer media under various environmental circumstances, and (ii) the combined toxicity of MPs and pathogens to organisms.

Author Contributions: W.Z. and H.H. conceived and supervised the current review. H.Z. conducted the literature review, collated and analyzed the data, and wrote the manuscript. X.H. conducted the literature review, collated, and analyzed the data. J.C., B.W., K.Z. and C.H. collated and analyzed the data. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Natural Science Foundation of China [grant number 42177047].

Conflicts of Interest: The authors declare no competing interests.

References

1. Thompson, R.C.; Olsen, Y.; Mitchell, R.P.; Davis, A.; Rowland, S.J.; John, A.W.G.; McGonigle, D.; Russell, A.E. Lost at Sea: Where Is All the Plastic? *Science* **2004**, *304*, 838. [CrossRef] [PubMed]
2. Arthur, C.; Baker, J.E.; Bamford, H.A. International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris. In Proceedings of the Conference Proceedings, Tacoma, WA, USA, 9–11 September 2008; University of Washington Tacoma: Tacoma, WA, USA, 2009.
3. Collignon, A.; Hecq, J.-H.; Galgani, F.; Collard, F.; Goffart, A. Annual variation in neustonic micro- and meso-plastic particles and zooplankton in the Bay of Calvi (Mediterranean–Corsica). *Mar. Pollut. Bull.* **2014**, *79*, 293–298. [CrossRef] [PubMed]
4. Gigault, J.; ter Halle, A.; Baudrimont, M.; Pascal, P.-Y.; Gauffre, F.; Phi, T.-L.; El Hadri, H.; Grassl, B.; Reynaud, S. Current opinion: What is a nanoplastic? *Environ. Pollut.* **2018**, *235*, 1030–1034. [CrossRef] [PubMed]
5. .He, L.; Wu, D.; Rong, H.; Li, M.; Tong, M.; Kim, H. Influence of Nano- and Microplastic Particles on the Transport and Deposition Behaviors of Bacteria in Quartz Sand. *Environ. Sci. Technol.* **2018**, *52*, 11555–11563. [CrossRef]

6. Auta, H.; Emenike, C.; Fauziah, S. Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environ. Int.* **2017**, *102*, 165–176. [CrossRef] [PubMed]
7. Su, Y.; Zhang, Z.; Wu, D.; Zhan, L.; Shi, H.; Xie, B. Occurrence of microplastics in landfill systems and their fate with landfill age. *Water Res.* **2019**, *164*, 114968. [CrossRef] [PubMed]
8. Boucher, J.; Friot, D. *Primary Microplastics in the Oceans: A Global Evaluation of Sources*; IUCN: Gland, Switzerland, 2017.
9. Yurtsever, M. Glitters as a Source of Primary Microplastics: An Approach to Environmental Responsibility and Ethics. *J. Agric. Environ. Ethics* **2019**, *32*, 459–478. [CrossRef]
10. Wang, L.; Wu, W.-M.; Bolan, N.S.; Tsang, D.C.; Li, Y.; Qin, M.; Hou, D. Environmental fate, toxicity and risk management strategies of nanoplastics in the environment: Current status and future perspectives. *J. Hazard. Mater.* **2021**, *401*, 123415. [CrossRef]
11. Bouwmeester, H.; Hollman, P.C.H.; Peters, R.J.B. Potential Health Impact of Environmentally Released Micro- and Nanoplastics in the Human Food Production Chain: Experiences from Nanotoxicology. *Environ. Sci. Technol.* **2015**, *49*, 8932–8947. [CrossRef]
12. da Costa, J.P.; Santos, P.S.; Duarte, A.C.; Rocha-Santos, T. (Nano)plastics in the environment–Sources, fates and effects. *Sci. Total Environ.* **2016**, *566–567*, 15–26. [CrossRef]
13. Birch, Q.T.; Potter, P.M.; Pinto, P.X.; Dionysiou, D.D.; Al-Abed, S.R. Sources, transport, measurement and impact of nano and microplastics in urban watersheds. *Rev. Environ. Sci. Bio/Technol.* **2020**, *19*, 275–336. [CrossRef] [PubMed]
14. Rochman, C.M.; Brookson, C.; Bikker, J.; Djuric, N.; Earn, A.; Bucci, K.; Athey, S.; Huntington, A.; McIlwraith, H.; Munno, K.; et al. Rethinking microplastics as a diverse contaminant suite. *Environ. Toxicol. Chem.* **2019**, *38*, 703–711. [CrossRef] [PubMed]
15. Deng, L.; Cai, L.; Sun, F.; Li, G.; Che, Y. Public attitudes towards microplastics: Perceptions, behaviors and policy implications. *Resour. Conserv. Recycl.* **2020**, *163*, 105096. [CrossRef]
16. Chia, R.W.; Lee, J.-Y.; Kim, H.; Jang, J. Microplastic pollution in soil and groundwater: A review. *Environ. Chem. Lett.* **2021**, *19*, 4211–4224. [CrossRef]
17. Chatziparaskeva, G.; Papamichael, I.; Zorpas, A.A. Microplastics in the coastal environment of Mediterranean and the impact on sustainability level. *Sustain. Chem. Pharm.* **2022**, *29*, 100768. [CrossRef]
18. Kyriakopoulos, G.L.; Zamparas, M.G.; Kapsalis, V.C. Investigating the Human Impacts and the Environmental Consequences of Microplastics Disposal into Water Resources. *Sustainability* **2022**, *14*, 828. [CrossRef]
19. Gerolin, C.R.; Pupim, F.N.; Sawakuchi, A.O.; Grohmann, C.H.; Labuto, G.; Semensatto, D. Microplastics in sediments from Amazon rivers, Brazil. *Sci. Total Environ.* **2020**, *749*, 141604. [CrossRef] [PubMed]
20. Selvam, S.; Jesuraja, K.; Venkatramanan, S.; Roy, P.D.; Kumari, V.J. Hazardous microplastic characteristics and its role as a vector of heavy metal in groundwater and surface water of coastal south India. *J. Hazard. Mater.* **2021**, *402*, 123786. [CrossRef]
21. Yan, M.; Nie, H.; Xu, K.; He, Y.; Hu, Y.; Huang, Y.; Wang, J. Microplastic abundance, distribution and composition in the Pearl River along Guangzhou city and Pearl River estuary, China. *Chemosphere* **2019**, *217*, 879–886. [CrossRef]
22. Samandra, S.; Johnston, J.M.; Jaeger, J.E.; Symons, B.; Xie, S.; Currell, M.; Ellis, A.V.; Clarke, B.O. Microplastic contamination of an unconfined groundwater aquifer in Victoria, Australia. *Sci. Total Environ.* **2022**, *802*, 149727. [CrossRef]
23. Chen, Y.; Shen, Z.; Li, G.; Wang, K.; Cai, X.; Xiong, X.; Wu, C. Factors affecting microplastic accumulation by wild fish: A case study in the Nandu River, South China. *Sci. Total Environ.* **2022**, *847*, 157486. [CrossRef]
24. Bošković, N.; Joksimović, D.; Bajt, O. Microplastics in mussels from the Boka Kotorska Bay (Adriatic Sea) and impact on human health. *Food Chem. Toxicol.* **2023**, *173*, 113641. [CrossRef] [PubMed]
25. Carlin, J.; Craig, C.; Little, S.; Donnelly, M.; Fox, D.; Zhai, L.; Walters, L. Microplastic accumulation in the gastrointestinal tracts in birds of prey in central Florida, USA. *Environ. Pollut.* **2020**, *264*, 114633. [CrossRef]
26. Muhib, I.; Uddin, K.; Rahman, M.; Malafaia, G. Occurrence of microplastics in tap and bottled water, and food packaging: A narrative review on current knowledge. *Sci. Total Environ.* **2023**, *865*, 161274. [CrossRef] [PubMed]
27. Cox, K.D.; Covernton, G.A.; Davies, H.L.; Dower, J.F.; Juanes, F.; Dudas, S.E. Human Consumption of Microplastics. *Environ. Sci. Technol.* **2019**, *53*, 7068–7074. [CrossRef] [PubMed]
28. Qiao, R.; Deng, Y.; Zhang, S.; Wolosker, M.B.; Zhu, Q.; Ren, H.; Zhang, Y. Accumulation of different shapes of microplastics initiates intestinal injury and gut microbiota dysbiosis in the gut of zebrafish. *Chemosphere* **2019**, *236*, 124334. [CrossRef] [PubMed]
29. Yong, C.Q.Y.; Valiyaveettil, S.; Tang, B.L. Toxicity of Microplastics and Nanoplastics in Mammalian Systems. *Int. J. Environ. Res. Public Health* **2020**, *17*, 1509. [CrossRef]
30. Khalid, N.; Aqeel, M.; Noman, A.; Hashem, M.; Mostafa, Y.S.; Alhaithloul, H.A.S.; Alghanem, S.M. Linking effects of microplastics to ecological impacts in marine environments. *Chemosphere* **2021**, *264*, 128541. [CrossRef]
31. Chai, B.; Li, X.; Liu, H.; Lu, G.; Dang, Z.; Yin, H. Bacterial communities on soil microplastic at Guiyu, an E-Waste dismantling zone of China. *Ecotoxicol. Environ. Saf.* **2020**, *195*, 110521. [CrossRef]
32. McCormick, A.; Hoellein, T.J.; Mason, S.A.; Schluep, J.; Kelly, J.J. Microplastic is an Abundant and Distinct Microbial Habitat in an Urban River. *Environ. Sci. Technol.* **2014**, *48*, 11863–11871. [CrossRef]
33. Zhang, E.; Kim, M.; Rueda, L.; Rochman, C.; VanWormer, E.; Moore, J.; Shapiro, K. Association of zoonotic protozoan parasites with microplastics in seawater and implications for human and wildlife health. *Sci. Rep.* **2022**, *12*, 6532. [CrossRef]
34. Rodrigues, A.; Oliver, D.M.; McCarron, A.; Quilliam, R.S. Colonisation of plastic pellets (nurdles) by *E. coli* at public bathing beaches. *Mar. Pollut. Bull.* **2019**, *139*, 376–380. [CrossRef]
35. Fabra, M.; Williams, L.; Watts, J.E.; Hale, M.S.; Couceiro, F.; Preston, J. The plastic Trojan horse: Biofilms increase microplastic uptake in marine filter feeders impacting microbial transfer and organism health. *Sci. Total Environ.* **2021**, *797*, 149217. [CrossRef]

36. Jiang, P.; Zhao, S.; Zhu, L.; Li, D. Microplastic-associated bacterial assemblages in the intertidal zone of the Yangtze Estuary. *Sci. Total Environ.* **2018**, *624*, 48–54. [CrossRef] [PubMed]
37. Nizzetto, L.; Futter, M.; Langaas, S. Are Agricultural Soils Dumps for Microplastics of Urban Origin? *Environ. Sci. Technol.* **2016**, *50*, 10777–10779. [CrossRef] [PubMed]
38. Othman, A.R.; Abu Hasan, H.; Muhamad, M.H.; Ismail, N.; Abdullah, S.R.S. Microbial degradation of microplastics by enzymatic processes: A review. *Environ. Chem. Lett.* **2021**, *19*, 3057–3073. [CrossRef]
39. Nizzetto, L.; Bussi, G.; Futter, M.N.; Butterfield, D.; Whitehead, P.G. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. *Environ. Sci. Process. Impacts* **2016**, *18*, 1050–1059. [CrossRef] [PubMed]
40. O'Connor, D.; Pan, S.; Shen, Z.; Song, Y.; Jin, Y.; Wu, W.M.; Hou, D. Microplastics undergo accelerated vertical migration in sand soil due to small size and wet-dry cycles. *Environ. Pollut.* **2019**, *249*, 527–534. [CrossRef] [PubMed]
41. Hou, L.; Kumar, D.; Yoo, C.G.; Gitsov, I.; Majumder, E.L.-W. Conversion and removal strategies for microplastics in wastewater treatment plants and landfills. *Chem. Eng. J.* **2021**, *406*, 126715. [CrossRef]
42. Li, N.; Han, Z.; Guo, N.; Zhou, Z.; Liu, Y.; Tang, Q. Microplastics spatiotemporal distribution and plastic-degrading bacteria identification in the sanitary and non-sanitary municipal solid waste landfills. *J. Hazard. Mater.* **2022**, *438*, 129452. [CrossRef]
43. Yu, F.; Wu, Z.; Wang, J.; Li, Y.; Chu, R.; Pei, Y.; Ma, J. Effect of landfill age on the physical and chemical characteristics of waste plastics/microplastics in a waste landfill sites. *Environ. Pollut.* **2022**, *306*, 119366. [CrossRef]
44. Liang, H.; Ji, Y.; Ge, W.; Wu, J.; Song, N.; Yin, Z.; Chai, C. Release kinetics of microplastics from disposable face masks into the aqueous environment. *Sci. Total Environ.* **2022**, *816*, 151650. [CrossRef]
45. Ziajahromi, S.; Neale, P.A.; Silveira, I.T.; Chua, A.; Leusch, F.D. An audit of microplastic abundance throughout three Australian wastewater treatment plants. *Chemosphere* **2021**, *263*, 128294. [CrossRef] [PubMed]
46. Sun, J.; Zhu, Z.-R.; Li, W.-H.; Yan, X.; Wang, L.-K.; Zhang, L.; Jin, J.; Dai, X.; Ni, B.-J. Revisiting Microplastics in Landfill Leachate: Unnoticed Tiny Microplastics and Their Fate in Treatment Works. *Water Res.* **2021**, *190*, 116784. [CrossRef] [PubMed]
47. Huang, Y.; Liu, Q.; Jia, W.; Yan, C.; Wang, J. Agricultural plastic mulching as a source of microplastics in the terrestrial environment. *Environ. Pollut.* **2020**, *260*, 114096. [CrossRef] [PubMed]
48. Jin, T.; Tang, J.; Lyu, H.; Wang, L.; Gillmore, A.B.; Schaeffer, S.M. Activities of Microplastics (MPs) in Agricultural Soil: A Review of MPs Pollution from the Perspective of Agricultural Ecosystems. *J. Agric. Food Chem.* **2022**, *70*, 4182–4201. [CrossRef] [PubMed]
49. Zhang, L.; Xie, Y.; Liu, J.; Zhong, S.; Qian, Y.; Gao, P. An Overlooked Entry Pathway of Microplastics into Agricultural Soils from Application of Sludge-Based Fertilizers. *Environ. Sci. Technol.* **2020**, *54*, 4248–4255. [CrossRef]
50. Carr, S.A.; Liu, J.; Tesoro, A.G. Transport and fate of microplastic particles in wastewater treatment plants. *Water Res.* **2016**, *91*, 174–182. [CrossRef] [PubMed]
51. van Den Berg, P.; Huerta-Lwanga, E.; Corradini, F.; Geissen, V. Sewage sludge application as a vehicle for microplastics in eastern Spanish agricultural soils. *Environ. Pollut.* **2020**, *261*, 114198. [CrossRef]
52. Wright, S.; Ulke, J.; Font, A.; Chan, K.; Kelly, F. Atmospheric microplastic deposition in an urban environment and an evaluation of transport. *Environ. Int.* **2020**, *136*, 105411. [CrossRef]
53. Zhou, D.; Cai, Y.; Yang, Z. Key factors controlling transport of micro- and nanoplastic in porous media and its effect on coexisting pollutants. *Environ. Pollut.* **2022**, *293*, 118503. [CrossRef]
54. Pang, Z.; Huang, T.; Chen, Y. Diminished groundwater recharge and circulation relative to degrading riparian vegetation in the middle Tarim River, Xinjiang Uygur, Western China. *Hydrol. Process.* **2010**, *24*, 147–159. [CrossRef]
55. Yeh, H.-F.; Lin, H.-I.; Lee, C.-H.; Hsu, K.-C.; Wu, C.-S. Identifying Seasonal Groundwater Recharge Using Environmental Stable Isotopes. *Water* **2014**, *6*, 2849–2861. [CrossRef]
56. He, B.; Wijesiri, B.; Ayoko, G.A.; Egodawatta, P.; Rintoul, L.; Goonetilleke, A. Influential factors on microplastics occurrence in river sediments. *Sci. Total Environ.* **2020**, *738*, 139901. [CrossRef] [PubMed]
57. Bian, P.; Liu, Y.; Zhao, K.; Hu, Y.; Zhang, J.; Kang, L.; Shen, W. Spatial variability of microplastic pollution on surface of rivers in a mountain-plain transitional area: A case study in the Chin Ling-Wei River Plain, China. *Ecotoxicol. Environ. Saf.* **2022**, *232*, 113298. [CrossRef] [PubMed]
58. Hu, E.; Sun, C.; Yang, F.; Wang, Y.; Hu, L.; Wang, L.; Li, M.; Gao, L. Microplastics in 48 wastewater treatment plants reveal regional differences in physical characteristics and shape-dependent removal in the transition zone between North and South China. *Sci. Total Environ.* **2022**, *834*, 155320. [CrossRef] [PubMed]
59. Li, Q.; Hu, Y.; Kou, D.; Yang, W.; Tang, W.; Chen, Q.; Que, S.; Zhao, X. Factors Impacting Microplastic Biofilm Community and Biological Risks Posed by Microplastics in Drinking Water Sources. *Water Air Soil Pollut.* **2022**, *233*, 179. [CrossRef]
60. Wang, L.; Luo, Z.; Zhen, Z.; Yan, Y.; Yan, C.; Ma, X.; Sun, L.; Wang, M.; Zhou, X.; Hu, A. Bacterial community colonization on tire microplastics in typical urban water environments and associated impacting factors. *Environ. Pollut.* **2020**, *265*, 114922. [CrossRef] [PubMed]
61. Ballent, A.; Corcoran, P.L.; Madden, O.; Helm, P.A.; Longstaffe, F.J. Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. *Mar. Pollut. Bull.* **2016**, *110*, 383–395. [CrossRef] [PubMed]
62. Besseling, E.; Quik, J.T.; Sun, M.; Koelmans, A.A. Fate of nano- and microplastic in freshwater systems: A modeling study. *Environ. Pollut.* **2017**, *220*, 540–548. [CrossRef]

63. Zuo, L.-Z.; Li, H.-X.; Lin, L.; Sun, Y.-X.; Diao, Z.-H.; Liu, S.; Zhang, Z.-Y.; Xu, X.-R. Sorption and desorption of phenanthrene on biodegradable poly(butylene adipate co-terephtalate) microplastics. *Chemosphere* **2019**, *215*, 25–32. [CrossRef]
64. Rühs, P.; Böcker, L.; Inglis, R.; Fischer, P. Studying bacterial hydrophobicity and biofilm formation at liquid–liquid interfaces through interfacial rheology and pendant drop tensiometry. *Colloids Surf. B Biointerfaces* **2014**, *117*, 174–184. [CrossRef]
65. Liu, H.; Yue, L.; Zhao, Y.; Li, J.; Fu, Y.; Deng, H.; Feng, D.; Li, Q.; Yu, H.; Zhang, Y.; et al. Changes in bacterial community structures in soil caused by migration and aging of microplastics. *Sci. Total Environ.* **2022**, *848*, 157790. [CrossRef]
66. Helmberger, M.S.; Tiemann, L.K.; Grieshop, M.J. Towards an ecology of soil microplastics. *Funct. Ecol.* **2020**, *34*, 550–560. [CrossRef]
67. Ding, L.; Zhang, S.; Wang, X.; Yang, X.; Zhang, C.; Qi, Y.; Guo, X. The occurrence and distribution characteristics of microplastics in the agricultural soils of Shaanxi Province, in north-western China. *Sci. Total Environ.* **2020**, *720*, 137525. [CrossRef] [PubMed]
68. Li, J.; Ouyang, Z.; Liu, P.; Zhao, X.; Wu, R.; Zhang, C.; Lin, C.; Li, Y.; Guo, X. Distribution and characteristics of microplastics in the basin of Chishui River in Renhuai, China. *Sci. Total Environ.* **2021**, *773*, 145591. [CrossRef] [PubMed]
69. Zhang, B.; Wu, D.; Yang, X.; Teng, J.; Liu, Y.; Zhang, C.; Zhao, J.; Yin, X.; You, L.; Liu, Y.; et al. Microplastic pollution in the surface sediments collected from Sishili Bay, North Yellow Sea, China. *Mar. Pollut. Bull.* **2019**, *141*, 9–15. [CrossRef] [PubMed]
70. Bradford, S.A.; Kim, H.; Shen, C.; Sasidharan, S.; Shang, J. Contributions of Nanoscale Roughness to Anomalous Colloid Retention and Stability Behavior. *Langmuir* **2017**, *33*, 10094–10105. [CrossRef] [PubMed]
71. He, L.; Rong, H.; Li, M.; Zhang, M.; Liu, S.; Yang, M.; Tong, M. Bacteria have different effects on the transport behaviors of positively and negatively charged microplastics in porous media. *J. Hazard. Mater.* **2021**, *415*, 125550. [CrossRef] [PubMed]
72. Cai, L.; Wu, D.; Xia, J.; Shi, H.; Kim, H. Influence of physicochemical surface properties on the adhesion of bacteria onto four types of plastics. *Sci. Total Environ.* **2019**, *671*, 1101–1107. [CrossRef]
73. Rodríguez-Hernández, A.G.; Muñoz-Tabares, J.A.; Godoy-Gallardo, M.; Juárez, A.; Gil, F.-J. *S. sanguinis* adhesion on rough titanium surfaces: Effect of culture media. *Mater. Sci. Eng. C* **2013**, *33*, 714–720. [CrossRef]
74. Cohen, N.; Radian, A. Microplastic Textile Fibers Accumulate in Sand and Are Potential Sources of Micro(nano)plastic Pollution. *Environ. Sci. Technol.* **2022**, *56*, 17635–17642. [CrossRef] [PubMed]
75. Neves, D.; Sobral, P.; Ferreira, J.L.; Pereira, T. Ingestion of microplastics by commercial fish off the Portuguese coast. *Mar. Pollut. Bull.* **2015**, *101*, 119–126. [CrossRef] [PubMed]
76. Li, J.-D.; Feng, W.; Gallup, M.; Kim, J.-H.; Gum, J.; Kim, Y.; Basbaum, C. Activation of NF-κB via a Src-dependent Ras-MAPK-pp90rsk pathway is required for *Pseudomonas aeruginosa* -induced mucin overproduction in epithelial cells. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 5718–5723. [CrossRef] [PubMed]
77. Cheesman, S.E.; Neal, J.T.; Mittge, E.; Seredick, B.M.; Guillemin, K. Epithelial cell proliferation in the developing zebrafish intestine is regulated by the Wnt pathway and microbial signaling via Myd88. *Proc. Natl. Acad. Sci. USA* **2010**, *108*, 4570–4577. [CrossRef] [PubMed]
78. Bates, J.M.; Mittge, E.; Kuhlman, J.; Baden, K.N.; Cheesman, S.E.; Guillemin, K. Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. *Dev. Biol.* **2006**, *297*, 374–386. [CrossRef] [PubMed]
79. Drzyzga, O. The strengths and weaknesses of *Gordonia*: A review of an emerging genus with increasing biotechnological potential. *Crit. Rev. Microbiol.* **2012**, *38*, 300–316. [CrossRef] [PubMed]
80. Arkatkar, A.; Juwarkar, A.A.; Bhaduri, S.; Uppara, P.V.; Doble, M. Growth of Pseudomonas and Bacillus biofilms on pretreated polypropylene surface. *Int. Biodeterior. Biodegrad.* **2010**, *64*, 530–536. [CrossRef]
81. Ren, L.; Lin, Z.; Liu, H.; Hu, H. Bacteria-mediated phthalic acid esters degradation and related molecular mechanisms. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 1085–1096. [CrossRef]
82. Zhang, W.; Chai, J.; Li, S.; Wang, X.; Wu, S.; Liang, Z.; Baloch, M.Y.J.; Silva, L.F.; Zhang, D. Physiological characteristics, geochemical properties and hydrological variables influencing pathogen migration in subsurface system: What we know or not? *Geosci. Front.* **2022**, *13*, 101346. [CrossRef]
83. Bao, R.; Pu, J.; Xie, C.; Mehmood, T.; Chen, W.; Gao, L.; Lin, W.; Su, Y.; Lin, X.; Peng, L. Aging of biodegradable blended plastic generates microplastics and attached bacterial communities in air and aqueous environments. *J. Hazard. Mater.* **2022**, *434*, 128891. [CrossRef]
84. Das, M.P. Effect of cell surface hydrophobicity in microbial biofilm formation. *Eur. J. Exp. Biol.* **2014**, *4*, 254–256.
85. Zhang, F.; Wang, Z.; Vijver, M.G.; Peijnenburg, W.J. Theoretical investigation on the interactions of microplastics with a SARS-CoV-2 RNA fragment and their potential impacts on viral transport and exposure. *Sci. Total Environ.* **2022**, *842*, 156812. [CrossRef] [PubMed]
86. Lu, J.; Yu, Z.; Ngiam, L.; Guo, J. Microplastics as potential carriers of viruses could prolong virus survival and infectivity. *Water Res.* **2022**, *225*, 119115. [CrossRef] [PubMed]
87. Liu, G.; Fang, Z.; Zhong, H.; Shi, L.; Yang, X.; Liu, Z. Transport of *Pseudomonas aeruginosa* in Porous Media Mediated by Low-Concentration Surfactants: The Critical Role of Surfactant to Change Cell Surface Hydrophobicity. *Water Resour. Res.* **2020**, *56*, 26103. [CrossRef]
88. Armanious, A.; Aeppli, M.; Jacak, R.; Refardt, D.; Sigstam, T.; Kohn, T.; Sander, M. Viruses at Solid–Water Interfaces: A Systematic Assessment of Interactions Driving Adsorption. *Environ. Sci. Technol.* **2016**, *50*, 732–743. [CrossRef] [PubMed]

89. Zhong, H.; Jiang, Y.; Zeng, G.; Liu, Z.; Liu, L.; Liu, Y.; Yang, X.; Lai, M.; He, Y. Effect of low-concentration rhamnolipid on adsorption of *Pseudomonas aeruginosa* ATCC 9027 on hydrophilic and hydrophobic surfaces. *J. Hazard. Mater.* **2015**, *285*, 383–388. [CrossRef] [PubMed]
90. Cheng, Y.; Chen, J.; Bao, M.; Zhao, L.; Li, Y. The proliferation and colonization of functional bacteria on amorphous polyethylene terephthalate: Key role of ultraviolet irradiation and nonionic surfactant polysorbate 80 addition. *Chemosphere* **2022**, *291*, 132940. [CrossRef] [PubMed]
91. Angelini, T.E.; Roper, M.; Kolter, R.; Weitz, D.A.; Brenner, M.P. *Bacillus subtilis* spreads by surfing on waves of surfactant. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 18109–18113. [CrossRef]
92. Gunning, P.; Mackie, A.; Gunning, A.; Wilde, P.; Woodward, N.; Morris, V. The effect of surfactant type on protein displacement from the air–water interface. *Food Hydrocoll.* **2004**, *18*, 509–515. [CrossRef]
93. Klein, B.; Bouriat, P.; Goulas, P.; Grimaud, R. Behavior of *Marinobacter hydrocarbonoclasticus* SP17 cells during initiation of biofilm formation at the alkane–water interface. *Biotechnol. Bioeng.* **2010**, *105*, 461–468. [CrossRef]
94. Wu, H.; Fang, H.; Xu, C.; Ye, J.; Cai, Q.; Shi, J. Transport and retention of copper oxide nanoparticles under unfavorable deposition conditions caused by repulsive van der Waals force in saturated porous media. *Environ. Pollut.* **2020**, *256*, 113400. [CrossRef]
95. Dika, C.; Duval, J.F.; Francius, G.; Perrin, A.; Gantzer, C. Isoelectric point is an inadequate descriptor of MS2, Phi X 174 and PRD1 phages adhesion on abiotic surfaces. *J. Colloid Interface Sci.* **2015**, *446*, 327–334. [CrossRef] [PubMed]
96. Zhang, M.; Zhao, Y.; Qin, X.; Jia, W.; Chai, L.; Huang, M.; Huang, Y. Microplastics from mulching film is a distinct habitat for bacteria in farmland soil. *Sci. Total Environ.* **2019**, *688*, 470–478. [CrossRef] [PubMed]
97. Campana, R.; Sabatini, L.; Giorgi, L.; Pettinari, G.; Valentini, L.; Gobbi, P. A Multidisciplinary Approach in Examining the Susceptibility to Microbial Attack of Polyacrylic and Polyurethane Resins Used in Art Restoration. *Int. J. Mol. Sci.* **2022**, *23*, 11725. [CrossRef] [PubMed]
98. Niu, L.; Zhao, S.; Chen, Y.; Li, Y.; Zou, G.; Tao, Y.; Zhang, W.; Wang, L.; Zhang, H. Diversity and potential functional characteristics of phage communities colonizing microplastic biofilms. *Environ. Res.* **2023**, *219*, 115103. [CrossRef] [PubMed]
99. Yoshida, S.; Hiraga, K.; Takehana, T.; Taniguchi, I.; Yamaji, H.; Maeda, Y.; Toyohara, K.; Miyamoto, K.; Kimura, Y.; Oda, K. A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science* **2016**, *351*, 1196–1199. [CrossRef] [PubMed]
100. Das, G.; Bordoloi, N.K.; Rai, S.K.; Mukherjee, A.K.; Karak, N. Biodegradable and biocompatible epoxidized vegetable oil modified thermostable poly(vinyl chloride): Thermal and performance characteristics post biodegradation with *Pseudomonas aeruginosa* and *Achromobacter* sp. *J. Hazard. Mater.* **2012**, *209–210*, 434–442. [CrossRef] [PubMed]
101. Mohan, A.J.; Sekhar, V.C.; Bhaskar, T.; Nampoothiri, K.M. Microbial assisted High Impact Polystyrene (HIPS) degradation. *Bioresour. Technol.* **2016**, *213*, 204–207. [CrossRef]
102. Sekhar, V.C.; Nampoothiri, K.M.; Mohan, A.J.; Nair, N.R.; Bhaskar, T.; Pandey, A. Microbial degradation of high impact polystyrene (HIPS), an e-plastic with decabromodiphenyl oxide and antimony trioxide. *J. Hazard. Mater.* **2016**, *318*, 347–354. [CrossRef]
103. El Othmany, R.; Zahir, H.; Ellouali, M.; Latrache, H. Current Understanding on Adhesion and Biofilm Development in Actinobacteria. *Int. J. Microbiol.* **2021**, *2021*, 6637438. [CrossRef] [PubMed]
104. Simmons, E.L.; Drescher, K.; Nadell, C.D.; Bucci, V. Phage mobility is a core determinant of phage–bacteria coexistence in biofilms. *ISME J.* **2018**, *12*, 532–543. [CrossRef] [PubMed]
105. Penrod, S.L.; Olson, T.M.; Grant, S.B. Deposition Kinetics of Two Viruses in Packed Beds of Quartz Granular Media. *Langmuir* **1996**, *12*, 5576–5587. [CrossRef]
106. Liu, J.; He, X.-X.; Lin, X.-R.; Chen, W.-C.; Zhou, Q.-X.; Shu, W.-S.; Huang, L.-N. Ecological Effects of Combined Pollution Associated with E-Waste Recycling on the Composition and Diversity of Soil Microbial Communities. *Environ. Sci. Technol.* **2015**, *49*, 6438–6447. [CrossRef] [PubMed]
107. Wu, W.; Dong, C.; Wu, J.; Liu, X.; Wu, Y.; Chen, X.; Yu, S. Ecological effects of soil properties and metal concentrations on the composition and diversity of microbial communities associated with land use patterns in an electronic waste recycling region. *Sci. Total Environ.* **2017**, *601–602*, 57–65. [CrossRef] [PubMed]
108. Zamparas, M.; Kyriakopoulos, G.L.; Kapsalis, V.C.; Drosos, M.; Kalavrouziotis, I.K. Application of novel composite materials as sediment capping agents: Column experiments and modelling. *Desalination Water Treat.* **2019**, *170*, 111–118. [CrossRef]
109. Jiang, B.; Adebayo, A.; Jia, J.; Xing, Y.; Deng, S.; Guo, L.; Liang, Y.; Zhang, D. Impacts of heavy metals and soil properties at a Nigerian e-waste site on soil microbial community. *J. Hazard. Mater.* **2019**, *362*, 187–195. [CrossRef]
110. Duan, J.; Bolan, N.; Li, Y.; Ding, S.; Atugoda, T.; Vithanage, M.; Sarkar, B.; Tsang, D.C.; Kirkham, M. Weathering of microplastics and interaction with other coexisting constituents in terrestrial and aquatic environments. *Water Res.* **2021**, *196*, 117011. [CrossRef]
111. Purwiyanto, A.I.S.; Suteja, Y.; Trisno; Ningrum, P.S.; Putri, W.A.E.; Rozirwan; Agustriani, F.; Fauziyah; Cordova, M.R.; Koropitan, A.F. Concentration and adsorption of Pb and Cu in microplastics: Case study in aquatic environment. *Mar. Pollut. Bull.* **2020**, *158*, 111380. [CrossRef]
112. Wang, X.; Zhang, R.; Li, Z.; Yan, B. Adsorption properties and influencing factors of Cu(II) on polystyrene and polyethylene terephthalate microplastics in seawater. *Sci. Total Environ.* **2022**, *812*, 152573. [CrossRef]
113. Park, Y.-W.; Lee, G.-S.; Kwak, J.-S. A study on compound contents for plastic injection molding products of metallic resin pigment. *J. Mech. Sci. Technol.* **2016**, *30*, 5673–5677. [CrossRef]
114. Cabrera, G.; Pérez, R.; Gómez, J.; Ábalos, A.; Cantero, D. Toxic effects of dissolved heavy metals on *Desulfovibrio vulgaris* and *Desulfovibrio* sp. strains. *J. Hazard. Mater.* **2006**, *135*, 40–46. [CrossRef] [PubMed]

115. Qian, H.; Zhang, M.; Liu, G.; Lu, T.; Qu, Q.; Du, B.; Pan, X. Effects of Soil Residual Plastic Film on Soil Microbial Community Structure and Fertility. *Water Air Soil Pollut.* **2018**, *229*, 261. [CrossRef]
116. Lengeler, J.W.; Drews, G.; Schlegel, H.G. *Biology of the Prokaryotes*; John Wiley & Sons: Hoboken, NJ, USA, 2009.
117. Yuan, Q.; Sun, R.; Yu, P.; Cheng, Y.; Wu, W.; Bao, J.; Alvarez, P.J. UV-aging of microplastics increases proximal ARG donor-recipient adsorption and leaching of chemicals that synergistically enhance antibiotic resistance propagation. *J. Hazard. Mater.* **2022**, *427*, 127895. [CrossRef] [PubMed]
118. Yu, X.; Du, H.; Huang, Y.; Yin, X.; Liu, Y.; Li, Y.; Liu, H.; Wang, X. Selective adsorption of antibiotics on aged microplastics originating from mariculture benefits the colonization of opportunistic pathogenic bacteria. *Environ. Pollut.* **2022**, *313*, 120157. [CrossRef] [PubMed]
119. Papamichael, I.; Voukkali, I.; Jeguirim, M.; Argirusis, N.; Jellali, S.; Sourkouni, G.; Argirusis, C.; Zorpas, A.A. End-of-Life Management and Recycling on PV Solar Energy Production. *Energies* **2022**, *15*, 6430. [CrossRef]
120. He, L.; Rong, H.; Wu, D.; Li, M.; Wang, C.; Tong, M. Influence of biofilm on the transport and deposition behaviors of nano- and micro-plastic particles in quartz sand. *Water Res.* **2020**, *178*, 115808. [CrossRef]
121. Lichter, J.A.; Thompson, M.T.; Delgadillo, M.; Nishikawa, T.; Rubner, M.F.; Van Vliet, K.J. Substrata Mechanical Stiffness Can Regulate Adhesion of Viable Bacteria. *Biomacromolecules* **2008**, *9*, 1571–1578. [CrossRef]
122. McGivney, E.; Cederholm, L.; Barth, A.; Hakkarainen, M.; Hamacher-Barth, E.; Ogonowski, M.; Gorokhova, E. Rapid Physicochemical Changes in Microplastic Induced by Biofilm Formation. *Front. Bioeng. Biotechnol.* **2020**, *8*, 205. [CrossRef]
123. Moresco, V.; Oliver, D.M.; Weidmann, M.; Matallana-Surget, S.; Quilliam, R.S. Survival of human enteric and respiratory viruses on plastics in soil, freshwater, and marine environments. *Environ. Res.* **2021**, *199*, 111367. [CrossRef]
124. Moresco, V.; Charatzidou, A.; Oliver, D.M.; Weidmann, M.; Matallana-Surget, S.; Quilliam, R.S. Binding, recovery, and infectiousness of enveloped and non-enveloped viruses associated with plastic pollution in surface water. *Environ. Pollut.* **2022**, *308*, 119594. [CrossRef]
125. Fries, E.; Dekiff, J.H.; Willmeyer, J.; Nuelle, M.-T.; Ebert, M.; Remy, D. Identification of polymer types and additives in marine microplastic particles using pyrolysis-GC/MS and scanning electron microscopy. *Environ. Sci. Process. Impacts* **2013**, *15*, 1949–1956. [CrossRef]
126. Chatziparaskeva, G.; Papamichael, I.; Voukkali, I.; Loizia, P.; Sourkouni, G.; Argirusis, C.; Zorpas, A.A. End-of-Life of Composite Materials in the Framework of the Circular Economy. *Microplastics* **2022**, *1*, 377–392. [CrossRef]
127. Agans, R.T.; Gordon, A.; Hussain, S.; Paliy, O. Titanium Dioxide Nanoparticles Elicit Lower Direct Inhibitory Effect on Human Gut Microbiota Than Silver Nanoparticles. *Toxicol. Sci.* **2019**, *172*, 411–416. [CrossRef] [PubMed]
128. Williams, K.; Milner, J.; Boudreau, M.D.; Gokulan, K.; Cerniglia, C.E.; Khare, S. Effects of subchronic exposure of silver nanoparticles on intestinal microbiota and gut-associated immune responses in the ileum of Sprague-Dawley rats. *Nanotoxicology* **2015**, *9*, 279–289. [CrossRef] [PubMed]
129. Niu, X.-Z.; Busetti, F.; Langsa, M.; Croué, J.-P. Roles of singlet oxygen and dissolved organic matter in self-sensitized photooxidation of antibiotic norfloxacin under sunlight irradiation. *Water Res.* **2016**, *106*, 214–222. [CrossRef] [PubMed]
130. Liu, C.; Nanaboina, V.; Korshin, G.V.; Jiang, W. Spectroscopic study of degradation products of ciprofloxacin, norfloxacin and lomefloxacin formed in ozonated wastewater. *Water Res.* **2012**, *46*, 5235–5246. [CrossRef] [PubMed]
131. Tang, C.Y.; Fu, Q.S.; Gao, D.; Criddle, C.S.; Leckie, J.O. Effect of solution chemistry on the adsorption of perfluorooctane sulfonate onto mineral surfaces. *Water Res.* **2010**, *44*, 2654–2662. [CrossRef] [PubMed]
132. Wang, Y.; Xu, L.; Chen, H.; Zhang, M. Retention and transport behavior of microplastic particles in water-saturated porous media. *Sci. Total Environ.* **2022**, *808*, 152154. [CrossRef]
133. Yu, J.; Zhang, D.; Ren, W.; Liu, B. Transport of Enterococcus faecalis in granular activated carbon column: Potential energy, migration, and release. *Colloids Surf. B Biointerfaces* **2019**, *183*, 110415. [CrossRef]
134. Schinner, T.; Letzner, A.; Liedtke, S.; Castro, F.D.; Eydelnant, I.A.; Tufenkji, N. Transport of selected bacterial pathogens in agricultural soil and quartz sand. *Water Res.* **2010**, *44*, 1182–1192. [CrossRef] [PubMed]
135. Chrysikopoulos, C.V.; Aravantinou, A.F. Virus attachment onto quartz sand: Role of grain size and temperature. *J. Environ. Chem. Eng.* **2014**, *2*, 796–801. [CrossRef]
136. Sasidharan, S.; Torkzaban, S.; Bradford, S.A.; Cook, P.G.; Gupta, V.V. Temperature dependency of virus and nanoparticle transport and retention in saturated porous media. *J. Contam. Hydrol.* **2017**, *196*, 10–20. [CrossRef] [PubMed]
137. Hou, D.; Hong, M.; Wang, Y.; Dong, P.; Cheng, H.; Yan, H.; Yao, Z.; Li, D.; Wang, K.; Zhang, D. Assessing the Risks of Potential Bacterial Pathogens Attaching to Different Microplastics during the Summer–Autumn Period in a Mariculture Cage. *Microorganisms* **2021**, *9*, 1909. [CrossRef] [PubMed]
138. Pinnell, L.J.; Turner, J.W. Temporal changes in water temperature and salinity drive the formation of a reversible plastic-specific microbial community. *FEMS Microbiol. Ecol.* **2020**, *96*, fiaa230. [CrossRef] [PubMed]
139. Cappello, S.; Guglielmino, S.P. Effects of growth temperature on polystyrene adhesion of *Pseudomonas aeruginosa* ATCC 27853. *Braz. J. Microbiol.* **2006**, *37*, 205–207. [CrossRef]
140. Bales, R.C.; Hinkle, S.R.; Kroeger, T.W.; Stocking, K.; Gerba, C.P. Bacteriophage adsorption during transport through porous media: Chemical perturbations and reversibility. *Environ. Sci. Technol.* **1991**, *25*, 2088–2095. [CrossRef]

141. Briandet, R.; Meylheuc, T.; Maher, C.; Bellon-Fontaine, M.N. *Listeria monocytogenes* Scott A: Cell Surface Charge, Hydrophobicity, and Electron Donor and Acceptor Characteristics under Different Environmental Growth Conditions. *Appl. Environ. Microbiol.* **1999**, *65*, 5328–5333. [CrossRef]
142. Ajaj, A.; J'bari, S.; Ononogbo, A.; Buonocore, F.; Bear, J.C.; Mayes, A.G.; Morgan, H. An Insight into the Growing Concerns of Styrene Monomer and Poly(Styrene) Fragment Migration into Food and Drink Simulants from Poly(Styrene) Packaging. *Foods* **2021**, *10*, 1136. [CrossRef]
143. Ye, X.; Wang, P.; Wu, Y.; Zhou, Y.; Sheng, Y.; Lao, K. Microplastic acts as a vector for contaminants: The release behavior of dibutyl phthalate from polyvinyl chloride pipe fragments in water phase. *Environ. Sci. Pollut. Res.* **2020**, *27*, 42082–42091. [CrossRef]
144. Murano, C.; Donnarumma, V.; Corsi, I.; Casotti, R.; Palumbo, A. Impact of Microbial Colonization of Polystyrene Microbeads on the Toxicological Responses in the Sea Urchin *Paracentrotus lividus*. *Environ. Sci. Technol.* **2021**, *55*, 7990–8000. [CrossRef] [PubMed]
145. Vroom, R.J.; Koelmans, A.A.; Besseling, E.; Halsband, C. Aging of microplastics promotes their ingestion by marine zooplankton. *Environ. Pollut.* **2017**, *231*, 987–996. [CrossRef]
146. Hansen, P.; von Bargen, K.; Jünger-Leif, A.; Haas, A. Laboratory Plasticware Induces Expression of a Bacterial Virulence Factor. *mSphere* **2022**, *7*, e0031122. [CrossRef]
147. Sun, C.; Teng, J.; Wang, D.; Zhao, J.; Shan, E.; Wang, Q. The adverse impact of microplastics and their attached pathogen on hemocyte function and antioxidative response in the mussel Mytilus galloprovincialis. *Chemosphere* **2023**, *325*, 138381. [CrossRef]
148. Al Akhrass, F.; Al Wohoush, I.; Chaftari, A.-M.; Reitzel, R.; Jiang, Y.; Ghannoum, M.; Tarrand, J.; Hachem, R.; Raad, I. Rhodococcus Bacteremia in Cancer Patients Is Mostly Catheter Related and Associated with Biofilm Formation. *PLoS ONE* **2012**, *7*, e32945. [CrossRef] [PubMed]

water

MDPI

Systematic Review

Application and Efficacy of Management Interventions for the Control of Microplastics in Freshwater Bodies: A Systematic Review

Suveshnee Munien [1,2,*], Puspa L. Adhikari [1], Kimberly Reycraft [3], Traci J. Mays [3], Trishan Naidoo [4,5], MacKenzie Pruitt [1], Jacqueline Arena [1] and Sershen [1,6]

[1] The Water School, Florida Gulf Coast University, Fort Myers, FL 33965, USA
[2] School of Agriculture, Earth and Environmental Sciences, University of KwaZulu-Natal, Durban 4041, South Africa
[3] Wilson G. Bradshaw Library, Florida Gulf Coast University, Fort Myers, FL 33967, USA; tmays@fgcu.edu (T.J.M.)
[4] Department of Zoology and Entomology, Rhodes University, Makhanda 6140, South Africa; trishan.naidoo@ru.ac.za
[5] South African Institute for Aquatic Biodiversity, Makhanda 6140, South Africa
[6] Department of Biodiversity and Conservation Biology, University of the Western Cape, Bellville 7530, South Africa
* Correspondence: smunien@fgcu.edu

Abstract: This systematic review represents one of the first attempts to compare the efficacy of the full suite of management interventions developed to control (prevent or remove) microplastics (MPs) in freshwater bodies, both man-made and natural. The review also traces the evolution of research on the topic in relation to the timing of key policy and regulatory events and investigates whether interventions are being applied within regions and freshwater bodies that represent concerns in terms of MP pollution. The review incorporated bibliometric analysis and meta-analysis of 124 original research articles published on the topic between 2012 and April 2023. To supplement the key findings, data were extracted from 129 review articles on the major knowledge gaps and recommendations. The number of articles on the topic increased with each year, coinciding with a range of global policy commitments to sustainability and mitigating plastic pollution. The majority of the studies focused on MPs in general, rather than any particular particle shape or polymer type, and were conducted at wastewater/sludge treatment plants. Upstream interventions accounted for the majority of studies reviewed (91.1%). A smaller proportion (4.8%) of studies involved reduction in production and physical removal at the point of production (1.6%); treatment-related objectives such as removal through filtration and separation and the combination of these with other technologies in hybrid systems were dominant. Of the physical, chemical and biological methods/technologies (and combinations thereof) employed, physical types (particularly membrane filtration) were most common. The majority of the studies within the wastewater/sludge, stormwater and in situ water/sediment categories exhibited removal efficacies >90%. Although new interventions are constantly being developed under laboratory conditions, their scalability and suitability across different settings are uncertain. Downstream interventions lack sustainability without effective upstream interventions. Though in situ methods are technically achievable, they may not be feasible in resource-limited settings.

Keywords: microplastics; freshwater; treatment efficacy; intervention; mitigation

Citation: Munien, S.; Adhikari, P.L.; Reycraft, K.; Mays, T.J.; Naidoo, T.; Pruitt, M.; Arena, J.; Sershen. Application and Efficacy of Management Interventions for the Control of Microplastics in Freshwater Bodies: A Systematic Review. *Water* **2024**, *16*, 176. https://doi.org/10.3390/w16010176

Academic Editors: Grigorios L. Kyriakopoulos, Antonis A. Zorpas, María Rocío Rodríguez Barroso and Vassilis J. Inglezakis

Received: 28 October 2023
Revised: 24 December 2023
Accepted: 25 December 2023
Published: 3 January 2024

1. Introduction

Microplastics (MPs) are emerging environmental pollutants that are ubiquitous in aquatic environments (freshwater and marine). MP pollution has raised much concern globally and consequently spurred voluminous studies on the prevalence, characterization, fate,

impacts and removal of MPs [1–3]. The phenomenon of MP pollution in aquatic environments reflects a much bigger challenge, namely, the global plastic waste burden, which was placed at 275 million tons (Mt) in 2010, of which 31.9 Mt were estimated to be mismanaged plastics that enter the environment [4]. In 2015, Jambeck et al. [4] approximated 8% (8 Mt) of the total plastics used globally to eventually enter the ocean via rivers, surface run-offs and other means. Furthermore, in 2017, Geyer et al. [5] approximated 12,000 Mt of plastics to have escaped from the waste management cycle and entered landfills or the environment directly over the last 50 years. After macroplastic debris enters the aquatic environment, it can undergo biological (degradation by microorganisms), mechanical (erosion, abrasion) and chemical (photo-oxidation, hydrolysis) modifications [6]. These modifications collectively lead to the weathering and the fragmentation of macroplastic debris into smaller and more abundant pieces (5 mm), what we refer to as secondary MPs [7]. In addition to the formation of MPs from the breaking down of the macroplastics, a large amount of plastics is manufactured as MPs (microfibers and microbeads), known as primary MPs, and used in various products, particularly in cosmetic products and textiles, and manufacturing processes [8,9].

Primary MPs are synthetic polymer particles produced as small-sized beads or pellets for further processing or addition to goods to act as 'scrubbers' in cosmetics and household cleaners, as industrial abrasives for sandblasting, or to manufacture feedstock pellets [8]. These particles (pellets and beads) are transported into water systems and then into natural rivers, eventually entering the ocean [9]. Secondary sources of MPs come about as a result of the unintentional introduction of plastic particles into water bodies from macroplastic pollution; macroplastics break down into MPs as discussed earlier [6]. Synthetic textiles and clothing are also significant sources of MPs because laundry physical and chemical abrasion leads to the production of smaller microfibers [10] which can enter the environment, particularly waterways (lakes, reservoirs, ponds, rivers, streams, wetlands), through the inappropriate/untreated release of wastewater [11].

The first comprehensive overview of the state of waste management globally in the 21st century [12] highlighted that waste management is still a challenge which is predicted to intensify given that the global quantity of mismanaged plastic waste has been projected to increase to 155–265 Mt per year in 2060, in comparison to 60–99 Mt in 2015 [13]. In commenting on the global waste management challenge in general, Wilson and Velis [12:1049] raised an important point: "Effective technologies required to 'solve' the waste problem are largely already available, and have been much written about". Waste management decisions may, therefore, need to focus on the implementation and suitability of waste management strategies, and, in the case of plastics, this needs to incorporate strategies (technologies/methods) for both (1) better management of the macroplastic waste that could potentially produce MPs and (2) the control (removal and degradation) of MPs after they enter water in the built and natural environment. However, this is not a simple task, given that decisions on the selection of strategies to control MP pollution are complicated by the fact that MPs are highly variable in terms of type, abundance, source and fate [3,14–16]. While there is a comprehensive summary of the sources of MPs to the environment [17], the multiplicity of MP sources has created a host of potential fates, hazards and remediation options that have been documented within freshwater bodies [18–20], though not as extensively as in marine environments [3].

Within a catchment, pollutants such as MPs can move across (out of or into) multiple compartments, accumulate in certain compartments and even return to previous compartments. Efforts to manage and enhance the built environment and water resources through strategies such as stormwater infrastructure and the creation of green infrastructure further complicate the movement and distribution patterns of MPs within a catchment. Freshwater bodies that are found to contain MPs include groundwater, freshwater lakes, rivers, dams [3] and both constructed and temporary wetlands [21]. Microplastics are most common in urban freshwater sources but have also been found in remote locations such as high-altitude streams [22]. The factors that could influence their relative concentra-

tion/distribution (vertical and horizontal) in freshwater bodies include the source inputs, duration of input and type of material (including size, shape and density), as well as transport mechanisms which are in turn influenced by hydrodynamic elements such as turbulence, turbidity and climatic conditions [19,21]. For example, water flow [3] and rainfall have been shown to influence the input and concentration of MPs in freshwater environments and bring about seasonal variations in concentrations [3]. Retention of particles in these riverine environments is also governed by the presence or absence of aquatic and fringing vegetation, as they may serve to trap particles in the higher reaches if present [23], which has pointed to the value of ecological infrastructure in managing MPs [24,25].

A major consideration when selecting interventions for the prevention and removal of MPs is the impacts that one seeks to mitigate. The nature of these impacts will dictate whether an established or bespoke intervention or combination of interventions is needed [26,27]. These impacts include reduced environmental quality [3,18,25] and negative impacts on aquatic organisms. A review of these impacts on organisms is beyond the scope of the current review but it is worth mentioning that they can be direct in the form of gut blockage [28,29] or indirect by vectoring other sorbed pollutants such as organic contaminants, heavy metals and microbial pathogens [30–32] that threaten the lives of biota [33–35]. In terms of their impacts on water and sediment quality, inherent plastic monomers and additive compounds that aid plastic function can be leached out over time [34]. In light of the above, the suite of MP pollution control interventions employed in any catchment compartment should ideally accommodate for the variety of impacts these plastic particles could have within the compartment(s) under consideration and the downstream water bodies.

Making decisions on the suitability of MP pollution control interventions for freshwater systems requires careful consideration of the location where they are to be applied and, more specifically, the catchment features. On this note, the definition of a catchment in the Anthropocene now accommodates the built environment as part of catchments, particularly in urban areas where stormwater runoff from buildings and roads [36], and other infrastructure such as wastewater treatment plants (WWTPs) [37,38] and ponds and reservoirs [39,40], can all receive and release MPs into natural water bodies, particularly freshwater systems such as rivers [41,42]. This threat has spurred increased implementation/commitment to policies, treaties and/or regulations focused on controlling MP pollution globally [43–45]. This increased interest in controlling MP pollution, together with the multiplicity, complexity and variability of the factors that contribute to the prevalence and fate of MPs in aquatic environments in general, has led to diversification in the types of interventions (e.g., source control) and associated technologies/methods (e.g., membrane filtration) used.

Traditionally, environmental management interventions include pollution control measures (which include implementing regulations and technologies to reduce or eliminate harmful emissions and pollutants into the air, water and soil), habitat conservation and restoration (protecting and restoring natural habitats to preserve biodiversity and ecosystem functions), sustainable resource management (i.e., promoting the sustainable and responsible use of natural resources to ensure their long-term availability and encouraging responsible consumption and waste management) and environmental education and awareness [3,46]. Solutions for MP pollution include interventions that prevent the release of plastics to the environment during their life cycle and physical methods of recovering or removing MPs from the natural environment (e.g., using pumps, mesh nets and other capturing devices) [3]. Strategies to recover or remove MPs have been extensively reviewed [38,47–50] but focus largely on those associated with wastewater treatment [11,51–54] or on specific types of interventions such as bioremediation [55]. Reviews on interventions to prevent the release of plastics to the environment focus largely on policies and regulations [43–45] or source control [25] and rarely focus specifically on freshwater. Reviews that do focus on MP removal in freshwater environments e.g., [56,57] either do not compare the relative efficacy of the variety of prevention and removal inter-

ventions that have been applied, or focus exclusively on chemical, biological and physical methods. The systematic review on management interventions for the control of MP pollution in freshwater bodies undertaken here, therefore, represents one of the first attempts to compare the efficacy of the full suite of interventions that have been developed to control (prevent or remove) MPs in freshwater bodies, both man-made and natural, based on the contemporary understanding of what constitutes a catchment. The review addresses the following research questions:

- What are the temporal, geographic and thematic trends in research on the application of interventions to prevent or remove MPs in freshwater bodies?
- How do these research trends relate to the policy and regulatory evolution of microplastic pollution control?
- What types of MPs and which catchment compartments represent research priorities?
- What types of interventions and combinations thereof are being prioritized (i.e., what are the comparative levels of uptake of different interventions), and where?
- What are the comparative levels of efficacy of the different interventions, and which combinations appear to be most effective?

We believe that the comparative approach adopted in this review can aid management decisions on MP pollution control in freshwater bodies, particularly in terms of the selection of fit-for-purpose and efficacious intervention types and appropriate methods/technologies. The knowledge gaps identified and recommendations made will help move research on MP pollution into a solution-oriented paradigm.

2. Methodology

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, 2020) guidelines were followed to document the literature review [58]. The PRISMA method provides a comprehensive set of guidelines for conducting systematic reviews [59,60], which were applied as shown in the PRISMA flow chart (Figure 1). The results of the identification, screening and inclusion process are described below.

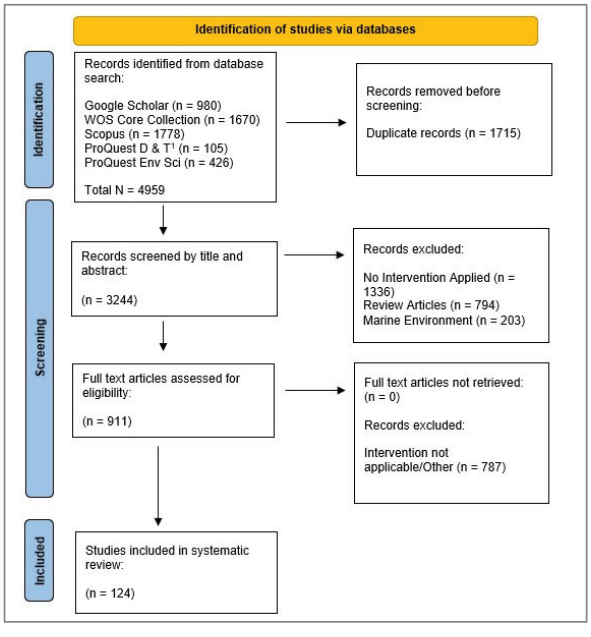


Figure 1. PRISMA flow chart showing steps and article counts at the various stages of the review process [[1] Database: ProQuest Dissertations and Theses Global and Environmental Science].

2.1. Search Strategy, Eligibility & Inclusion

A search strategy was devised to cover the three main topics: MPs, freshwater and management/mitigation. The full search strategy, which included multiple search strings and five databases, is presented in Appendix A. Google Scholar, Web of Science Core Collection (Clarivate Analytics, London, UK) and Scopus (Elsevier, Amsterdam, The Netherlands) were searched on 27 April 2023. Dissertations & Theses Global and Environmental Science, both on the ProQuest platform, were searched on 28 April 2023. All searches except for Google Scholar were limited to the period 2012 to April 2023. For studies to meet the inclusion criteria, they had to be peer-reviewed, original research articles or book chapters or dissertations published in English from 2012 to April 2023, and, most importantly, had to report on the application of an intervention to control MP pollution in a freshwater body/system/habitat. Studies were excluded if they reported on MPs in the marine environment, nanoplastics or macroplastics, or if they represented conference proceedings or review articles. Studies were imported into Rayyan [61] and assessed for eligibility by two independent reviewers. Any conflict based on the inclusion and exclusion criteria was marked and resolved by discussion with the entire research team. The initial search identified peer-reviewed articles on the control, mitigation, prevention and management of MP pollution in freshwater systems and identified 3244 articles after de-duplication. Of these 3244 articles, 911 were deemed suitable for a full-text review after title and abstract screening. Of these 911 articles, only 124 were found to involve the application of an intervention to control MP pollution in a freshwater system and subsequent assessment of efficacy—our primary criterion for inclusion after full-text screening.

In addition to the systematic literature review, we extracted data on the major recommendations and knowledge gaps on controlling MP pollution in freshwater bodies from a selection of review articles. From the original search of 3244 articles used for the systematic review, 794 review articles were screened, yielding 129 articles that were deemed suitable for inclusion after a full-text screening based on the fact that they contained specific recommendations and identified knowledge gaps related to controlling MP pollution in freshwater bodies or aquatic habitats in general.

2.2. Data Extraction & Management

The abstracts for all records identified via the initial search (n = 3244) were retrieved and imported into Rayyan [61] for screening. The 3244 records were then screened (two independent reviewers per article) using the title and abstract, and separated into three categories: 'include', 'exclude' and 'maybe'. The 'maybe' category served as the holding folder for review articles, which were subjected to a separate data extraction and analysis process (described below) by two independent reviewers per article, separate from the 124 original research articles ultimately included in the systematic review. The full text of all articles identified for inclusion was obtained, and the text was critiqued for eligibility by two independent reviewers per article as shown in the PRISMA flow diagram. Any conflicts were identified by Rayyan. All identified conflicts were resolved by discussion between the reviewers. If a resolution could not be obtained, then the article was assessed and discussed by the entire research team.

Using a modified data extraction spreadsheet created in Excel (see Supplementary File S1), two independent reviewers extracted data on the following for each of the 124 articles included in the systematic review: full citation, article country of origin, MP types, type(s) of intervention, objective(s) of intervention, method/technology employed, location/habitat/environment in which the intervention was applied and efficacy (based on percentage removal/reduction). If multiple interventions were reported in a single article, then data were extracted for each type of intervention separately. Additionally, recommendations and knowledge gaps on the subject were extracted from 129 review articles into an Excel spreadsheet and used for the supplemental analysis.

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It should be noted that this review process was subjected to multistage quality checks. As mentioned above, during the screening and full-text review phases, a minimum of two authors were assigned to each record. In cases where both authors yielded different results/outcomes, a third author examined the record and served as an arbitrator. Authors' profiles and disciplinary focus differed, resulting in a multidisciplinary group of reviewers, which ensured the reduction of bias.

2.3. Data Analysis

Data on MPs type and the number, type, objective, methods/technologies and efficacy(ies) of the interventions were coded based on predefined categories to generate a comparative matrix (Supplementary File S1). These data were disaggregated into the main thematic areas by crosstabulations (SPSS, Version 27). Publication dates were analyzed. The citation and keyword network analyses were generated in VOSViewer version 1.6.19, a tool for blending and visualizing bibliometric networks based on citation and journal data extracted from a robust body of scientific literature [62]. Recommendations and knowledge gaps extracted from the review articles were arranged thematically and scored for frequency (i.e., how many articles they appeared in). Using the frequencies, each theme was then ranked to identify the most frequent recommendations and knowledge gaps, and the top five for each category were selected for discussion.

3. Results & Discussion

The results of almost all the analyses described in this section were based on the database compiled for the study (Supplementary File S1), which contains data on the year in which the article was published; country where the intervention(s) was/were applied; the MP type(s) targeted (e.g., microbeads/pellets/nurdles); whether single or multiple interventions were applied; what type(s) of intervention(s) was/were applied (e.g., source control measures or wastewater/sludge treatment); what the objective of the intervention was (e.g., reduction in production or degradation); the method/technology (e.g., filtration or flocculation) employed; the location (habitat/environment/setting) where the intervention was applied (e.g., river/stream); and, finally, the normalized percentage efficacy of the intervention to allow for comparisons (given that the same method of quantification was used before and after application of each intervention). The database represents what we believe could be the beginnings of a decision-making tool for practitioners, managers and researchers to compare strategies for MP control in freshwater systems and access relevant literature on these strategies.

3.1. Scope of Publications on MP Interventions in Freshwater Systems

Our initial search yielded a total of 3244 articles but only 124 papers [3,4,63–183] were ultimately selected for review based on the exclusion criteria. The initial search yield (based on title and abstract) is reflective of the overwhelming number of publications on MPs over the last decade [2,184], but the marked difference between the number of papers in the initial search yield and the final number of papers selected for review is possibly a consequence of an over-emphasis on MPs in the marine environment. For example, Blettler et al.'s [185] analysis of journal databases for publications over an unspecified duration until May 2018 showed 440 (~87%) marine MP studies that fulfilled their search criteria in comparison to only 64 (~13%) in freshwater habitats. When the authors expressed these numbers in terms of publication rate, they showed that publication rates of studies of plastics in the

marine and freshwater environments were 41 and 7 papers per year, respectively. The underrepresentation of studies on the application of interventions for the management, prevention and mitigation of MP pollution in freshwater environments in the literature is of particular concern; for instance, in the present study, only 3.24% of the studies that met the initial search criteria (Figure 1) focused on the application of an intervention to manage, prevent or remove MPs in freshwater bodies.

All studies included in the systematic review [3,4,63–183] were published in 2016 or later, with an increase in the number of studies published each year, except in 2023. We believe that the 'false' decline in 2023 is simply a consequence of the analysis only including articles published up until April 2023. Therefore, while the set of articles analyzed for this paper represents a focused sample of research published on MPs, it can be inferred from this sample that the number of papers being published on MPs is continuously growing. In terms of the chosen timeline, 33.1% of publications used in this systematic review were generated in the year 2022. This is reflective of publication trends in the literature on MPs in general [2], where research on MP pollution is seen to have gained momentum after the publication of the global Paris Agreement (UNFCCC, 2015) and the listing of MPs as one of the top 10 environmental issues in 2014 (Figure 2). Even though our analysis begins in 2016, there may have been several factors promoting research on MP removal/degradation in freshwater systems before this timeframe. For example, the significant rise in publications may have been spurred by the global commitment to the Sustainable Development Goals (SDGs) in 2015. This thrust may have been sustained by subsequent events such as the G7 signing of the Ocean Plastic Charter in 2018 and China's release of a document strengthening plastic pollution control by its National Development and Reform Commission and Ministry of Energy in 2020.

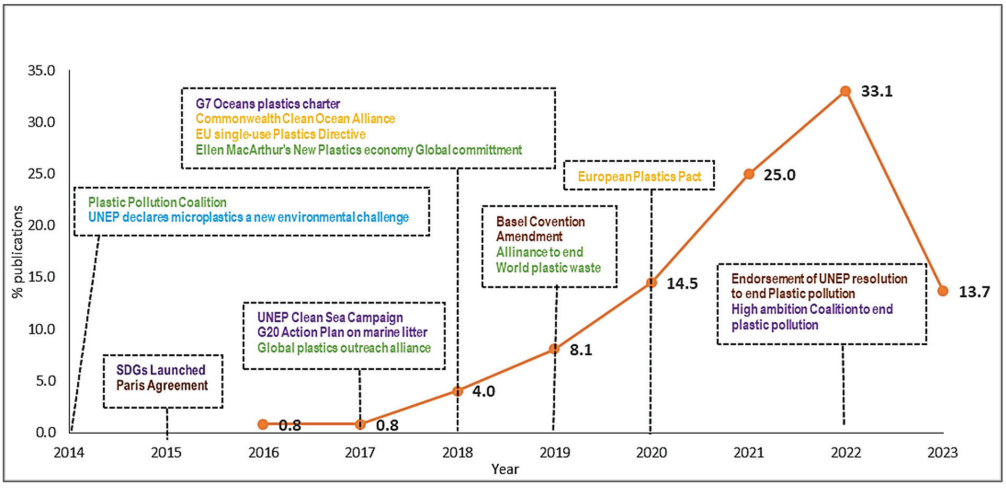

Figure 2. Timeline giving publications per year [3,4,63–183] and key events in the evolution of microplastic pollution control.

To truly appreciate this increased momentum, one has to only consider that our analysis showed the number of publications on the control of MPs in freshwater bodies to increase by 1700% between 2016 and 2020 and by 127.8% between 2020 and 2022. Similarly, Al et al. [2], in their bibliometric analysis of emerging trends in research on MP pollution in the post-Paris Agreement and post-COVID-19 pandemic world, showed that the number of articles in 2020 increased by 1770.0% relative to 2015. Based on the trends they observed, Ali et al. [2] predicted a much higher number by the end of 2021. The trends we see here in terms of the increase in the number of studies on management interventions for MP pollution control in freshwater bodies are also very likely due to the increased generation

and access to published research on MPs, as evidenced by an analysis by Sorensen and Jovanović [184] which showed that the number of MP publications increased by 2323.1% in 2019 relative to 2009. Furthermore, their analysis showed that 2019 was the year with the highest total number of citations (42,000 citations), followed by 2018 (25,000 citations) and 2017 (13,000 citations). However, in comparison, freshwater interventions for MP control remains under-researched across most of the world when compared to research on marine systems. This may change in the foreseeable future, given the endorsement of the first-ever legally binding UNEP resolution to end plastic pollution in 2022 and the continuous improvement in detection methods [186].

The minimum number of co-occurrences between the keywords was set at five, which produced a total of 53 keywords (Figure 3). In Figure 3, the larger the 'dot 'underneath the keyword, the more often the keyword occurs, and, based on this, the top keywords include microplastics, pollution, particles, fate, removal and identification. There are four clusters of keywords indicated in red, green, blue and yellow. The blue cluster reveals themes of pollution, particles and identification, with a focus on fibers, debris, WWTPs and sewage sludge. The red cluster includes MPs, marine environment and sediments with a variety of other terms like degradation and biodegradation coagulation, polymers, drinking water, polyethylene and polystyrene. The green cluster represents not only fate and wastewater-related keywords but also the natural environment with terms like fish and river. A small yellow cluster includes the removal and activated-sludge process. This cluster analysis reflects the importance of the types of data extracted from the 124 selected articles: The blue cluster highlighted the need to discriminate among the types of particles targeted across different studies; the red cluster highlighted the need to extract data on the methods/technologies used; the blue and green clusters highlighted the importance of looking at the application of interventions upstream (e.g., wastewater/sludge treatment plants (upstream) and downstream (e.g., rivers); the yellow cluster highlighted the importance of interventions focused on the removal of MPs (Figure 3). Numerous articles have pointed towards the importance of considering the type(s) of MP targeted, the source of the MPs and the method/technology used for their removal/degradation/reduction when designing MP control interventions for freshwater systems [49,170,187].

The article with the highest number of citations was Carr [71], with 1043 citations (Figure 4). This paper discussed MPs in wastewater treatment plants. Other articles with 200 or more citations include Auta [65,66] discussing microbial MP degradation, as well as Edo [81], Conley [75] and Lv [119] discussing WWTPs. These results highlight the emphasis placed on interventions focused on the removal of MPs from wastewater (i.e., upstream interventions) globally, a trend evidenced by the large number of articles on wastewater treatment in this study (74.2%) and the voluminous number of articles on the removal of MPs from wastewater/sludge reviewed elsewhere [37,38,51].

The publications reviewed in this paper reflect studies on the application of interventions to control MP pollution in freshwater bodies from across the globe; however, the majority of articles originate in the People's Republic of China, the United States of America and Australia (Supplementary File S1). Likewise, articles with the highest citations were also from the People's Republic of China and the United States of America, each with two distinct authors contributing to the metric (Supplementary File S1). The possible reasons for this geographical bias are discussed in Section 3.3, where we examine the geographic patterns in terms of the application of interventions for the control of MPs in freshwater bodies. However, it should be mentioned that these two nations host several premier academic institutions that have been driving the research agenda on environmental pollution for many years. Moreover, they have been actively involved in developing and lobbying for improved policies and regulations for MP pollution, specifically along the MP pollution control timeline shown in Figure 2.

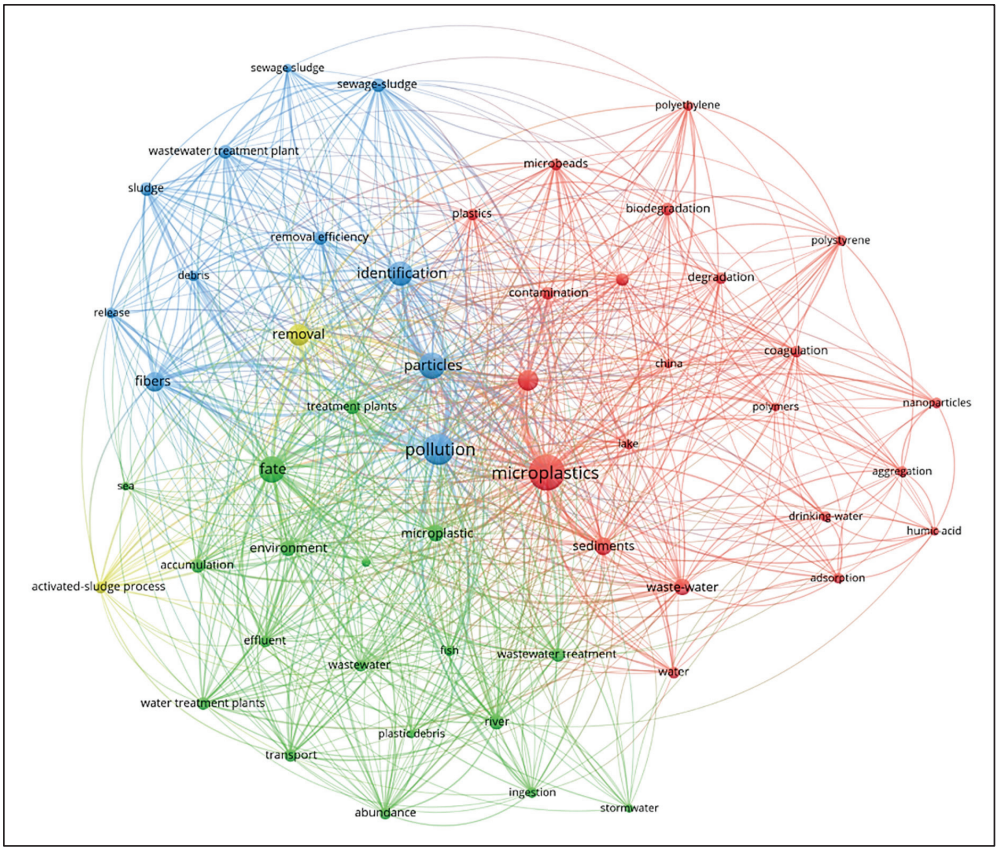

Figure 3. Map of the network of co-occurring keywords from the included articles [3,4,63–183] generated in the visualization software VOSViewer version 1.6.19 (n = 124).

3.2. Location of Studies

A country-level classification shows that 36.3% of the publications were conducted in China, while USA (8%), Germany and UK (5.7%), Australia and Thailand (4%), India (3.2%) and Canada, Denmark, Iran, Malaysia, Spain and Sweden (2.4%) comprised the main geographic focus. Further analysis revealed that the research foci varied across these main countries (Table 1): China, Germany and the UK produced publications across several categories of interventions while countries such as the USA and India focused on wastewater/ sludge, bioremediation and stormwater treatment interventions (Table 1). Evidently, publications on wastewater/sludge treatments were the dominant research focus in terms of intervention type, with China contributing 29.8% of the articles in this category.

However, more important in this systematic review is habitat/environment/setting in which these interventions were applied, as this will help shed light on the catchment compartments that are being prioritized and the degree to which interventions are being applied in real-world settings. Of the 18 potential habitat/environment/setting types used to extract the data (see Supplementary File S1), nine were represented in the articles reviewed here (Figure 5). As expected, a large proportion of the studies were conducted at treatment plants (36.3%). However, it was interesting to note that the majority of the studies (44.4%) were conducted ex situ, under laboratory conditions, aiming to develop or refine interventions. These two locations were followed by constructed/natural wetlands in terms of frequency, with a handful of studies (8.8%) taking place in real-world settings such as

stormwater drainage, roads/streets/highways and households/homes [86]. Importantly, none of the studies reviewed were applied at the catchment scale and there were no studies on other freshwater catchment compartments known to be impacted by MPs, such as lakes and dams, that met the search criteria. Even though this suggests that future research should aim to close these gaps in terms of under-researched catchment compartments, they may also point to the fact that the freshwater systems that are currently the foci of research in the field (e.g., rivers/streams, stormwater systems and WWTPs), represent the major threats/priorities. The results also suggest that new methods are constantly being developed and refined under laboratory conditions before they are implemented (at scale) in situ [112]. However, many of these lab-based studies use manufactured MPs [99,171,176] and appear to ignore weathering/aging effects and diversity in MPs (shape and polymer types) that exist in natural settings [132]. This can result in the development of interventions that are not fit-for-purpose (i.e., not scalable and/or inefficient in situ).

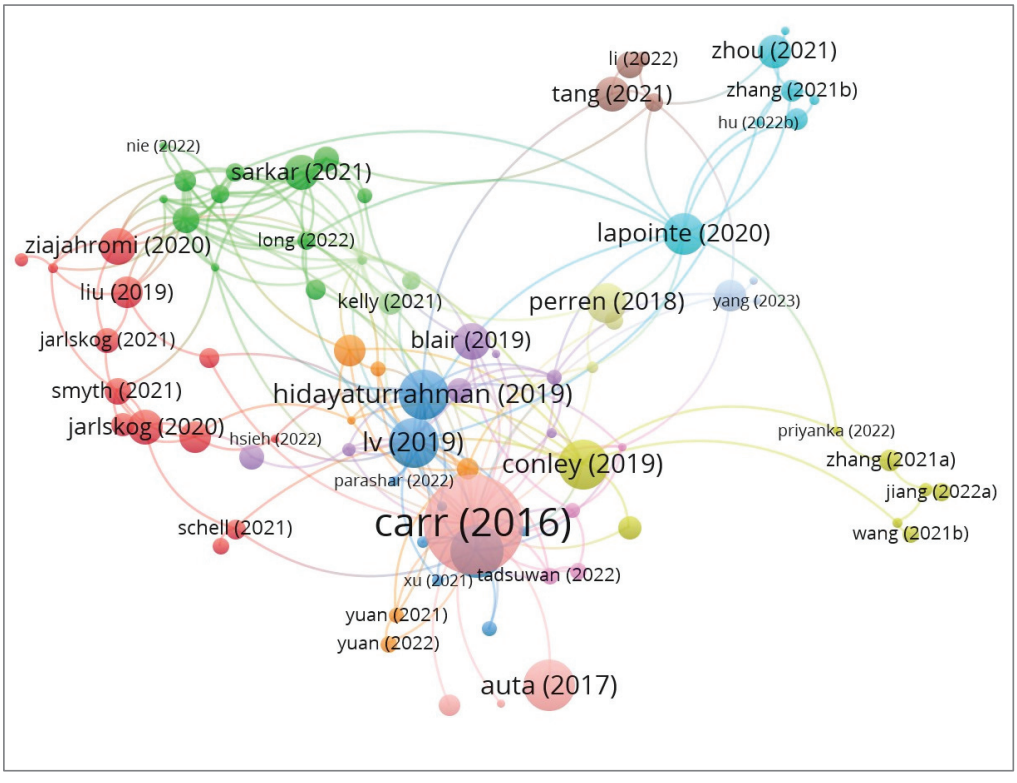

Figure 4. Results of citation analysis (up until July 2023) of the included articles [3,4,63–183] generated in VOSViewer (n = 124).

Table 1. Intervention types researched by top-producing countries (n = 124) [3,4,63–183].

Country *	1	2	3	4	5
China	3.2	29.8	2.4	-	0.8
USA	-	5.7	-	2.4	-
Germany	0.8	4	-	0.8	-
UK	0.8	4	.8	-	-
Australia	-	4	-	-	-
Thailand	-	4	-	-	-
India	-	2.4	0.8	-	-

1 = Source Control; 2 = Wastewater/Sludge treatment; 3 = Bioremediation; 4 = Stormwater treatment; 5 = In-situ sediment/water treatment; * Only showing results for the main countries contributing to research highlighted in the systematic review.

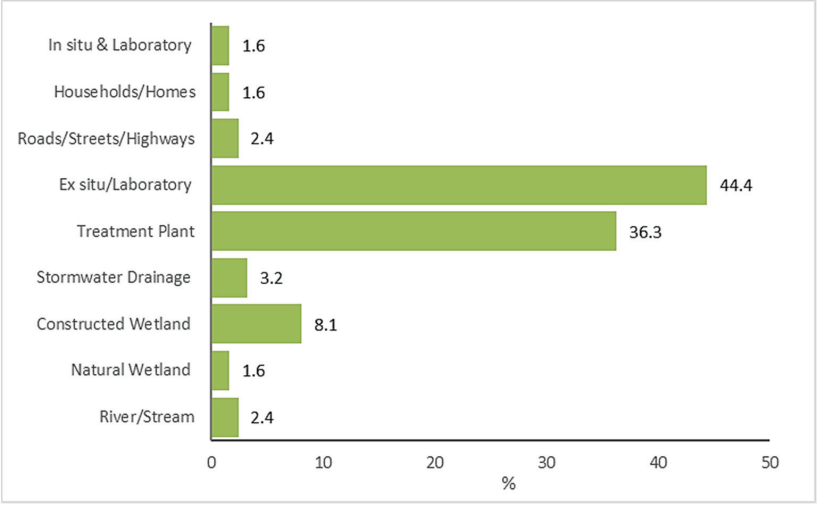

Figure 5. Habitat/environment/setting in which interventions were applied [3,4,63–183] (n = 124; Multiple entries observed in some records).

3.3. Types of MP

Of the 124 studies reviewed, the majority (89.5%) focused on interventions targeting MPs in general rather than any particle shape (fibers, films, foams and fragments) or polymer type (polyethylene, polypropylene, polystyrene, polyamide, polyester and acrylic). There was a small proportion of studies that focused on microbead and microfiber removal (~4.8% each). Fibers are the most dominant group of MPs, especially in urban landscapes and originate from the washing of clothes/textiles [180,188]. Microbeads have also been identified as a major threat to many aquatic environments, given their widespread use in cosmetics and personal care products [8,189]. These MP prevalence patterns do not appear to be reflected in the literature on interventions to control MP pollution, though. For example, there was a very limited focus on interventions designed to capture microfibers using laundry technologies [120] and microbeads [127] specifically. Targeted interventions such as these do not seem to be the norm since the size, ubiquity and indiscriminate release of MPs into freshwater environments would make such an approach unnecessary and/or ineffective in most instances [190].

3.4. Type and Application of Interventions for Freshwater Systems

Based on the logic of process principles, by reducing the loss of plastics to the environment, the input of MPs into freshwater environments could be subsequently reduced. Solutions that focus on the prevention of plastics release to the environment during their

life cycle can be termed 'upstream solutions', while 'downstream solutions' refer to the physical methods of recovering or removing MPs from the natural (freshwater) environment (e.g., using pumps, mesh nets and other capturing devices) [3]. Our review revealed a suite of upstream and downstream solutions (termed 'interventions' henceforth) that could be discriminated at the highest level in terms of 'intervention type' using the following typology (Supplementary File S1):

(1) Source control—measures to prevent MPs from coming into contact with (stormwater) runoff in natural and/or built environments; this includes clean technologies—any technology-based process, product or service that reduces/prevents MP inputs into the environment [74];
(2) Wastewater/sludge treatment—physical water treatment, biological water treatment, chemical treatment, and sludge treatment aimed at removing MPs and other pollutants [113];
(3) Bioremediation—use of either naturally occurring or deliberately introduced microorganisms or other forms of life to consume and break down MPs, to clean up a chronically or episodically polluted site [77];
(4) Stormwater treatment—the installation of structural controls primarily designed to remove MPs from stormwater runoff before this water is released into natural freshwater bodies [86];
(5) In situ water/sediment treatment—the physical removal of MPs from water or sediment in a natural freshwater body [99].

It was clear that the majority of the studies reviewed (95.2%) involved a singular intervention type, with only 4.8% of the studies involving a combination of interventions. Upstream interventions, namely wastewater/sludge, source control and stormwater interventions (collectively 91.1%) appear to be the dominant approaches adopted, with downstream interventions (bioremediation and in situ water/sediment interventions) accounting for a total of 12 studies reviewed. Wong et al. [3], in their review of the prevalence, fates, impacts and sustainable solutions to MPs in freshwater and terrestrial environments, observed that downstream solutions lack sustainability without effective upstream solutions. Though physical (e.g., mesh nets, pumps and other capturing devices) [100] and microbe-based [142] methods of recovering or removing MPs from freshwater bodies are technically achievable, they are often not logistically feasible due to the large number of MPs that are constantly entering these environments. Given that these methods require the installation of infrastructure or the introduction of foreign organisms, they also have the potential to disrupt local ecosystems.

In terms of the ultimate objective(s) of the interventions, a very small proportion of the studies involved reduction in MP production and physical removal at the point of production [120]; far more emphasis seems to be placed on treatment-related objectives such as removal through filtration and separation, capture and attachment and a combination of these technologies in hybrid systems (Table 2). However, it should be noted that interventions that involve the actual degradation of MPs appear to be uncommon (only 8.1% of the studies investigated). Interventions that involved the use of biodegradation were limited [126,159]. Similarly, research on bioplastics was limited [163], pointing to the technical challenges associated with this type of research: the rate at which bioplastics degrade is affected by different environmental conditions such as temperature, moisture, pH, oxygen content and, importantly, the availability of microorganisms [191]. The aquatic environment appears to be less suited than the terrestrial one for the degradation of bioplastics due to the lower availability of diverse microorganisms that enable higher biodegradability as compared to other environments such as soil [3].

Table 2. Description of ultimate objective(s) of the interventions/studies investigated (n = 124) [3,4,63–183].

Intervention Objective	% of the Articles Reviewed
Reduction in production	4.8
Removal–Filtration & Separation	21.6
Removal–Capture & Surface attachment	16.1
Removal–hybrid	48.4
Degradation	8.1
Physical removal at production	1.6

The high frequency of removal through the use of a combination of technologies (60; labeled 'Removal–hybrid' in Table 2) is a consequence of the fact that studies dealing with WWTPs constituted the majority (74.2%) of the articles reviewed here. This sampling bias, which was unavoidable as it reflects the literature on the subject over the last decade (evidenced by multiple reviews) [38,192], influenced other trends that emerged in our data analysis which are discussed later in this paper. This is largely because WWTPs are viewed as perhaps the most important source control points within catchments since they represent the solution in cases where they are efficient but quickly turn into problems when they experience failures and/or are based on inefficient technologies/methods [193].

Our analysis revealed that a wide range of techniques/methods are being employed/explored for controlling MP pollution in freshwater bodies (Table 3). A brief description of the technologies that were identified in the systematic review is given below. However, it should be noted that this list is not exhaustive and some of these technologies are evolving very rapidly, with a single method/technology sometimes displaying multiple design, application and combination variations, so we have also highlighted articles that offer a more detailed technical description of these technologies.

Table 3. Description of technologies adopted for MP control in freshwater systems (n = 124) [3,4,63–183].

MP Control	Descriptions	Citation/s	% of Articles Reviewed
Filtration membrane	Using different types of membrane filters to remove MPs during water treatment.	[50,123,147]	36.3
Constructed/natural wetland	Using natural or engineered (constructed) wetland systems to capture and remove MPs from wastewater and non-point source pollution. These include various designs of constructed wetlands, with a variety of materials and plants.	[70,74,132,156,181,182]	15.3
Coagulation/electrocoagulation	Using different types of coagulants such as polyacrylamide (PAM) and alum, or electrical charge to allow MPs in water to form an agglomeration. The coagulation is followed by flocculation and then settling (sedimentation) of the particles, after which they are physically removed.	[26,63,82,104,109,127]	15.3

Table 3. *Cont.*

MP Control	Descriptions	Citation/s	% of Articles Reviewed
Flocculation and sedimentation	Using flocculation of MPs, followed by their sedimentation, and then physical removal of the particles during water treatment. Various types of chemical coagulants and/or electrocoagulation have been used to speed up the natural flocculation and sedimentation process.	[109,111,128]	15.3
Adsorption	Utilizing different types of sorbents (e.g., activated carbon, biochar, zeolites, sponges) or electrical charges to facilitate sorption of MPs onto these particles, followed by their sedimentation and physical removal.	[48,172]	6.5
Magnetization	Utilizing magnetisms (e.g., via binding with nano-Fe_3O_4 particles) to magnetize the hydrophobic surface of MPs, followed by their separation and removal under the influence of a magnetic field.	[141,148]	2.4
Micromachines	Utilizing novel approaches like microscale particles with magnetic properties, e.g., magnetic field, to create a continuous motion to facilitate transportation and then separation/removal of MPs in aquatic environments [48]. Micromachines can also include utilizing a bubble barrier device to collect [175] and surface-functionalize microbubbles to accumulate and remove MPs in aquatic systems [176].	[48,175,176]	0.8
Superhydrophobic materials	Using various chemicals, with superhydrophobic surfaces, to functionalize MP surfaces which results in a change in the surface chemistry of the particle, facilitating removal (e.g., via sorption, flocculation and sedimentation).	[63] and references therein	1.6
Microorganism aggregation	Using microorganisms (e.g., micro-algae and bacterial films) to facilitate aggregation (via biofilm formation) of MPs in aquatic/treatment systems, which increases the density and promotes sedimentation of particles for ultimate physical removal.	[63,76,77]	4.0

Table 3. *Cont.*

MP Control	Descriptions	Citation/s	% of Articles Reviewed
Photocatalytic	Using light irradiation to excite photocatalysts, a pair of electrons and holes are produced in the redox reaction, and then this process degrades MPs into smaller inorganic molecules, such as carbon dioxide and water.	[47]	0.8
Microorganism degradation	Using microorganisms (e.g., bacteria) to physically degrade MPs as natural and/or engineered remediation of MPs in aquatic systems.	[48] and references therein [65,66]	10.5
Thermal degradation	Using various thermal processes, often hydrothermal hydrolysis combined with the use of various chemical treatments (e.g., Thermal Fenton Reaction), to remove MPs in water bodies or WWTPs.	[72,93]	4.0
Oxidation ditch	Exposing MPs to an oxidizing environment enriched with bacteria (e.g., during activated sludge system in WWTPs) increases their oxidation, which will increase their hydrophilicity. The increased hydrophilicity of MPs assists with their removal via froth flotation in the presence of cationic and anionic surfactants.	[90,100]	8.9
Sedimentation	Removal of MPs from aquatic systems (including WWTPs) via vertical sinking (often combined with coagulation and flocculation) and deposition onto the bottom, which can be followed by physical removal.	[26,48,63]	6.5
Mechanical manual removal	Mechanical/manual removal of MPs via flotation, sedimentation and filtration, using various filtration techniques such as screening, sand/membrane filtration and reverse osmosis.	[26,48]	4.0
Agglomeration	Natural and/or enhanced (using chemical and/or electrical coagulation) aggregation of MPs into a large mass. The agglomerated particles are larger and thus sink and accumulate on the bottom, after which they can be physically removed.	[88,89,139]	1.6

Table 3. *Cont.*

MP Control	Descriptions	Citation/s	% of Articles Reviewed
Sand filtration column	Using a sand filtration system that traps MPs between sand grains is often enhanced with the addition of various filtration aids such as biochar.	[26] and references therein [92]	1.6
Laundry technology	Using various technologies in washing machines (e.g., filtration system or removable fiber attracting innovations as part of the wash) for source control of MPs.	[79,120,121]	0.8

Based on the descriptions given above, our analysis revealed physical methods to be the most widely used, including membrane filtration, separation via phyto-capture, grit/primary sedimentation, density separation, coagulation and flocculation and combinations thereof. This finding is supported by other recent reviews [194] and is largely a consequence of the dominance of wastewater/sludge treatment-related studies in the dataset, as discussed earlier. Membrane filtration, for example, is the most frequent technology/method employed in wastewater/sludge treatment but is almost always used in combination with other methods (as evidenced by other reviews [52,195]), most frequently coagulation/electrocoagulation, the combination of flocculation and sedimentation and oxidation ditch. The employment of other technologies traditionally associated with wastewater/sludge treatment, such as adsorption, magnetization, superhydrophobic, thermal degradation, and agglomeration, appears to be less frequent (all between 1.6–5.6%). Technologies/methods that involved microorganisms either for aggregation or degradation of MPs (14.5% in total) and the use of constructed/natural wetlands (15.3%) for separation were less frequent than traditional physical methods/technologies. However, it should be noted that both microorganisms and wetlands were observed to be applied in isolation or integrated into wastewater/sludge treatment plants. More rudimentary (combination of sand filtration and sedimentation, and sedimentation in isolation) and highly technological (micromachines) methods/techniques were recorded, but these were less frequently used (all $\leq$ 6.5%).

3.5. Levels of Efficacy across Interventions

When efficacy was assessed within each category, we noted that the majority of the studies within the wastewater/sludge, stormwater and in situ water/sediment interventions all exhibited reported efficacies of >90% (Table 4). Furthermore, though a significant proportion of studies in the wastewater/sludge category (19.8%) exhibited efficacies of 76–90%, efficacies for this intervention type were spread across the lower, middle and upper ranges. This can be attributed to variations in the number of treatment steps, type and operating conditions of treatment technologies and, possibly, differences in the age and/or quality of facilities within and across different studies. Similarly, efficacies for studies within the bioremediation category were spread across the lower and upper ranges; the majority (30%) of these studies exhibited efficacies <25%. In this case, differences in the efficacy of any biological solution can be expected given that changes in climatic/environmental conditions acting on aquatic environments cannot be controlled/accommodated for and most often influence the performance/physiology of the organisms used.

Table 4. Comparison of MP prevention/removal efficacy rates (%) across intervention types [3,4,63–183].

Type of Intervention	<25%	26–50%	51–75%	76–90%	>90%	Effective: Rate Not Disclosed
Source control (*n* = 14)	-	-	14.3	21.4	14.3	50.0
Wastewater/sludge treatment (*n* = 91)	4.4	1.1	12.1	19.8	41.8	20.9
Bioremediation (*n* = 10)	30.0	10.0	-	20	10.0	30.0
Stormwater treatment (*n* = 7)	-	-	14.3	14.3	42.6	28.6
In situ water/sediment treatment (*n* = 2)	50.0	-	-	-	50.0	-
Total (*n* = 124)	7.4	1.6	11.3	19.4	36.3	25.1

Efficacies for studies in the source control category were spread across the upper three ranges (51–75, 76–90 and >90%) but the design of these studies often did not require the quantification of efficacy, which may explain why efficacy was not reported in 50% of the studies of this type. Other intervention categories included a significant number of studies where the intervention was reported to be effective, but the efficacy rate was not reported (20.9–50%); in total, 25.1% of the 124 studies reviewed did not report efficacy. Importantly, when data for all intervention types were pooled for analysis, 55.7% of the studies reviewed exhibited efficacies >76%, of which 36.3% exhibited efficacies >90% (Table 4).

When we compare the level of efficacy of different interventions in terms of their main objective, it was evident that the removal of MPs using hybrid systems, which refer to wastewater/sludge treatment plants, was the most effective, with 50.8% of the studies in this category displaying efficacy rates of >90% and 16.9% of studies reporting rates of between 75–90% (Table 5). Other reviews [192,196] have also concluded that during the wastewater/sludge treatment process, most of the MPs are removed. Systems that employed a grease-skimming method during the preliminary treatment process seemed to have a large proportion of MPs removed from the treatment process, while filtration and membrane technologies seemed to be the most effective during the final stages of treatment [192]. While other reviews have shown that high efficacy rates for wastewater [51,196] and sludge [197] treatment plants are common, it should be said that very few of these treatment plants remove MPs with 100% efficacy, which implies that treatment plants are also a significant secondary source of MPs [194]. WWTPs have for some time now been recognized as perhaps one of the most significant sources of MP pollution globally [193,198,199]. Closer analysis of the wastewater-related studies suggests that the high levels of efficacy can be attributed to significant advances in membrane technologies such as ultrafiltration (UF), microfiltration, reverse osmosis and membrane bioreactors over the last decade [50,147] and the adoption of other treatment technologies/methods such as the combination of a porous membrane with a biological process [49,200]. These supplemental/alternate wastewater treatment options have been born of necessity. One of the major drawbacks of membrane filtration is the fouling phenomenon which is the result of the adsorption of particles on the membrane surface. This fouling leads to reduced membrane filtration performance and consequently higher energy costs, operation time and maintenance [201]. Also, the efficacy of WWTPs is based on the systematic and accurate detection of MPs to keep track of how effectively the treatment process is in removing MP particles [192]; in this regard, during the screening of articles for our review, we encountered a large number of studies on MP detection methods/technologies.

Table 5. Comparison of MP prevention/removal efficacy rates across intervention objectives (in %) [3,4,63–183].

Intervention Objective	<25%	26–50%	51–75%	76–90%	>90%	Effective: Rate Not Disclosed
Reduction in production (*n* = 6)	-	-	1.7	33.3	1.7	33.3
Removal–Filtration & separation (*n* = 26)	7.7	-	26.9	15.4	26.9	23.1
Removal–Capture & surface attachment (*n* = 21)	4.8	9.5	4.8	28.6	28.6	23.8
Removal–hybrid (*n* = 59)	3.4	-	8.5	16.9	50.8	20.3
Degradation (*n* = 10)	30.0	-	-	20.0	10.0	40.0
Physical removal at point of production (*n* = 2)	-	-	-	-	-	100.0
Total (*n* = 124)	5.6	1.6	11.3	19.4	36.3	24.2

Even though studies on interventions with other objectives, specifically reduction in production, removal–filtration and separation and removal–capture and surface attachment and degradation, comprised a far smaller proportion of the studies reviewed, there were a significant number of reports within these categories where efficacy rates were between 75–90% and even >90% (Table 5). Additionally, 33.3% of the studies that had the objective of reduction in production reported efficacies of between 76–90%. It was also apparent that MPs of higher density can be removed effectively by coagulation, flocculation and then sedimentation (a finding supported by other review articles [22]).

Studies focused on the removal of MPs at the point of production were the exception, with efficacy rates not being reported for both the studies reviewed. On this note, it was worrying that 24.2% of the studies investigated (including all categories in the table below) indicated that the intervention was effective but did not report an efficacy rate; most notable was the 40% of studies on degradation that did not report levels of efficacy. In terms of the lowest levels of efficacy, interventions that focused on degradation (30%) appeared to be by far the least effective.

As mentioned earlier, our review revealed a wide variety of technologies/methods (*n* = 18) that have been applied/investigated to control MP pollution of freshwater bodies. However, the frequency with which these methods/technologies are used independently and/or in combination with each other varies widely (Table 6), necessitating careful interpretation of their efficacy. For example, 100% of the studies involving the independent use of CSA–micromachines, CSA–superhydrophobic, CSA–adsorption, agglomeration, sand filtration column and laundry technologies/methods exhibited efficacies of 100%; however, all these categories were represented by just 1–4 articles. Similarly, a significant proportion of the studies that involved the independent use of sedimentation, agglomeration and thermal degradation reported efficacies of >50% but these categories were represented by 2–3 articles. When we look at the technologies/methods that were used more frequently, either independently and/or in combination, the following stand out in terms of significantly high levels of efficacy: Filtration–membrane was used in a total of 42 studies and 72.4% of these exhibited efficacies >76%; separation–constructed/natural wetlands was a feature of 16 studies, of which 50.1% exhibited efficacies >76%; CSA–coagulation/electrocoagulation, CSA–flocculation & sedimentation and microorganism degradation were all featured in 5–9 studies, with 40–66.6% of these exhibiting efficacies >76%. The pros and cons of membrane filtration technologies have already been discussed above but it is worth noting that an oxidation ditch was used eight times in combination with a filtration membrane, pointing to the efficiency of this combination in the hybrid systems used in wastewater/sludge treatment. Our review also revealed some very novel approaches to microorganism-aided degradation of MPs [142,145], which is encouraging despite the relatively lower efficacies.

Table 6. Comparison of MP prevention/removal efficacy rates (%) across intervention methods/technologies applied independently and/or in combination (n = 124) [3,4,63–183].

Method/Technology	<25%	26–50%	51–75%	76–90%	>90%	Effective: Rate Not Disclosed
1 (n =29)	-	-	10.3	17.2	51.7	20.7
1,11 (n = 2)	-	-	-	-	50.0	50.0
1,13 (n = 4)	-	-	-	-	75.0	25.0
1,13,14 (n = 1)	-	-	-	-	-	100.0
1,13,14,15 (n = 1)	-	-	100.0	-	-	-
1,14 (n = 2)	-	-	-	50.0	50.0	-
1,2 (n = 2)	-	-	100.0	-	-	-
1,2,4,9,11,14 (n = 1)	-	-	-	-	-	100.0
1,3,4,13 (n = 1)	-	-	-	-	100.0	-
1,4 (n = 3)	-	-	33.3	-	33.3	33.3
1,4,11,13 (n = 1)	-	-	-	100	-	-
1,4,13 (n = 2)	-	-	-	50	50	-
1,4,13,14 (n = 1)	-	-	-	100	-	-
1,9,13 (n = 1)	-	-	-	-	100	-
2 (n = 16)	12.5	-	18.8	18.8	31.3	18.8
2,3,6 (n = 1)	-	-	-	100	0	0
3 (n = 9)	22.2	-	11.1	22.2	33.3	11.1
3,4 (n = 4)	25	-	-	25	50	-
3,4,5,11 (n = 1)	-	-	-	-	-	100
3,5 (n = 1)	-	-	-	100	-	-
3,6(n = 1)	-	-	-	100	-	-
4 (n = 5)	-	-	-	60	40	-
5 (n = 3)	-	-	-	-	33.3	66.7
5,12 n = 1)	-	-	-	-	100	-
6 (n = 2)	-	-	-	50	-	50
6,12 (n = 1)	-	-	-	-	100	0
7 (n = 1)	-	-	-	-	100	-
8 (n = 2)	-	-	-	-	100	-
9 (n = 2)	-	100	-	-	-	-
11(n = 8)	37.5	-	-	12.5	-	50
12 (n = 3)	-	-	-	33.3	-	
13 (n = 2)	-	-	50	-	-	50
14 (n = 4)	-	-	25	-	-	75
15 (n = 2)	-	-	-	-	-	100
16 (n = 2)	-	-	-	-	100	-
17 (n = 1)	-	-	-	-	-	100
18 (n = 1)	-	-	100	-	-	-

1 = Filtration-membrane; 2 = Separation-constructed/natural wetland; 3 = Coagulation/electrocoagulation; 4 = Flocculation & sedimentation; 5 = Adsorption; 6 = Magnetization; 7 = Micromachines; 8 = Superhydrophobic; 9 = Microorganism aggregation; 10 = Photocatalytic degradation; 11 = Microorganism degradation; 12 = Thermal degradation; 13 = Oxidation ditch; 14 = Sedimentation; 15 = Mechanical manual removal; 16 = Agglomeration; 17 = Sand filtration column; 18 = Laundry technology.

The results described above confirm that filtration technologies such as UF, sand filtration and granular filtration are the most popular choices—no doubt related to their effective, economic and energy-efficient application in WWTPs of different sizes [53,192]. In most cases in the articles reviewed here, the WWTP-related studies employed membrane bioreactor technologies, which is not surprising since membrane bioreactors have become the most popular and most effective (usually >99% removal rate) treatment technology among all of the biological treatment methods for MP removal [71]. Similarly, the combination of filtration with other biological treatment methods (e.g., activated sludge process, aerobic digestion, anaerobic digestion, biological degradation and constructed wetlands) was more common than combinations with chemical methods (e.g., oxidation, photo-oxidation, photo-catalytic degradation, coagulation, Fenton, photo-Fenton and acid–alkali treatment). Electrochemical methods such as electrocoagulation [127] and electro-Fenton processes [93]

have added a new dimension to chemical methods to improve their efficiency but were less frequently combined with other technologies. It was also interesting to note that pyrolysis and co-pyrolysis technologies which were touted as promising approaches for MP removal over the last decade based on their extra advantages of low-cost fuel production [202,203] were virtually absent from the technology/method combinations identified in this review.

3.6. Overview of Knowledge Gaps & Recommendations Extracted from Review Articles

To supplement the results of the systematic review, the recommendations and knowledge gaps were extracted from 129 review articles and subjected to thematic analysis which showed that these could be separated into the following categories: source control, wastewater and sludge treatments, bioremediation, stormwater treatment, behavior, education and awareness, and policy and regulatory frameworks (Table 7). However, there was sufficient overlap across categories for us to generate a set of overarching recommendations and knowledge gaps (five each, Table 7) based on the frequency of occurrence across the articles reviewed.

Table 7. Top five recommendations and knowledge gaps on management interventions for controlling MP pollution in freshwater bodies, ranked in descending frequency (F) of occurrence across 129 review articles published between 2012 and 2023 [1,3,11,14–16,18–20,25–27,36,38,41,43,45,47–56,189,192–200,204–296].

Rank	Knowledge Gaps	F (n = 129)	Citations
1	Data on sources, diversity, transport and fates of MPs, particularly within developing countries.	30	[3,20,27,45,49,56,189,198,205,206,209, 215,218,219,223,226,234,235,238–240, 244,248,277,278,280,282,286,290,292]
2	Exposure pathways and biological/toxicological effects of MPs for humans and environments.	24	[14,15,20,26,49,51,213,215,218,220,222, 224,229,231,237,242,278,284,285,289, 290,292–294]
3	Standardized MP analytical methods: Quantification and characterization.	24	[15,16,18,37,41,189,193,199,211,214, 219,220,222,227,233,246,254,255,264, 274,280,283,289,293]
4	MP weathering, degradation and removal (e.g., via biodegradation).	12	[1,49,50,53,195,196,246,247,262,266, 272,274]
5	Abilities of MP to interact with and eventually release associated pollutants.	7	[3,197,217,221,225,226,252]
	Recommendations		
1	Develop standardized detection and analytical methods to study and monitor MPs.	37	[14,26,27,49,50,56,189,192,197,199, 204–206,208,209,215,225– 227,234,235,239,240,242–244,251,252, 255,256,268,282,284,288,294–296]
2	Conduct more research on sources, transport pathways, fates, trophic interactions, toxicity, removal (e.g., biodegradation, electrocoagulation) and ecological impacts of MPs.	28	[16,25,36,47,49,52,53,189,205– 207,209,212,216– 223,226,231,238,239,243,258,295]
3	Implement comprehensive policies/legislation/regulations at local, national and international levels to prevent or remove MPs, and foster research collaboration and cooperation.	24	[14–16,18,41,193,216,219,224,227,229, 230,232–234,242,277–279,290–294]

Table 7. *Cont.*

Rank	Knowledge Gaps	F (n = 129)	Citations
4	Conduct extensive public education, training and awareness programs on MP pollution mitigation.	16	[18,51,204,223,229,231,233,242,244, 245,267,279,282,283,285,292]
5	Optimize secondary and tertiary MP treatments (e.g., with membrane bioreactors) at wastewater/sludge treatment plants.	12	[11,51,53,195,200,237,248,251,252,255, 257,258]

In terms of knowledge gaps, it appears that the application and efficacy of interventions could benefit from increased availability of data on sources, diversity, transport and fates of MPs, particularly within developing countries. Fit-for-purpose interventions could also be designed if exposure pathways and biological/toxicological effects of MPs for humans and environments are better characterized. In general, interventions need to be informed by/based on more robust and standardized MP analytical methods (for quantification and characterization). Management interventions appear to be hampered by insufficient knowledge of MP weathering, degradation, removal (e.g., via biodegradation) and the abilities of MPs to act as vectors of other pollutants.

Based on the 129 review articles included in the analysis, the major recommendations largely speak to the knowledge gaps identified in that they call for the development of standardized detection and analytical methods to study and monitor MPs and generate more data on sources, transport pathways, fate, trophic interactions, toxicity, removal (e.g., biodegradation, electrocoagulation) and ecological impacts of MPs. Additionally, the risks posed by WWTPs need to be addressed by optimizing or improving secondary and trtiary (e.g., with membrane bioreactors) MP treatments at wastewater/sludge treatment plants. Importantly, the recommendations do speak to the need to bring about behavioral change for reduced plastic use, improved plastic waste management and mitigation of MP pollution through the implementation of comprehensive policies/legislation/regulations, research collaboration and cooperation, and extensive public education, training and awareness raising (Table 7).

4. Concluding Remarks & Recommendations

To protect freshwater bodies from MP pollution, we must seek to develop and implement fit-for-purpose interventions. This can best be achieved by an evidence-based approach toward intervention design, selection and implementation. Irrespective of the intervention(s) selected, they must strike the balance between resource availability and environmental sensitivity. On this note, lab-based studies aimed at developing management interventions need to be more environmentally relevant and focus on treatment technologies that can be taken to scale in both the developed and developing world. Highly effective technologies used for the removal of MPs at wastewater/sludge treatment plants such as UF are a case in point; these must be made more accessible and affordable to developing countries [10].

To better protect freshwater bodies from MP pollution, we must increase awareness around the fact that ecosystems and human systems are connected within a catchment (though not always at the primary level), which implies that what happens in one compartment in terms of MP pollution can have knock-on effects on others. These knock-on effects can be accommodated when management interventions for MP pollution are planned and implemented at the catchment scale using participatory approaches such as catchment management forums.

Reducing the discharge of MPs from WWTPs into freshwater systems represents an immediate priority. Though wastewater, and, by implication, the MPs that cannot be removed, may be transferred to marine environments by deep sea discharge, it is now

well-established in the literature that WWTPs represent the dominant discharge pathway of MPs into freshwater environments [56,151,193,239]. In fact, Wang et al. [239] reported that 85% of the studies they reviewed on discharge pathways into freshwater ecosystems involved discharge from WWTPs.

New interventions are constantly being developed and refined under laboratory conditions but their scalability and suitability across different settings are uncertain. For improved efficacy, the application of these interventions must also be strategically tailored to local hydrogeological and climatic conditions. Downstream interventions are not sustainable without effective upstream interventions. Though in situ methods are technically achievable, they may not be feasible in resource-limited settings. On this note, although it did not emerge as part of the findings of this study, cost-benefit comparisons of the different types of interventions reviewed here represent a major knowledge gap that should be addressed in future studies. These types of analyses are a major consideration, and, in resource-limited settings, perhaps the basis, for management decisions related to pollution control.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w16010176/s1, Supplementary File S1: Meta-data on interventions applied to freshwater bodies extracted from 124 articles included in the systematic review.

Author Contributions: Conceptualization, S.M., P.L.A. and S.; Methodology, S.M., P.L.A., S., T.J.M. and K.R.; Software, T.J.M. and K.R.; Validation, S.M., P.L.A. and S.; Formal Analysis, All authors; Data Curation, All authors; Writing—Original Draft Preparation, S.M., P.L.A., S., T.N., T.J.M. and K.R.; Writing—Review & Editing, S.M., P.L.A. and S. All authors have read and agreed to the published version of the manuscript.

Funding: S.M. acknowledges funding received from Florida Gulf Coast University to cover publication costs.

Data Availability Statement: All the meta-data used for the analyses carried out are included in Supplementary File S1.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A Search Strategies

Google Scholar

Limits: None

Microplastic* AND (freshwater OR river* OR wetland* OR estuar* OR catchment* OR drainage OR basin* OR reservoir* OR stream*) AND (intervention* OR manage* OR mitigation OR *remediation OR reduction OR treatment OR removal OR regulation* OR law* OR polic*)

Web of Science Core Collection (Clarivate Analytics)

Limits: 2012–2023

(microplastic* OR microbead*) AND (freshwater OR river* OR wetland* OR estuar* OR catchment* OR drainage OR basin* OR reservoir* OR stream* OR lake* OR pond* OR "inland water bod*" OR dam OR bay* OR lagoon*) AND (intervention* OR manage* OR mitigation OR *remediation OR reduction* OR treat* OR removal OR regulation* OR law* OR policy OR policies OR "land use" OR prevent*)

Scopus

Limits: 2012–2023

(TITLE-ABS-KEY ((microplastic* OR microbead*)) AND TITLE-ABS-KEY ((freshwater OR river* OR wetland* OR estuar* OR catchment* OR drainage OR basin* OR reservoir* OR stream* OR lake* OR pond* OR "inland water bod*" OR dam OR bay* OR lagoon*)) AND TITLE-ABS-KEY ((intervention* OR manage* OR mitigation OR *remediation OR reduction* OR treat* OR removal OR regulation* OR law* OR policy OR policies OR "land use" OR prevent*))) AND PUBYEAR > 2011 AND PUBYEAR < 2024

Dissertations and Theses Global (ProQuest) and Environmental Science (ProQuest)

Limits: 2012–2023

noft ((microplastic* OR microbead*)) AND noft ((freshwater OR river* OR wetland* OR estuar* OR catchment* OR drainage OR basin* OR reservoir* OR stream* OR lake* OR pond* OR "inland water bod*" OR dam OR bay* OR lagoon*)) AND noft ((intervention* OR manage* OR mitigation OR remediation OR reduction* OR treat* OR removal OR regulation* OR law* OR policy OR policies OR "land use" OR prevent*))

References

1. Ahmad, I.; Abdullah, N.; Koji, I.; Yuzir, A.; Mohamad, S.E. Potential of Microalgae in Bioremediation of Wastewater. *Bull. Chem. React. Eng. Catal.* **2021**, *16*, 413–429. [CrossRef]
2. Ali, S.; Faizi, B.; Waqas, H.; Asghar, M.; Zarei, N.; Hashmi, M.Z.; Anjum, S. Bibliometric Analysis of Emerging Trends in Research on Microplastic Pollution in Post-Paris Agreement and Post-COVID-19 Pandemic World. In *Soren Microplastic Pollution: Environmental Occurrence and Treatment Technologies, Emerging Contaminants and Associated Treatment Technologies*; Hashmi, M.Z., Ed.; Springer International Publishing: Cham, Switzerland, 2022; pp. 511–538. [CrossRef]
3. Wong, J.K.H.; Lee, K.K.; Tang, K.H.D.; Yap, P.-S. Microplastics in the freshwater and terrestrial environments: Prevalence, fates, impacts and sustainable solutions. *Sci. Total Environ.* **2020**, *719*, 137512. [CrossRef] [PubMed]
4. Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K.L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347*, 768–771. [CrossRef] [PubMed]
5. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci. Adv.* **2017**, *3*, e1700782. [CrossRef] [PubMed]
6. Andrady, A.L.; Hamid, S.H.; Hu, X.; Torikai, A. Effects of increased solar ultraviolet radiation on materials. *J. Photochem. Photobiol. B Biol.* **1998**, *46*, 96–103. [CrossRef] [PubMed]
7. Arthur, C.; Baker, J.E.; Bamford, H.A. *Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris, September 9–11, 2008, University of Washington Tacoma, Tacoma, WA, USA*; National Oceanic and Atmospheric Administration: Washington, DC, USA, 2023. Available online: https://repository.library.noaa.gov/view/noaa/2509 (accessed on 29 July 2023).
8. Juliano, C.; Magrini, G.A. Cosmetic Ingredients as Emerging Pollutants of Environmental and Health Concern. A Mini-Review. *Cosmetics* **2017**, *4*, 11. [CrossRef]
9. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. Microplastics as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* **2011**, *62*, 2588–2597. [CrossRef]
10. Browne, M.A.; Crump, P.; Niven, S.J.; Teuten, E.; Tonkin, A.; Galloway, T.; Thompson, R. Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. *Environ. Sci. Technol.* **2011**, *45*, 9175–9179. [CrossRef]
11. Sol, D.; Laca Amanda Laca Adriana Diaz, M. Wastewater treatment approaches to remove microplastics: Microfibre incidence and fate. In *Polluting Textiles: The Problem with Microfibres, Routledge Explorations in Environmental Studies*; Routledge by Taylor & Francis: Oxford, UK, 2022.
12. Wilson, D.C.; Velis, C.A. Waste management—Still a global challenge in the 21st century: An evidence-based call for action. *Waste Manag. Res.* **2015**, *33*, 1049–1051. [CrossRef]
13. Lebreton, L.; Andrady, A. Future scenarios of global plastic waste generation and disposal. *Palgrave Commun.* **2019**, *5*, 6. [CrossRef]
14. Peng, J.; Wang, J.; Cai, L. Current understanding of microplastics in the environment: Occurrence, fate, risks, and what we should do. *Integr. Environ. Assess. Manag.* **2017**, *13*, 476–482. [CrossRef] [PubMed]
15. Rose, P.K.; Jain, M.; Kataria, N.; Sahoo, P.K.; Garg, V.K.; Yadav, A. Microplastics in multimedia environment: A systematic review on its fate, transport, quantification, health risk, and remedial measures. *Groundw. Sustain. Dev.* **2023**, *20*, 100889. [CrossRef]
16. Yusuf, A.; Sodiq, A.; Giwa, A.; Eke, J.; Pikuda, O.; Eniola, J.O.; Ajiwokewu, B.; Sambudi, N.S.; Bilad, M.R. Updated review on microplastics in water, their occurrence, detection, measurement, environmental pollution, and the need for regulatory standards. *Environ. Pollut.* **2022**, *292*, 118421. [CrossRef] [PubMed]
17. Wagner, M.; Lambert, S. Microplastics Are Contaminants of Emerging Concern in Freshwater Environments: An Overview. In *Freshwater Microplastics: Challenges for Regulation and Management, The Handbook of Environmental Chemistry*; Springer Open: Oslo, Norway; Berlin/Heidelberg, Germany, 2018; pp. 1–23. [CrossRef]
18. Raza, M.; Lee, J.-Y.; Cha, J. Microplastics in soil and freshwater: Understanding sources, distribution, potential impacts, and regulations for management. *Sci. Prog.* **2022**, *105*, 00368504221126676. [CrossRef] [PubMed]
19. Sun, Q.; Li, J.; Wang, C.; Chen, A.; You, Y.; Yang, S.; Liu, H.; Jiang, G.; Wu, Y.; Li, Y. Research progress on distribution, sources, identification, toxicity, and biodegradation of microplastics in the ocean, freshwater, and soil environment. *Front. Environ. Sci. Eng.* **2021**, *16*, 1. [CrossRef]
20. Xu, Y.; Chan, F.K.S.; He, J.; Johnson, M.; Gibbins, C.; Kay, P.; Stanton, T.; Xu, Y.; Li, G.; Feng, M.; et al. A critical review of microplastic pollution in urban freshwater environments and legislative progress in China: Recommendations and insights. *Crit. Rev. Environ. Sci. Technol.* **2021**, *51*, 2637–2680. [CrossRef]
21. Atugoda, T.; Piyumali, H.; Liyanage, S.; Mahatantila, K.; Vithanage, M. Fate and behavior of microplastics in freshwater systems. In *Handbook of Microplastics in the Environment*; Springer: Cham, Switzerland, 2022; pp. 781–811. [CrossRef]

22. Brennholt, N.; He, B.M.; Reifferscheid, G. Freshwater Microplastics: Emerging Environmental Contaminants? In *Freshwater Microplastics: Challenges for Regulation and Management, The Handbook of Environmental Chemistry*; Springer Open: Oslo, Norway; Berlin/Heidelberg, Germany, 2018; pp. 239–272. [CrossRef]
23. Yin, L.; Wen, X.; Huang, D.; Zeng, G.; Deng, R.; Liu, R.; Zhou, Z.; Tao, J.; Xiao, R.; Pan, H. Microplastics retention by reeds in freshwater environment. *Sci. Total Environ.* **2021**, *790*, 148200. [CrossRef]
24. Meera, S.P.; Bhattacharyya, M.; Nizam, A.; Kumar, A. A review on microplastic pollution in the mangrove wetlands and microbial strategies for its remediation. *Environ. Sci. Pollut. Res.* **2022**, *29*, 4865–4879. [CrossRef]
25. Zhong, L.; Wu, T.; Sun, H.-J.; Ding, J.; Pang, J.-W.; Zhang, L.; Ren, N.-Q.; Yang, S.-S. Recent advances towards micro(nano)plastics research in wetland ecosystems: A systematic review on sources, removal, and ecological impacts. *J. Hazard. Mater.* **2023**, *452*, 131341. [CrossRef]
26. Park, H.; Park, B. Review of Microplastic Distribution, Toxicity, Analysis Methods, and Removal Technologies. *Water* **2021**, *13*, 2736. [CrossRef]
27. Tang, S.; Gao, L.; Gao, H.; Chen, Z.; Zou, D. Microplastics pollution in China water ecosystems: A review of the abundance, characteristics, fate, risk and removal. *Water Sci. Technol.* **2020**, *82*, 1495–1508. [CrossRef] [PubMed]
28. Naidoo, T.; Glassom, D. Decreased growth and survival in small juvenile fish, after chronic exposure to environmentally relevant concentrations of microplastic. *Mar. Pollut. Bull.* **2019**, *145*, 254–259. [CrossRef]
29. Murray, F.; Cowie, P.R. Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus, 1758). *Mar. Pollut. Bull.* **2011**, *62*, 1207–1217. [CrossRef] [PubMed]
30. Zettler, E.R.; Mincer, T.J.; Amaral-Zettler, L.A. Life in the "Plastisphere": Microbial Communities on Plastic Marine Debris. *Environ. Sci. Technol.* **2013**, *47*, 7137–7146. [CrossRef] [PubMed]
31. Zhang, H.; Fei, Y.; Wang, H.; Chen, Y.; Huang, S.; Yu, B.; Wang, J.; Tong, Y.; Wen, D.; Zhou, B.; et al. Interaction of Microplastics and Organic Pollutants: Quantification, Environmental Fates, and Ecological Consequences. In *Microplastics in Terrestrial Environments: Emerging Contaminants and Major Challenges, The Handbook of Environmental Chemistry*; He, D., Luo, Y., Eds.; Springer International Publishing: Cham, Switzerland, 2020; pp. 161–184. [CrossRef]
32. Hildebrandt, L.; Nack, F.L.; Zimmermann, T.; Pröfrock, D. Microplastics as a Trojan horse for trace metals. *J. Hazard. Mater. Lett.* **2021**, *2*, 100035. [CrossRef]
33. Fendall, L.S.; Sewell, M.A. Contributing to marine pollution by washing your face: Microplastics in facial cleansers. *Mar. Pollut. Bull.* **2009**, *58*, 1225–1228. [CrossRef] [PubMed]
34. Gunaalan, K.; Fabbri, E.; Capolupo, M. The hidden threat of plastic leachates: A critical review on their impacts on aquatic organisms. *Water Res.* **2020**, *184*, 116170. [CrossRef]
35. Naidoo, T.; Rajkaran, A.; Sershen. Impacts of plastic debris on biota and implications for human health: A South African perspective. *South Afr. J. Sci.* **2020**, *116*, 1–8. [CrossRef]
36. Stang, C.; Mohamed, B.A.; Li, L.Y. Microplastic removal from urban stormwater: Current treatments and research gaps. *J. Environ. Manag.* **2022**, *317*, 115510. [CrossRef]
37. Abeynayaka, A.; Werellagama, I.; Ngoc-Bao, P.; Hengesbaugh, M.; Gajanayake, P.; Nallaperuma, B.; Karkour, S.; Bui, X.-T.; Itsubo, N. Chapter 11—Microplastics in wastewater treatment plants. In *Current Developments in Biotechnology and Bioengineering*; Bui, X.-T., Nguyen, D.D., Nguyen, P.-D., Ngo, H.H., Pandey, A., Eds.; Elsevier: Amsterdam, The Netherlands, 2022; pp. 311–337. [CrossRef]
38. Ngo, P.L.; Pramanik, B.K.; Shah, K.; Roychand, R. Pathway, classification and removal efficiency of microplastics in wastewater treatment plants. *Environ. Pollut.* **2019**, *255*, 113326. [CrossRef]
39. Liu, F.; Olesen, K.B.; Borregaard, A.R.; Vollertsen, J. Microplastics in urban and highway stormwater retention ponds. *Sci. Total Environ.* **2019**, *671*, 992–1000. [CrossRef]
40. Moruzzi, R.B.; Speranza, L.G.; da Conceição, F.T.; de Souza Martins, S.T.; Busquets, R.; Campos, L.C. Stormwater Detention Reservoirs: An Opportunity for Monitoring and a Potential Site to Prevent the Spread of Urban Microplastics. *Water* **2020**, *12*, 1994. [CrossRef]
41. Feilin, H.; Mingwei, S. Ecofriendly removing microplastics from rivers: A novel air flotation approach crafted with positively charged carrier. *Process Saf. Environ. Prot.* **2022**, *168*, 613–623. [CrossRef]
42. Narciso-Ortiz, L.; Coreno-Alonso, A.; Mendoza-Olivares, D.; Lucho-Constantino, C.; Lizardi-Jimenez, M. Baseline for plastic and hydrocarbon pollution of rivers, reefs, and sediment on beaches in Veracruz State, Mexico, and a proposal for bioremediation. *Environ. Sci. Pollut. Res.* **2020**, *27*, 23035–23047. [CrossRef] [PubMed]
43. Barceló, D.; Picó, Y. Microplastics in the global aquatic environment: Analysis, effects, remediation and policy solutions. *J. Environ. Chem. Eng.* **2019**, *7*, 103421. [CrossRef]
44. McDevitt, J.P.; Criddle, C.S.; Morse, M.; Hale, R.C.; Bott, C.B.; Rochman, C.M. Addressing the Issue of Microplastics in the Wake of the Microbead-Free Waters Act—A New Standard Can Facilitate Improved Policy. *Environ. Sci. Technol.* **2017**, *51*, 6611–6617. [CrossRef]
45. Whitehead, P.G.; Bussi, G.; Hughes, J.M.R.; Castro-Castellon, A.T.; Norling, M.D.; Jeffers, E.S.; Rampley, C.P.N.; Read, D.S.; Horton, A.A. Modelling Microplastics in the River Thames: Sources, Sinks and Policy Implications. *Water* **2021**, *13*, 861. [CrossRef]
46. Eerkes-Medrano, D.; Thompson, R.C.; Aldridge, D.C. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res.* **2015**, *75*, 63–82. [CrossRef]

47. Günes-Durak, S. Investigation of microplastics removal methods from aquatic environments. *Herit. Sustain. Dev.* **2021**, *3*, 58–63. [CrossRef]
48. Hanif, M.A.; Ibrahim, N.; Dahalan, F.A.; Ali, U.F.M.; Hasan, M.; Jalil, A.A. Microplastics and nanoplastics: Recent literature studies and patents on their removal from aqueous environment. *Sci. Total. Environ.* **2022**, *810*, 152115. [CrossRef]
49. Miloloža, M.; Cvetnić, M.; Grgić, D.K.; Bulatović, V.O.; Ukić, Š.; Rogošić, M.; Dionysiou, D.D.; Kušić, H.; Bolanča, T. Biotreatment strategies for the removal of microplastics from freshwater systems. *A review. Environ. Chem. Lett.* **2022**, *20*, 1377–1402. [CrossRef]
50. Mishra, S.; Das, A.P. Chapter 11—Current Treatment Technologies for Removal of Microplastic and Microfiber Pollutants from Wastewater. In *Wastewater Treatment*; Shah, M.P., Sarkar, A., Mandal, S., Eds.; Elsevier: Amsterdam, The Netherlands, 2021; pp. 237–251. [CrossRef]
51. Khadir, A.; Negarestani, M.; Kheradmand, A.; Sillanpää, M. Microplastic Pollution in Water and Their Removal in Various Wastewater Treatment Plants. In *Microplastics Pollution in Aquatic Media: Occurrence, Detection, and Removal, Environmental Footprints and Eco-Design of Products and Processes*; Sillanpää, M., Khadir, A., Muthu, S.S., Eds.; Springer: Singapore, 2022; pp. 247–271. [CrossRef]
52. Mao, Y.; Zhao, Y.; Cotterill, S. Examining Current and Future Applications of Electrocoagulation in Wastewater Treatment. *Water* **2023**, *15*, 1455. [CrossRef]
53. Mishra, S.; Singh, R.P.; Rout, P.K.; Das, A.P. Chapter 3—Membrane bioreactor (MBR) as an advanced wastewater treatment technology for removal of synthetic microplastics. In *Development in Wastewater Treatment Research and Processes*; Shah, M., Rodriguez-Couto, S., Biswas, J., Eds.; Elsevier: Amsterdam, The Netherlands, 2022; pp. 45–60. [CrossRef]
54. Shen, M.; Xiong, W.; Song, B.; Zhou, C.; Almatrafi, E.; Zeng, G.; Zhang, Y. Microplastics in landfill and leachate: Occurrence, environmental behavior and removal strategies. *Chemosphere* **2022**, *305*, 135325. [CrossRef] [PubMed]
55. Chellasamy, G.; Kiriyanthan, R.M.; Maharajan, T.; Radha, A.; Yun, K. Remediation of microplastics using bionanomaterials: A review. *Environ. Res.* **2022**, *208*, 112724. [CrossRef] [PubMed]
56. Li, C.; Busquets, R.; Campos, L.C. Assessment of microplastics in freshwater systems: A review. *Sci. Total. Environ.* **2020**, *707*, 135578. [CrossRef] [PubMed]
57. Padervand, M.; Lichtfouse, E.; Robert, D.; Wang, C. Removal of microplastics from the environment. *A review. Environ. Chem. Lett.* **2020**, *18*, 807–828. [CrossRef]
58. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *Int. J. Surg.* **2010**, *8*, 336–341. [CrossRef]
59. Paul, J.; Lim, W.M.; O'cass, A.; Hao, A.W.; Bresciani, S. Scientific procedures and rationales for systematic literature reviews (SPAR-4-SLR). Int. *J. Consum. Stud.* **2021**, *45*, O1–O16. [CrossRef]
60. Sajid, S.; Alam, M.S.; Kok, J.K.; Rehman, M. Women's participation in Science, Technology Engineering and Mathematics (STEM) education: A review of literature. *Asia Proc. Soc. Sci.* **2020**, *6*, 230–234. [CrossRef]
61. Ouzzani, M.; Hammady, H.; Fedorowicz, Z.; Elmagarmid, A. Rayyan—A web and mobile app for systematic reviews. *Syst. Rev.* **2016**, *5*, 210. [CrossRef]
62. Van Eck, N.J.; Waltman, L. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics* **2009**, *84*, 523–538. [CrossRef] [PubMed]
63. Adib, D.; Mafigholami, R.; Tabeshkia, H.; Walker, T. Optimization of Polypropylene Microplastics Removal Using Conventional Coagulants in Drinking Water Treatment Plants via Response Surface Methodology. *J. Environ. Health Sci. Eng.* **2022**, *20*, 565–577. [CrossRef] [PubMed]
64. Alvim, C.; Valiente, S.; Bes-Pia, M.; Mendoza-Roca, J. Methodology for Removing Microplastics and Other Anthropogenic Microparticles from Sludge Dewatering System. *J. Environ. Manag.* **2022**, *314*, 115010. [CrossRef] [PubMed]
65. Auta, H.S.; Emenike, C.U.; Fauziah, S.H. Screening of Bacillus Strains Isolated from Mangrove Ecosystems in Peninsular Malaysia for Microplastic Degradation. *Environ. Pollut.* **2017**, *231*, 1552–1559. [CrossRef] [PubMed]
66. Auta, H.S.; Emenike, C.U.; Jayanthi, B.; Fauziah, S.H. Growth Kinetics and Biodeterioration of Polypropylene Microplastics by *Bacillus* Sp. and *Rhodococcus* Sp. Isolated from Mangrove Sediment. *Mar. Pollut. Bull.* **2018**, *127*, 15–21. [CrossRef] [PubMed]
67. Ben-David, E.; Habibi, M.; Haddad, E.; Hasanin, M.; Angel, D.; Booth, A.; Sabbah, I. Microplastic Distributions in a Domestic Wastewater Treatment Plant: Removal Efficiency, Seasonal Variation and Influence of Sampling Technique. *Sci. Total Environ.* **2021**, *752*, 141880. [CrossRef]
68. Blair, R.; Waldron, S.; Gauchotte-Lindsay, C. Average Daily Flow of Microplastics through a Tertiary Wastewater Treatment Plant over a Ten-Month Period. *Water Res.* **2019**, *163*, 114909. [CrossRef]
69. Brooks, J.M.; Stewart, C.J.; Haberstroh, C.J.; Arias, M.E. Characteristics and Fate of Plastic Pollution in Urban Stormwater Ponds. *Environ. Pollut.* **2023**, *320*, 121052. [CrossRef]
70. Bydalek, F.; Ifayemi, D.; Reynolds, L.; Barden, R.; Kasprzyk-Hordern, B.; Wenk, J. Microplastic Dynamics in a Free Water Surface Constructed Wetland. *Sci. Total Environ.* **2023**, *858*, 160113. [CrossRef]
71. Carr, S.; Liu, J.; Tesoro, A. Transport and Fate of Microplastic Particles in Wastewater Treatment Plants. *Water Res.* **2016**, *91*, 174–182. [CrossRef]
72. Chand, R.; Kohansal, K.; Toor, S.; Pedersen, T.; Vollertsen, J. Microplastics degradation through hydrothermal liquefaction of wastewater treatment sludge. *J. Clean. Prod.* **2022**, *335*, 130383. [CrossRef]

73. Chen, B.; Chen, Z.; Liu, Y.; Zhu, S.; Cai, X. Effects of Garbage Salvaging and Suspended Crossbar on Microplastic Pollution along a Typical Urban River. *Environ. Geochem. Health* **2022**, *44*, 3239–3248. [CrossRef] [PubMed]
74. Chen, Y.; Li, T.; Hu, H.; Ao, H.; Xiong, X.; Shi, H.; Wu, C. Transport and Fate of Microplastics in Constructed Wetlands: A Microcosm Study. *J. Hazard. Mater.* **2021**, *415*, 125615. [CrossRef] [PubMed]
75. Conley, K.; Clum, A.; Deepe, J.; Lane, H.; Beckingham, B. Wastewater Treatment Plants as a Source of Microplastics to an Urban Estuary: Removal Efficiencies and Loading per Capita over One Year. *Water Res. X* **2019**, *3*, 100030. [CrossRef] [PubMed]
76. Cunha, C.; Silva, L.; Paulo, J.; Faria, M.; Nogueira, N.; Cordeiro, N. Microalgal-Based Biopolymer for Nano- and Microplastic Removal: A Possible Biosolution for Wastewater Treatment. *Environ. Pollut.* **2020**, *263*, 114385. [CrossRef] [PubMed]
77. Cunha, C.; Faria, M.; Nogueira, N.; Ferreira, A.; Cordeiro, N. Marine vs Freshwater Microalgae Exopolymers as Biosolutions to Microplastics Pollution. *Environ. Pollut.* **2019**, *249*, 372–380. [CrossRef] [PubMed]
78. Darvishi, G.; Ehteshami, M.; Mehrdadi, N.; Abedini, R. Identification and Analysis of Plastic Microparticles in the Inlet and Outlet of the Wastewater Treatment Plant and Investigation of the Relationship between Different Seasons of the Year with the Amount of Production and Emission of Particles. *Adv. Mater. Sci. Eng.* **2022**, *2022*, 8527899. [CrossRef]
79. De Falco, F.; Di Pace, E.; Avella, M.; Gentile, G.; Errico, M.; Krzan, A.; ElKhiar, H.; Zupan, M.; Cocca, M. Development and Performance Evaluation of a Filtration System for Washing Machines to Reduce Microfiber Release in Wastewater. *Water Air Soil Pollut.* **2021**, *232*, 406. [CrossRef]
80. Dou, Y.; Cheng, X.; Miao, M.; Wang, T.; Hao, T.; Zhang, Y.; Li, Y.; Ning, X.; Wang, Q. The Impact of Chlorination on the Tetracycline Sorption Behavior of Microplastics in Aqueous Solution. *Sci. Total Environ.* **2022**, *849*, 157800. [CrossRef]
81. Edo, C.; González-Pleiter, M.; Leganés, F.; Fernández-Piñas, F.; Rosal, R. Fate of Microplastics in Wastewater Treatment Plants and Their Environmental Dispersion with Effluent and Sludge. *Environ. Pollut.* **2020**, *259*, 113837. [CrossRef]
82. Elkhatib, D.; Oyanedel-Craver, V.; Carissimi, E. Electrocoagulation Applied for the Removal of Microplastics from Wastewater Treatment Facilities. *Sep. Purif. Technol.* **2021**, *276*, 118877. [CrossRef]
83. Erdle, L. Microfibers in a Freshwater Ecosystem: Sources, Fate, Effects, and Mitigation. Ph.D. Thesis, University of Toronto, Toronto, ON, Canada, 2022.
84. Fan, L.; Mohseni, A.; Schmidt, J.; Evans, B.; Murdoch, B.; Gao, L. Efficiency of Lagoon-Based Municipal Wastewater Treatment in Removing Microplastics. *Sci. Total Environ.* **2023**, *876*, 162714. [CrossRef] [PubMed]
85. Gallitelli, L.; Di Lollo, G.; Adduce, C.; Maggi, M.R.; Trombetta, B.; Scalici, M. Aquatic Plants Entrap Different Size of Plastics in Indoor Flume Experiments. *Sci. Total Environ.* **2023**, *863*, 161051. [CrossRef]
86. Gilbreath, A.; McKee, L.; Shimabuku, I.; Lin, D.; Werbowski, L.; Zhu, X.; Grbic, J.; Rochman, C. Multiyear Water Quality Performance and Mass Accumulation of PCBs, Mercury, Methylmercury, Copper, and Microplastics in a Bioretention Rain Garden. *J. Sustain. Water Built Environ.* **2019**, *5*, 04019004–1. [CrossRef]
87. Gkanasos, A.; Tsiaras, K.; Triantaphyllidis, G.; Panagopoulos, A.; Pantazakos, G.; Owens, T.; Karametsis, C.; Pollani, A.; Nikoli, E.; Katsafados, N.; et al. Stopping Macroplastic and Microplastic Pollution at Source by Installing Novel Technologies in River Estuaries and Waste Water Treatment Plants: The CLAIM Project. *Front. Mar. Sci.* **2021**, *8*, 738876. [CrossRef]
88. Herbort, A.F.; Sturm, M.T.; Schuhen, K. A New Approach for the Agglomeration and Subsequent Removal of Polyethylene, Polypropylene, and Mixtures of Both from Freshwater Systems—A Case Study. *Environ. Sci. Pollut. Res.* **2018**, *25*, 15226–15234. [CrossRef] [PubMed]
89. Herbort, A.; Sturm, M.; Fiedler, S.; Abkai, G.; Schuhen, K. Alkoxy-Silyl Induced Agglomeration: A New Approach for the Sustainable Removal of Microplastic from Aquatic Systems. *J. Polym. Environ.* **2018**, *26*, 4258–4270. [CrossRef]
90. Hidayaturrahman, H.; Lee, T.-G. A Study on Characteristics of Microplastic in Wastewater of South Korea: Identification, Quantification, and Fate of Microplastics during Treatment Process. *Mar. Pollut. Bull.* **2019**, *146*, 696–702. [CrossRef]
91. Hongprasith, N.; Kittimethawong, C.; Lertluksanaporn, R.; Eamchotchawalit, T.; Kittipongvises, S.; Lohwacharin, J. IR Microspectroscopic Identification of Microplastics in Municipal Wastewater Treatment Plants. *Environ. Sci. Pollut. Res.* **2020**, *27*, 18557–18564. [CrossRef]
92. Hsieh, L.; He, L.; Zhang, M.; Lv, W.; Yang, K.; Tong, M. Addition of Biochar as Thin Preamble Layer into Sand Filtration Columns Could Improve the Microplastics Removal from Water. *Water Res.* **2022**, *221*, 118783. [CrossRef]
93. Hu, K.; Zhou, P.; Yang, Y.; Hall, T.; Nie, G.; Yao, Y.; Duan, X.; Wang, S. Degradation of Microplastics by a Thermal Fenton Reaction. *ACS ES&T Eng.* **2021**, *2*, 110–120. [CrossRef]
94. Hu, P.; Su, K.; Sun, Y.; Li, P.; Cai, J.; Yang, H. Efficient Removal of Nano- and Micro- Sized Plastics Using a Starch-Based Coagulant in Conjunction with Polysilicic Acid. *Sci. Total Environ.* **2022**, *850*, 157829. [CrossRef] [PubMed]
95. Järlskog, I.; Strömvall, A.-M.; Magnusson, K.; Gustafsson, M.; Polukarova, M.; Galfi, H.; Aronsson, M.; Andersson-Sköld, Y. Occurrence of Tire and Bitumen Wear Microplastics on Urban Streets and in Sweepsand and Washwater. *Sci. Total Environ.* **2020**, *729*, 138950. [CrossRef] [PubMed]
96. Järlskog, I.; Strömvall, A.-M.; Magnusson, K.; Galfi, H.; Björklund, K.; Polukarova, M.; Garção, R.; Markiewicz, A.; Aronsson, M.; Gustafsson, M.; et al. Traffic-Related Microplastic Particles, Metals, and Organic Pollutants in an Urban Area under Reconstruction. *Sci. Total Environ.* **2021**, *774*, 145503. [CrossRef] [PubMed]
97. Jiang, F.; Wang, M.; Ding, J.; Cao, W.; Sun, C. Occurrence and Seasonal Variation of Microplastics in the Effluent from Wastewater Treatment Plants in Qingdao, China. *J. Mar. Sci. Eng.* **2022**, *10*, 58. [CrossRef]

98. Jiang, H.; Bu, J.; Bian, K.; Su, J.; Wang, Z.; Sun, H.; Wang, H.; Zhang, Y.; Wang, C. Surface Change of Microplastics in Aquatic Environment and the Removal by Froth Flotation Assisted with Cationic and Anionic Surfactants. *Water Res.* **2023**, *233*, 119794. [CrossRef] [PubMed]
99. Jiang, H.; Zhang, Y.; Bian, K.; Wang, C.; Xie, X.; Wang, H.; Zhao, H. Is It Possible to Efficiently and Sustainably Remove Microplastics from Sediments Using Froth Flotation? *Chem. Eng. J.* **2022**, *448*, 137692. [CrossRef]
100. Jiang, L.; Chen, M.; Huang, Y.; Peng, J.; Zhao, J.; Chan, F.; Yu, X. Effects of Different Treatment Processes in Four Municipal Wastewater Treatment Plants on the Transport and Fate of Microplastics. *Sci. Total Environ.* **2022**, *831*, 154946. [CrossRef]
101. Jiang, L.; Yin, M.; Tang, Y.; Dai, R.; Mo, L.; Yang, W.; Liang, Y.; Huang, K. Microfibers Shed from Synthetic Textiles during Laundry: Flow to Wastewater Treatment Plants or Release to Receiving Waters through Storm Drains? *Process Saf. Environ. Prot.* **2022**, *168*, 689–697. [CrossRef]
102. Johnson, A.C.; Ball, H.; Cross, R.; Horton, A.A.; Jürgens, M.D.; Read, D.S.; Vollertsen, J.; Svendsen, C. Identification and Quantification of Microplastics in Potable Water and Their Sources within Water Treatment Works in England and Wales. *Environ. Sci. Technol.* **2020**, *54*, 12326. [CrossRef]
103. Kara, N.; Erkan, H.; Engin, G. Characterization and Removal of Microplastics in Landfill Leachate Treatment Plants in Istanbul, Turkey. *Anal. Lett.* **2023**, *56*, 1535–1548. [CrossRef]
104. Keawchouy, S.; Na-Phatthalung, W.; Keaonaborn, D.; Jaichuedee, J.; Musikavong, C.; Sinyoung, S. Enhanced Coagulation Process for Removing Dissolved Organic Matter, Microplastics, and Silver Nanoparticles. *J. Environ. Sci. Health—Part A Toxic/Hazard. Subst. Environ. Eng.* **2022**, *57*, 1084–1098. [CrossRef] [PubMed]
105. Kelly, J.J.; London, M.G.; McCormick, A.R.; Rojas, M.; Scott, J.W.; Hoellein, T.J. Wastewater Treatment Alters Microbial Colonization of Microplastics. *PLoS ONE* **2021**, *16*, e0244443. [CrossRef] [PubMed]
106. Kittipongvises, S.; Phetrak, A.; Hongprasith, N.; Lohwacharin, J. Unravelling Capability of Municipal Wastewater Treatment Plant in Thailand for Microplastics: Effects of Seasonality on Detection, Fate and Transport. *J. Environ. Manag.* **2022**, *302*, 113990. [CrossRef] [PubMed]
107. Kılıç, E.; Yücel, N.; Şahutoğlu, S.M. Microplastic Composition, Load and Removal Efficiency from Wastewater Treatment Plants Discharging into Orontes River. *Int. J. Environ. Res.* **2023**, *17*, 25. [CrossRef]
108. Kwon, H.J.; Hidayaturrahman, H.; Shaik Gouse Peera; Lee, T.G. Elimination of Microplastics at Different Stages in Wastewater Treatment Plants. *Water* **2022**, *14*, 2404. [CrossRef]
109. Lapointe, M.; Farner, J.; Hernandez, L.M. Understanding and Improving Microplastic Removal during Water Treatment: Impact of Coagulation and Flocculation. *Environ. Sci. Technol.* **2020**, *54*, 8719–8727. [CrossRef]
110. Le, T.; Truong, T.; Nguyen, P.; Le, Q.; Tran, Q.; Le, T.; Nguyen, Q.; Kieu-Le, T.; Strady, E. Evaluation of Microplastic Removal Efficiency of Wastewater-Treatment Plants in a Developing Country, Vietnam. *Environ. Technol. Innov.* **2023**, *29*, 102994. [CrossRef]
111. Li, B.; Zhao, J.; Ge, W.; Li, W.; Yuan, H. Coagulation-Flocculation Performance and Floc Properties for Microplastics Removal by Magnesium Hydroxide and PAM. *J. Environ. Chem. Eng.* **2022**, *10*, 107263. [CrossRef]
112. Liu, B.; Gao, Y.; Yue, Q.; Guo, K.; Gao, B. The Suitability and Mechanism of Polyaluminum-Titanium Chloride Composite Coagulant (PATC) for Polystyrene Microplastic Removal: Structural Characterization and Theoretical Calculation. *Water Res.* **2023**, *232*, 119690. [CrossRef]
113. Liu, F.; Nord, N.; Bester, K.; Vollertsen, J. Microplastics Removal from Treated Wastewater by a Biofilter. *Water* **2020**, *12*, 1085. [CrossRef]
114. Liu, F.; Vianello, A.; Vollertsen, J. Retention of Microplastics in Sediments of Urban and Highway Stormwater Retention Ponds. *Environ. Pollut.* **2019**, *255*, 113335. [CrossRef] [PubMed]
115. Lofty, J.; Muhawenimana, V.; Wilson, C.; Ouro, P. Microplastics Removal from a Primary Settler Tank in a Wastewater Treatment Plant and Estimations of Contamination onto European Agricultural Land via Sewage Sludge Recycling. *Environ. Pollut.* **2022**, *304*, 119198. [CrossRef] [PubMed]
116. Long, Y.; Zhou, Z.; Yin, L.; Wen, X.; Xiao, R.; Du, L.; Zhu, L.; Liu, R.; Xu, Q.; Li, H.; et al. Microplastics Removal and Characteristics of Constructed Wetlands WWTPs in Rural Area of Changsha, China: A Different Situation from Urban WWTPs. *Sci. Total Environ.* **2022**, *811*, 152352. [CrossRef] [PubMed]
117. Long, Y.; Zhou, Z.; Wen, X.; Wang, J.; Xiao, R.; Wang, W.; Li, X.; Lai, X.; Zhang, Y.; Deng, C.; et al. Microplastics Removal and Characteristics of a Typical Multi-Combination and Multi-Stage Constructed Wetlands Wastewater Treatment Plant in Changsha, China. *Chemosphere* **2023**, *312*, 137199. [CrossRef] [PubMed]
118. Lu, H.; Ziajahromi, S.; Locke, A.; Neale, P.; Leusch, F. Microplastics Profile in Constructed Wetlands: Distribution, Retention and Implications. *Environ. Pollut.* **2022**, *313*, 120079. [CrossRef]
119. Lv, X.; Dong, Q.; Zuo, Z.; Liu, Y.; Huang, X.; Wu, W.-M. Microplastics in a Municipal Wastewater Treatment Plant: Fate, Dynamic Distribution, Removal Efficiencies, and Control Strategies. *J. Clean. Prod.* **2019**, *225*, 579–586. [CrossRef]
120. Masselter, T.; Schaumann, U.; Kampowski, T.; Ulrich, K.; Thielen, M.; Bold, G.; Speck, T. Improvement of a microfiber filter for domestic washing machines. *Bioinspir. Biomim.* **2022**, *18*, 016017. [CrossRef]
121. Napper, I.; Barrett, A.; Thompson, R. The Efficiency of Devices Intended to Reduce Microfibre Release during Clothes Washing. *Sci. Total Environ.* **2020**, *738*, 140412. [CrossRef]

122. Nie, C.; Yang, J.; Sang, C.; Xia, Y.; Huang, K. Reduction Performance of Microplastics and Their Behavior in a Vermi-Wetland during the Recycling of Excess Sludge: A Quantitative Assessment for Fluorescent Polymethyl Methacrylate. *Sci. Total Environ.* **2022**, *832*, 155005. [CrossRef]
123. Nkosi, S.D.; Malinga, S.P.; Mabuba, N. Microplastics and Heavy Metals Removal from Fresh Water and Wastewater Systems Using a Membrane. *Separations* **2022**, *9*, 166. [CrossRef]
124. Okoffo, E.; Rauert, C.; Thomas, K. Mass Quantification of Microplastic at Wastewater Treatment Plants by Pyrolysis-Gas Chromatography-Mass Spectrometry. *Sci. Total Environ.* **2023**, *856*, 159251. [CrossRef]
125. Parashar, N.; Hait, S. Occurrence and Removal of Microplastics in a Hybrid Growth Sewage Treatment Plant from Bihar, India: A Preliminary Study. *J. Clean. Prod.* **2022**, *18*, 807–828. [CrossRef]
126. Pathak, V.M. Exploitation of Bacterial Strains for Microplastics (LDPE) Biodegradation. *Chemosphere* **2023**, *316*, 137845. [CrossRef]
127. Perren, W.; Wojtasik, A.; Cai, Q. Removal of Microbeads from Wastewater Using Electrocoagulation. *ACS Omega* **2018**, *3*, 3357–3364. [CrossRef] [PubMed]
128. Peydayesh, M.; Suta, T.; Usuelli, M.; Handschin, S.; Canelli, G.; Bagnani, M.; Mezzenga, R. Sustainable Removal of Microplastics and Natural Organic Matter from Water by Coagulation–Flocculation with Protein Amyloid Fibrils. *Environ. Sci. Technol.* **2021**, *55*, 8848. [CrossRef]
129. Priyanka, M.; Saravanakumar, M.P. A Sustainable Approach for Removal of Microplastics from Water Matrix Using Colloidal Gas Aphrons: New Insights on Flotation Potential and Interfacial Mechanism. *J. Clean. Prod.* **2022**, *334*, 130198. [CrossRef]
130. Radityaningrum, A.; Trihadiningrum, Y.; Mar'atusholihah; Soedjono, E.; Herumurti, W. Microplastic Contamination in Water Supply and the Removal Efficiencies of the Treatment Plants: A Case of Surabaya City, Indonesia. *J. Water Process Eng.* **2021**, *43*, 102195. [CrossRef]
131. Ren, P.; Dou, M.; Wang, C.; Li, G.; Jia, R. Abundance and Removal Characteristics of Microplastics at a Wastewater Treatment Plant in Zhengzhou. *Environ. Sci. Pollut. Res.* **2020**, *27*, 36295–36305. [CrossRef]
132. Rozman, U.; Klun, B.; Kalčíková, G. Distribution and Removal of Microplastics in a Horizontal Sub-Surface Flow Laboratory Constructed Wetland and Their Effects on the Treatment Efficiency. *Chem. Eng. J.* **2023**, *461*, 142076. [CrossRef]
133. Rusthoven, I.R.; O'Reilly, C. *The Effects of Sand, Cloth, and No Tertiary Filtration on Microplastic Particles*; Illinois State University: Normal, IL, USA, 2019.
134. Salmi, P.; Ryymin, K.; Karjalainen, A.K.; Mikola, A.; Uurasjärvi, E.; Talvitie, J. Particle Balance and Return Loops for Microplastics in a Tertiary-Level Wastewater Treatment Plant. *Water Sci. Technol.* **2021**, *84*, 89–100. [CrossRef] [PubMed]
135. Sarcletti, M.; Park, H.; Wirth, J.; Englisch, S.; Eigen, A.; Drobek, D.; Vivod, D.; Friedrich, B.; Tietze, R.; Alexiou, C.; et al. The Remediation of Nano-/Microplastics from Water. *Mater. Today* **2021**, *48*, 38–46. [CrossRef]
136. Sarkar, D.J.; Das Sarkar, S.; Das, B.K.; Sahoo, B.K.; Das, A.; Nag, S.K.; Manna, R.K.; Behera, B.K.; Samanta, S. Occurrence, Fate and Removal of Microplastics as Heavy Metal Vector in Natural Wastewater Treatment Wetland System. *Water Res.* **2021**, *192*, 116853. [CrossRef] [PubMed]
137. Schell, T.; Hurley, R.; Nizzetto, L.; Rico, A.; Vighi, M. Spatio-Temporal Distribution of Microplastics in a Mediterranean River Catchment: The Importance of Wastewater as an Environmental Pathway. *J. Hazard. Mater.* **2021**, *420*, 126481. [CrossRef] [PubMed]
138. Schmidt, C.; Kumar, R.; Yang, S.; Büttner, O. Microplastic Particle Emission from Wastewater Treatment Plant Effluents into River Networks in Germany: Loads, Spatial Patterns of Concentrations and Potential Toxicity. *Sci. Total Environ.* **2020**, *737*, 139544. [CrossRef] [PubMed]
139. Schmidtmann, J.; Elagami, H.; Gilfedder, B.S.; Fleckenstein, J.H.; Papastavrou, G.; Mansfeld, U.; Peiffer, S. Heteroaggregation of PS Microplastic with Ferrihydrite Leads to Rapid Removal of Microplastic Particles from the Water Column. *Environ. Sci. Process. Impacts* **2022**, *24*, 1782–1789. [CrossRef]
140. Shen, M.; Hu, T.; Huang, W.; Song, B.; Qin, M.; Yi, H.; Zeng, G.; Zhang, Y. Can Incineration Completely Eliminate Plastic Wastes? An Investigation of Microplastics and Heavy Metals in the Bottom Ash and Fly Ash from an Incineration Plant. *Sci. Total Environ.* **2021**, *779*, 146528. [CrossRef]
141. Shi, X.; Zhang, X.; Gao, W.; Zhang, Y.; He, D. Removal of Microplastics from Water by Magnetic Nano-Fe_3O_4. *Sci. Total Environ.* **2022**, *802*, 149838. [CrossRef]
142. Shnada, A.H. Bioremediation of Microplastic using Microbes Isolated from Mangrove Sediments. Master's Thesis, Universiti Malaya, Kuala Lumpur, Malaysia, 2018. Available online: http://studentsrepo.um.edu.my/ (accessed on 29 July 2023).
143. Smyth, K.; Drake, J.; Li, Y.; Rochman, C.; Van Seters, T.; Passeport, E. Bioretention Cells Remove Microplastics from Urban Stormwater. *Water Res.* **2021**, *191*, 116785. [CrossRef]
144. Sotiropoulou, M.; Stefanatou, A.; Schiza, S.; Petousi, I.; Stasinakis, A.S.; Fountoulakis, M.S. Removal of Microfiber in Vertical Flow Constructed Wetlands Treating Greywater. *Sci. Total Environ.* **2023**, *858*, 159723. [CrossRef]
145. Sun, X.; Chen, Z.Y.; Kong, T.; Chen, Z.; Dong, Y.; Kolton, M.; Cao, Z.; Zhang, X.; Zhang, H.; Liu, G.; et al. Mycobacteriaceae Mineralizes Micropolyethylene in Riverine Ecosystems. *Environ. Sci. Technol.* **2022**, *56*, 15705–15717. [CrossRef] [PubMed]
146. Swart, B.; Pihlajamaki, A.; Chew, Y.; Wenk, J. Microbubble-Microplastic Interactions in Batch Air Flotation. *Chem. Eng. J.* **2022**, *449*, 137866. [CrossRef]
147. Tadsuwan, K.; Babel, S. Microplastic Abundance and Removal via an Ultrafiltration System Coupled to a Conventional Municipal Wastewater Treatment Plant in Thailand. *J. Environ. Chem. Eng.* **2022**, *10*, 107142. [CrossRef]

148. Tang, Y.; Zhang, S.; Su, Y.; Wu, D.; Zhao, Y.; Xie, B. Removal of Microplastics from Aqueous Solutions by Magnetic Carbon Nanotubes. *Chem. Eng. J.* **2021**, *406*, 126804. [CrossRef]
149. Tapeh, N.G.; Fataei, E.; Naji, A.; Imani, A.A.; Nasehi, F. Municipal Wastewater Efluent as a Source of Microplastic Pollution Crisis in the Qarasu River Water, Ardabil Province, Iran. *Health Emerg. Disaster Q.* **2022**, *7*, 117–126. [CrossRef]
150. Thaiyal Nayahi, N.; Ou, B.; Liu, Y.; Janjaroen, D. Municipal Solid Waste Sanitary and Open Landfills: Contrasting Sources of Microplastics and Its Fate in Their Respective Treatment Systems. *J. Clean. Prod.* **2022**, *380*, 135095. [CrossRef]
151. Uddin, S.; Behbehani, M.; Habibi, N.; Faizuddin, M.; Al-Murad, M.; Martinez-Guijarro, K.; Al-Sarawi, H.A.; Karam, Q. Microplastics in Kuwait's Wastewater Streams. *Sustainability* **2022**, *14*, 15817. [CrossRef]
152. Uoginte, I.; Pleskyte, S.; Pauraite, J.; Lujaniene, G. Seasonal Variation and Complex Analysis of Microplastic Distribution in Different WWTP Treatment Stages in Lithuania. *Environ. Monit. Assess.* **2022**, *194*, 829. [CrossRef]
153. Venghaus, D.; Neupert, J.W.; Barjenbruch, M. Evaluation of a Modular Filter Concept to Reduce Microplastics and Other Solids from Urban Stormwater Runoff. *Water* **2023**, *15*, 506. [CrossRef]
154. Wan, X.; Huang, H.; Liao, Z.; He, H.; Yue, Q.; Zhao, F.; Huang, H.; Huang, B.; Pan, X. The Distribution and Risk of Microplastics Discharged from Sewage Treatment Plants in Terrestrial and Aquatic Compartment. *J. Environ. Manag.* **2022**, *314*, 115067. [CrossRef]
155. Wang, F.; Wang, B.; Duan, L.; Zhang, Y.; Zhou, Y.; Sui, Q.; Xu, D.; Qu, H.; Yu, G. Occurrence and Distribution of Microplastics in Domestic, Industrial, Agricultural and Aquacultural Wastewater Sources: A Case Study in Changzhou, China. *Water Res.* **2020**, *182*, 115956. [CrossRef] [PubMed]
156. Wang, Q.; Hernandez-Crespo, C.; Du, B.; Van Hulle, S.; Rousseau, D. Fate and Removal of Microplastics in Unplanted Lab-Scale Vertical Flow Constructed Wetlands. *Sci. Total Environ.* **2021**, *778*, 146152. [CrossRef] [PubMed]
157. Wang, Q.; Hernandez-Crespo, C.; Santoni, M.; Van Hulle, S.; Rousseau, D. Horizontal Subsurface Flow Constructed Wetlands as Tertiary Treatment: Can They Be an Efficient Barrier for Microplastics Pollution? *Sci. Total Environ.* **2020**, *721*, 137785. [CrossRef] [PubMed]
158. Wang, R.; Ji, M.; Zhai, H.; Liu, Y. Occurrence of Phthalate Esters and Microplastics in Urban Secondary Effluents, Receiving Water Bodies and Reclaimed Water Treatment Processes. *Sci. Total Environ.* **2020**, *737*, 140219. [CrossRef] [PubMed]
159. Wang, S.; Shi, W.; Huang, Z.; Zhou, N.; Xie, Y.; Tang, Y.; Tang, Y. Complete Digestion/Biodegradation of Polystyrene Microplastics by Greater Wax Moth (*Galleria Mellonella*) Larvae: Direct in Vivo Evidence, Gut Microbiota independence, and potential metabolic pathways. *J. Hazard. Mater.* **2022**, *423*, 127213. [CrossRef] [PubMed]
160. Wang, Y.; Li, Y.; Tian, L.; Ju, L.; Liu, Y. The Removal Efficiency and Mechanism of Microplastic Enhancement by Positive Modification Dissolved Air Flotation. *Water Environ. Res.* **2021**, *93*, 693–702. [CrossRef] [PubMed]
161. Watkins, L.; Sullivan, P.J.; Walter, M.T. A Case Study Investigating Temporal Factors That Influence Microplastic Concentration in Streams under Different Treatment Regimes. *Environ. Sci. Pollut. Res.* **2019**, *26*, 21797–21807. [CrossRef]
162. Wei, S.; Luo, H.; Zou, J.; Chen, J.; Pan, X.; Rousseau, D.; Li, J. Characteristics and Removal of Microplastics in Rural Domestic Wastewater Treatment Facilities of China. *Sci. Total Environ.* **2020**, *739*, 139935. [CrossRef]
163. Wei, X.-F.; Bohlén, M.; Lindblad, C.; Hedenqvist, M.; Hakonen, A. Microplastics Generated from a Biodegradable Plastic in Freshwater and Seawater. *Water Res.* **2021**, *198*, 117123. [CrossRef]
164. Werbowski, L.; Gilbreath, A.; Munno, K.; Zhu, X.; Grbic, J.; Wu, T.; Sutton, R.; Sedlak, M.; Deshpande, A.; Rochman, C. Urban Stormwater Runoff: A Major Pathway for Anthropogenic Particles, Black Rubbery Fragments, and Other Types of Microplastics to Urban Receiving Waters. *ACS EST Water* **2021**, *1*, 1420–1428. [CrossRef]
165. White, E.M.; Horn, J.; Wang, S.; Crawford, B.; Ritchie, B.W.; Carraway, D.; Locklin, J. Comparative Study of the Biological Degradation of Poly(3-Hydroxybutyrate-Co-3-Hydroxyhexanoate) Microbeads in Municipal Wastewater in Environmental and Controlled Laboratory Conditions. *Environ. Sci. Technol.* **2021**, *55*, 11646–11656. [CrossRef] [PubMed]
166. Xia, F.; Liu, H.; Zhang, J.; Wang, D. Migration Characteristics of Microplastics Based on Source-Sink Investigation in a Typical Urban Wetland. *Water Res.* **2022**, *213*, 118154. [CrossRef] [PubMed]
167. Xu, X.; Zhang, L.; Jian, Y.; Xue, Y.; Gao, Y.; Peng, M.; Jiang, S.; Zhang, Q. Influence of Wastewater Treatment Process on Pollution Characteristics and Fate of Microplastics. *Mar. Pollut. Bull.* **2021**, *169*, 112448. [CrossRef] [PubMed]
168. Xu, X.; Jian, Y.; Xue, Y.; Hou, Q.; Wang, L. Microplastics in the Wastewater Treatment Plants (WWTPs): Occurrence and Removal. *Chemosphere* **2019**, *235*, 1089–1096. [CrossRef] [PubMed]
169. Xu, Y.; Ou, Q.; Wang, X.; Hou, F.; Li, P.; van der Hoek, J.P.; Liu, G. Assessing the Mass Concentration of Microplastics and Nanoplastics in Wastewater Treatment Plants by Pyrolysis Gas Chromatography-Mass Spectrometry. *Environ. Sci. Technol.* **2023**, *57*, 3114–3123. [CrossRef]
170. Yang, J.; Monnot, M.; Sun, Y.; Asia, L.; Wong-Wah-Chung, P.; Doumenq, P.; Moulin, P. Microplastics in Different Water Samples (Seawater, Freshwater, and Wastewater): Removal Efficiency of Membrane Treatment Processes. *Water Res.* **2023**, *232*, 119673. [CrossRef]
171. Yang, J.; Chen, Z.; Xu, D.; Liu, P.; Li, L. Enhanced Interfacial Adhesion of Polystyrene Bead Foams by Microwave Sintering for Microplastics Reduction. *Ind. Eng. Chem. Res.* **2021**, *60*, 8812–8820. [CrossRef]
172. Yuan, F.; Yue, L.; Zhao, H.; Wu, H. Study on the Adsorption of Polystyrene Microplastics by Three-Dimensional Reduced Graphene Oxide. *Water Sci. Technol.* **2020**, *81*, 2163–2175. [CrossRef]

173. Yuan, F.; Zhao, H.; Sun, H.; Zhao, J.; Sun, Y. Abundance, Morphology, and Removal Efficiency of Microplastics in Two Wastewater Treatment Plants in Nanjing, China. *Environ. Sci. Pollut. Res.* **2021**, *28*, 9327–9337. [CrossRef]
174. Yuan, F.; Zhao, H.; Sun, H.; Sun, Y.; Zhao, J.; Xia, T. Investigation of Microplastics in Sludge from Five Wastewater Treatment Plants in Nanjing, China. *J. Environ. Manag.* **2022**, *301*, 113793. [CrossRef]
175. Zhang, E.; Stocchino, A.; De Leo, A.; Fang, J.K.-H. Performance Assessment of Bubbles Barriers for Microplastic Remediation. *Sci. Total Environ.* **2022**, *844*, 157027. [CrossRef] [PubMed]
176. Zhang, M.; Yang, J.; Kang, Z.; Wu, X.; Tang, L.; Qiang, Z.; Zhang, D.; Pan, X. Removal of Micron-Scale Microplastic Particles from Different Waters with Efficient Tool of Surface-Functionalized Microbubbles. *J. Hazard. Mater.* **2021**, *404*, 124095. [CrossRef]
177. Zhang, Y.; Zhou, G.; Yue, J.; Xing, X.; Yang, Z.; Wang, X.; Wang, Q.; Zhang, J. Enhanced Removal of Polyethylene Terephthalate Microplastics through Polyaluminum Chloride Coagulation with Three Typical Coagulant Aids. *Sci. Total Environ.* **2021**, *800*, 149589. [CrossRef] [PubMed]
178. Zhang, Y.; Zhao, J.; Liu, Z.; Tian, S.; Lu, J.; Mu, R.; Yuan, H. Coagulation Removal of Microplastics from Wastewater by Magnetic Magnesium Hydroxide and PAM. *J. Water Process Eng.* **2021**, *43*, 102250. [CrossRef]
179. Zhou, G.; Wang, Q.; Li, J.; Li, Q.; Xu, H.; Ye, Q.; Wang, Y.; Shu, S.; Zhang, J. Removal of Polystyrene and Polyethylene Microplastics Using PAC and FeCl3 Coagulation: Performance and Mechanism. *Sci. Total Environ.* **2021**, *752*, 141837. [CrossRef] [PubMed]
180. Zhou, H.; Zhou, L.; Ma, K. Microfiber from Textile Dyeing and Printing Wastewater of a Typical Industrial Park in China: Occurrence, Removal and Release. *Sci. Total Environ.* **2020**, *739*, 140329. [CrossRef] [PubMed]
181. Zhou, X.; Zhao, Y.; Pang, G.; Jia, X.; Song, Y.; Guo, A.; Wang, A.; Zhang, S.; Ji, M. Microplastic Abundance, Characteristics and Removal in Large-Scale Multi-Stage Constructed Wetlands for Effluent Polishing in Northern China. *Chem. Eng. J.* **2022**, *430*, 132752. [CrossRef]
182. Ziajahromi, S.; Drapper, D.; Hornbuckle, A.; Rintoul, L.; Leusch, F.D.L. Microplastic Pollution in a Stormwater Floating Treatment Wetland: Detection of Tyre Particles in Sediment. *Sci. Total Environ.* **2020**, *713*, 136356. [CrossRef]
183. Zou, Y.; Ye, C.; Pan, Y. Abundance and Characteristics of Microplastics in Municipal Wastewater Treatment Plant Effluent: A Case Study of Guangzhou, China. *Environ. Sci. Pollut. Res.* **2021**, *28*, 11572–11585. [CrossRef]
184. Sorensen, R.M.; Jovanović, B. From nanoplastic to microplastic: A bibliometric analysis on the presence of plastic particles in the environment. *Mar. Pollut. Bull.* **2021**, *163*, 111926. [CrossRef]
185. Blettler, M.C.; Abrial, E.; Khan, F.R.; Sivri, N.; Espinola, L.A. Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. *Water Res.* **2018**, *143*, 416–424. [CrossRef] [PubMed]
186. Li, J.; Liu, H.; Chen, J.P. Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water Res.* **2018**, *137*, 362–374. [CrossRef] [PubMed]
187. Horton, A.A.; Walton, A.; Spurgeon, D.J.; Lahive, E.; Svendsen, C. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total. Environ.* **2017**, *586*, 127–141. [CrossRef] [PubMed]
188. Zhou, Y.; Li, Y.; Yan, Z.; Wang, H.; Chen, H.; Zhao, S.; Zhong, N.; Cheng, Y.; Acharya, K. Microplastics discharged from urban drainage system: Prominent contribution of sewer overflow pollution. *Water Res.* **2023**, *236*, 119976. [CrossRef] [PubMed]
189. Zhou, Y.; Ashokkumar, V.; Amobonye, A.; Bhattacharjee, G.; Sirohi, R.; Singh, V.; Flora, G.; Kumar, V.; Pillai, S.; Zhang, Z.; et al. Current research trends on cosmetic microplastic pollution and its impacts on the ecosystem: A review. *Environ. Pollut.* **2023**, *320*, 121106. [CrossRef] [PubMed]
190. Rani, A. Types and Sources of Microplastics; The Ubiquitous Environment Contaminant: A Review. *J. Polym. Mater.* **2022**, *39*, 17–35. [CrossRef]
191. Emadian, S.M.; Onay, T.T.; Demirel, B. Biodegradation of bioplastics in natural environments. *Waste Manag.* **2017**, *59*, 526–536. [CrossRef]
192. Priya, A.; Anusha, G.; Thanigaivel, S.; Karthick, A.; Mohanavel, V.; Velmurugan, P.; Balasubramanian, B.; Ravichandran, M.; Kamyab, H.; Kirpichnikova, I.M.; et al. Removing microplastics from wastewater using leading-edge treatment technologies: A solution to microplastic pollution—A review. *Bioprocess. Biosyst. Eng.* **2023**, *46*, 309–321. [CrossRef]
193. Vercauteren, M.; Semmouri, I.; Van Acker, E.; Pequeur, E.; Janssen, C.R.; Asselman, J. Toward a Better Understanding of the Contribution of Wastewater Treatment Plants to Microplastic Pollution in Receiving Waterways. *Environ. Toxicol. Chem.* **2023**, *42*, 642–654. [CrossRef]
194. Ahmed, M.B.; Rahman, S.; Alom, J.; Hasan, S.; Johir, M.; Mondal, M.I.H.; Lee, D.-Y.; Park, J.; Zhou, J.L.; Yoon, M.-H. Microplastic particles in the aquatic environment: A systematic review. *Sci. Total. Environ.* **2021**, *775*, 145793. [CrossRef]
195. Estahbanati, M.K.; Kiendrebeogo, M.; Mostafazadeh, A.K.; Drogui, P.; Tyagi, R. Treatment processes for microplastics and nanoplastics in waters: State-of-the-art review. *Mar. Pollut. Bull.* **2021**, *168*, 112374. [CrossRef] [PubMed]
196. Bodzek, M.; Pohl, A. Removal of microplastics in unit processes used in water and wastewater treatment: A review. *Arch. Environ. Prot.* **2022**, *48*, 102–128. [CrossRef]
197. Hatinoğlu, M.D.; Sanin, F.D. Sewage sludge as a source of microplastics in the environment: A review of occurrence and fate during sludge treatment. *J. Environ. Manag.* **2021**, *295*, 113028. [CrossRef] [PubMed]
198. Blair, R.M.; Waldron, S.; Phoenix, V.; Gauchotte-Lindsay, C. Micro- and Nanoplastic Pollution of Freshwater and Wastewater Treatment Systems. *Springer Sci. Rev.* **2017**, *5*, 19–30. [CrossRef]

199. Uddin, S.; Fowler, S.W.; Behbehani, M. An assessment of microplastic inputs into the aquatic environment from wastewater streams. *Mar. Pollut. Bull.* **2020**, *160*, 111538. [CrossRef] [PubMed]
200. Masiá, P.; Sol, D.; Ardura, A.; Laca, A.; Borrell, Y.J.; Dopico, E.; Laca, A.; Machado-Schiaffino, G.; Díaz, M.; Garcia-Vazquez, E. Bioremediation as a promising strategy for microplastics removal in wastewater treatment plants. *Mar. Pollut. Bull.* **2020**, *156*, 111252. [CrossRef] [PubMed]
201. Malankowska, M.; Echaide-Gorriz, C.; Coronas, J. Microplastics in marine environment: A review on sources, classification, and potential remediation by membrane technology. *Environ. Sci. Water Res. Technol.* **2021**, *7*, 243–258. [CrossRef]
202. Ni, B.-J.; Zhu, Z.-R.; Li, W.-H.; Yan, X.; Wei, W.; Xu, Q.; Xia, Z.; Dai, X.; Sun, J. Microplastics Mitigation in Sewage Sludge through Pyrolysis: The Role of Pyrolysis Temperature. *Environ. Sci. Technol. Lett.* **2020**, *7*, 961–967. [CrossRef]
203. Zulkafli, A.H.; Hassan, H.; Ahmad, M.A.; Din, A.T.M.; Wasli, S.M. Co-pyrolysis of biomass and waste plastics for production of chemicals and liquid fuel: A review on the role of plastics and catalyst types. *Arab. J. Chem.* **2023**, *16*, 104389. [CrossRef]
204. Coffin, S.; Wyer, H.; Leapman, J.C. Addressing the environmental and health impacts of microplastics requires open collaboration between diverse sectors. *PLoS Biol.* **2021**, *19*, e3000932. [CrossRef]
205. Yardy, L.D.; Al-Jaibachi, R.; Callaghan, A. Chapter 9—Microplastics in freshwater ecosystems with special reference to tropical systems: Detection, impact, and management. In *Emerging Freshwater Pollutants*; Dalu, T., Tavengwa, N.T., Eds.; Elsevier: Amsterdam, The Netherlands, 2022; pp. 151–169. [CrossRef]
206. Xu, D.; Yin, X.; Zhou, S.; Jiang, Y.; Xi, X.; Sun, H.; Wang, J. A review on the remediation of microplastics using constructed wetlands: Bibliometric, co-occurrence, current trends, and future directions. *Chemosphere* **2022**, *303*, 134990. [CrossRef] [PubMed]
207. Weis, J.S.; De Falco, F. Microfibers: Environmental Problems and Textile Solutions. *Microplastics* **2022**, *1*, 626–639. [CrossRef]
208. Wang, T.; Li, B.; Zou, X.; Wang, Y.; Li, Y.; Xu, Y.; Mao, L.; Zhang, C.; Yu, W. Emission of primary microplastics in mainland China: Invisible but not negligible. *Water Res.* **2019**, *162*, 214–224. [CrossRef] [PubMed]
209. Wang, C.; O'Connor, D.; Wang, L.; Wu, W.-M.; Luo, J.; Hou, D. Microplastics in urban runoff: Global occurrence and fate. *Water Res.* **2022**, *225*, 119129. [CrossRef]
210. Tirkey, A.; Pandey, M.; Tiwari, A.; Sahu, R.L.; Kukkar, D.; Dubey, R.; Kim, K.; Pandey, S.K. Global distribution of microplastic contaminants in aquatic environments and their remediation strategies. *Water Environ. Res.* **2022**, *94*, e10819. [CrossRef]
211. Tan, Y.; Dai, J.; Wu, X.; Wu, S.; Zhang, J. Characteristics, occurrence and fate of non-point source microplastic pollution in aquatic environments. *J. Clean. Prod.* **2022**, *341*, 130766. [CrossRef]
212. Sajid, M.; Ihsanullah, I.; Khan, M.T.; Baig, N. Nanomaterials-based adsorbents for remediation of microplastics and nanoplastics in aqueous media: A review. *Sep. Purif. Technol.* **2022**, *305*, 122453. [CrossRef]
213. Saini, A.; Sharma, J.G. Emerging microplastic contamination in ecosystem: An urge for environmental sustainability. *J. Appl. Biol. Biotechnol.* **2022**, *10*, 66–75. [CrossRef]
214. Ramasamy, R.; Subramanian, R.B. Synthetic textile and microfiber pollution: A review on mitigation strategies. *Environ. Sci. Pollut. Res.* **2021**, *28*, 41596–41611. [CrossRef]
215. Muthuvairavasamy, R. Mitigation, Management, and the Challenges Ahead. In *Microplastics: Footprints on The Earth and Their Environmental Management*; Springer International Publishing: Cham, Switzerland, 2022; pp. 67–72. [CrossRef]
216. Mitrano, D.M.; Wohlleben, W. Microplastic regulation should be more precise to incentivize both innovation and environmental safety. *Nat. Commun.* **2020**, *11*, 5324. [CrossRef]
217. Mishra, S.R.; Ahmaruzzaman, M. Microplastics: Identification, Toxicity and Their Remediation from Aqueous Streams. *Sep. Purif. Rev.* **2022**, *52*, 283–304. [CrossRef]
218. Meng, Y.; Kelly, F.J.; Wright, S.L. Advances and challenges of microplastic pollution in freshwater ecosystems: A UK perspective. *Environ. Pollut.* **2020**, *256*, 113445. [CrossRef] [PubMed]
219. Meegoda, J.N.; Hettiarachchi, M.C. A Path to a Reduction in Micro and Nanoplastics Pollution. *Int. J. Environ. Res. Public Heal.* **2023**, *20*, 5555. [CrossRef] [PubMed]
220. Luo, Z.; Zhou, X.; Su, Y.; Wang, H.; Yu, R.; Zhou, S.; Xu, E.G.; Xing, B. Environmental occurrence, fate, impact, and potential solution of tire microplastics: Similarities and differences with tire wear particles. *Sci. Total. Environ.* **2021**, *795*, 148902. [CrossRef] [PubMed]
221. López, A.F.; Fabiani, M.; Lassalle, V.; Spetter, C.; Severini, F. Critical review of the characteristics, interactions, and toxicity of micro/nanomaterials pollutants in aquatic environments. *Mar. Pollut. Bull.* **2022**, *174*, 113276. [CrossRef] [PubMed]
222. Liu, Q.; Chen, Y.; Chen, Z.; Yang, F.; Xie, Y.; Yao, W. Current status of microplastics and nanoplastics removal methods: Summary, comparison and prospect. *Sci. Total. Environ.* **2022**, *851*, 157991. [CrossRef] [PubMed]
223. Leone, G.; Moulaert, I.; Devriese, L.I.; Sandra, M.; Pauwels, I.; Goethals, P.L.; Everaert, G.; Catarino, A.I. A comprehensive assessment of plastic remediation technologies. *Environ. Int.* **2023**, *173*, 107854. [CrossRef]
224. Lamichhane, G.; Acharya, A.; Marahatha, R.; Modi, B.; Paudel, R.; Adhikari, A.; Raut, B.K.; Aryal, S.; Parajuli, N. Microplastics in environment: Global concern, challenges, and controlling measures. *Int. J. Environ. Sci. Technol.* **2023**, *20*, 4673–4694. [CrossRef]
225. Kurniawan, T.A.; Haider, A.; Ahmad, H.M.; Mohyuddin, A.; Aslam, H.M.U.; Nadeem, S.; Javed, M.; Othman, M.H.D.; Goh, H.H.; Chew, K.W. Source, occurrence, distribution, fate, and implications of microplastic pollutants in freshwater on environment: A critical review and way forward. *Chemosphere* **2023**, *325*, 138367. [CrossRef]

226. Kumar, M.; Chen, H.; Sarsaiya, S.; Qin, S.; Liu, H.; Awasthi, M.K.; Kumar, S.; Singh, L.; Zhang, Z.; Bolan, N.S.; et al. Current research trends on micro- and nano-plastics as an emerging threat to global environment: A review. *J. Hazard. Mater.* **2021**, *409*, 124967. [CrossRef]
227. Kristanti, R.A.; Hadibarata, T.; Wulandari, N.F.; Sibero, M.T.; Darmayati, Y.; Hatmanti, A. Overview of microplastics in the environment: Type, source, potential effects and removal strategies. *Bioprocess. Biosyst. Eng.* **2023**, *46*, 429–441. [CrossRef]
228. Krantzberg, G. Plastic Pollution in the Great Lakes and Marine Waters: Sources, Effects and Policy Responses. *J. Waste Resour. Recycl.* **2019**, *1*, 107.
229. Karbalaei, S.; Hanachi, P.; Walker, T.R.; Cole, M. Occurrence, sources, human health impacts and mitigation of microplastic pollution. *Environ. Sci. Pollut. Res.* **2018**, *25*, 36046–36063. [CrossRef] [PubMed]
230. Kanwal, H.; Nadeem, H.; Azeem, F.; Rasul, I.; Muzammil, S.; Zubair, M.; Imran, M.; Afzal, M.; Siddique, M.H. Chapter 5—Generation and impact of microplastics and nanoplastics from bioplastic sources. In *Biodegradability of Conventional Plastics*; Sarkar, A., Sharma, B., Shekhar, S., Eds.; Elsevier: Amsterdam, The Netherlands, 2023; pp. 83–99. [CrossRef]
231. Chaudhry, A.K.; Sachdeva, P. Microplastics' origin, distribution, and rising hazard to aquatic organisms and human health: Socio-economic insinuations and management solutions. *Reg. Stud. Mar. Sci.* **2021**, *48*, 102018. [CrossRef]
232. Geyer, R.; Gavigan, J.; Jackson, A.M.; Saccomanno, V.R.; Suh, S.; Gleason, M.G. Quantity and fate of synthetic microfiber emissions from apparel washing in California and strategies for their reduction. *Environ. Pollut.* **2022**, *298*, 118835. [CrossRef] [PubMed]
233. Grünzner, M.; Pahl, S.; White, M.P.; Thompson, R.C. Exploring expert perceptions about microplastics: From sources to potential solutions. *Microplast. Nanoplast.* **2023**, *3*, 7. [CrossRef]
234. Gupta, D.K.; Choudhary, D.; Vishwakarma, A.; Mudgal, M.; Srivastava, A.K.; Singh, A. Microplastics in freshwater environment: Occurrence, analysis, impact, control measures and challenges. *Int. J. Environ. Sci. Technol.* **2023**, *20*, 6865–6896. [CrossRef]
235. Hann, S.; Sherrington, C.; Jamieson, O.; Hickman, M.; Kershaw, P.; Bapasola, A.; Cole, G. *Investigating Options for Reducing Releases in the Aquatic Environment of Microplastics Emitted by (But Not Intentionally Added In) Products Final Report*; Report for DG Environment of the European Commission; European Commission: Brussels, Belgium, 2018.
236. Helinski, O.K.; Poor, C.J.; Wolfand, J.M. Ridding our rivers of plastic: A framework for plastic pollution capture device selection. *Mar. Pollut. Bull.* **2021**, *165*, 112095. [CrossRef]
237. Zhang, Y.; Li, Y.; Su, F.; Peng, L.; Liu, D. The life cycle of micro-nano plastics in domestic sewage. *Sci. Total. Environ.* **2022**, *802*, 149658. [CrossRef]
238. Fei, X.; He, H.; Pi, X.; Lu, X.; Chen, Q.; Ma, J.; Wang, Y.; Fang, M.; Wu, C.; Feng, S. The distribution, behavior, and release of macro- and micro-size plastic wastes in solid waste disposal sites. *Crit. Rev. Environ. Sci. Technol.* **2023**, *53*, 366–389. [CrossRef]
239. Wang, J.; Bucci, K.; Helm, P.A.; Hoellein, T.; Hoffman, M.J.; Rooney, R.; Rochman, C.M. Runoff and discharge pathways of microplastics into freshwater ecosystems: A systematic review and meta-analysis. *FACETS* **2022**, *7*, 1473–1492. [CrossRef]
240. Vaid, M.; Sarma, K.; Gupta, A. Microplastic pollution in aquatic environments with special emphasis on riverine systems: Current understanding and way forward. *J. Environ. Manag.* **2021**, *293*, 112860. [CrossRef]
241. Ray, S.S.; Lee, H.K.; Huyen, D.T.T.; Chen, S.-S.; Kwon, Y.-N. Microplastics waste in environment: A perspective on recycling issues from PPE kits and face masks during the COVID-19 pandemic. *Environ. Technol. Innov.* **2022**, *26*, 102290. [CrossRef] [PubMed]
242. Mishra, S.; Singh, R.P.; Rath, C.C.; Das, A.P. Synthetic microfibers: Source, transport and their remediation. *J. Water Process. Eng.* **2020**, *38*, 101612. [CrossRef]
243. Cera, A.; Cesarini, G.; Scalici, M. Microplastics in freshwater: What is the news from the world? *Diversity* **2020**, *12*, 276. [CrossRef]
244. Kiran, B.R.; Kopperi, H.; Venkata Mohan, S.V. Micro/nano-plastics occurrence, identification, risk analysis and mitigation: Challenges and perspectives. *Rev. Environ. Sci. Biotechnol.* **2022**, *21*, 169–203. [CrossRef] [PubMed]
245. Katare, Y.; Singh, P.; Singh Sankhla, M.; Singhal, M.; Jadhav, E.B.; Parihar, K.; Nikalje, B.T.; Trpathi, A.; Bhardwaj, L. Microplastics in Aquatic Environments: Sources, Ecotoxicity, Detection, & Remediation. *Biointerface Res. Appl. Chem.* **2021**, *12*, 3407–3428. [CrossRef]
246. Adegoke, K.A.; Adu, F.A.; Oyebamiji, A.K.; Bamisaye, A.; Adigun, R.A.; Olasoji, S.O.; Ogunjinmi, O.E. Microplastics toxicity, detection, and removal from water/wastewater. *Mar. Pollut. Bull.* **2023**, *187*, 114546. [CrossRef]
247. Azizi, N.; Pirsaheb, M.; Jaafarzadeh, N.; Nodehi, R.N. Microplastics removal from aquatic environment by coagulation: Selecting the best coagulant based on variables determined from a systematic review. *Heliyon* **2023**, *9*, e15664. [CrossRef]
248. Barchiesi, M.; Chiavola, A.; Di Marcantonio, C.; Boni, M.R. Presence and fate of microplastics in the water sources: Focus on the role of wastewater and drinking water treatment plants. *J. Water Process. Eng.* **2021**, *40*, 101787. [CrossRef]
249. Freeman, S.; Booth, A.M.; Sabbah, I.; Tiller, R.; Dierking, J.; Klun, K.; Rotter, A.; Ben-David, E.; Javidpour, J.; Angel, D.L. Between source and sea: The role of wastewater treatment in reducing marine microplastics. *J. Environ. Manag.* **2020**, *266*, 110642. [CrossRef]
250. Golmohammadi, M.; Musavi, S.F.; Habibi, M.; Maleki, R.; Golgoli, M.; Zargar, M.; Dumée, L.F.; Baroutian, S.; Razmjou, A. Molecular mechanisms of microplastics degradation: A review. *Sep. Purif. Technol.* **2023**, *309*, 122906. [CrossRef]
251. El Hajjaji, S.; Dahchour, A.; Mabrouki, J.; Assou, Y.; Alanssari, N.; Elfarissi, L.; Benqlilou, H. Investigation of Microplastics in Water and Wastewater: A Review. In *Water and Wastewater Management: Global Problems and Measures, Water and Wastewater Management*; Bahadir, M., Haarstrick, A., Eds.; Springer International Publishing: Cham, Switzerland, 2022; pp. 207–219. [CrossRef]
252. Hamidian, A.H.; Ozumchelouei, E.J.; Feizi, F.; Wu, C.; Zhang, Y.; Yang, M. A review on the characteristics of microplastics in wastewater treatment plants: A source for toxic chemicals. *J. Clean. Prod.* **2021**, *295*, 126480. [CrossRef]

253. Ho, L.; Goethals, P.L.M. Municipal wastewater treatment with pond technology: Historical review and future outlook. *Ecol. Eng.* **2020**, *148*, 105791. [CrossRef]
254. Hou, L.; Kumar, D.; Yoo, C.G.; Gitsov, I.; Majumder, E.L.-W. Conversion and removal strategies for microplastics in wastewater treatment plants and landfills. *Chem. Eng. J.* **2021**, *406*, 126715. [CrossRef]
255. Hu, Y.; Gong, M.; Wang, J.; Bassi, A. Current research trends on microplastic pollution from wastewater systems: A critical review. *Rev. Environ. Sci. Biotechnol.* **2019**, *18*, 207–230. [CrossRef]
256. Iqbal, R.; Khan, M.T.; Bilal, H.; Aslam, M.M.; Khan, I.A.; Raja, S.; Arslan, M.; Nguyen, P.M. Microplastics as vectors of environmental contaminants: Interactions in the natural ecosystems. *Hum. Ecol. Risk Assess. Int. J.* **2022**, *28*, 1022–1042. [CrossRef]
257. Jagadeesh, N.; Sundaram, B. A review of microplastics in wastewater, their persistence, interaction, and fate. *J. Environ. Chem. Eng.* **2021**, *9*, 106846. [CrossRef]
258. Junaid, M.; Liu, S.; Liao, H.; Liu, X.; Wu, Y.; Wang, J. Wastewater plastisphere enhances antibiotic resistant elements, bacterial pathogens, and toxicological impacts in the environment. *Sci. Total. Environ.* **2022**, *841*, 156805. [CrossRef]
259. Zhou, Y.; Kumar, M.; Sarsaiya, S.; Sirohi, R.; Awasthi, S.K.; Sindhu, R.; Binod, P.; Pandey, A.; Bolan, N.S.; Zhang, Z.; et al. Challenges and opportunities in bioremediation of micro-nano plastics: A review. *Sci. Total. Environ.* **2022**, *802*, 149823. [CrossRef]
260. Wani, A.K.; Akhtar, N.; Naqash, N.; Rahayu, F.; Djajadi, D.; Chopra, C.; Singh, R.; Mulla, S.I.; Sher, F.; Américo-Pinheiro, J.H.P. Discovering untapped microbial communities through metagenomics for microplastic remediation: Recent advances, challenges, and way forward. *Environ. Sci. Pollut. Res.* **2023**, *30*, 81450–81473. [CrossRef]
261. Thakur, S.; Mathur, S.; Patel, S.; Paital, B. Microplastic Accumulation and Degradation in Environment via Biotechnological Approaches. *Water* **2022**, *14*, 4053. [CrossRef]
262. Tang, K.H.D.; Hadibarata, T. The application of bioremediation in wastewater treatment plants for microplastics removal: A practical perspective. *Bioprocess Biosyst. Eng.* **2022**, *45*, 1865–1878. [CrossRef]
263. Sutradhar, M. A review of microplastics pollution and its remediation methods: Current scenario and future aspects. *Arch. Agric. Environ. Sci.* **2022**, *7*, 288–293. [CrossRef]
264. Sun, X.-L.; Xiang, H.; Xiong, H.-Q.; Fang, Y.-C.; Wang, Y. Bioremediation of microplastics in freshwater environments: A systematic review of biofilm culture, degradation mechanisms, and analytical methods. *Sci. Total. Environ.* **2023**, *863*, 160953. [CrossRef]
265. Solanki, S.; Sinha, S.; Singh, R. Myco-degradation of microplastics: An account of identified pathways and analytical methods for their determination. *Biodegradation* **2022**, *33*, 529–556. [CrossRef]
266. Singh, S.P.; Sharma, P.; Bano, A.; Nadda, A.K.; Varjani, S. Microbial communities in plastisphere and free-living microbes for microplastic degradation: A comprehensive review. *Green Anal. Chem.* **2022**, *3*, 100030. [CrossRef]
267. Singh, R.P.; Mishra, S.; Das, A.P. Synthetic microfibers: Pollution toxicity and remediation. *Chemosphere* **2020**, *257*, 127199. [CrossRef]
268. Pico, Y.; Alfarhan, A.; Barcelo, D. Nano- and microplastic analysis: Focus on their occurrence in freshwater ecosystems and remediation technologies. *TrAC Trends Anal. Chem.* **2019**, *113*, 409–425. [CrossRef]
269. Othman, R.; Ramya, R.; Latif, N.H.M.; Sulaiman, W.S.H.W.; Hatta, F.A.M.; Ali, Q.A.M.; Jusoh, N.H.M. Mitigation of the Micro- and Nanoplastic Using Phycoremediation Technology. In *Impact of Plastic Waste on the Marine Biota*; Shahnawaz, M., Sangale, M.K., Daochen, Z., Ade, A.B., Eds.; Springer Nature: Singapore, 2022; pp. 183–208. [CrossRef]
270. Magalhães, S.; Alves, L.; Medronho, B.; Romano, A.; Rasteiro, M.d.G. Microplastics in Ecosystems: From Current Trends to Bio-Based Removal Strategies. *Molecules* **2020**, *25*, 3954. [CrossRef]
271. Anand, U.; Dey, S.; Bontempi, E.; Ducoli, S.; Vethaak, A.D.; Dey, A.; Federici, S. Biotechnological methods to remove microplastics: A review. *Environ. Chem. Lett.* **2023**, *21*, 1787–1810. [CrossRef]
272. Barone, G.D.; Ferizović, D.; Biundo, A.; Lindblad, P. Hints at the Applicability of Microalgae and Cyanobacteria for the Biodegradation of Plastics. *Sustainability* **2020**, *12*, 10449. [CrossRef]
273. Dey, S.; Rout, A.K.; Behera, B.K.; Ghosh, K. Plastisphere community assemblage of aquatic environment: Plastic-microbe interaction, role in degradation and characterization technologies. *Environ. Microbiome* **2022**, *17*, 32. [CrossRef]
274. Gaur, V.K.; Gupta, S.; Sharma, P.; Gupta, P.; Varjani, S.; Srivastava, J.K.; Chang, J.-S.; Bui, X.-T. Metabolic Cascade for Remediation of Plastic Waste: A Case Study on Microplastic Degradation. *Curr. Pollut. Rep.* **2022**, *8*, 30–50. [CrossRef]
275. Hadian-Ghazvini, S.; Hooriabad Saboor, F.; Safaee Ardekani, L. Bioremediation Techniques for Microplastics Removal. In *Microplastics Pollution in Aquatic Media: Occurrence, Detection, and Removal, Environmental Footprints and Eco-Design of Products and Processes*; Sillanpää, M., Khadir, A., Muthu, S.S., Eds.; Springer: Singapore, 2022; pp. 327–377. [CrossRef]
276. Iroegbu, A.O.; Sadiku, R.E.; Ray, S.S.; Hamam, Y. Plastics in municipal drinking water and wastewater treatment plant effluents: Challenges and opportunities for South Africa—A review. *Environ. Sci. Pollut. Res.* **2020**, *27*, 12953–12966. [CrossRef]
277. Yang, S.; Zhou, M.; Chen, X.; Hu, L.; Xu, Y.; Fu, W.; Li, C. A comparative review of microplastics in lake systems from different countries and regions. *Chemosphere* **2022**, *286*, 131806. [CrossRef]
278. Yalwaji, B.; John-Nwagwu, H.O.; Sogbanmu, T.O. Plastic pollution in the environment in Nigeria: A rapid systematic review of the sources, distribution, research gaps and policy needs. *Sci. Afr.* **2022**, *16*, e01220. [CrossRef]
279. Sarkar, B.; Dissanayake, P.D.; Bolan, N.S.; Dar, J.Y.; Kumar, M.; Haque, N.; Mukhopadhyay, R.; Ramanayaka, S.; Biswas, J.K.; Tsang, D.C.; et al. Challenges and opportunities in sustainable management of microplastics and nanoplastics in the environment. *Environ. Res.* **2022**, *207*, 112179. [CrossRef]

280. Qiu, Y.; Zhou, S.; Zhang, C.; Qin, W.; Lv, C. A framework for systematic microplastic ecological risk assessment at a national scale. *Environ. Pollut.* **2023**, *327*, 121631. [CrossRef]
281. Prata, J.C.; Silva, A.L.P.; da Costa, J.P.; Mouneyrac, C.; Walker, T.R.; Duarte, A.C.; Rocha-Santos, T. Solutions and Integrated Strategies for the Control and Mitigation of Plastic and Microplastic Pollution. *Int. J. Environ. Res. Public Health* **2019**, *16*, 2411. [CrossRef]
282. Narloch, I.; Gackowska, A.; Wejnerowska, G. Microplastic in the Baltic Sea: A review of distribution processes, sources, analysis methods and regulatory policies. *Environ. Pollut.* **2022**, *315*, 120453. [CrossRef]
283. Lozoya, J.P.; Carranza, A.; Lenzi, J.; Machin, E.; Teixeira de Mello, F.; Gonzalez, S.; Hernandez, D.; Lacerot, G.; Martinez, G.; Scarabino, F.; et al. Management and research on plastic debris in Uruguayan Aquatic Systems: Update and perspectives. *J. Integr. Coast. Zone Manag.* **2015**, *15*, 377–393. [CrossRef]
284. Kumar, R.; Sharma, P.; Manna, C.; Jain, M. Abundance, interaction, ingestion, ecological concerns, and mitigation policies of microplastic pollution in riverine ecosystem: A review. *Sci. Total. Environ.* **2021**, *782*, 146695. [CrossRef]
285. Kentin, E.; Battaglia, G. Policies and Perspectives on Regulating Microplastic Fibre Pollution. In *Polluting Textiles: The Problem with Microfibres, Routledge Explorations in Environmental Studies*; Routledge by Taylor & Francis: Oxford, UK, 2022.
286. Barcelo, D.; Pico, Y. Case studies of macro- and microplastics pollution in coastal waters and rivers: Is there a solution with new removal technologies and policy actions? *Case Stud. Chem. Environ. Eng.* **2020**, *2*, 100019. [CrossRef]
287. Campanale, C.; Massarelli, C.; Bagnuolo, G.; Savino, I.; Uricchio, V.F. The Problem of Microplastics and Regulatory Strategies in Italy. In *Plastics in the Aquatic Environment—Part II: Stakeholders' Role Against Pollution, The Handbook of Environmental Chemistry*; Stock, F., Reifferscheid, G., Brennholt, N., Kostianaia, E., Eds.; Springer International Publishing: Cham, Switzerland, 2022; pp. 255–276. [CrossRef]
288. Coffin, S. The emergence of microplastics: Charting the path from research to regulations. *Environ. Sci. Adv.* **2023**, *2*, 356–367. [CrossRef]
289. Deme, G.G.; Ewusi-Mensah, D.; Olagbaju, O.A.; Okeke, E.S.; Okoye, C.O.; Odii, E.C.; Ejeromedoghene, O.; Igun, E.; Onyekwere, J.O.; Oderinde, O.K.; et al. Macro problems from microplastics: Toward a sustainable policy framework for managing microplastic waste in Africa. *Sci. Total. Environ.* **2022**, *804*, 150170. [CrossRef]
290. Fu, D.; Chen, C.M.; Qi, H.; Fan, Z.; Wang, Z.; Peng, L.; Li, B. Occurrences and distribution of microplastic pollution and the control measures in China. *Mar. Pollut. Bull.* **2020**, *153*, 110963. [CrossRef]
291. Golwala, H.; Zhang, X.; Iskander, S.M.; Smith, A.L. Solid waste: An overlooked source of microplastics to the environment. *Sci. Total. Environ.* **2021**, *769*, 144581. [CrossRef]
292. Gong, J.; Xie, P. Research progress in sources, analytical methods, eco-environmental effects, and control measures of microplastics. *Chemosphere* **2020**, *254*, 126790. [CrossRef]
293. Halfar, J.; Brožová, K.; Čabanová, K.; Heviánková, S.; Kašpárková, A.; Olšovská, E. Disparities in Methods Used to Determine Microplastics in the Aquatic Environment: A Review of Legislation, Sampling Process and Instrumental Analysis. *Int. J. Environ. Res. Public Heal.* **2021**, *18*, 7608. [CrossRef]
294. Jung, Y.S.; Sampath, V.; Prunicki, M.; Aguilera, J.; Allen, H.; LaBeaud, D.; Veidis, E.; Barry, M.; Erny, B.; Patel, L.; et al. Characterization and regulation of microplastic pollution for protecting planetary and human health. *Environ. Pollut.* **2022**, *315*, 120442. [CrossRef]
295. Österlund, H.; Blecken, G.; Lange, K.; Marsalek, J.; Gopinath, K.; Viklander, M. Microplastics in urban catchments: Review of sources, pathways, and entry into stormwater. *Sci. Total. Environ.* **2023**, *858*, 159781. [CrossRef]
296. Monira, S.; Bhuiyan, M.A.; Haque, N.; Shah, K.; Roychand, R.; Hai, F.I.; Pramanik, B.K. Understanding the fate and control of road dust-associated microplastics in stormwater. *Process. Saf. Environ. Prot.* **2021**, *152*, 47–57. [CrossRef]

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Release of Microplastics from Urban Wastewater Treatment Plants to Aquatic Ecosystems in Acapulco, Mexico

Enrique J. Flores-Munguía [1], José Luis Rosas-Acevedo [1,*], Aurelio Ramírez-Hernández [2], Alejandro Aparicio-Saguilan [3], Rosa M. Brito-Carmona [1] and Juan Violante-González [4]

[1] Centro de Ciencias de Desarrollo Regional, Universidad Autónoma de Guerrero, Privada de Laurel No. 13, Col. El Roble, Acapulco C.P. 39640, Mexico; enriqueflores@uagro.mx (E.J.F.-M.); rosabrito@uagro.mx (R.M.B.-C.)
[2] Instituto de Química Aplicada, Universidad del Papaloapan, Circuito Central 200, Col. Parque Industrial, Tuxtepec C.P. 68301, Mexico; chino_raha@hotmail.com
[3] Instituto de Biotecnología, Universidad del Papaloapan, Circuito Central 200, Col. Parque Industrial, Tuxtepec C.P. 68301, Mexico; alejandrosag@hotmail.com
[4] Facultad de Ecología Marina, Universidad Autónoma de Guerrero, Gran vía Tropical No. 20, Col. Fraccionamiento Las Playas, Acapulco C.P. 39390, Mexico; viojuang@yahoo.com.mx
* Correspondence: jlrosas@uagro.mx; Tel.: +52-744-1727932

Abstract: Contamination of aquatic ecosystems by microplastics (MPs) is mainly due to the release of high levels of MP particles from treated effluents by wastewater treatment plants (WWTPs). Due to the lack of policies and regulations establishing criteria for the control and elimination of MPs from WWTP effluents, this research evaluates the presence of 38 and 150 μm MPs in influents and effluents from three WWTPs in the port of Acapulco, Mexico. Optical microscopy and Fourier transform infrared spectroscopy revealed that the MPs were polyethylene, polypropylene, polyethylene terephthalate, and polyvinyl chloride. MP removal efficiencies of 82.5–98.7% (38 μm) and 86.8–97.5% (150 μm) were achieved. Moreover, the MP average daily emissions to the receiving bodies of the three WWTPs ranged from 9.5×10^6 to 4.70×10^8 particles, while the annual emissions ranged from 3.05×10^9 to 1.72×10^{11} particles. This work reveals the urgency of implementing regulatory policies to avoid the continuous emission of MPs into aquatic ecosystems from WWTPs in Acapulco, Mexico.

Keywords: microplastics; removal; contamination; FTIR; regulatory standards; tourist city

Citation: Flores-Munguía, E.J.; Rosas-Acevedo, J.L.; Ramírez-Hernández, A.; Aparicio-Saguilan, A.; Brito-Carmona, R.M.; Violante-González, J. Release of Microplastics from Urban Wastewater Treatment Plants to Aquatic Ecosystems in Acapulco, Mexico. *Water* **2023**, *15*, 3643. https://doi.org/10.3390/w15203643

Academic Editors: Grigorios L. Kyriakopoulos, Antonis A. Zorpas, María Rocío Rodríguez Barroso and Vassilis J. Inglezakis

Received: 10 September 2023
Revised: 7 October 2023
Accepted: 15 October 2023
Published: 17 October 2023

1. Introduction

Approximately 6.3 billion metric tons of plastics are consumed worldwide each year, ultimately discarded into the environment, with a high potential to generate large amounts of microplastics (MPs) [1,2]. MPs have different morphologies and polymeric compositions and range in size from 5 mm to the nanometric scale. MPs originate from industrial production (primary MPs) or the fragmentation of larger plastic waste (secondary MPs) [3]. Indeed, they have been identified in soil, air, and water, with those composed of polypropylene (PP), polyethylene (PE), polyethylene terephthalate (PET), and polyvinyl chloride (PVC) being the most common [4]. These MPs constitute emerging pollutants as they have not yet been incorporated into regulatory environmental standards. However, due to their multidimensional nature, they pose a potential risk to the health of aquatic and terrestrial ecosystems as all living organisms are exposed to them [5,6]. Recent sex-based estimates revealed that girls, women, boys, and men consume or inhale 74,060, 98,305, 81,331, and 121,664 MP particles each year, respectively [7]. Additionally, MPs have been identified in the human placenta, feces, intestinal tract, and blood [8–11]. Meanwhile, PET reportedly alters the intestinal fauna and affects the genomic stability of human lymphocytes [10,11].

Due to the complexity and polymeric diversity of MPs, standardized protocols have yet to be established to precisely and accurately determine their accurate concentration in

aquatic and terrestrial ecosystems. Meanwhile, their emissions and transfer greatly hinder the implementation of policies and regulations regarding the maximum permissible limits to control and eliminate MPs in the environment [12]. Nevertheless, recent research has adopted various protocols to sample, treat, quantify, and identify MPs. The most widely used analytical techniques for their identification include optical microscopy, Fourier transform infrared (FTIR) spectroscopy, Raman spectroscopy, and Pyr/GC/MS [13]. In particular, FTIR is the most commonly employed technique for identifying MPs originating from wastewater treatment plants (WWTPs) [14]. For example, FTIR spectroscopy coupled with optical microscopy can determine the polymeric composition of $\geq$20 µm MPs [12].

According to the physicochemical characteristics of wastewater (municipal or industrial), different types of WWTPs (secondary or tertiary) eliminate pollutants according to the policies and regulatory criteria of each country for their control and removal [15]. The removal efficiencies of MPs in WWTPs (secondary and tertiary) are approximately 90 to 98%. Nonetheless, the treated effluents continue to carry MPs that are transferred to the receiving bodies. Accordingly, WWTP discharge is considered an emission and exposure source for MPs to aquatic and terrestrial ecosystems [16–23]. However, the current standards that establish the quality criteria applied to treated wastewater do not include MPs within their reference framework; hence, MPs continue to contaminate aquatic and terrestrial ecosystems. Regarding Mexico, the official Mexican standards (NOM-001-SEMARNAT-2021; NOM-002-SEMARNAT-1996; NOM-SEMARNAT-1997) do not consider MPs within their regulatory framework. Instead, only the maximum permissible limits are established regarding the quality of discharge from WWTPs with effluents destined for aquatic and terrestrial receiving bodies [24–27].

Due to the lack of standards to regulate and establish maximum permissible limits in Mexico, the current study sought to assess the levels of MPs in WWTP emissions and the associated pollution of the environment in Mexico. To this end, we applied optical microscopy and FTIR spectroscopy to detect, quantify, and characterize MPs (150–38 µm) in wastewater and wastewater treated in three urban secondary WWTPs in Acapulco. Moreover, we estimated the removal percentages for these pollutants based on the MP concentrations in the influents and effluents of each WWTP to project their daily and annual emissions to receiving bodies. Collectively, the results of this study provide insights for the development of technical and methodological criteria to create policies and regulatory norms to eliminate MPs from effluents and mitigate future damage to the environment.

2. Materials and Methods

2.1. WWTP Evaluation

Sampling was carried out at three WWTPs in Acapulco (16°51′46″ N 99°53′13″ W), Guerrero (17°36′47″ N 99°57′00″ W), Mexico (19°25′10″ N 99°08′44″ W) (Figure 1). These WWTPs are operated by the para-municipal body; their purification systems comprise activated sludge (secondary treatment). WWTP A (16°48′04″ N 99°48′03″ W) is in the eastern part of the city with an operating capacity of 23 L/s for a population of 10,948 inhabitants; the receiving body is the Tres Palos lagoon. WWTP B (16°53′03″ N 99°49′12″ W) is in the suburban area of the city with a 350 L/s operating capacity for a population of 166,600 inhabitants; its effluents are discharged into the Sabana River—a tributary of the Tres Palos lagoon. WWTP C (16°51′34″ N 99°54′30″ W) is in the western part of the city and has an operating capacity of 650 L/s for a population of 309,400 inhabitants; its effluents are discharged into the sea through Olvidada beach.

Influent and effluent samples from the three WWTPs were collected via point sampling during June and July (dry season) and October and November (rainy season) of 2022. The influent samples (4 L each) were collected from the intake pit at the WWTP after screening. Effluent samples (20 L each) were collected at the WWTP outlet. After collection, the samples were transported to the laboratory and stored at 15 °C.

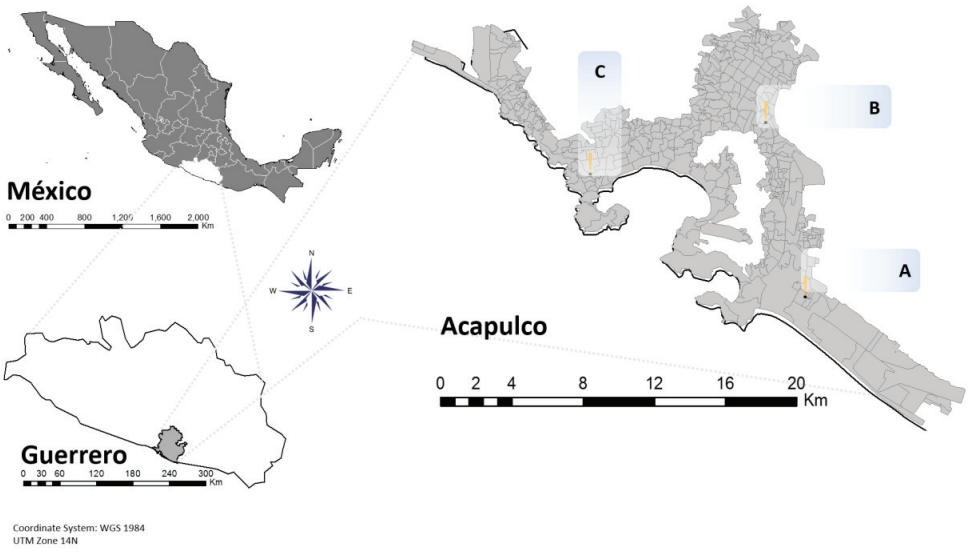

Figure 1. WWTP monitored locations for this study: WWTP (**A**) 23 L/s; WWTP (**B**) 350 L/s, and WWTP (**C**) 650 L/s.

2.2. MP Separation

MPs were separated from influents and effluents based on the methodologies reported by the National Oceanic and Atmospheric Administration (NOAA) and other researchers [18,28–33]. Influent samples were successively passed through mesh with 300 µm (to retain the larger solids), 150 µm, and 38 µm pores. The sieves were rinsed with distilled water to remove MP particles and deposit them in a beaker. MPs were dried in an oven at ±80 °C for 12 h and digested by adding 30 mL of 30% H_2O_2 and incubating at 60 °C for 2 h. To influent samples, 30 mL of H_2O_2 was added and incubated for 2 h to improve the digestion of organic matter. Once digestion was complete, the solutions were cooled to room temperature. Subsequently, density-based separation was carried out by adding 60 mL of $ZnCl_2$ (1.62 g/cm^3) to the samples in separation funnels, allowing them to settle for 12 h. Under vacuum, the supernatant was passed through 47-mm glass fiber filters that were later placed in Petri dishes for MP counting and identification.

2.3. Quality Control

Cotton lab coats, gloves, and face masks were used to avoid cross-contamination. Prior to sample processing, all areas of the laboratory were cleaned using distilled water and 30% (*v*/*v*) ethanol. All reagents were vacuum filtered through Whatman® filters (grade 41, 125 diameter). Blanks (distilled water) were collected and analyzed according to the same protocol used for the influent and effluent samples.

To assess the atmospheric quality of the laboratory, open Petri dishes with Whatman® filter paper (grade 41, 125 in diameter) were placed on laboratory surfaces throughout the experimental process. Subsequently, the exposed discs were analyzed by light microscopy. Additionally, commercial PVC, PE, PET, and PP plastics were included as references to determine the composition of the MPs within the influents and effluents of the WWTPs. The glass microfiber filter used during sample filtration was analyzed by FTIR to rule out interference in the spectra.

2.4. MP Characterization

MP characterization was carried out based on the classification criteria proposed by Hidalgo-Ruz et al. [5], which considers color and morphology. MPs were quantified

and identified using a SWIFT® M10 Series optical microscope with 4X and 40X objectives. Infrared spectra of the MPs were recorded at room temperature in a Perkin-Elmer Spectrum 100FT-IR (ATR) spectrometer with a 4 cm^{-1} resolution and were averaged over 4 scans in the 4000–650 cm^{-1} range.

2.5. Daily and Annual Projections of Removal Efficiencies

Removal efficiencies were calculated based on the MP abundance in the influents and effluents, according to the method described by Talvitie et al. [34]. Additionally, the daily and annual projections of MP release to the receiving bodies were determined by multiplying the MP concentration in effluents (MP/L) by the average daily operating flow in each WWTP (L/day). Annual concentrations were determined by multiplying the total daily MP quantities by 365 [20].

2.6. Statistical Analysis

One factor analysis of variance (ANOVA) was performed to compare the three WWTPs with respect to their MP removal efficiency. A $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1. MP Detection

Optical microscopy of the influents and effluents collected from the three WWTPs, revealed 38–150 μm MPs (Figure 2).

The MPs detected in the secondary treatment WWTPs were similar in morphology to those previously reported but of different sizes [17–21,35–37]. MPs from 10 to 1000 μm [31], 20 to ≥500 μm [38], 43 to 355 μm [13], 10 to 5000 μm [19], 20 to 200 μm [20], and 20 to ≥300 μm [33] have been reported. Regarding WWTPs with tertiary treatment systems [29,34], the MP morphology and appearance were similar to those in the current study. Therefore, unitary wastewater treatment systems do not affect the appearance or morphology of MPs in influents and effluents.

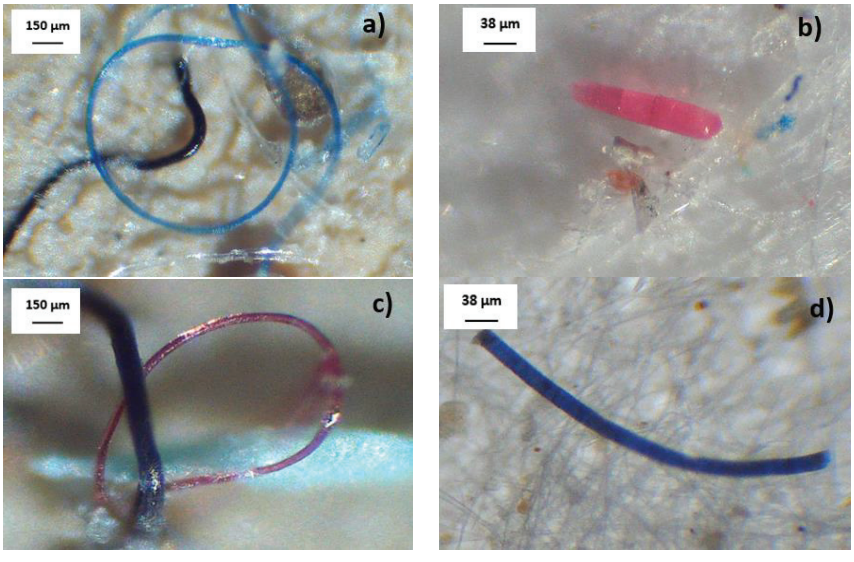

Figure 2. *Cont.*

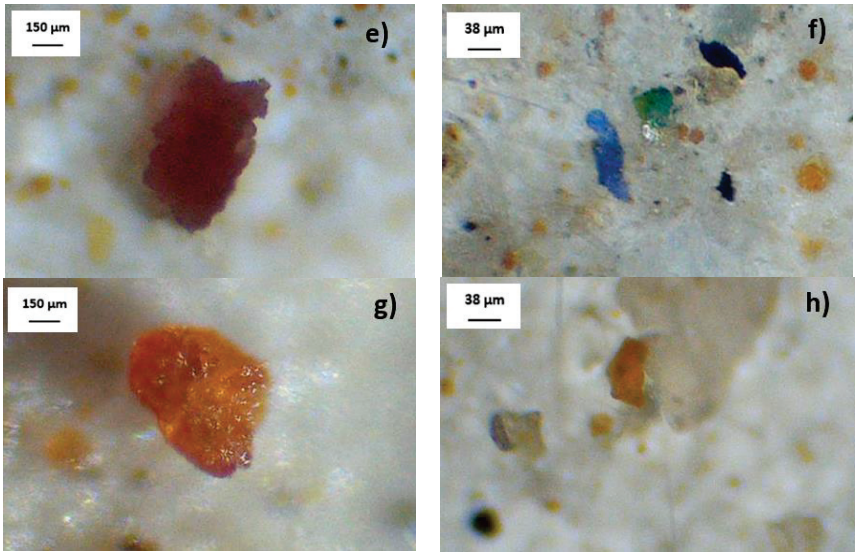

Figure 2. MP filaments in influents (**a**,**b**) and effluents (**c**,**d**); MP fragments in influents (**e**,**f**) and effluents (**g**,**h**). 150 µm size (**a**,**c**,**e**,**g**) and 38 µm (**b**,**d**,**f**,**h**).

3.2. MP Physical Characterization

3.2.1. MP Color

The MPs detected in a four-month sampling period from WWTP influents and effluents were diverse colors according to the classification of Hidalgo-Ruz et al. [5] (Figure 3).

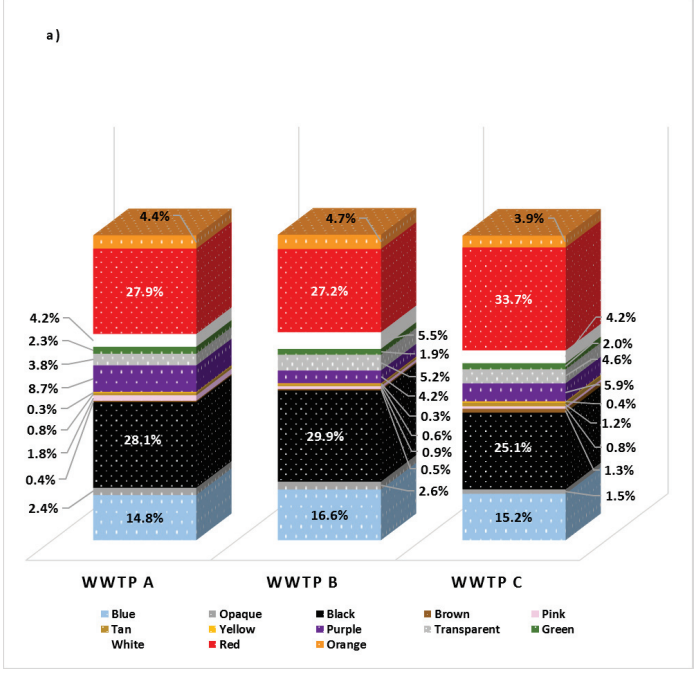

Figure 3. *Cont.*

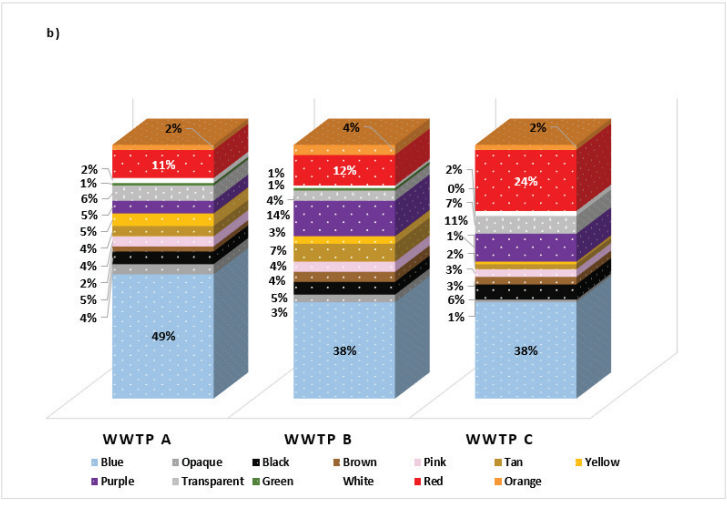

Figure 3. Average percentage distribution of MPs according their colors identified in influents (**a**) and effluents (**b**) in the three study WWTPs. Red MPs were more abundant in the influent than the effluent; blue MPs were more abundant in the effluent.

In the influents, black MPs were the most abundant in WWTP A (28.1%) and B (29.9%), while in WWTP C, red MPs were the most abundant (33.7%). Conversely, blue MPs predominated all effluents (WWTP A: 49%, WWTPs B and C: 38%), followed by red and purple MP. Meanwhile, the proportion of transparent plastics in the influents of WWTP A, B, and C was 6, 4, and 7%, respectively (Figure 4). Hence, black MPs predominated the influents, followed by transparent and blue [18]. In Scotland, red MPs were the most abundant (26.7%), followed by blue (25.4%) and green (19.1%) [20]. In China, white PMs represent 27.3% of the quantified plastics [13]. It should be noted that different MP colors are due to additives used to alter the plastic pigmentation [39]. Since chemical additives in polymers, such as PE, PVC, and PA, can be toxic, MPs pose an environmental risk. However, this toxicity is often due not only to the presence of these polymers but also to the leachates from their additives [40]. For example, the blue coloration in plastics may be due to the use of cobalt (II) diacetate ($C_4H_6CoO_4$) in PET; to achieve red pigmentation, various additives are used, including cadmium selenide (CdSe), lead sulfate ($PbSO_4$) and lead molybdate ($PbMoO_4$), which are applied to various plastics [41]. Indeed, the chemical production of additives is constantly increasing, with an estimated 20,000 million tons of plastics containing additives produced by 2050 [22]. Accordingly, aquatic and terrestrial ecosystems will continue to be exposed to constant MP emissions and other associated pollutants (heavy metals) if adequate regulations for using additives in the plastic industry are not implemented.

The abundance of MPs of different colors in the influents and effluents of the analyzed WWTPs reflects the complexity of these polymers that, when transferred to hydrological bodies, pose potential risks to environmental health. Meanwhile, the lack of regulatory policies and standards does not prevent the deliberate emission of MPs, which is a potentially greater threat to the health of ecosystems. Furthermore, not only can the degree of toxicity be attributable to MPs, but their association with chemical additives and heavy metals can represent a toxicological danger [41]. Accordingly, the implementation of regulatory norms should consider the coloration of MPs as an additional criterion for evaluating the quality of the treated effluents discharged into aquatic and terrestrial ecosystems.

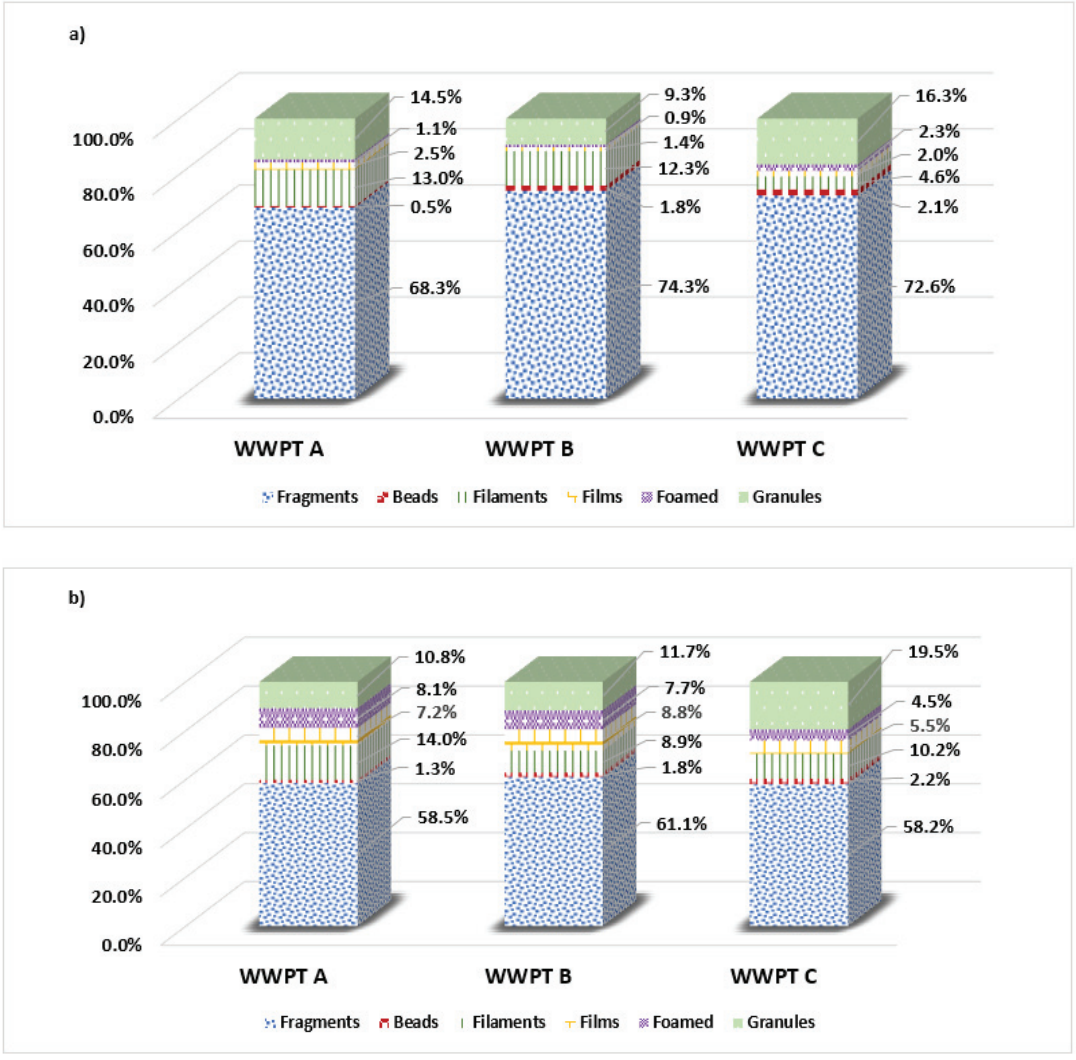

Figure 4. Average percentage distribution of MPs according to their morphology identified in the (**a**) influents and (**b**) effluents of the three study WWTPs.

In Mexico, there are currently no standards or criteria that regulate the manufacture of plastics and the use of additives, which has a considerable impact on the generation of MPs and their emission and transfer to the environment. Instead, regulations only exist that identify plastic types to guide their selection, separation, collection, and recycling [42].

3.2.2. MP Morphology

Various MP forms were detected in the influents and effluents of the three WWTPs, with the fragment-type morphology being the most abundant, followed by filaments (Figure 4). There were no differences between the three WWTPs regarding MP morphological distribution in the treated effluents (one factor ANOVA, $F = 0.11$, $p > 0.05$) (Table 1). Therefore, it can be deduced that the MPs entering the three WWTPs and those subsequently released to the receiving bodies present similar morphological characteristics.

Table 1. One factor ANOVA ($p < 0.05$) of the morphology of MPs in the effluents of three WWTPs.

Group	*Count*	*Sum*	*Average*	*Variance*		
WWPT A	6	564.77	94.13	13,993.38		
WWPT B	6	736.8	122.80	26,269.83		
WWPT C	6	538.04	89.67	13,066.88		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*p value*	*F crit*
Between Groups	3878.58	2	1939.29	0.11	0.90	3.68
Within Groups	266,650.44	15	17,776.70			
Total	270,529.01	17				

In contrast, other studies have reported higher proportions of filaments [18,21,29,43], granules [13], fragments [30], and scales [20]. The morphological classification of MPs informs their degradation mechanism and possible mechanisms of transfer and cellular absorption in organisms of the trophic chain. In this sense, MPs have been defined as vectors of other contaminants and pathogenic microorganisms. Therefore, their morphology offers information on the potential risks of transporting more dangerous pollutants, such as the chemical additives discussed in the previous section.

From the data obtained in this study, it can be deduced that most MPs that enter and leave the WWTPs are secondary; however, significant amounts of filaments have also been detected, suggesting the presence of many primary MPs. Similarly, fragments are the most abundant MP in secondary treatment WWTPs in Korea [44]. MP morphological characterization also provides insights regarding the changes they undergo in their shape and size during purification processes [45]. This informs the establishment of maximum permissible limits of control and the development of technologies and complementary unit operations (tertiary treatment) to be incorporated into current systems in accordance with the morphological typification to effectively eliminate these pollutants from the treated effluents [44,46–48]. Countries such as China, Kenya, and the European Union have implemented standards and public policies to reduce plastic pollutant generation. Moreover, in 2020 international agreements were established to reduce single-use plastic products and packaging by up to 20% by 2025 [5]. Despite these measures, there is an urgency to incorporate and establish criteria for MP evaluation and control in effluents treated at WWTPs.

The diverse sizes, colors, and shapes of the MPs destined for various receiving bodies can have different impacts on living organisms [5]. Moreover, the additives used during plastic manufacturing add another pollutant factor to the MPs. Hence, the characterization and classification of these chemical compounds will provide basic theoretical insights to inform the development of regulatory standards [16].

3.3. MP Characterization by FTIR

In the influent and effluent samples, FTIR analysis detected four types of polymers: PVC, PE, PET, and PP (Figure 5). To verify their identification, the spectra were compared with the reference spectra of conventional polymers.

The functional groups were compared according to the different characteristic vibration signals in the spectra from the MPs and reference samples (Table 2). Based on these results, PET, PE, PP, and PVC were the most frequently detected polymers in the influents and effluents of the three WWTPs (Figure 5). Notably, the signals corresponding to the glass fiber paper used to filter the samples did not interfere with the vibration signals of the MP spectra (Figure 5).

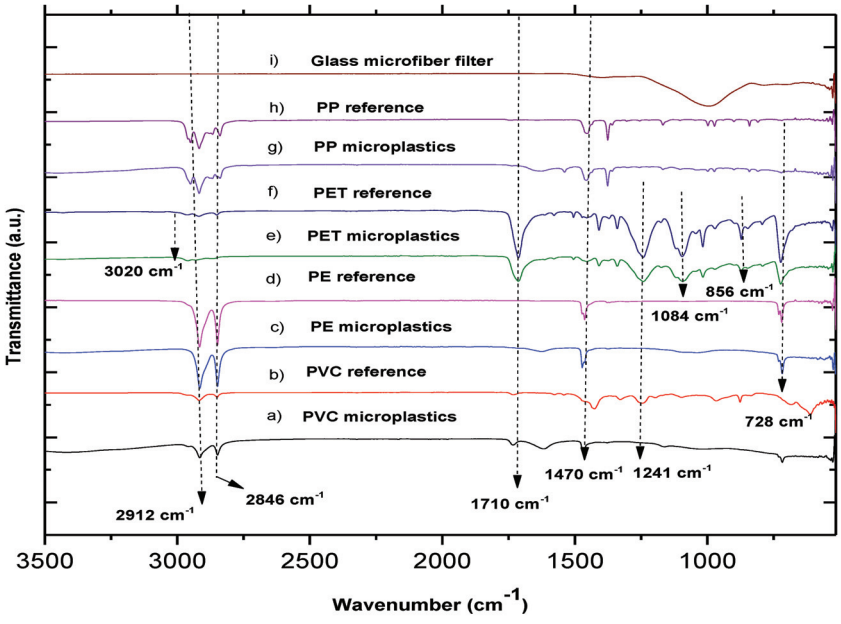

Figure 5. FTIR spectra of MP obtained in the samples of the study WWTPs.

Table 2. Wavenumber and functional groups detected in the FTIR spectra of the three WWTPs in Acapulco.

Functional Group	OH	CH_2 (Stretch)	CH	C=O	CH_2 (Scissor)	C-O-C	C-O	CH_2 (Rock)
Wavenumber (cm^{-1})	3020	2900	2800	1713	1448	1247	1084	728
	PET	PET	PET	PET	PET	PET	PET	-
	-	PVC	PVC	-	PVC	-	-	-
	-	PP	PP	-	PP	-	-	PP
	-	PE	-	-	PE	-	-	PE

Similar to our results, others have also reported the four polymers (PET, PVC, PP, and PE) as the most abundant [18–20,37,38,49–53]. However, additionally, other studies have detected the presence of polyurethane (PU), polyphenylene oxide (PPO), nylon (PA), phenolic epoxide (PER), polyacrylonitrile (PAN), and polyvinyl alcohol (PVA), among others [18]. The detection of other MPs in different WWTPs across the world depends on the particular plastic-type consumption in each country and city. Moreover, the source of wastewater that enters the WWTPs, for instance those originating from a combination of industrial and domestic waste, contains more types of polymers in domestic use discharge [19,29]. One limitation of this work is that the samples obtained (by sieve sizes) and analyzed (equipment used) were only 38 and 150 μm; thus, other polymers may have been present in different particle size fractions. Additionally, the WWTPs analyzed in this research receive only domestic wastewater; thus, when comparing the results with similar WWTPs, consistencies are observed in the identified polymers. For example, in WWTPs in Colombia, low-density polyethylene (LDPE), PP, PET, and PS were reported [31]. In another similar case, the MPs identified in a WWTP in a Chinese province comprised primarily PP, PE, PS, and PET [50]; however, unlike this study, neither previous study reported PVC, and both identified PS.

Furthermore, the type of discharge (industrial, domestic, or mixed) can determine the predominance of PMs in influents and effluents [32]. Plastics, such as those detected in

this study, are the basis for establishing mid- and long-term classification criteria on the types of discharge that enter the WWTPs and their discharge to the receiving bodies in cities with a tourist vocation. Therefore, considering only the chemical characterization of MPs is insufficient as it is necessary also to quantify the number of particles that enter and leave the WWTPs to determine the associated removal percentages and, thus, objectively evaluate the operating conditions and emission of MPs whose permanent destination is receiving bodies [34].

3.4. MP Concentration and Removal Efficiencies

The MP particles in influents and effluents from the three WWTPs presented different amounts and removal percentages concerning the sampling months (Table 3). Removal efficiency from monthly sampling was calculated for each MP particle size (38 μm and 150 μm). The obtained MP removal efficiencies for the WWTPs in Acapulco were similar to other investigations of secondary treatment systems. For example, in Italy, 95% of MPs were removed by WWTPs [18,30], while in China, 79.33–84% [18], in Colombia, 93.2–94.19% [31], in Canada, 99% [43] and in Scotland, 98% [20] was achieved. Hence, only the influents and effluents were analyzed for the three WWTPs, while the retained MPs were presumed to be in the sludge of the secondary clarifiers following biological treatment. According to different studies, most retained MPs are detected in biological sludge, so it is considered that the largest amount of MPs is intercepted in primary and secondary sludge [13,18,30,35–37,44,53–55]. Therefore, secondary treatment systems for biological sludge retain and concentrate most MPs in the primary and secondary sedimentation.

Temporality is a factor that influences the concentration, distribution, and removal of MPs in WWTPs [45,56]. However, these studies did not specify whether rainwater can affect the removal efficiency of MPs during treatment. According to the data obtained in this study, weather conditions are a variable factor impacting MP entry and emission. Thus, during the rainy season, the entry of MPs to the WWTPs increased, decreasing the removal efficiencies of the three WWTPs. The samples collected in June (dry season) presented the highest removal efficiencies (98.7%), while in November (rainy season), the lowest removal efficiencies were obtained (82.5%). This difference is due to the pluvial currents interfering with the influents during the rainy seasons; it is inferred that they significantly influence the transfer, transport, and emission of MPs from WWTPs due to the connection of the pluvial channels with the network plumbing in Acapulco. Considering the ANOVA results ($p < 0.05$), there were significant variations in MP concentrations between the dry and rainy months in influents and effluents (Table 4) (F calculated > F critical). Similarly, in Korea, higher concentrations of MP were reported in the treated effluents from a WWTP during the rainy season than in the dry season [56]. In contrast, in WWTPs in China, the MP concentrations and removal efficiencies were higher in dry seasons than in rainy seasons [45]. We posit that factors such as exposure of urban solid waste to the elements in storm channels and hydro-sanitary operating conditions contribute to the increase in MPs in urban wastewater treatment systems.

Table 3. MP removal percentages from influents and effluents of three WWTPs in Acapulco, Gro., Mex. in 2022.

	WWTP A						**WWTP B**						**WWTP C**					
	MP (Ítems/L) 38 µm			**MP (Ítems/L) 150 µm**			**MP (Ítems/L) 38 µm**			**MP (Ítems/L) 150 µm**			**MP (Ítems/L) 38 µm**			**MP (Ítems/L) 150 µm**		
	I	**E**	**% r**	**I**	**E**	**% r**	**I**	**E**	**% r**	**I**	**E**	**% r**	**I**	**E**	**%r**	**I**	**E**	**%r**
June	76.0 ± 1.62	1.0 ± 0.61	**98.7**	20.7 ± 0.48	1.73 ± 0.36	**91.7**	57.7 ± 2.25	0.8 ± 0.34	**98.5**	45.50 ± 1.64	1.1 ± 0.23	**97.4**	44.2 ± 2.92	0.7 ± 0.17	**98.3**	33.4 ± 2.53	0.8 ± 0.18	**97.5**
July	96.1 ± 1.26	2.8 ± 0.55	**97.1**	68.5 ± 0.93	2.00 ± 1.65	**97.1**	80.6 ± 2.67	2.8 ± 0.74	**96.4**	48.33 ± 1.91	1.8 ± 0.81	**96.3**	104 ±6.09	2.4 ± 0.20	**97.7**	39.4 ± 3.76	0.9 ± 0.64	**97.5**
October	174.9 ± 2.63	9.0 ± 0.71	**94.8**	66.7 ± 2.23	4.82 ± 0.53	**92.8**	159.3 ± 7.72	13.0 ± 1.93	**91.8**	103.67 ± 4.04	8.0 ± 0.56	**92.3**	150 ±8.67	13.5 ± 1.16	**91.0**	67.9 ± 4.64	6.2 ± 0.48	**90.8**
November	218.8 ± 3.29	24.3 ± 1.04	**88.9**	88.4 ± 0.53	10.73 ± 0.79	**87.9**	194.7 ± 13.51	34 ± 0.90	**82.5**	122.75 ± 4.76	13.3 ± 1.08	**89.2**	200 ±1.01	16.8 ± 0.41	**91.6**	88.3 ± 5.0	11.6 ± 0.38	**86.8**

Notes: Dry season: June and July; rainy season: October and November.

Table 4. One factor ANOVA ($p < 0.05$) according to the temporality in influents (a) and effluents (b) of three WWTPs in Acapulco, Gro. Mex.

(**a**)

Influent

Group	*Count*	*Sum*	*Average*	*Variance*
June (dry season)	3	277.58	92.53	178.11
July (dry season)	3	437.50	145.83	321.10
October (rainy season)	3	723.09	241.03	497.15
November (rainy season)	3	912.92	304.31	221.56

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p value*	*F crit*
Between Groups	80,944.31	3	26,981.44	88.61	1.8×10^{-6}	4.07
Within Groups	2435.85	8	304.48			
Total	83,380.16	11				

(**b**)

Effluent

Group	*Count*	*Sum*	*Average*	*Variance*
June (dry season)	3	6.41	2.14	0.36
July (dry season)	3	12.82	4.27	0.65
October (rainy season)	3	54.67	18.22	14.60
November (rainy season)	3	110.81	36.94	91.59

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*p value*	*F crit*
Between Groups	2314.55	3	771.52	28.79	0.0001	4.07
Within Groups	214.37	8	26.80			
Total	2528.93	11				

Notes: Dry season: June and July; rainy season: October and November.

Similar MP removal percentages were obtained among the WWTPs in Acapulco: WWTP (A), 93.6%; WWTP (B), 93.06%; WWTP (C), 93.91%. One factor ANOVA ($p > 0.05$) further revealed that there were no significant differences in the MP purification processes (Table 5); hence, the three WWTPs presented similarities in MP elimination for secondary treatment systems.

It has been documented that WWTPs with tertiary treatment systems have higher removal percentages than primary and secondary systems, reaching approximately 99.9% [32,46]. This is an alternative to reduce MP concentrations in treated effluents further. As the WWTPs analyzed in Acapulco do not present tertiary treatment, it is suggested to consider incorporating advanced systems to purify MP in the treated effluents before they are sent to the receiving bodies.

In this sense, complementary technological alternatives can be implemented to purify treated effluents. For example, in effluent treatments treated by sand filtration, 99.2−99.4% removal percentages were obtained [47], and 89.7% was achieved using disk filters [48]. Meanwhile, in Germany, an advanced oxidation system has been developed in a pilot plant coupled to a municipal WWTP induced by organosilanes in parallel to a filtration system using granular activated carbon; they obtained 60.9% MP removal [57]. In China,

the application of biofilters resulted in MP removal percentages of 79% and 89% by mass from treated effluents [58].

Table 5. One factor ANOVA ($p < 0.05$) according to the removal efficiencies of three WWTPs in Acapulco, Gro., Mex.

Effluent						
Group	Count	Sum	Average	Variance		
WWTP A	4	56.49	14.12	217.32		
WWTP B	4	75.02	18.76	433.26		
WWTP C	4	53.20	13.30	169.34		
ANOVA						
Source of Variation	SS	df	MS	F	*p* value	F crit
Between Groups	69.19	2	34.60	0.13	0.88	4.26
Within Groups	2459.74	9	273.30			
Total	2528.93	11				

The secondary treatment systems in the three Acapulco WWTPs present high MP removal rates; however, significant amounts of MP continue to be released into water bodies [33]. It is, therefore, necessary to implement regulatory norms and policies that establish technical criteria to increase the removal of MPs and prevent their entry into aquatic ecosystems. Hence, it is important to extrapolate the values obtained in this study concerning the capacities of WWTP operation to determine the daily and annual concentrations of MPs that will enter the aquatic environments (rivers, coastal lagoons, wetlands, and sea).

3.5. Daily and Annual MP Emission Projections

The average MP concentrations during the four-month study (Figure 6) reveal that the effluents from WWTP B presented higher concentrations of MPs (Figure 6a). The three WWTPs released a greater quantity of 38 μm MP particles. According to the operating capacity, WWTP C released the most MPs per day to hydrological bodies with values of 2.77×10^8 (150 μm) and 4.70×10^8 (38 μm) particles (Figure 6b). For example, contrasting the treatment systems in similar works, the approximate daily emission in Scotland was 65×10^6 particles [20]. In Italy, the emissions from one of its largest WWTPs (for a population serving 1,200,000 inhabitants) was 160×10^6 PM per day [19], while the average emission per WWTP in China amounts to 650×10^6 PM per day [13].

Regarding other studies carried out in tertiary treatment systems, average MP emissions of 2.2×10^7 particles/day have been recorded [29]. In Spain, the estimate of MPs released according to the particular conditions of the evaluated WWTPs was 1.6×10^7/day [4]. The daily projections of the three WWTPs in the current study indicate that their secondary treatment systems require other complementary technologies to reduce the MP concentrations in their effluents and prevent their spread to different receiving bodies.

In Acapulco, as the largest studied WWTP (650 L/s), WWTP C had the highest projected annual MP emission value (Figure 6c). However, these values were estimated based on the concentrations of MPs obtained from specific samplings, so that the annual concentration may present significant variations estimates based on composite samplings. Despite this limitation, the values obtained demonstrate the MP contamination of municipal WWTPs in Mexico, specifically in a tourist city.

Despite the various works on the presence of MPs in WWTPs, several have not reported annual projections [8,18,21,29–32,44,46,53,54,59]. However, in others that included projections, the annual MP emissions from WWTPs were ~0.3×10^9 particles [44], 9×10^7 and 4×10^9 particles [38] and 1.56×10^{14} particles [36]. The data obtained in the current study are within these other estimated values. Hence, it can be deduced that the issue of removing MPs in WWTPs is a global problem requiring the establishment of

regulatory standards and protocols to standardize sampling and laboratory analysis to reduce the emission of MP particles and subsequent environmental degradation caused by the emerging pollutants [60–62].

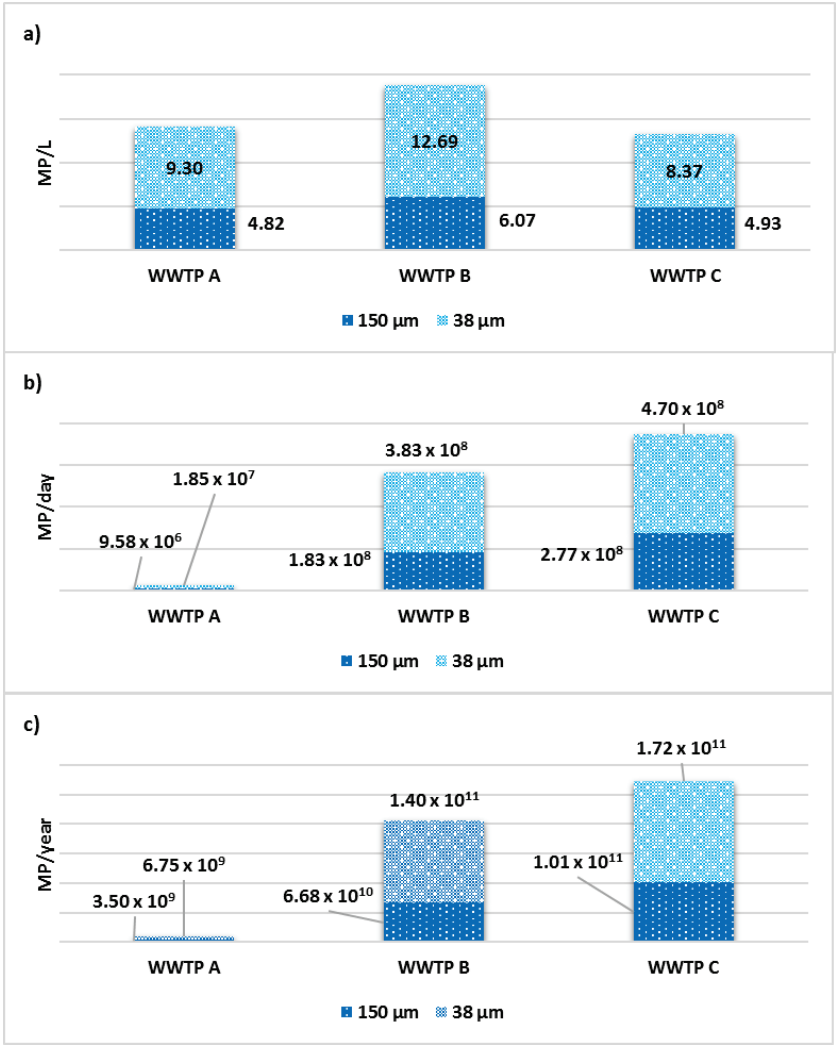

Figure 6. MP average concentrations per liter (**a**), daily projections (**b**), annual projections (**c**) for 150 and 38 µm particles of three WWTPs in Acapulco, Gro. Mex.

4. Conclusions

MPs were detected and characterized in three secondary-type municipal wastewater treatment plants in Acapulco, Mexico, that were monitored for four months in 2022. Regarding polymeric particle diversity according to their pigmentation, black was the most abundant in the influents and red in the effluents. Hence, the MP coloring must be considered in the regulatory criteria as an indicator of other associated pollutants, such as heavy metals within additives used in the plastic industry, that represent a serious threat to living organisms, including humans.

The diversity of sizes and shapes in MPs represents a challenge for developing standardized analytical methodologies due to the multidimensional nature of these polymeric particles. Therefore, it is suggested to carry out studies in other size ranges to expand the knowledge on MPs' qualitative and quantitative composition. Additionally, standardized analytical protocols, policies, and regulatory standards must be developed in the immediate future, and complementary technologies must be designed and implemented in the current wastewater treatment systems in Acapulco to retain and eliminate MPs in their different dimensions effectively.

This study shows the role of WWTPs in the retention and elimination of MPs, given the alarming global issue regarding plastic use and degradation, which are considered emerging pollutants. Within this context and the current lack of regulations and policies related to small particle plastic (e.g., MP) degradation in Mexico, the results of this study demonstrate the prevalence of MPs in three secondary municipal WWTPs in Acapulco, Mexico.

The results also indicate high MP removal efficiency by the three WWTPs; however, the effluents contain considerable amounts of MPs continuously released to receiving bodies. In this sense, temporality is a factor that influences MP quantity and removal. During rainy months, the highest MP load and lowest removal efficiencies were detected. However, the removal percentages only considered 150 and 38 μm particles and were, thus, only based on this size parameter. Hence, the removal efficiencies for other MP sizes may differ. Accordingly, it is suggested to incorporate finer meshes than those used in this study in future research. The annual MP load estimates indicate that aquatic ecosystems are highly exposed to the inappropriate use, consumption, and disposal of plastics. Moreover, there is a general lack of knowledge regarding the degradation of plastics to smaller sizes, such as MPs. Hence, the implementation of regulatory policies and standards must be promoted.

To date, no official Mexican standards establish maximum permissible limits for MPs in treated effluents discharged to receiving bodies. Based on the results of this study, it is recommended to continue advancing with work in other WWTPs at the local and national levels to incorporate MPs into the national agenda to update current regulations and policies in the medium term.

Author Contributions: Conceptualization, E.J.F.-M. and J.L.R.-A.; methodology, E.J.F.-M., J.L.R.-A., A.R.-H. and A.A.-S.; software, A.R.-H. and A.A.-S.; validation, E.J.F.-M., J.L.R.-A., A.R.-H. and A.A.-S.; formal analysis, E.J.F.-M., A.H-R. and J.L.R.-A.; data curation, E.J.F.-M., J.L.R.-A., A.R.-H. and A.A.-S.; writing—original draft preparation, E.J.F.-M. and J.L.R.-A.; writing—review and editing, E.J.F.-M., J.L.R.-A., A.R.-H. and A.A.-S.; visualization, E.J.F.-M., J.L.R.-A., A.R.-H., A.A.-S., R.M.B.-C. and J.V.-G.; supervision, J.L.R.-A., A.R.-H. and A.A.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by CONAHCYT, Mexico, through a graduate scholarship received by the first author (registration number 758303).

Data Availability Statement: All data presented in this research are contained in the article.

Acknowledgments: The authors thank the Acapulco Municipality Drinking Water and Sewerage Commission (CAPAMA) for allowing access to their facilities, especially Q.F.B. Gabriel Cejudo Blanco with the wastewater treatment systems. The authors also thank the Research Center of the Universidad del Papaloapan, Tuxtepec campus for the equipment for polymer chemical identification and Engineering in Water Treatment Systems S.A. de C.V. by microscopy.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci. Adv.* **2017**, *3*, e1700782. [CrossRef] [PubMed]
2. Meijer, L.J.J.; van Emmerik, T.; van der Ent, R.; Schmidt, C.; Lebreton, L. More than 1000 rivers account for 80% of global riverine plastic emissions into the ocean. *Sci. Adv.* **2021**, *7*, eaaz5803. [CrossRef]
3. Frias, J.P.G.L.; Nash, R. Microplastics: Finding a consensus on the definition. *Mar. Pollut. Bull.* **2019**, *138*, 145–147. [CrossRef]

4. Thompson, R.C.; Olsen, Y.; Mitchell, R.P.; Davis, A.; Rowland, S.J.; John, A.W.G.; McGonigle, D.; Russell, A.E. Lost at sea: Where is all the plastic? *Science* **2004**, *304*, 838. [CrossRef] [PubMed]
5. Hidalgo-Ruz, V.; Gutow, L.; Thompson, R.C.; Thiel, M. Microplastics in the marine environment: A review of the methods used for identification and quantification. *Environ. Sci. Technol.* **2012**, *46*, 3060–3075. [CrossRef]
6. Bucci, K.; Rochman, C.M. Microplastics: A multidimensional contaminant requires a multidimensional framework for assessing risk. *Microplastics Nanoplastics* **2022**, *2*, 7. [CrossRef]
7. Cox, K.D.; Covernton, G.A.; Davies, H.L.; Dower, J.F.; Juanes, F.; Dudas, S.E. Human Consumption of Microplastics. *Environ. Sci. Technol.* **2019**, *53*, 7068–7074. [CrossRef]
8. Ragusa, A.; Svelato, A.; Santacroce, C.; Catalano, P.; Notarstefato, V.; Carnevali, O.; Papa, F.; Rongioletti, M.C.A.; Baiocco, F.; Draghi, S.; et al. Plasticenta: First evidence of microplastics in human placenta. *Environ. Int.* **2021**, *146*, 106274. [CrossRef]
9. Schwabl, P.; Köppel, S.; Königshofer, P.; Bucsics, T.; Trauner, M.; Reiberger, T.; Liebmann, B. Detection of various Microplastics in Human Stool. *Ann. Intern. Med.* **2019**, *171*, 453–457. [CrossRef] [PubMed]
10. Tamargo, A.; Molinero, N.; Reinosa, J.J.; Alcolea-Rodriguez, V.; Portela, R.; Bañares, M.A.; Fernández, J.F.; Moreno-Arribas, M.V. PET microplastics affect human gut microbiota communities during simulated gastrointestinal digestion, first evidence of plausible polymer biodegradation during human digestion. *Sci. Rep.* **2022**, *12*, 528. [CrossRef] [PubMed]
11. Çobanoğlu, H.; Belivermiş, M.; Sıkdokur, E.; Kılıç, Ö.; Çayır, A. Genotoxic and cytotoxic effects of polyethylene microplastics on human peripheral blood lymphocytes. *Chemosphere* **2021**, *272*, 129805. [CrossRef]
12. Elkhatib, D.; Oyanedel-Craver, V. A critical review of extraction and identification methods of microplátics in wastewater and drinking wáter. *Environ. Sci. Technol.* **2020**, *54*, 7037–7049. [CrossRef] [PubMed]
13. Park, H.; Park, B. Review of microplastic distribution, toxicity, análisis methods, and removal technologies. *Water* **2021**, *13*, 2736. [CrossRef]
14. Sun, J.; Dai, X.; Wang, Q.; van Loosdrecht, M.C.M.; Ni, B.J. Microplastics in wastewater treatment plants: Detection, occurrence and removal. *Water Res.* **2019**, *152*, 21–37. [CrossRef] [PubMed]
15. Hernández, A. *Depuración y Desinfección de Aguas Residuales*, 5th ed.; Colegio de Ingenieros de Caminos, Canales y Puertos: Madrid, Spain, 2001; pp. 88–120.
16. Calero, M.; Gody, V.; Quesada, L.; Martín-Lara, M.A. Green strategies for microplastics reduction. *Green Sustain. Chem.* **2021**, *28*, 100442. [CrossRef]
17. Alvim, C.B.; Bes-Piá, M.A.; Mendoza-Roca, J.A. Separation and identification of microplastics from primary and secondary effluents and activated sludge from wastewater treatment plants. *Chem. Eng. J.* **2020**, *402*, 126293. [CrossRef]
18. Yang, L.; Li, K.; Cui, S.; Kang, Y.; An, L.; Lei, K. Removal of microplastics in municipal sewage from China's largest water reclamation plant. *Water Res.* **2019**, *155*, 175–181. [CrossRef] [PubMed]
19. Magni, S.; Binelli, A.; Pittura, L.; Avio, C.G.; Della Torre, C.; Parenti, C.C.; Gorbi, S.; Regoli, F. The fate of microplastics in an italian wastewater treatment plant. *Sci. Total Environ.* **2019**, *652*, 602–610. [CrossRef]
20. Murphy, F.; Ewins, C.; Carbonnier, F.; Quinn, B. Wastewater treatment works (WwTW) as a source of microplastics in the aquatic environment. *Environ. Sci. Technol.* **2016**, *50*, 5800–5808. [CrossRef]
21. Talvitie, J.; Heinonen, M.; Pääkkönen, J.P.; Vahtera, E.; Mikola, A.; Setälä, O.; Vahala, R. Do wastewater treatment plants act as a potential point source of microplastics? Preliminary study in the coastal Gulf of Finland, Baltic Sea. *Water Sci. Technol.* **2015**, *72*, 1495–1504. [CrossRef] [PubMed]
22. Mato, Y.; Isobe, T.; Takada, H.; Kanehiro, H.; Ohtake, C.; Kaminuma, T. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environ. Sci. Technol.* **2001**, *35*, 318–324. [CrossRef]
23. Ricciardi, M.; Pironti, C.; Motta, O.; Miele, Y.; Proto, A.; Montano, L. Microplastics in the aquatic environment: Occurrence, persistence, análisis, and human exposure. *Water* **2021**, *13*, 973. [CrossRef]
24. *NOM-001-SEMARNAT-2021*; Norma Oficial Mexicana—Que Establece los Límites Permisibles de Contaminantes en las Descargas de Aguas Residuales en Cuerpos Receptores Propiedad de la Nación. Comisión Nacional del Agua, Diario Oficial de la Federación: Ciudad de México, México, 2022.
25. *NOM-002-SEMARNAT-1996*; Norma Oficial Mexicana—Que Establece los Límites Máximos Permisibles de Contaminantes en las Descargas de Aguas Residuales a los Sistemas de Alcantarillado Urbano o Municipal. Comisión Nacional del Agua, Diario Oficial de la Federación: Ciudad de México, México, 1998.
26. *NOM-003-SEMARNAT-1997*; Norma Oficial Mexicana—Que Establece los Límites Máximos Permisibles de Contaminantes para las Aguas Residuales Tratadas que se Reúsen en Servicios al Público. Comisión Nacional del Agua, Diario Oficial de la Federación: Ciudad de México, México, 1998.
27. Cámara de Diputados del H. Congreso de la Unión. *Ley de Aguas Nacionales, Constitución Política Mexicana—Tiene por Objeto Regular la Explotación, Uso o Aprovechamiento de Dichas Aguas, su Distribución y Control, Así Como la Preservación de su Cantidad y Calidad para Lograr su Desarrollo Integral Sustentable*; Cámara de Diputados del H. Congreso de la Unión, Diario Oficial de la Federación: Ciudad de México, México, 2023.
28. Masura, J.; Baker, J.; Foster, G.; Arthur, C. Laboratory Methods for the Analysis of Microplastics in the Marine Environment; NOAA Marine Debris Program, 2015, Technical Memorandum NOS-OR&R-48. Available online: https://marinedebris.noaa.gov/sites/default/files/publications-files/noaa_microplastics_methods_manual.pdf (accessed on 1 October 2023).

29. Blair, R.M.; Waldron, S.; Gauchotte-Lindsay, C. Average daily flow of microplastics through a tertiary wastewater treatment plant over a ten-month period. *Water Res.* **2019**, *163*, 114909. [CrossRef]
30. Galafassi, S.; Di Cesare, A.; Di Nardo, L.; Sabatino, R.; Valsesia, A.; Fumagalli, F.S.; Corno, G.; Volta, P. Microplastic retention on small and medium municipal wastewater treatment plants and the role of the desinfection. *Environ. Sci. Pollut. Res.* **2022**, *29*, 10535–10546. [CrossRef] [PubMed]
31. Hernández Fernández, J.; Cano, H.; Guerra, Y.; Puello Polo, E.; Ríos-Rojas, J.F.; Vivas-Reyes, R.; Oviedo, J. Identification and quantification of microplastics in effluents of wastewater treatment plant by differential scanning calorimetry (DSC). *Sustainability* **2022**, *14*, 4920. [CrossRef]
32. Talvitie, J.; Mikola, A.; Koinstinen, A.; Setälä, O. Solutions to microplastic pollution -Removal of microplastics from wastewater effluent with advanced wastewater treatment technologies. *Water Res.* **2017**, *123*, 401–407. [CrossRef] [PubMed]
33. Lee, H.; Kim, Y. Treatment characteristics of microplastics at biological sewage treatment facilities in Korea. *Mar. Pollut. Bull.* **2018**, *137*, 1–8. [CrossRef]
34. Talvitie, J.; Mikola, A.; Setälä, O.; Heinonen, M.; Koistinen, A. How well is microlitter purified from wastewater?—A detailed study on the stepwise removal of microlitter in a tertiary level wastewater treatmetn plant. *Water Res.* **2017**, *109*, 164–172. [CrossRef]
35. Asamoah, B.O.; Salmi, P.; Räty, J.; Ryymin, K.; Talvitie, J.; Karjalainen, A.K.; Kukkonen, J.V.K.; Roussey, M.; Peiponen, K.E. Optical monitoring of microplastics filtrated from wastewater sludge and suspended in etanol. *Polymers* **2021**, *13*, 871. [CrossRef]
36. Li, X.; Chen, L.; Mei, Q.; Dong, B.; Dai, X.; Ding, G.; Zeng, E.Y. Microplastics in sewage sludge from the wastewater plants in China. *Water Res.* **2018**, *142*, 75–85. [CrossRef]
37. Mahon, A.M.; O'Conell, B.; Healy, M.G.; O'Connor, I.; Officer, R.; Nash, R.; Morrison, L. Microplastics in sewage sludge: Effects of treatment. *Environ. Sci. Technol.* **2017**, *51*, 810–818. [CrossRef]
38. Mintenig, S.M.; Int-Veen, I.; Löder, M.G.J.; Primpke, S.; Gerdts, G. Identification of microplastics in effluents of waste water treatment plants using focal plane-based micro-Fourier transform infrared imaging. *Water Res.* **2017**, *108*, 365–372. [CrossRef]
39. Hahladakis, J.N.; Velis, C.A.; Weber, R.; Iacovidou, E.; Purnell, P. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. *J. Hazard. Mater.* **2018**, *344*, 179–199. [CrossRef]
40. Beiras, R.; Verdejo, E.; Campoy-López, P.; Vidal-Liñán, L. Aquatic toxicity of chemically defined microplastics can be explained by functional additives. *J. Hazard. Mater.* **2021**, *406*, 124388. [CrossRef] [PubMed]
41. Turner, A.; Filella, M. Hazardous metal additives in plastics and their environmental impacts. *Environ. Int.* **2021**, *156*, 106622. [CrossRef]
42. *NMX-E-232-CNCP-2011*; Norma Oficial Mexicana—Industria del Plástico. Símbolos de Identificación de Plásticos. Dirección General de Normas, Diario Oficial de la Federación: Ciudad de México, México, 2011.
43. Gies, E.A.; LeNoble, J.L.; Noël, M.; Etemadifar, A.; Bishay, F.; Hall, E.R.; Ross, P.S. Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada. *Mar. Pollut. Bull.* **2018**, *133*, 553–561. [CrossRef] [PubMed]
44. Kwon, H.J.; Hidayaturrahman, H.; Peera, S.G.; Lee, T.G. Elimination of microplastics at different stages in wastewater treatment plants. *Water* **2022**, *14*, 2404. [CrossRef]
45. Meng, X.; Bao, T.; Hong, L.; Wu, K. Occurrence characterization and contamination risk evaluation of microplastics om Hefei's urban wastewater treatment plant. *Water* **2023**, *15*, 686. [CrossRef]
46. Bayo, J.; Olmos, S.; López-Castellanos, J. Assesment of microplastics in a municipal wastewater treatment plant with tertiary treatment: Removal efficiencies and loading per day into the environment. *Water* **2021**, *13*, 1339. [CrossRef]
47. Wolff, S.; Weber, F.; Kerpen, J.; Winklhofer, M.; Engelhart, M.; Barkmann, L. Elimination of microplastics by downstream sand filters in wastewater treatment. *Water* **2020**, *13*, 33. [CrossRef]
48. Simon, M.; Vianello, A.; Vollertsen, J. Removal of >10 µm microplastic particles from treated wastewater by a disc filter. *Water* **2019**, *11*, 1935. [CrossRef]
49. Syberg, K.; Nielsen, M.B.; Clausen, L.P.W.; van Calster, G.; van Wezel, A.; Rochman, C.; Koelmans, A.A.; Cronin, R.; Pahl, S.; Hansen, S.F. Regulation of plastic from a circular economy perspective. *Green Sustain. Chem.* **2021**, *29*, 100462. [CrossRef]
50. Lv, X.; Dong, Q.; Zuo, Z.; Liu, Y.; Huang, X.; Wu, W.M. Microplastics in a municipal wastewater treatment plant: Fate, dynamic distribution, removal efficiences, and control strategies. *J. Clean. Prod.* **2019**, *225*, 579–586. [CrossRef]
51. Rasmussen, L.A.; Iordachescu, L.; Tumlin, S.; Vollersten, J. A complete mass balance for plastics in a wastewater treatment plant -Macroplastics contributes more than microplastics. *Water Res.* **2021**, *2021*, 117307. [CrossRef] [PubMed]
52. Liu, W.; Zhang, J.; Liu, H.; Guo, X.; Zhang, X.; Yao, X.; Cao, Z.; Zhang, T. A review of the removal of microplastics in global wastewater treatment plants: Characteristics and mechanims. *Environ. Int.* **2021**, *146*, 106277. [CrossRef]
53. Liu, X.; Yuan, W.; Di, M.; Li, Z.; Wang, J. Transfer and fate of microplastics during the conventional activated sludge process in one wastewater tratment plant of China. *Chem. Eng. J.* **2019**, *362*, 176–182. [CrossRef]
54. Lares, M.; Ncibi, M.C.; Sillanpää, M.; Sillanpää, M. Ocurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. *Water Res.* **2018**, *133*, 236–246. [CrossRef]
55. Collivignarelli, M.C.; Miino, M.C.; Caccamo, F.M.; Milanese, C. Microplastics in sewage sludge: A known but underrated pathway in wastewater treatment plants. *Sustainability* **2021**, *13*, 12591. [CrossRef]
56. Park, T.J.; Lee, S.H.; Lee, M.S.; Lee, J.K.; Park, J.H.; Zoh, K.D. Distributions of microplastics in surface wáter, fish, and sediment in the vicinity of a sewage treatment plant. *Water* **2020**, *12*, 3333. [CrossRef]

57. Sturm, M.T.; Myers, E.; Schober, D.; Korzin, A.; Thege, C.; Schuhen, K. Comparision of AOP, GAC, and novel organosilane-based process for the removal of microplastics at a municipal wastewater treatment plant. *Water* **2023**, *15*, 1164. [CrossRef]
58. Liu, F.; Nord, N.B.; Bester, K.; Vollertsen, J. Microplastics removal from treated wastewater by a biofilter. *Water* **2020**, *12*, 1085. [CrossRef]
59. Anderson, Z.T.; Cundy, A.B.; Croudace, I.W.; Warwick, P.E.; Celis-Hernández, O.; Stead, J.L. A rapid method for assessing the accumulation of microplastics in the sea surface microlayer (SML) of estuarine systems. *Sci. Rep.* **2018**, *8*, 9428. [CrossRef] [PubMed]
60. Li, X.; Bao, L.; Wei, Y.; Zhao, W.; Wang, F.; Liu, W.; Su, H.; Zhang, R. Occurrence, bioacumulation, and risk assessment of microplastics in the aquatic environment: A review. *Water* **2023**, *15*, 1768. [CrossRef]
61. Haque, F.; Fan, C. Fate of microplastics under the influence of climate change. *iScience* **2023**, *26*, 107649. [CrossRef] [PubMed]
62. Horton, A.A.; Walton, A.; Spurgeon, D.J.; Lahive, E.; Svendsen, C. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total Environ.* **2017**, *586*, 127–141. [CrossRef] [PubMed]

toxics

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Article

Influence of Microplastics on Morphological Manifestations of Experimental Acute Colitis

Natalia Zolotova, Dzhuliia Dzhalilova *, Ivan Tsvetkov and Olga Makarova

Department of Immunomorphology of Inflammation, Avtsyn Research Institute of Human Morphology of Federal State Budgetary Scientific Institution "Petrovsky National Research Centre of Surgery", 117418 Moscow, Russia; natashazltv@gmail.com (N.Z.); davedm66@gmail.com (I.T.); makarov.olga2013@yandex.ru (O.M.)
* Correspondence: juliajal93@mail.ru

Abstract: Microplastic pollution poses a threat to human health. It is possible that the increase in the incidence of inflammatory bowel disease is associated with exposure to microplastics. We investigated the effect of the consumption of polystyrene microparticles with a diameter of 5 μm at a dose of 2.3 mg/kg/day for 6 weeks on morphological changes in the colons of healthy male C57BL/6 mice and of mice with acute colitis induced by a 1% dextran sulfate sodium solution (DSS). In healthy mice, microplastics caused an increase in the number of endocrine cells, an increase in the content of highly sulfated mucins in goblet cells, an increase in the number of cells in the lamina propria, and a decrease in the volume fraction of macrophages. Microplastic consumption caused more severe acute colitis, which is characterized by a greater prevalence of ulcers and inflammation and a decrease in the content of neutral mucins in goblet cells.

Keywords: microplastics; polystyrene; colon; colitis; dextran sulfate sodium; mice

Citation: Zolotova, N.; Dzhalilova, D.; Tsvetkov, I.; Makarova, O. Influence of Microplastics on Morphological Manifestations of Experimental Acute Colitis. *Toxics* **2023**, *11*, 730. https://doi.org/10.3390/toxics11090730

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

Received: 1 August 2023
Revised: 22 August 2023
Accepted: 23 August 2023
Published: 25 August 2023

1. Introduction

Plastic pollution is a global environmental problem. Of particular concern are small plastic particles with a diameter of less than 5 mm—microplastics (MPs). Due to the small size of the particles, MPs are intensively distributed in the environment via water and wind. MPs are found all over the globe: in the air, soil, and water, in polar ice, at the depths of the seas, and in living organisms [1–3]. In this regard, the question of the impact of MPs on human health is pertinent. To study the toxic effects of various substances, the pathogenesis of human diseases, and the preclinical evaluation of drugs, laboratory mice are most often used as model organisms. Therefore, in recent years, active work has been carried out to study the effects of MPs on the mouse organism.

It was demonstrated that orally consumed MPs accumulate in the large intestine of mice, causing damage to the epithelial barrier of the colon and changes in the composition of the intestinal microflora. MPs penetrate into the liver and kidneys of mice, causing inflammatory changes in these organs, a decrease in the relative weight of the liver, disturbances in carbohydrate and lipid metabolism, and oxidative stress. MPs, due to the induction of oxidative stress and damage to mitochondria, can cause the death of cardiomyocytes and the development of myocardial fibrosis. Moreover, MPs cause cognitive impairment and affect the behavior of animals, impair reproductive function, and cause disturbances in the development of offspring [1,4–11].

People consume MPs mainly via water and food, and the first target of their action is the gastrointestinal tract [3]. It is possible that the worldwide increase in the incidence of inflammatory bowel diseases (IBDs), including ulcerative colitis, is associated with an increase in the number of MP particles in the environment [12]. It was demonstrated that, in Taiwan, where the basis of people's diet is seafood from heavily polluted waters, the incidence of ulcerative colitis over 10 years has increased by more than 1.5 times—from

0.61 per 100,000 people in 1998 to 0.98 per 100,000 in 2008 [13]. Z. Yan et al. (2022) found that the concentration of MP particles in the feces of patients with IBDs is significantly higher than that of healthy people and that there is a positive correlation between the concentration of MPs in feces and the severity of IBDs [14].

To date, only three studies on the effect of MPs on the course of experimental colitis have been published [15–17]. According to these studies, exposure to MPs in mice with experimental colitis causes a more pronounced shortening of the colon, increases the severity of structural damage and inflammation, reduces mucus secretion, increases colon permeability and levels of pro-inflammatory cytokines in the blood serum, and exacerbates pathological changes in the liver. However, morphological changes in the colon mucosa during colitis against the background of MP consumption have not been studied.

Therefore, the aim of this study was to evaluate the effect of microplastic consumption on morphological changes in the colon during experimental acute colitis.

2. Materials and Methods

The study was performed on 32 adult male C57BL/6 mice obtained from the "Stolbovaya" branch of the Federal State Budgetary Institution of Science's "Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency", Russia. The mice were 1.5 months old and weighed 20–24 g. The mice were kept as 8 animals per cage in an open system at a temperature of 18–21 °C with natural light and had free access to water and feed. All efforts were made to decrease suffering and possible stress for the animals; the study was performed in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

The animals were divided into 4 groups of 8 mice each (Figure 1): K—the control group, in which, throughout the experiment, the mice received distilled water; P—the model of MP consumption; D—the model of acute DSS colitis; PD—the model of acute DSS colitis against the background of MP consumption. The drinking water of the animals in groups P and PD was substituted for 6 weeks with a suspension of polystyrene (PS) microparticles of 5 μm in diameter in distilled water at a concentration of 10 mg/L (79633, Sigma-Aldrich, St. Louis, MO, USA). The plastic type, particle size, and slurry concentration were consistent with previous studies [6,18]. Glass drinking vessels were used to avoid foreign plastic particles entering the water. The average weight of the mice was 22 g, the animals drank about 5 mL of the suspension per day, respectively, and the MP dose was about 2.3 mg/kg/day. Senathirajah K. et al. (2021) estimated that globally, on average, humans may ingest 0.1–5 g of MPs weekly through various exposure pathways, corresponding to approximately 0.2–10 mg/kg/day [19]. For the induction of acute colitis in the animals of groups D and PD, for 5 days (from days 36 to 40 of the experiment), dextran sulfate sodium (DSS) with a molecular weight of 40 kDa (AppliChem) was added to the drinking vessels with a final concentration of 1%. For 5 days, each animal consumed approximately 0.25 g of DSS [20]. The animals were taken out of the experiment on the 43rd day via the method of cervical dislocation under ether anesthesia.

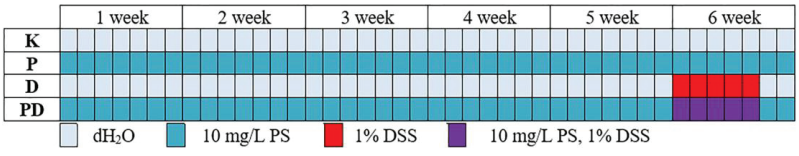

Figure 1. Experimental design.

In DSS-induced colitis, the most pronounced morphological changes are observed in the distal colon [20]. Therefore, the distal colon was taken. It was opened along the mesentery, washed with phosphate-buffered saline at pH 7.4, straightened on a filter, and fixed in 10% buffered formalin (Biovitrum, St. Petersburg, Russia) for a day. Histological

sections were made with a thickness of 5 μm. The histological sections were stained with hematoxylin and eosin, alcian blue at pH 1.0, and PAS reaction and immunofluorescence staining with antibodies to macrophage marker CD68 (DF7518 Affinity Biosciences, Jhubei City, Hsinchu County, Taiwan, dilution 1/100) and endocrine cell marker chromogranin A (ab15160, Abcam Inc, Boston, MA, USA, 1/200). The fluorescently labeled secondary antibodies Goat anti-Rabbit IgG (H + L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 (A11008, Invitrogen, Waltham, MA, USA, 1/300) were used. A morphological study was carried out using the program ImageJ.

To assess the prevalence of ulcers and inflammation, longitudinal sections of the distal intestine were scanned along the entire length at a magnification of 100. The total length of the section along the lamina muscularis mucosa and the length of its sections, along which ulcers and inflammatory infiltrates occurred, were measured. The percentage of the length of the intestine with ulcers and inflammation was calculated.

To evaluate inflammatory infiltration, the sections stained with hematoxylin and eosin were photographed at a magnification of 320 in 2 fields of view. We measured the area of the connective tissue of the lamina propria and counted the number of nuclei. The number of cellular elements per 1 mm^2 of the lamina propria area was calculated.

To assess the volume fraction of macrophages, the preparations stained with antibodies to CD68 were photographed at a magnification of 200 in 2 fields of view. The images were binarized, the area with correctly oriented crypts was circled from the lamina muscularis mucosa to the lumen, and its area and the area of the macrophages were determined. The volume fraction of macrophages was calculated as the ratio of the area of CD68-positive cells to the area of the mucosa.

To assess the number of endocrine cells, the sections stained with antibodies to chromogranin A were photographed at a magnification of 100 in 2 fields of view. The area of the mucosa with correctly oriented crypts was measured. The number of chromogranin A-positive cells in the isolated area was counted. The number of chromogranin A-positive cells per 1 mm^2 of the mucosa was calculated.

To assess the volume fraction of goblet cells and the content of highly sulfated and neutral mucins in them, the sections stained with alcian blue at pH 1.0 and with the PAS reaction were photographed at a magnification of 200 in 3 fields of view under the same lighting conditions. Binarization section images with the PAS reaction were obtained, setting the threshold so that only goblet cells were isolated. The area with correctly oriented crypts from the lamina muscularis mucosa to the lumen was outlined, and its area and the area of goblet cells were determined. The volume fraction of goblet cells was calculated as the ratio of the area of goblet cells to the area of the mucosa. On the images of the sections stained with alcian blue and after the PAS reaction, the average brightness of the goblet cell points and the background (the image area without tissue) was measured. The optical density of goblet cells was calculated as a decimal logarithm of the ratio of the average brightness of background dots to the average brightness of goblet cell dots. The higher the optical density, the higher the content of highly sulfated (alcian blue) or neutral (PAS reaction) mucins in goblet cells detected.

The obtained data were statistically processed using STATISTICA 6.0 (StatSoft, Inc., Tulsa, OK, USA). Nonparametric statistics methods were used since the samples were small (8 animals per group) and the parameter values were not normally distributed (χ^2 criterion). The samples were described in terms of the median and IQR (25%; 75%). To compare the two groups, the Mann–Whitney U-test was used, and differences were considered statistically significant at $p < 0.05$. To compare the four groups, the Kruskal–Wallis test was used; at $p < 0.05$, a posteriori pairwise comparisons were made according to the Mann–Whitney U-test with Bonferroni correction. Differences were considered statistically significant at $p < 0.0085$.

3. Results

After the histological study of the distal colon of control group mice (group K) and those who consumed PS particles with a diameter of 5 μm at a dose of 2.3 mg/kg/day for 6 weeks in drinking water (group P), no pronounced differences were observed (Figure 2A,B). In the distal colon of all animals, the epithelium was preserved throughout the mucosa. The crypts were deep, their openings narrow. The lamina propria and the submucosa contained a small amount of evenly distributed cellular elements—fibrocytes, fibroblasts, lymphocytes, and single histiocytes. For the animals with acute DSS-induced colitis, both the animals without MP exposure (group D) and the mice that consumed MPs (group PD), the morphological picture of the colon was mosaic. The most pronounced pathological changes in the colon were represented by extensive ulcers extending to the lamina muscularis mucosa. In areas with preserved epithelia and crypts, areas with severe inflammatory infiltration were identified. In these areas, the number of goblet cells was sharply reduced (Figure 2C–F). There were also areas that did not differ from the control group.

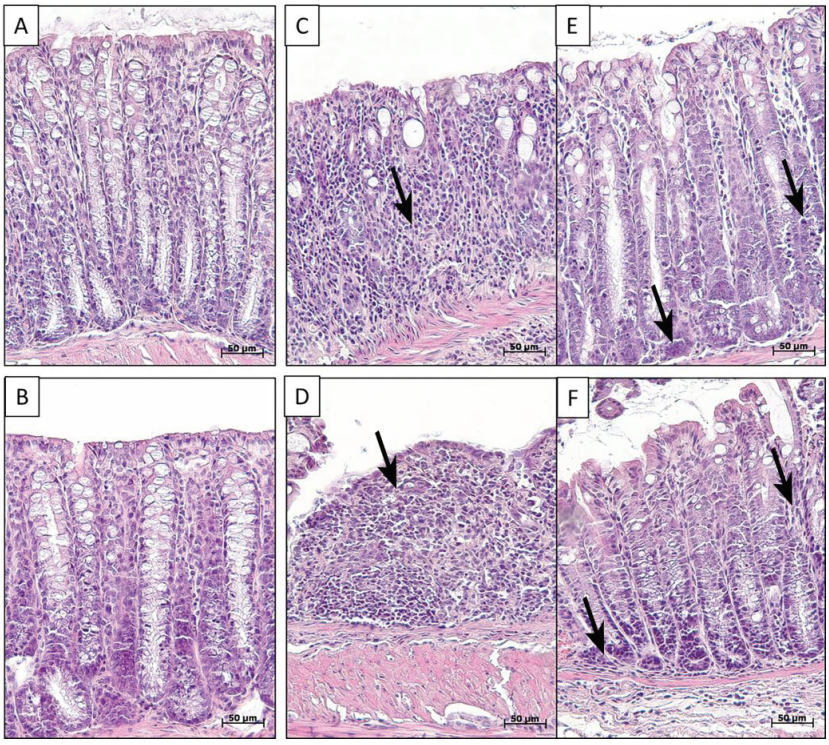

Figure 2. Distal colon of control mice (**A**), MP-treated mice (**B**), and mice with acute colitis that did not receive (**C**,**E**) and did receive MPs (**D**,**F**). In figures (**C**) and (**D**)—ulcers; (**E**,**F**)—inflammation (arrows). Hematoxylin and eosin staining. In control (**A**) and MP-treated (**B**) mice, mucus was normal: epi-thelium was preserved throughout the mucosa, the crypts were deep, and there were a lot of goblet cells and a small amount of immune cells. In mice with colitis which did not receive (**C**,**E**) and did receive MPs (**D**,**F**), there were acute ulcers and inflammation areas (arrows) with reduced goblet cell numbers that were infiltrated with neutrophils and lymphocytes.

The prevalence of the ulcerative inflammatory process varied greatly between animals with colitis, even within the same group (Figures 3 and 4); however, in the group of animals

treated with MPs, the prevalence of the ulcerative inflammatory process was statistically significantly higher (Figure 5).

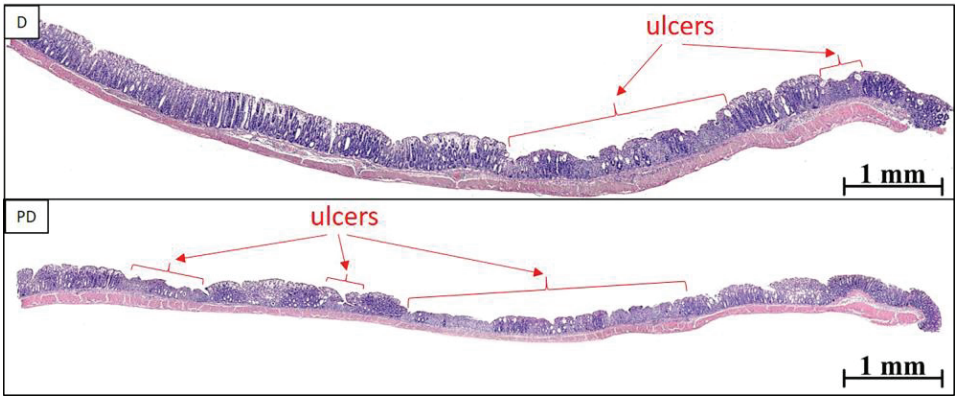

Figure 3. Ulcerative inflammatory changes in the distal colon of mice with acute colitis that did not receive (D) and did receive MPs (PD). Ulcers—arrows.

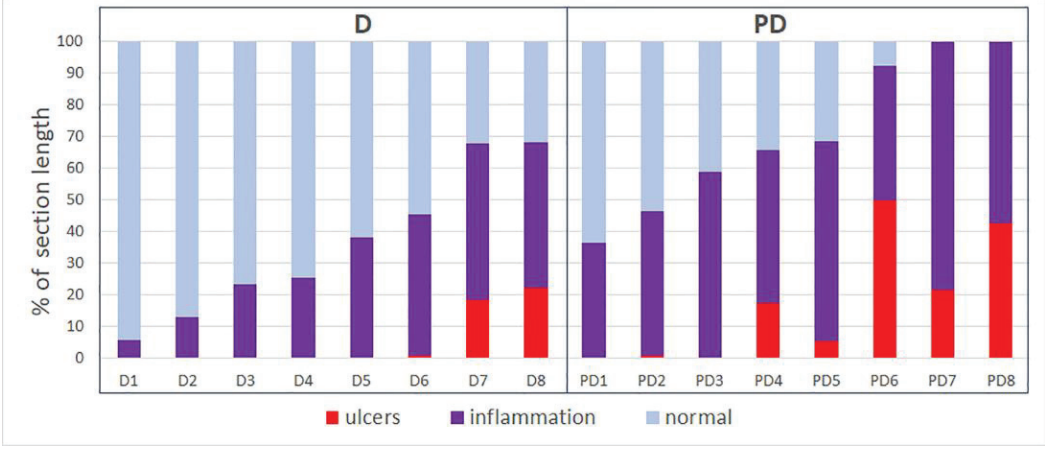

Figure 4. The prevalence of ulcers and inflammatory infiltration in the distal colon of mice with acute colitis that did not receive (D) and did receive MPs (PD).

In group P, compared with the control group, the content of cells in the lamina propria of the mucosa increased, but the volume fraction of macrophages in the mucosa decreased. The number of chromogranin A-positive endocrine cells and the content of highly sulfated mucins in goblet cells increased. There was a tendency to decrease the volume fraction of goblet cells (Figures 6 and 7).

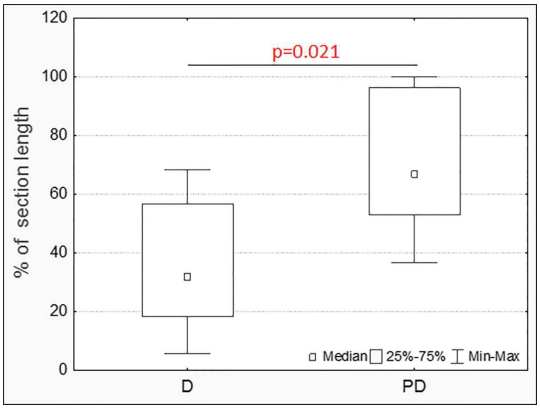

Figure 5. The prevalence of ulcers and inflammatory infiltration in the distal colon of mice with acute colitis that did not receive (D) and did receive MPs (PD) (Mann–Whitney test).

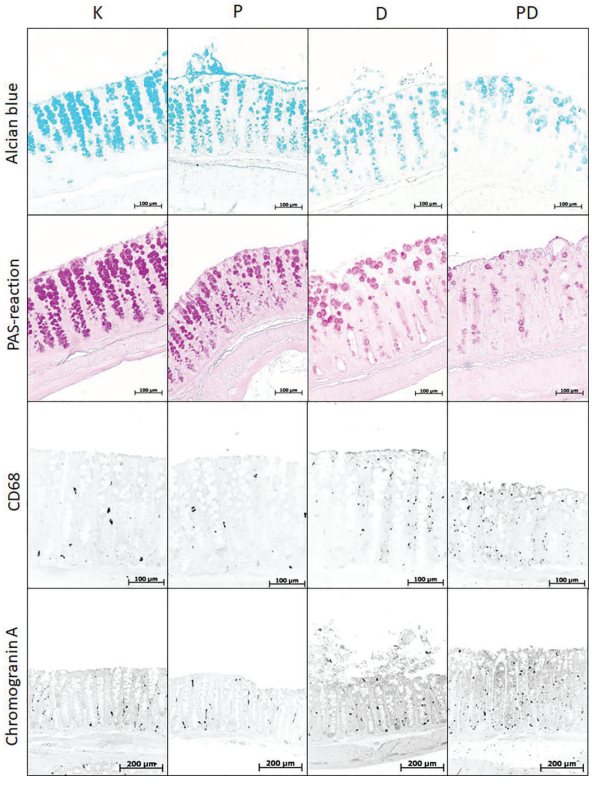

Figure 6. Distal colon of mice in the control group (K), consuming microplastics (P) with acute DSS-induced colitis (D) and with acute colitis against the background of microplastic consumption (PD), stained with alcian blue at pH 1.0 (highly sulfated mucins), the PAS reaction (neutral mucins), immunofluorescent staining with antibodies to CD68 (macrophages) and chromogranin A (endocrine cells). Photographs of sections with immunofluorescent staining are discolored and inverted for better contrast.

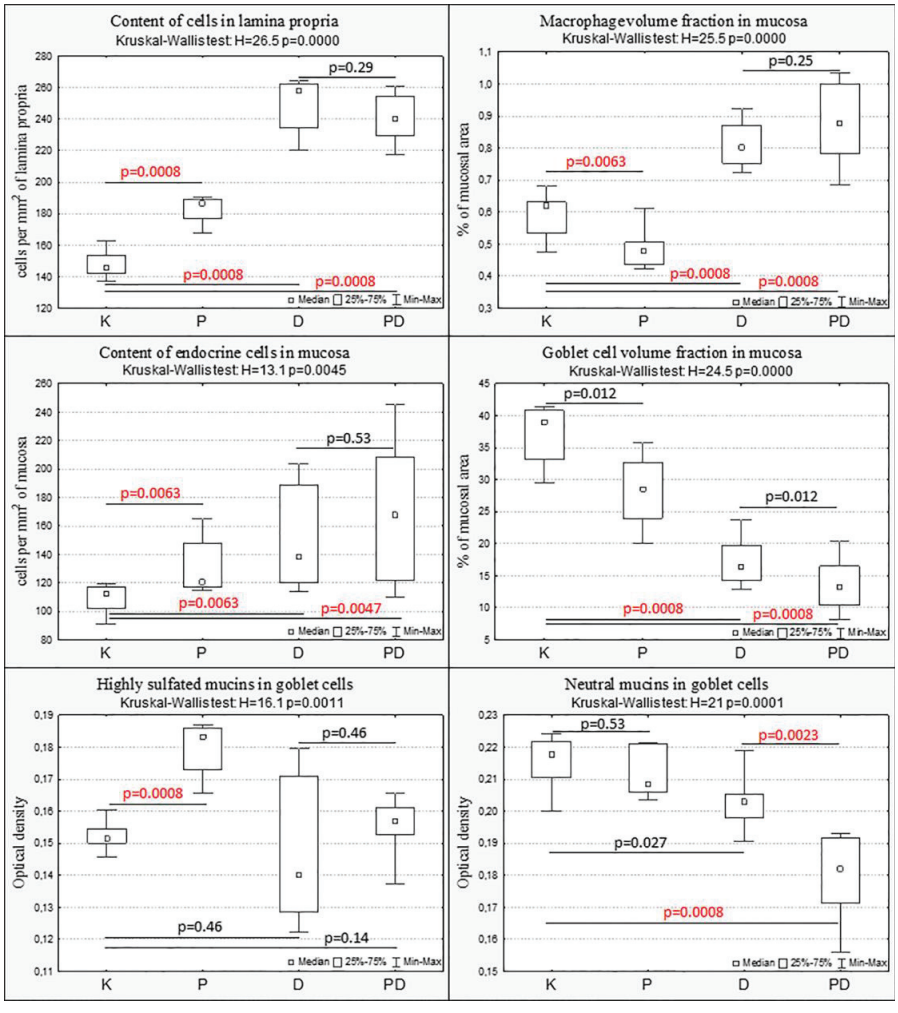

Figure 7. Changes in the mucosa of the distal colon with MP consumption (P), acute colitis (D), and acute colitis with MP consumption (PD) compared with the control group (K). Kruskal–Wallis test, post hoc comparisons—Mann–Whitney U-test with Bonferroni correction, statistically significant differences $p < 0.0085$.

In animals with colitis, changes in the colon mucosa were assessed in areas without ulcers but with pronounced inflammatory changes. In groups D and PD, a pronounced inflammatory infiltration of the mucosa was observed, especially in the basal part of the lamina propria of the mucosa. Compared with the control group, in mice with acute colitis that consumed and did not consume MP, the content of cellular elements in the lamina propria mucosa, the volume fraction of macrophages, and the number of endocrine cells in the mucosa increased, and the volume fraction of goblet cells decreased. In the PD group, the content of neutral mucins in goblet cells also decreased (Figures 6 and 7). In the PD group, compared with the D group, the content of neutral mucins in goblet cells was statistically significantly reduced (Figures 6 and 7).

4. Discussion

4.1. The Effect of MPs on the Colon Mucosa in Normal Mice

To date, about 20 experimental studies using mice have been published that have evaluated the MP effect on colon structure and function. The earliest of these works came out in 2018. Dysbiosis of the intestinal microflora was revealed in mice treated with MPs [21–25]. According to a number of authors, exposure to MPs causes mild or moderate inflammation in the colon, characterized by weak inflammatory infiltration of the mucosa, the activation of pro-inflammatory signaling pathways, and the increased expression of pro-inflammatory cytokines [17,23,24,26–30]. Violations of antioxidant defense and the development of oxidative stress were revealed [28–31]. MPs cause damage to the epithelial barrier of the colon. The stimulation of apoptosis [30] and the proliferation [17] of intestinal epithelial cells were observed. A decrease in the number of goblet cells [17,26,29], mucin expression [24,31], and mucus secretion [21,22,25,30,31] were revealed. There is a decrease in the expression of tight junction protein genes [9,30] and an increase in the permeability of the intestinal barrier [28].

We found an increase in the content of cellular elements in the lamina propria of the colon mucosa in mice that consumed PS microparticles with a diameter of 5 μm at a dose of 2.3 mg/kg/day during exposure for 6 weeks, which indirectly indicates an increase in the permeability of the epithelial barrier for luminal antigens and the activation of the local compartment of the immune system. At the same time, the volume fraction of macrophages in the mucosa decreased, which was probably due to its edema. According to Li et al. (2020), C57BL/6 mice treated for 5 weeks with 600 μg/day of 10–150 μm polyethylene (PE) particles showed inflammation in the colon and a higher expression of TLR4, AP-1, and IRF5 [23]. Xie S et al. (2023) noted an increase in the levels of IL-1β and IL-6 in the colon of C57BL/6 mice fed a suspension of 5 μm PS particles at a concentration of 100 μg/L for 6 weeks [17]. Jia R et al. (2023) reported mild inflammatory infiltration of the colon, an increase in the concentration of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, and a decrease in anti-inflammatory IL-10 in C57BL/6 mice fed a suspension of polypropylene (PP) particles with a diameter of 8 microns at a concentration of 10 mg/mL for 4 weeks [30]. Rawle et al. (2022), using C57BL/6J mice treated with 1 μm PS particles at a dose of 80 μg/kg/day for 33 days via the RNA-Seq method, revealed an increase in the expression of a number of genes associated with inflammation [27]. Xie L et al. (2022) studied the effect of various types of plastic particles with a diameter of 150–300 microns on Kunming mice and found that, in animals that received 0.2 mL of a microplastic suspension at a concentration of 20 mg/mL per day for 7 days, inflammatory infiltration of the colon mucosa and its severity depended on the type of plastic: PS > PVC > PET > PE > PP [29].

We observed an increase in the content of chromogranin A-positive endocrine cells in the distal colon mucosa of mice that consumed MP. There are no published data on the effect of MPs on endocrine cells in the colon. In mice, about half of the endocrine cells in the colon are serotonin-secreting Ec-cells. [32]. Serotonin promotes mucus secretion, accelerates the release of digestive enzymes, controls the acidity of the stomach contents, slows down the absorption of water and electrolytes in the intestine, and increases its contractile activity [33]. In addition, serotonin is involved in immune responses: it stimulates the production of pro-inflammatory cytokines and the differentiation of dendritic cells, and it attracts mast cells, eosinophils, and neutrophils to the focus of inflammation [34]. It is likely that these effects of serotonin contributed to the observed increase in the number of cellular elements in the lamina propria.

We revealed an increase in the content of highly sulfated mucins in goblet cells under the influence of MPs in the distal colon. The volume fraction of goblet cells and the content of neutral mucins in them did not change statistically significantly, although we noted a trend towards a decrease in the volume fraction of goblet cells. According to the literature, exposure to different types and sizes of MPs causes a decrease in the number of goblet cells, mucin expression, and mucus secretion [17,21,22,24–26,29–31]. There are no data in the literature on the effect of microplastics on the ratio of acidic and neutral mucins in the

colon. The mucus secreted by goblet cells is involved in protecting the body from internal and external stimuli, moistening the mucosa surface, promoting hummus, and parietal digestion. It forms the outer and inner layers. The outer layer is inhabited by commensal microflora and has a loose structure, while the inner layer is dense and impervious to particles larger than 0.5 μm in diameter. A key component of mucus is the mucin Muc2, which is a highly glycosylated protein. The mucin molecule has terminal carbohydrate groups which can be either neutral or acidic. The neutral groups are not modified (-CH_2OH) and are detected using the PAS reaction, while acidic groups are the modified residues of sulfuric (-CH_2SO_3-) or sialic acids and are detected using alcian blue (at pH 1.0, it stains the sulfo-groups). It is assumed that acidic mucins, especially sulfated ones, protect against bacterial translocation better than neutral ones since they are less susceptible to destruction by bacterial hydrolases [35]. Therefore, we also consider the increase in their production as a protective adaptive reaction of the organism.

4.2. The Effect of MPs on the Severity of the Ulcerative Inflammatory Process and Changes in the Epithelial Barrier in Acute Colitis

There are only three studies in the literature that have evaluated the effect of MPs on the severity of acute experimental colitis. According to Zheng H. et al. (2021), in male C57 mice with acute DSS colitis treated for 28 days with a suspension of PS particles with a diameter of 5 μm, more pronounced histopathological liver damage, increased intestinal permeability, and higher levels of the pro-inflammatory cytokines IL-1β, TNF-α, and INF-γ in serum compared to mice with colitis without MPs were observed [15]. Luo T. et al. (2022) studied the effect of PS microparticles with a diameter of 5 μm on the course of acute and chronic DSS colitis. Exposure to MPs caused a more pronounced shortening of the length of the colon, exacerbated histopathological damage and inflammation, decreased mucus secretion, and increased colonic permeability. In addition, MP exposure also increased the risk of secondary liver damage [16]. Xie S. et al. (2023) induced DSS colitis in male C57BL/6 mice treated with a suspension of PS microparticles with a diameter of 5 μm for 42 days. The impact of MPs accelerated the development of colitis and led to more pronounced weight loss, diarrhea, and inflammatory changes in the colon and liver [17].

According to our data, in acute DSS-induced colitis, the prevalence of the ulcerative inflammatory process in the distal colon was statistically significantly higher in mice that consumed MP. Also, in the group of animals with colitis against the background of the consumption of MPs, the content of neutral mucins in goblet cells was lower compared to the group with colitis without MPs and the control group. Therefore, MPs lead to a more severe course of colitis. However, we did not reveal differences in the content of cellular elements in the lamina propria of the mucosa, the number of endocrine cells, the volume fraction of goblet cells, or the content of highly sulfated mucins in mice with colitis between animals treated with and not treated with MPs. Probably, the absence of differences is due to the fact that the morphological picture of the intestine was mosaic, and these parameters were not evaluated along the entire length of the section, but areas of the colon mucosa with approximately the same severity of inflammatory changes were selected. Only Xie S et al. (2023) have considered such a parameter as the number of goblet cells. The authors noted a more pronounced decrease in the number of goblet cells under the influence of MPs [17]. The remaining parameters were estimated by us for the first time.

It should be noted that in mice with colitis, the prevalence of the ulcerative inflammatory process varied greatly between animals, even within the same group. Significant variations in ulcer-inflammatory process severity between animals of one group are associated with relatively low DSS concentrations (usually, a 1.5–5% DSS solution is used for acute colitis, but we used 1%) and the individual differences in animals. We chose low concentrations of DSS to cause mild symptoms because, with severe colitis, it would have been impossible to detect the influence of MPs. Even linear animals have distinct individual differences—in the population of adult male C57BL/6 mice, it is always possible to identify 10–40% of animals with low and high resistance to hypoxia. We have previously

demonstrated that, in susceptible-to-hypoxia animals, the course of acute and chronic DSS-induced colitis was much more pronounced than in tolerant-to-hypoxia mice [36,37].

4.3. Proposed Mechanisms of MP Action

To date, the mechanisms of the damaging action of MPs have not been studied enough. According to a review by Hirt N. and Body-Malapel M. (2020), exposure to nano- and microplastics leads to impairments of the oxidative and inflammatory intestinal balance and disruption of the gut's epithelial permeability. Other effects of nano- and microplastic exposure include dysbiosis (changes in the gut microbiota) and immune cell toxicity. Moreover, microplastics contain additives, adsorb contaminants, and may promote the growth of bacterial pathogens on their surfaces: they are potential carriers of intestinal toxicants and pathogens that can potentially lead to further adverse effects [38].

In our work, we used sterile polypropylene spheres without additives. The particle size was 5 μm. According to Pelaseyed T. et al. (2014) [39], particles with a diameter of more than 0.5 microns cannot penetrate through the dense layer of colon mucus. Therefore, it is possible that in mice without colitis, the effects of MPs are predominantly due to changes in the microflora. In addition, we suggest that the high ratio of the surface area to the volume of MP particles can act as a sorbent and wash out the mucus layer, which reduces the protective properties of the mucus barrier and leads to an increase in its permeability. In colitis, in addition to these mechanisms, MPs can penetrate through ulcers into the intestinal mucosa and directly interact with immune cells, stimulating inflammatory reactions.

5. Conclusions

Polystyrene microparticles with a diameter of 5 μm at a dose of 2.3 mg/kg/day, when exposed for 6 weeks, cause changes in the colon mucosa, characterized by an increase in the number of endocrine cells in the mucosa, an increase in the content of highly sulfated goblet cell mucins, an increase in the number of cells in the lamina propria of the mucosa, and a decrease in the volume fraction of macrophages in the mucosa. The consumption of polystyrene microparticles leads to a more severe course of acute DSS-induced colitis, which is characterized by a greater prevalence of the ulcerative inflammatory process and a decrease in the content of neutral mucins in goblet cells.

Author Contributions: Conceptualization, N.Z. and O.M.; methodology, N.Z., D.D. and I.T.; investigation, N.Z., D.D. and I.T.; writing—original draft preparation, N.Z.; writing—review and editing, D.D. and O.M.; visualization, N.Z.; project administration, N.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Russian Science Foundation (No. 22-24-00232).

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the Federal State Budgetary Scientific Institution the "Avtsyn Research Institute of Human Morphology" (protocol code 31(7), date 3 December 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zolotova, N.; Kosyreva, A.; Dzhalilova, D.; Makarova, O.; Fokichev, N. Harmful Effects of the Microplastic Pollution on Animal Health: A Literature Review. *Peerj* **2022**, *10*, e13503. [CrossRef]
2. Rodrigues, A.C.B.; de Jesus, G.P.; Waked, D.; Gomes, G.L.; Silva, T.M.; Yariwake, V.Y.; da Silva, M.P.; Magaldi, A.J.; Veras, M.M. Scientific Evidence about the Risks of Micro and Nanoplastics (MNPLs) to Human Health and Their Exposure Routes through the Environment. *Toxics* **2022**, *10*, 308. [CrossRef]
3. Pironti, C.; Ricciardi, M.; Motta, O.; Miele, Y.; Proto, A.; Montano, L. Microplastics in the Environment: Intake through the Food Web, Human Exposure and Toxicological Effects. *Toxics* **2021**, *9*, 224. [CrossRef]

4. Deng, Y.; Yan, Z.; Shen, R.; Huang, Y.; Ren, H.; Zhang, Y. Enhanced Reproductive Toxicities Induced by Phthalates Contaminated Microplastics in Male Mice (Mus Musculus). *J. Hazard. Mater.* **2021**, *406*, 124644. [CrossRef]
5. Estrela, F.N.; Guimarães, A.T.B.; da Costa Araújo, A.P.; Silva, F.G.; da Luz, T.M.; Silva, A.M.; Pereira, P.S.; Malafaia, G. Toxicity of Polystyrene Nanoplastics and Zinc Oxide to Mice. *Chemosphere* **2021**, *271*, 129476. [CrossRef] [PubMed]
6. Hou, B.; Wang, F.; Liu, T.; Wang, Z. Reproductive Toxicity of Polystyrene Microplastics: In Vivo Experimental Study on Testicular Toxicity in Mice. *J. Hazard. Mater.* **2021**, *405*, 124028. [CrossRef]
7. Jin, H.; Ma, T.; Sha, X.; Liu, Z.; Zhou, Y.; Meng, X.; Chen, Y.; Han, X.; Ding, J. Polystyrene Microplastics Induced Male Reproductive Toxicity in Mice. *J. Hazard. Mater.* **2021**, *401*, 123430. [CrossRef]
8. Park, E.-J.; Han, J.-S.; Park, E.-J.; Seong, E.; Lee, G.-H.; Kim, D.-W.; Son, H.-Y.; Han, H.-Y.; Lee, B.-S. Repeated-Oral Dose Toxicity of Polyethylene Microplastics and the Possible Implications on Reproduction and Development of the next Generation. *Toxicol. Lett.* **2020**, *324*, 75–85. [CrossRef]
9. Qiao, J.; Chen, R.; Wang, M.; Bai, R.; Cui, X.; Liu, Y.; Wu, C.; Chen, C. Perturbation of Gut Microbiota Plays an Important Role in Micro/Nanoplastics-Induced Gut Barrier Dysfunction. *Nanoscale* **2021**, *13*, 8806–8816. [CrossRef]
10. Shengchen, W.; Jing, L.; Yujie, Y.; Yue, W.; Shiwen, X. Polystyrene Microplastics-Induced ROS Overproduction Disrupts the Skeletal Muscle Regeneration by Converting Myoblasts into Adipocytes. *J. Hazard. Mater.* **2021**, *417*, 125962. [CrossRef]
11. Wei, J.; Wang, X.; Liu, Q.; Zhou, N.; Zhu, S.; Li, Z.; Li, X.; Yao, J.; Zhang, L. The Impact of Polystyrene Microplastics on Cardiomyocytes Pyroptosis through NLRP3/Caspase-1 Signaling Pathway and Oxidative Stress in Wistar Rats. *Environ. Toxicol.* **2021**, *36*, 935–944. [CrossRef] [PubMed]
12. Molodecky, N.A.; Soon, I.S.; Rabi, D.M.; Ghali, W.A.; Ferris, M.; Chernoff, G.; Benchimol, E.I.; Panaccione, R.; Ghosh, S.; Barkema, H.W.; et al. Increasing Incidence and Prevalence of the Inflammatory Bowel Diseases with Time, Based on Systematic Review. *Gastroenterology* **2012**, *142*, 46–54.e42. [CrossRef]
13. Tong, M.; Li, X.; Wegener Parfrey, L.; Roth, B.; Ippoliti, A.; Wei, B.; Borneman, J.; McGovern, D.P.B.; Frank, D.N.; Li, E.; et al. A Modular Organization of the Human Intestinal Mucosal Microbiota and Its Association with Inflammatory Bowel Disease. *PLoS ONE* **2013**, *8*, e80702. [CrossRef] [PubMed]
14. Yan, Z.; Liu, Y.; Zhang, T.; Zhang, F.; Ren, H.; Zhang, Y. Analysis of Microplastics in Human Feces Reveals a Correlation between Fecal Microplastics and Inflammatory Bowel Disease Status. *Environ. Sci. Technol.* **2022**, *56*, 414–421. [CrossRef]
15. Zheng, H.; Wang, J.; Wei, X.; Chang, L.; Liu, S. Proinflammatory Properties and Lipid Disturbance of Polystyrene Microplastics in the Livers of Mice with Acute Colitis. *Sci. Total Environ.* **2021**, *750*, 143085. [CrossRef]
16. Luo, T.; Wang, D.; Zhao, Y.; Li, X.; Yang, G.; Jin, Y. Polystyrene Microplastics Exacerbate Experimental Colitis in Mice Tightly Associated with the Occurrence of Hepatic Inflammation. *Sci. Total Environ.* **2022**, *844*, 156884. [CrossRef]
17. Xie, S.; Zhang, R.; Li, Z.; Liu, C.; Chen, Y.; Yu, Q. Microplastics Perturb Colonic Epithelial Homeostasis Associated with Intestinal Overproliferation, Exacerbating the Severity of Colitis. *Environ. Res.* **2023**, *217*, 114861. [CrossRef]
18. Zou, H.; Qu, H.; Bian, Y.; Sun, J.; Wang, T.; Ma, Y.; Yuan, Y.; Gu, J.; Bian, J.; Liu, Z. Polystyrene Microplastics Induce Oxidative Stress in Mouse Hepatocytes in Relation to Their Size. *Int. J. Mol. Sci.* **2023**, *24*, 7382. [CrossRef] [PubMed]
19. Senathirajah, K.; Attwood, S.; Bhagwat, G.; Carbery, M.; Wilson, S.; Palanisami, T. Estimation of the Mass of Microplastics Ingested—A Pivotal First Step towards Human Health Risk Assessment. *J. Hazard. Mater.* **2021**, *404*, 124004. [CrossRef]
20. Perše, M.; Cerar, A. Dextran Sodium Sulphate Colitis Mouse Model: Traps and Tricks. *J. Biomed. Biotechnol.* **2012**, *2012*, 718617. [CrossRef]
21. Lu, L.; Wan, Z.; Luo, T.; Fu, Z.; Jin, Y. Polystyrene Microplastics Induce Gut Microbiota Dysbiosis and Hepatic Lipid Metabolism Disorder in Mice. *Sci. Total Environ.* **2018**, *631–632*, 449–458. [CrossRef]
22. Jin, Y.; Lu, L.; Tu, W.; Luo, T.; Fu, Z. Impacts of Polystyrene Microplastic on the Gut Barrier, Microbiota and Metabolism of Mice. *Sci. Total Environ.* **2019**, *649*, 308–317. [CrossRef] [PubMed]
23. Li, B.; Ding, Y.; Cheng, X.; Sheng, D.; Xu, Z.; Rong, Q.; Wu, Y.; Zhao, H.; Ji, X.; Zhang, Y. Polyethylene Microplastics Affect the Distribution of Gut Microbiota and Inflammation Development in Mice. *Chemosphere* **2020**, *244*, 125492. [CrossRef] [PubMed]
24. Sun, H.; Chen, N.; Yang, X.; Xia, Y.; Wu, D. Effects Induced by Polyethylene Microplastics Oral Exposure on Colon Mucin Release, Inflammation, Gut Microflora Composition and Metabolism in Mice. *Ecotoxicol. Environ. Saf.* **2021**, *220*, 112340. [CrossRef] [PubMed]
25. Chen, X.; Zhuang, J.; Chen, Q.; Xu, L.; Yue, X.; Qiao, D. Polyvinyl Chloride Microplastics Induced Gut Barrier Dysfunction, Microbiota Dysbiosis and Metabolism Disorder in Adult Mice. *Ecotoxicol. Environ. Saf.* **2022**, *241*, 113809. [CrossRef] [PubMed]
26. Choi, Y.J.; Kim, J.E.; Lee, S.J.; Gong, J.E.; Jin, Y.J.; Seo, S.; Lee, J.H.; Hwang, D.Y. Inflammatory Response in the Mid Colon of ICR Mice Treated with Polystyrene Microplastics for Two Weeks. *Lab. Anim. Res.* **2021**, *37*, 31. [CrossRef]
27. Rawle, D.J.; Dumenil, T.; Tang, B.; Bishop, C.R.; Yan, K.; Le, T.T.; Suhrbier, A. Microplastic Consumption Induces Inflammatory Signatures in the Colon and Prolongs a Viral Arthritis. *Sci. Total Environ.* **2022**, *809*, 152212. [CrossRef]
28. Wen, S.; Zhao, Y.; Liu, S.; Chen, Y.; Yuan, H.; Xu, H. Polystyrene Microplastics Exacerbated Liver Injury from Cyclophosphamide in Mice: Insight into Gut Microbiota. *Sci. Total Environ.* **2022**, *840*, 156668. [CrossRef]
29. Xie, L.; Chen, T.; Liu, J.; Hou, Y.; Tan, Q.; Zhang, X.; Li, Z.; Farooq, T.H.; Yan, W.; Li, Y. Intestinal Flora Variation Reflects the Short-Term Damage of Microplastic to the Intestinal Tract in Mice. *Ecotoxicol. Environ. Saf.* **2022**, *246*, 114194. [CrossRef]

30. Jia, R.; Han, J.; Liu, X.; Li, K.; Lai, W.; Bian, L.; Yan, J.; Xi, Z. Exposure to Polypropylene Microplastics via Oral Ingestion Induces Colonic Apoptosis and Intestinal Barrier Damage through Oxidative Stress and Inflammation in Mice. *Toxics* **2023**, *11*, 127. [CrossRef]
31. Liang, B.; Zhong, Y.; Huang, Y.; Lin, X.; Liu, J.; Lin, L.; Hu, M.; Jiang, J.; Dai, M.; Wang, B.; et al. Underestimated Health Risks: Polystyrene Micro- and Nanoplastics Jointly Induce Intestinal Barrier Dysfunction by ROS-Mediated Epithelial Cell Apoptosis. *Part. Fibre Toxicol.* **2021**, *18*, 20. [CrossRef] [PubMed]
32. Billing, L.J.; Larraufie, P.; Lewis, J.; Leiter, A.; Li, J.; Lam, B.; Yeo, G.S.; Goldspink, D.A.; Kay, R.G.; Gribble, F.M.; et al. Single Cell Transcriptomic Profiling of Large Intestinal Enteroendocrine Cells in Mice—Identification of Selective Stimuli for Insulin-like Peptide-5 and Glucagon-like Peptide-1 Co-Expressing Cells. *Mol. Metab.* **2019**, *29*, 158–169. [CrossRef] [PubMed]
33. Mawe, G.M.; Hoffman, J.M. Serotonin Signaling in the Gastrointestinal Tract. *Nat. Rev. Gastroenterol. Hepatol.* **2013**, *10*, 473–486. [CrossRef] [PubMed]
34. Shajib, M.S.; Khan, W.I. The Role of Serotonin and Its Receptors in Activation of Immune Responses and Inflammation. *Acta Physiol.* **2015**, *213*, 561–574. [CrossRef]
35. Zolotova, N.A.; Akhrieva, K.M.; Zayratyants, O.V. Epithelial barrier of the colon in health and patients with ulcerative colitis. *Exp. Clin. Gastroenterol.* **2019**, *2*, 4–13. [CrossRef]
36. Dzhalilova, D.S.; Polyakova, M.A.; Diatroptov, M.E.; Zolotova, N.A.; Makarova, O.V. Morphological changes in the colon and composition of peripheral blood lymphocytes in acute colitis in mice with different resistance to hypoxia. *Mol. Med.* **2018**, *163*, 681–686. [CrossRef]
37. Dzhalilova, D.S.; Zolotova, N.S.; Polyakova, M.A.; Diatroptov, M.E.; Dobrynina, M.T.; Makarova, O.V. Morphological features of the inflammatory process and subpopulation pattern of peripheral blood lymphocytes during chronic colitis in mice exhibiting different responses to hypoxia. *Clin. Exp. Morph.* **2018**, *28*, 13–20. [CrossRef]
38. Hirt, N.; Body-Malapel, M. Immunotoxicity and intestinal effects of nano- and microplastics: A review of the literature. *Part. Fibre Toxicol.* **2020**, *17*, 57. [CrossRef]
39. Pelaseyed, T.; Bergström, J.H.; Gustafsson, J.K.; Ermund, A.; Birchenough, G.M.; Schütte, A.; van der Post, S.; Svensson, F.; Rodríguez-Piñeiro, A.M.; Nyström, E.E.; et al. The mucus and mucins of the goblet cells and enterocytes provide the first defense line of the gastrointestinal tract and interact with the immune system. *Immunol. Rev.* **2014**, *260*, 8–20. [CrossRef]

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Environmental Assessment of Microplastic Pollution Induced by Solid Waste Landfills in the Akmola Region (North Kazakhstan)

Natalya S. Salikova [1], Javier Rodrigo-Ilarri [2,*], María-Elena Rodrigo-Clavero [2], Saltanat E. Urazbayeva [1], Aniza Zh. Askarova [1] and Kuandyk M. Magzhanov [1]

[1] Department of Ecology, Life Safety and Environmental Protection, Abay Myrzakhmetov Kokshetau University, Kokshetau 020000, Kazakhstan; natsal66@mail.ru (N.S.S.); lily06_95@mail.ru (S.E.U.); aniza.askarova@mail.ru (A.Z.A.); magzhanovkuandyk99@mail.ru (K.M.M.)

[2] Instituto de Ingeniería del Agua y del Medio Ambiente (IIAMA), Universitat Politècnica de València, 46022 Valencia, Spain; marodcla@upv.es

* Correspondence: jrodrigo@upv.es

Abstract: This paper presents the outcomes derived from an environmental assessment of microplastic pollution resulting from solid waste landfills in the Akmola Region, situated in North Kazakhstan. This research represents a pioneering investigation conducted on microplastics within this specific region. This study encompasses a comprehensive examination of plastic waste disposal sites across the Akmola region, with a particular emphasis on evaluating the status of the municipal solid waste management system. To characterize the plastic content within the waste present at the landfill sites, quantitative techniques were employed. Through experimental means, the composition and fractionation of plastics within the municipal solid waste (MSW) at the landfills were determined. These data were subjected to a comparative analysis, aligning them with official statistics and previously published scientific data from both Kazakhstan and other regions globally. The methodologies employed focused on the "soft" removal of organic substances through the use of oxidants which do not damage plastics, and were tested using a water-bath therapeutic treatment. Furthermore, an analysis of soil samples taken from the landfills unveiled the ultimate retention of microplastic particles, attributed to leachate and rainwater runoff. Extracts were obtained from the subsoil samples using a density-based separation process, involving a three-step extraction followed by subsequent filtration of the resulting supernatants. In addition, the soil samples underwent examination through dry-phase particle fractional separation. The particles were meticulously enumerated and classified, and their dimensions were measured employing microscopic techniques coupled with photographic documentation. The outcomes stemming from these diverse tests will serve as fundamental input for the forthcoming numerical modeling endeavor, which aims to simulate the behavior of microplastics within both soil and water. This endeavor represents a continuation of the research project, the preliminary findings of which are expounded upon in this paper.

Keywords: microplastics; municipal solid waste landfills; fractional composition; analysis methods; aging of plastics

Citation: Salikova, N.S.; Rodrigo-Ilarri, J.; Rodrigo-Clavero, M.-E.; Urazbayeva, S.E.; Askarova, A.Z.; Magzhanov, K.M. Environmental Assessment of Microplastic Pollution Induced by Solid Waste Landfills in the Akmola Region (North Kazakhstan). *Water* **2023**, *15*, 2889. https://doi.org/10.3390/w15162889

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas, María Rocío Rodríguez Barroso and Jian Liu

Received: 30 June 2023
Revised: 7 August 2023
Accepted: 8 August 2023
Published: 10 August 2023

1. Introduction

Microplastics are found in nature as a result of the degradation of macroplastics included in household waste, insufficiently treated wastewater containing synthetic fabric fibers, and tire wear products, among many others. The influence of temperature, wind, water, and ultraviolet radiation causes macroplastics to degrade over time into micro- and nanoparticles, thereby increasing the total amount of plastic particles in the environment [1,2]. Recent estimates indicate that each year, 1.15 to 2.41 million tons of

plastic waste enter the oceans from rivers, with 67% of this waste originating from the 20 most polluting rivers [3].

Microplastics have garnered attention across various scientific disciplines as they are regarded as new, persistent environmental pollutants [4,5]. In the last few decades, research on microplastic pollution in the environment has primarily concentrated on marine ecosystems [6]. Within the scope of marine microplastic research, comprehensive analyses of pollution protocols, classifications, and global impacts have been conducted [5,7,8]. However, the unique characteristics of the open-ocean aquatic environment, combined with the logistical challenges of remote oceanic locations, have resulted in higher concentrations of microplastics in coastal areas and inland water bodies compared to the open ocean or freshwater bodies.

Only recently have scientists begun to investigate soil and groundwater contamination caused by microplastics [9–12]. This increased focus could be attributed to the intentional or unintentional disposal of plastics on land, which is estimated to be 4–23 times greater than in marine environments [6,13].

Microplastic particles deposited on land can seep vertically into groundwater, which serves as the primary source of drinking water in many countries. As a consequence, microplastic deposition and soil contamination have been detected in certain plants, ultimately entering the food chain and posing a threat to soil properties and human health [6].

Given the recent emphasis on microplastic contamination in soil and groundwater, the number of international studies has gradually increased. These studies include the development of sampling, analysis, and identification protocols for microplastics in soil and groundwater [14–16]. However, many of these protocols are still awaiting recognition or approval from the global scientific community [6].

Global plastic production reached 4.9 billion metric tons in 2015 and is projected to escalate to 12 billion metric tons per year by 2050 [17]. Plastics released into the environment undergo degradation processes that reduce their size, further exacerbating the management of plastic waste. Depending on their size, plastics can be categorized into macro- (>25 mm), meso- (<25–5 mm), micro- (5 mm to 0.1 μm), or nano- (<0.1 μm) plastics [18].

Microplastics can be further classified into primary microplastics and secondary microplastics. Primary microplastics are intentionally produced in microsizes for various purposes (e.g., microgranules in personal care products, glitter). On the other hand, secondary microplastics are formed from the breakdown of macro- and mesoplastics through photo-oxidative, mechanical, chemical, and/or biological interactions (e.g., microfibers derived from synthetic clothing) [19]. Microplastics can take various forms, such as foams, fragments, shafts, fibers, and flakes [20]. The fate and degradation of microplastics in the environment are influenced by their size and shape.

Microplastics are ubiquitous, occurring in seas, lakes, rivers, estuaries, air, sediment, landfills, and sewage treatment plants as a result of humans' improper disposal of plastics and inadequate waste management [21,22]. Solid waste represents a significant source of microplastics in the environment, even though it has been understudied compared to the above-mentioned sources [23].

This paper presents the results obtained from an environmental assessment of microplastic pollution induced by solid waste landfills in the Akmola Region (North Kazakhstan). It is the first-ever research on microplastics performed in the region. This study focuses on the state of the municipal solid waste management system, using quantitative techniques to characterize the content of plastics in the waste at the landfills.

Landfills, the most common solid waste management system, are major repositories and distributors of microplastics [19]. They are estimated to store 21–42% of the world's plastic waste production [24]. Plastic waste disposed of in landfills is exposed to much harsher environmental conditions, due to its enclosure in relatively tightly sealed containers which undergo complex biochemical reactions and physical changes. These conditions include varying pH levels of leachate (ranging from 4.5 to 9), high salinity, temperature fluctuations, gas generation (e.g., CO_2 and CH_4), physical stress, and microbial degrada-

tion [19]. All of these factors can lead to the fragmentation of plastics into microplastics. Additionally, fine plastic debris can migrate through leachate discharge.

Plastics in landfills can be broken down into secondary microplastics through complex biochemical reactions and physical changes [25], resulting in irregular shapes and structures [20], indicating that the breakdown of plastic debris is a major source of microplastics. Furthermore, landfills directly receive primary microplastics in various forms (e.g., recycled sludge) [26]. The high concentrations of microplastics in waste make landfills the main absorber of microplastics. Moreover, microplastics have been found in water bodies, sediment, and aquatic animals (e.g., mussels) near landfills [27], making landfills a source of microplastic contamination of the environment in their immediate vicinity.

Microplastics can spread from landfills to the environment via the air pathway [28]. Wind and precipitation significantly contribute to the transport of landfilled microplastics to neighboring areas [29]. Additionally, leachate from landfills contains abundant contaminants, including heavy metals and organic pollutants [30]. Thus, microplastics potentially carried by leachate can act as vectors for other contaminants and exacerbate adverse environmental impacts upon leachate release.

To better understand and control microplastics pollution, it is important to properly understand all the sources of microplastics and their associated pathways and degradation mechanisms. While solid waste is a major source of microplastics in the environment, the fate, treatment, and degradation of microplastics in various solid waste sources remain poorly understood [23].

Once soil or groundwater samples have been collected from the field, they must undergo a cleaning process [16,31,32]. In many soil microplastics studies and several groundwater studies, researchers have utilized 30% hydrogen peroxide (H_2O_2) to clean the samples before microplastics analysis. Hydrogen peroxide is a popular choice due to its ability to break down any organic material present in the field soil or groundwater samples that might be mistaken for microplastics or filtration materials [33]. In some cases, researchers have opted for using either KOH [34] or NaCl [35,36], based on the specific objectives of their study.

It is essential for any study to report not only the oxidant used for organic matter breakdown, but also its concentration [6]. After the physical processing of the soil or wastewater samples, including the sorting and removal of contaminants, as well as the chemical digestion of any organic pollutants that may have been picked up along with the plastics during analysis, the next step is to filter the supernatants.

The choice of filter paper and its pore size for filtration is crucial; it should be of sufficient size to capture the minimum particle size required while ensuring it is not a potential source of contamination [14].

To avoid potential contamination by microplastics or confusion during microscopic counting, it is recommended to use a non-plastic filter, such as glass or gold-coated filter paper, as cellulose in filters can sometimes resemble microplastics, especially in the form of cellulose fibers [6]. Microplastics are complex environmental pollutants that require proper classification [37–39]. The common classification method involves grouping them into different forms, such as films, spheres, pellets, foams, and fibers [6].

Numerous methods have been proposed for characterizing and quantifying microplastics [40]. These methods range from simple ones, like optical microscopy after sieving and/or density separation, to more complex combinations, like thermal extraction and desorption combined with gas chromatography–mass spectroscopy (TDS-GC/MS). Molecular vibrational techniques, such as Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy, are commonly used to identify microplastics extracted from environmental samples.

A typical approach involves initially identifying suspected microplastics using a microscope and then confirming their identity using spectroscopy and thermodynamic techniques, such as Fourier transform infrared spectroscopy (FTIR) or Raman spectroscopy, as well as pyrolysis gas chromatography–mass spectrometry [32,41,42].

Optical microscopes, especially stereomicroscopes, play a crucial role in recording the physical properties of microplastics [43]. Morphological characteristics, such as color, shape, and surface texture, serve as the primary basis for determining whether a particle is indeed a microplastic [44]. The research community continues to refine these criteria based on the analysis of more environmental samples [32].

2. Materials and Methods

2.1. Research Methods

Methodologies aimed at the extraction of microplastics with water, high-density saline solutions, as well as those aimed at the removal of organic substances using oxidizing agents (iron (II) salts, 3% H_2O_2, 30% H_2O_2) with thermostating in a water bath, were tested in this work.

The list of used equipment and materials includes: a DTX 500 LCD Levenhuk microscope with photo- and video-recording capability (Levenhuk Inc., Tampa, FL, USA); AX-200 Shimadzu analytical electronic scales with a measurement accuracy of 0.0001 g (Shymadzu Inc., Kyoto, Japan); stainless-steel sieves with mesh sizes of 3, 2, 1, 0.3, and 0.175 mm; a TS-1/80 SPU dry-air electric thermostat (Smolensk Special Design and Technology Office, Smolensk, Russia) for which the maximum deviation of the average temperature of any point in the working volume from the set temperature in steady-state thermal conditions was no more than ±1 °C (the maximum deviation of temperature at any point in the working chamber from the average was ±0.4 °C); an Ekros-4310 water bath (Ekros Inc., Moscow, Russia); Whatman 42 filters for the quantitative analysis (Little Chalfont Inc., Buckinghamshire, UK); Sefar polyamide filters with a mesh size of 300 microns (Sefar Inc., Heiden, Switzerland); an Opn-3.01 «Dastan» laboratory centrifuge with rotation speeds of 1000, 1500, and 3000 rpm (Dastan Inc., Bishkek, Kyrgyzstan); a set of hydrometers; a 5.32 M NaCl solution; and a 5.75M $ZnCl_2$ solution.

2.2. Description of the Study Area

Figure 1 shows the location of the Akmola Region inside the Republic of Kazakhstan. This region is located inside Northern Kazakhstan, with the city of Kokshetau being the administrative center and one of the most dynamically developing regions of Kazakhstan in recent years. It is characterized by an increasing population and employment in both agricultural and industrial production.

Figure 1. Location of the Akmola Region inside the Republic of Kazakhstan.

The relevance of introducing an effective local plastic waste management system is confirmed by the observation of the widespread detection of plastic waste in unauthorized locations. In Kokshetau, as well as in most regions of Kazakhstan, there is no component sorting of waste. According to national statistics, a total of 4.6 million tons of municipal solid waste (MSW) was generated in the Republic of Kazakhstan in 2020, of which 2.8 million tons were municipal waste collected by 625 units of specialized enterprises and individual entrepreneurs for waste collection and transportation. The main share belongs to household waste (71.4%), 14.6% to production waste (equated to household wastes), 9.9% to street waste, and 2.2% to market waste. Of the total amount of collected and transported waste, 5.8% was collected by the public, 93.6% by private entities, and 0.6% by foreign entities.

In 2020, 3.7 million tons of waste, representing 80.4% of the total amount of waste generated, was delivered to officially operating landfills and municipal waste sorting and recycling facilities. Of this amount, only 30.3% was sorted and 68.6% was received for further deposit. Of the waste deposited in landfills, 61.8% was mixed municipal waste and 30.9% was residual waste after recycling. At the end of 2020, more than 45.7 million tons of waste had accumulated in officially operating landfills. The average MSW generation per capita according to the 2020 data was 242.7 kg [45]. Supplementary Material Table S1 shows the available data about waste production in Kazakhstan and the recycling rates by region.

Despite the fact that, on average in Kazakhstan, only 19% of the generated MSW is recycled, as shown in Table S1, in the Akmola region only 10% of the solid waste generated in the region is sent for recycling. Table 1 shows the data on MSW sources in the Akmola region compared to the average for Kazakhstan [46], where the share of household waste contributes the most to MSW by plastics, at 71.5%. In the Akmola region, household waste accounts for 54.8% of the total amount of MSW generated. A significant contribution to the production of municipal solid waste in the Akmola region is made by industries. The share of production waste equated to domestic waste in Akmola region is 28.8%.

Table 1. Sources of municipal solid waste generation in 2020 (data in t/year).

Region	Household Waste	Park Waste	Construction Waste	Industrial Waste (Household Waste)	Street Waste	Market Waste
Kazakhstan	2,009,342	8595	41,473	411,450	278,850	61,324
Akmola region	52,985	2550	2722	27,839	4822	5717

The potential of the Akmola region to increase the recycling of municipal solid waste is not high. Table 2 shows the volume of MSW transferred for recycling, including the contribution of third-party organizations and waste-recycling companies. Most of the municipal solid waste in Kazakhstan and the Akmola region is sent to landfills for final disposal. On average, its share is 58.6% in Kazakhstan and significantly higher—81.95%—in the Akmola region.

Table 2. Volume of municipal solid waste transferred for recycling (data in t/year).

Region	Total MSW Transferred to Recycling	To Landfill Sites for Municipal Solid Waste	Transferred to Third Parties/Recycling Plants	Others
Kazakhstan	2,812,240	1,649,217	1,084,028	78,995
Akmola region	96,643	79,202	4825	12,616

The waste-component analysis (Table S2) reveals that in the Akmola region, there are no data available on the assortment of household waste sent to landfills, including food waste, electronic and electrical equipment, tires, clothes, and textiles.

According to the official data from the Republic of Kazakhstan Department of Statistics for 2020, plastic waste accounted for 12.26% of the total waste, while other unsorted waste made up 63.24%.

However, in the Akmola region, the proportion of plastic waste was significantly lower, accounting for only 1.30%, with other waste of unknown content making up 69.9%. This high percentage of waste classified as 'other waste' both on average in Kazakhstan and specifically in the Akmola region suggests a lack of reliable data on the characterization of municipal solid waste (MSW).

Existing scientific studies can provide information on the content of plastics in MSW. For example, a study of solid waste sorting in the capital city of Kazakhstan, Nur-Sultan (formerly Astana), noted that the main component of MSW in the city was food waste (46.3%), followed by plastic (15.2%), with low-density polyethylene being the predominant plastic type (4.5%). The official statistics on the content of plastics in MSW (12.26%) on average in Kazakhstan (Table S2) align with the available data [47–49]. However, there is a notable difference in the content of plastic in MSW according to the statistical data for the Akmola region (1.3%), which requires further verification.

The average plastic content in MSW in Kazakhstan is comparable to similar data from other countries. For instance, in Spain, the average plastic content is 12.6%, and in Brazil it is 16.8%, based on the analysis of municipal solid waste samples from 186 municipalities [50,51]. The data from Brazil may be more suitable for a comparative analysis with the Kazakh data, as both countries consider the total waste mass without pre-selective sorting.

As of 2022, there are no published statistical data on the plastic waste stored in landfills in the Akmola region or in Kazakhstan as a whole. The new Environmental Code of the Republic of Kazakhstan, enacted in 2021, bans the placement of plastic waste in landfills, but proper procedures for sorting MSW with plastic separation have not been fully implemented yet.

Visits to landfills in Shchuchinsk and Stepnogorsk indicated that plastic waste is still entering the landfills. Only the landfill in Shchuchinsk has organized the separation of plastic waste, and only non-deformed liquid containers are separated from the entire mass of plastic.

Despite at least 130 landfills operating in the Akmola region in 2022, only 24 landfills were licensed. Out of the total 243,000 tons of MSW generated in 2020, only 109,128 thousand tons (44.85%) is stored in licensed landfills. Supplementary Materials Figure S1 shows the locations of these licensed landfills and their associated populations, while Figure S2 depicts the annual waste disposal in each landfill for 2022. Further information about the landfill locations in the Akmola region, the socio-economic characteristics of the municipalities, and statistical data about the landfill sites are provided in Tables S3 and S4.

3. Results and Discussion

3.1. Survey of Plastic Waste Disposal Sites in the Akmola Region

During this study, field trips were conducted to assess the presence of plastics in two MSW landfills in the Akmola region: the Shchuchinsk landfill (located on the Shchuchinsk–Zerenda highway) and the Stepnogorsk landfill. Figures 2 and 3 display images depicting the current state of both landfills.

The Shchuchinsk landfill is situated along the Shchuchinsk–Zerenda highway, approximately 2 km away from the city border. This landfill was chosen due to its proximity to the Shchuchinsk–Borovsk resort area, which attracts a high number of tourists to the region. Consequently, a considerable amount of MSW, including plastic waste, was expected to be present within the landfill.

Figure 2. Current state of the Shchuchinsk MSW landfill.

Figure 3. Current state of the Stepnogorsk MSW landfill.

On the other hand, the Stepnogorsk landfill is located at a significant distance from heavily frequented tourist areas and is situated in an area of intense industrial development of mountain deposits. Therefore, it was anticipated that the Stepnogorsk landfill would contain a lower amount of plastic waste. The landfill is positioned in the Stepnogorsk communal storage area, around 1.0 km away from the residential area. It comprises various facilities, such as a landfill site for MSW storage, a checkpoint, and a disinfection bath. In January 2019, a waste-sorting line, an enclosed hangar for the temporary storage of incoming solid waste, and an open concrete pad for the temporary storage of sorted waste were introduced. However, during the visit to the landfill site, it was observed that these facilities were not operational.

A visual inspection of the Shchuchinsk and Stepnogorsk landfills showed a lack of compliance with the requirements of Kazakh Sanitary Rules ("Sanitary and Epidemiological Requirements to Collection, Use, Utilization, Decontamination, Transportation, Storage and Disposal of Production and Consumption Waste") [52]. It was seen that, at these landfills, the following requirements are currently absent:

- Design solutions to collect and prevent MSW leachate from entering the groundwater;
- Division of the landfill into individual operation cells;
- Equipment for weighing the incoming waste;
- Specific measures for disinfecting the wheels of waste trucks;
- Fencing and dewatering trenches, as well as earthen berms not more than 2 m high, around the perimeter of the entire landfill area;
- Collection drains to prevent leachate and rainwater from entering the soil;
- Landscaping of the sanitary protection zones of the landfills.

A survey of the landfills revealed that the Shchuchinsk landfill has a pre-sorting system in place, where non-deformed plastic containers are removed from the waste. However, the Stepnogorsk landfill lacks such a system, and there is no separate collection of MSW at the time of original intake from the public (no separate collection sites were found during the visit to the town). Additionally, a visual inspection of the Stepnogorsk landfill indicated that the predominant waste at the site consisted of plastic and clothing waste, as well as packaging, rubber, and tires.

The calculated MSW waste composition of both Shchuchinsk and Stepnogorsk landfills, as per their environmental permits, aligns with the statistical data for Akmola Oblast. According to this data, the plastic content in the landfills must not exceed 1.3%. The composition of the waste includes paper and cardboard (14.7%), kitchen and food waste (14.4%), wood (4.2%), textiles (3.55%), leather and rubber (0.5%), stones (5.1%), metal (3.4%), drop-off materials (38.6%), glass (4.27%), plastic (0.8%), wool (0.5%), hay, straw, and leaves (2.0%), and organic matter (6.45%).

To analyze the proportion of plastic in the waste disposed of at both landfills, six waste samples weighing up to 2 kg each were randomly collected. The sampling points were determined using GPS and are detailed in Table 3 and illustrated in Figures S3 and S4 (Supplementary Materials).

Table 3. GPS coordinates of sampling points.

Sample Number	Stepnogorsk		Shchuchinsk	
	Latitude	**Longitude**	**Latitude**	**Longitude**
1	52.359220	71.923026	52.903334	70.111852
2	52.359510	71.925249	52.905588	70.108644
3	52.358966	71.928161	52.907846	70.102070
4	52.358576	71.929435	52.904524	70.102409
5	52.357261	71.928710	52.905813	70.100141
6	52.357305	71.930410	52.902452	70.103571

Each sample was weighed on an electronic scale to the nearest 1 g, then sorted, and the plastic waste was separated from the other waste. The selected plastic was also weighed on scales with the same accuracy. The fraction of plastic waste was calculated as the ratio of the mass of plastic to the mass of the respective sample (Equation (1)):

$$\gamma_{pl} = \frac{m_{pl}}{m_{sa}} \ (\%) \quad (1)$$

where γ_{pl} is the fraction of plastic waste in the sample, m_{pl} is the mass of dirty plastic in the sample (g), and m_{sa} is the weight of the waste sample (g). The results of the plastic content in each landfill site are shown in Table 4.

Table 4. Plastic content in each landfill site.

Sample Number	Shchuchinsk Landfill Samples			Stepnogorsk Landfill Samples		
	m_{pl} **(g)**	m_{sa} **(g)**	γ_{pl} **(%)**	m_{pl} **(g)**	m_{sa} **(g)**	γ_{pl}**(%)**
1	143	1403	10.19	307	1254	24.48
2	186	1112	16.72	294	1564	18.80
3	195	1089	17.91	302	1367	22.09
4	176	1142	15.41	187	983	19.02
5	105	521	20.15	206	907	22.71
6	268	810	33.09	281	1064	26.41
Average			19.25			22.25

The content of plastic in Stepnogorsk's landfill is not significantly higher than the content of plastic in Shchuchinsk's landfill. This can likely be attributed to the randomness

of the sampling as well as the presence of plastic sorting at the Shchuchinsk landfill. The average plastic content in the waste disposed of at both landfills was calculated to be 20.75%, which surpasses the average value for Kazakhstan (12.26%), and also greatly exceeds the officially available statistics for the Akmola region (1.3%—Table S2). This significant difference more accurately reflects the actual state of plastic recycling in the region.

The largest fraction (measured in weight) of sorted plastics from both landfills in Shchuchinsk and Stepnogorsk is liquid ware, which includes beverage and synthetic detergent containers, with an average of 62.5%. Packaging for dairy products and disposable tableware (glasses, plates) accounts for approximately 28.6%. Polyethylene packaging makes up around 8.9%. Most of the packaging containers were found in a deformed but unbroken condition. A smaller proportion of the packaging, disposable utensils, and containers were in deformed fractions, including packaging made of polyethylene. Degraded plastic can be divided into fractions: less than 5 cm, 5–10, 10–15, 15–20, and 20–25 cm. The largest share of deformed plastic is for the fractions larger than 5 cm—up to 85%. The fractions less than 5 cm account for 15% of the total mass of the deformed plastic.

3.2. Application of Cleaning Methods to MSW Plastic Waste

A literature search for methods to isolate and purify microplastics allowed for the adaptation of these methods to determine the most optimal way to separate plastic particles from organic contaminants. Since the experiment was conducted on plastic particles previously extracted from samples of plastic waste, the degree of separation of microplastics from organic impurities was assessed through a microscopic examination of the cleanliness of the plastic surface and by measuring the weight of the samples using the weight method. Based on an analysis of the literature, it was found that the purification of plastic particles could be achieved by mixing them with water at a 1:10 ratio at room temperature (23–25 °C), using warm water (60 °C) and utilizing chemical reagents [53–57].

In this study, different methodologies for the "soft" removal of organic substances from plastics using various oxidants (iron salts (II), 3% H_2O_2, 30% H_2O_2) were tested using a thermostatic water bath. However, we rejected the use of strongly acidic and strongly alkaline solutions, as they caused significant destruction and fragmentation of soft plastics [58–63]. Consequently, the total amount of plastic particles and their fractional composition may not accurately represent the actual content. Published research results did not show a better performance for cleaning plastic contaminants with warm water compared to cold water; in fact, the opposite was observed. Nevertheless, the authors of those studies claimed that hot water contributed to a better removal of grease and adhesive residues from labels [64,65]. In our experiment, the purification of plastic substances from organic impurities was conducted with a preliminary treatment (while stirring) using warm distilled water heated to 60 °C, in a ratio of not less than 1:10 by mass. Special attention was given to removing label residue from the plastic particles, as it could account for up to 10–14% of the total weight of contaminants.

After washing the plastic particles with warm water for 30 min, the removal of dirt was carried out through liquid oxidation using hydrogen peroxide. Different variations with varying ratios of hydrogen peroxide and a catalyst (iron salts) were tested, ranging from 4:1 to 1:4. It was observed that decreasing the ratio of hydrogen peroxide negatively affected the destruction of organic matter, and reducing the content of Fe (II) salt in the mixture slowed down the process of organic pollutant degradation. Thus, the process for the oxidation of organic impurities on the surface of the microplastics was carried out at the ratio of hydrogen peroxide: Fe (II) salt—1:1, with an oxidation time of 30 min, at a temperature of 50 °C (thermostating in a water bath). The sequence of analysis was as follows:

- The plastic particles selected by random sampling were weighed to within 0.0001 g, cleaned of heavy dirty contamination with warm distilled water, and stirred with a magnetic stirrer.

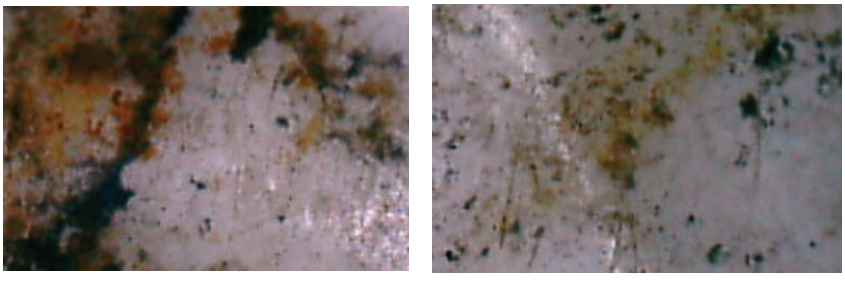
- Afterward, they were immersed in a heat-resistant beaker (600–800 mL), a 0.05 M solution of $FeSO_4$ was added, and then a 30% hydrogen peroxide solution (in a 1:1 ratio) was slowly added.
- The resulting mixture with plastic was maintained in a water bath with constant stirring for 30 min (50 °C).
- If residual (organic) contaminants were visually observed on the plastic, the procedure was repeated by adding an additional amount of a 30% hydrogen peroxide solution.
- After cleaning, the plastic was removed, washed with distilled water, dried at 35 °C [66] to a constant weight, and weighed.

The specific gravity of the contamination was calculated as the ratio of the mass difference of the contaminated plastic (original sample) and the cleaned sample of plastic to the mass of the original (contaminated) plastic, according to Equation (2):

$$\gamma_{fr} = \frac{m_{raw} - m_{cle}}{m_{raw}} \ (\%) \quad (2)$$

where γ_{fr} is the contamination fraction, m_{cle} is the mass of cleaned plastic in the sample (g), and m_{eaw} is the weight of the contaminated (raw) plastic sample (g).

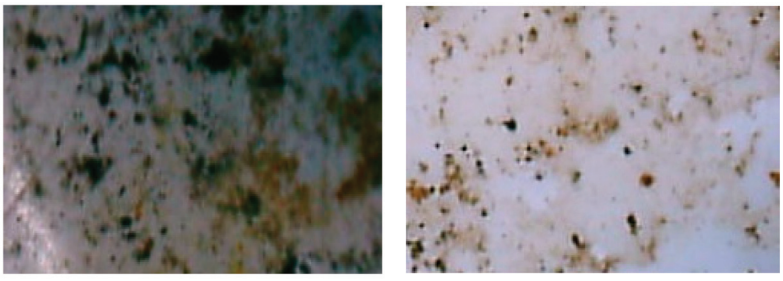
A total of six samples were collected from each landfill, and different cleaning methods were applied to them, with each sample being taken from different locations within the respective landfill. To ensure comparable results, particles with similar sizes were selected for the experiment. The aim was to avoid obtaining results that fell outside the sensitivity limits of the measuring instruments. Hence, contaminated plastic particles with a maximum length of at least 1 cm on one side were chosen. The treatment involved cleaning the contaminated plastic particles using heated distilled water (at 60 °C) and stirring them for 30 min. However, this method showed a low degree of contamination removal, with an average of 1.02% by mass. Upon inspecting the surface of the plastic particles after cleaning, it was evident that a significant amount of residual contamination remained (as depicted in Figure 4).



(**a**) (**b**)

Figure 4. Microscopic surface inspection of (**a**) contaminated plastic and (**b**) cleaned plastic when cleaned with H_2O at 60 °C.

In the next step of the first experiment, heated distilled water and iron salts (II) and 3% H_2O_2 were used to treat the contaminated plastic particles. The content of the removed contaminated particles averaged up to 2.6%. A visual inspection of the surface for residual contamination using an electron microscope revealed areas of significant contamination (Figure 5).

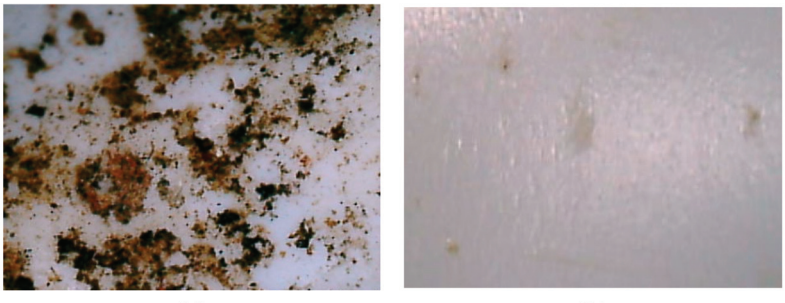


(**a**) (**b**)

Figure 5. Microscopic surface inspection of (**a**) contaminated plastics and (**b**) cleaned plastics when cleaned with a 3% H_2O_2 solution and pre-treated with heated water.

The method involving a 30% H_2O_2 solution and a 0.05 M $FeSO_4$ solution, along with pre-treatment using H_2O at 60 °C and temperature control in a water bath at 50 °C, demonstrated the highest efficiency in cleaning organic impurities from the plastics.

Figure 6 displays images of the surface of the (a) contaminated plastic and (b) cleaned plastic of sample No. 3 from Shchuchinsk's landfill.



(**a**) (**b**)

Figure 6. Surface of the (**a**) contaminated plastic and (**b**) cleaned plastic of sample No. 3 from Shchuchinsk's landfill, observed microscopically (30% H_2O_2 solution and 0.05 M $FeSO_4$ solution), with pre-treatment with heated water.

The results from calculating the content of organic contaminants removed from the plastics are presented in Table 5. These findings suggest that municipal solid waste plastics can accumulate between 1.70% and 10.40% of their mass from organic contaminants.

Table 5. Experimental results for cleaning the plastic of organic contaminants at each landfill site.

Sample Number	Shchuchinsk Landfill Samples			Stepnogorsk Landfill Samples		
	m_{raw} (g)	m_{cle} (g)	γ_{fr}(%)	m_{raw} (g)	m_{cle} (g)	γ_{fr}(%)
1	0.2251	0.2119	5.86	0.1934	0.1891	2.22
2	0.3287	0.3195	2.80	0.1631	0.1575	3.43
3	0.0875	0.0784	10.40	0.1387	0.1346	2.95
4	0.1597	0.1502	5.95	0.2988	0.2768	7.36
5	0.2854	0.2779	2.63	0.3057	0.3005	1.70
6	0.1844	0.1812	1.74	0.3323	0.3124	5.98
Average			4.89			3.94

The difference in the extent to which plastics accumulate contaminants depends on how long the plastic has been in the landfill and the type of plastic material.

Thus, in the course of the experiments, the mass ratio of the contaminated plastics, the oxidizing agent solutions necessary for cleaning, the duration of oxidation, and the temperature range of exposure were established.

The results of this experiment will be used in further research for the monitoring of microplastics in environmental objects in the Akmola region and their purification after extraction.

3.3. Determination of Macro- and Microplastic Particles in Soil Samples

The surveyed landfills, namely Shchuchinsk and Stepnogorsk, like all landfills in the Akmola region, lack collection headers to prevent leachate and rainfall runoff from entering the underlying soils. As a result, the underlying soils become the ultimate destination for the migration and retention of microplastic particles. These soils were selected for analysis to determine the presence of microplastic particles. Soil samples were collected from each landfill at the same locations where the waste samples were taken (see Table 5). For both landfills, soil samples weighing at least 2 kg were gathered using metal scoops and placed into glass jars. Each soil sample was taken to a depth of at least 15–20 cm. A total of six soil samples from each landfill site were analyzed.

3.3.1. Determination of Soil Moisture

For each landfill site, the soil moisture was determined by analyzing three parallel samples from each sampling point. The moisture content in the soil was determined following the guidelines of the Kazakh State obligatory standard GOST 28268–89 [67].

Pre-numbered aluminum bags were first dried to a constant weight and then weighed on analytical scales (AX-200 Shimadzu) with an accuracy of 0.0001 g. Soil samples weighing 3–5 g were placed into the bags and weighed with an accuracy of 0.0001 g. The weighed bags, along with the soil and with the lid left open, were then placed in a TC-1/80 SPU drying oven heated to 105 ± 1 °C. The initial drying time was set at 3 h, followed by subsequent drying periods of 1 h. After each drying session, the bags with the soil were covered with lids, allowed to cool in a desiccator with calcium chloride, and then weighed on the same analytical scales after cooling. The drying and weighing process was halted if the difference between subsequent weightings did not exceed 0.0002 g.

The mass moisture content, W (%), was calculated according to Equation (3):

$$W = \frac{m_l - m_0}{m_0 - m} \ (\%) \quad (3)$$

where m_l is the weight of the moist soil with cup and lid (g), m_0 is the mass of the dried soil with cup and lid (g), and m is the weight of the empty beaker with lid (g). The arithmetic average of the three parallel measurements, with a relative error between the three measurements not larger than 0.01%, was taken as the result of one sample moisture content. The average value of the soil moisture at Shchuchinsk's landfill was 13.6%, while at Stepnogorsk's landfill it was 17.8%.

3.3.2. Analysis of Microplastic Particles in Soil Samples and Leachates

To analyze the content of microplastic particles in the soil and to separate them by size, a dry separation method was employed. The soil collected from the MSW landfills was air-dried at room temperature until it reached a constant mass. Large debris was removed from the soil, and any substantial clods were crushed. The prepared soil was then sifted through a series of polyamide sieves with a metal base, each with different mesh sizes: 3 mm, 2 mm, 1 mm, 300 μm, and 175 μm. The resulting fractions were visually and microscopically examined. The second method utilized soil fractionation through dry separation with stainless sieves of known sizes (5 mm, 2 mm, and 1 mm). The soil fractions obtained through this process were also visually and microscopically analyzed (refer to Figure 7 for further details).

Figure 7. Dry separation of soil into fractions.

In order to enhance the efficiency of microplastic extraction, a "wet" detection approach was employed by preparing soil extracts. It is known that the density of plastic is determined by its chemical composition and, in most cases, plastics without heavy modifiers have a density lower than that of water. This is why so much research has focused on analyzing floating debris on water surfaces or plastics washed ashore by waves. Lightweight plastics commonly found include disposable tableware, packaging made of polyethylene, and polypropylene.

However, due to the sorption processes of plastics on the surface of suspended solids and the influence of biofouling, these particles can increase in mass and eventually settle to the bottom. Moreover, certain polymers, such as polycarbonate, polyethylene terephthalate, and polymers with modifiers, have a density greater than that of water.

As there is no standardized procedure for quantifying microplastics in soil samples and leachates, two methods of soil analysis have been used based on the properties of plastic particles, relying on previously published results:

- Most plastics have a density lower than that of water, so the plastic microparticles must pass into the water phase during separation [68];
- To extract weighted plastic, it is necessary to use solutions of higher density [69,70].

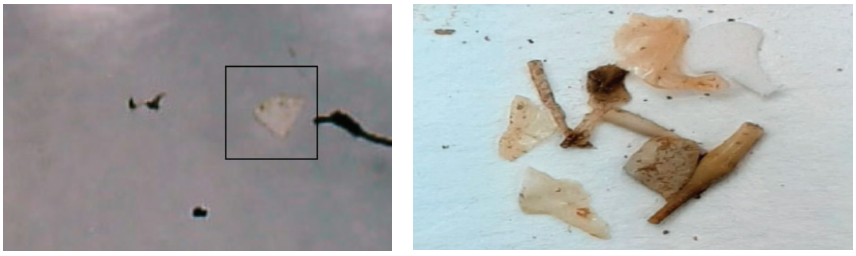
Thus, the analysis of the plastic particles was performed using the "wet" method, based on the preparation of the soil filtrate, the isolation of microplastic particles by density separation, the flotation of microplastic particles, the filtration of the supernatant through a filter for quantitative analysis (Sefar polyamide mesh with a diameter of 100–300 μm or Whatman filters No. 42 with a particle retention rate of more than 8 μm), and the analysis of the particles retained by the filter using a microscopic method (Figure 8).

Figure 8. Density separation of plastic particles from soil leachate.

Samples of 2 g to 5 g of soil were analyzed, adding 10–20 mL of distilled water, stirring for 15 min, centrifuging at 1500 rpm, and filtering the supernatant through a filter for quantification. A similar amount (10–20 mL) of 5.32 M NaCl (]ρ = 1.20 g/cm^3) solution was added to the precipitate in step 2, and the procedure was repeated. In step 3, 10–20 mL of 5.75 M $ZnCl_2$ (]ρ = 1.57 g/cm^3) solution was added to the precipitate and the procedure was repeated with the supernatant.

The filters with retained particles were examined using a digital microscope, specifically the Levenhuk DTX 500 LCD microscope with 20×–200×–500× magnification, which was coupled to an intense illuminator consisting of eight dimmable LEDs. Microplastic samples detected using both methods were recorded in .jpeg/.avi format.

During the identification of microplastics, particles with shiny surfaces, bright colors, and sharp geometric shapes were taken into consideration [63,71,72]. These particles were then classified based on their shape, such as fibers and non-fibrous materials like debris (angular and hard), films (flexible and thin), or pellets (rounded and hard), along with their size (as shown in Figure 9).



(**a**) (**b**)

Figure 9. Plastic particles detected in (**a**) dry and (**b**) liquid landfill samples.

The analysis found that plates and films were present in 89% of the samples, while the remaining particles were fibers, and no pellets were detected. The results are presented as the number of microplastic particles per 1 g of dry soil and average 0.81 particles per gram.

The granulometric composition was determined by classifying the microplastic particles by their largest size (Figure 10).

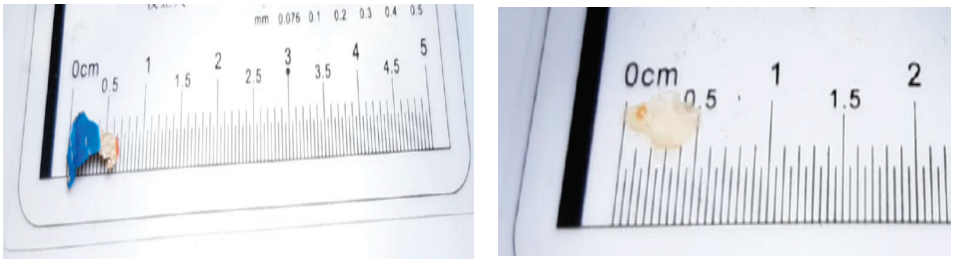


Figure 10. Classification of plastic particles by size.

The size classification of the identified plastic particles showed that 50.00% of them were in the 5–10 mm size range, 32.14% were smaller than 5 mm, and 14.29% were in the 10–20 mm size range. The smallest fraction comprised particles in the 20–50 mm size range. Particles larger than 50 mm were not included in the analysis.

4. Summary and Conclusions

This work conducted on the waste samples from the Akmola region's landfill sites provides valuable insights into the role of plastic waste in the formation of microparticles

and its impact on the natural environment. The landfill survey results offer up-to-date information on plastic inputs. A comprehensive database, including landfill characteristics, waste content and composition, and socio-economic characteristics of the regions, will enable the forecasting of the impact of MSW and plastics in the area.

Through surveys and expeditions to the landfills, the state of the landfills were assessed for compliance with sanitary norms. The content and fractional composition of plastics in MSW at the landfills were experimentally determined, and a comparative analysis of this data with official statistics and previously published scientific data in Kazakhstan and abroad was conducted.

To develop effective methods for separating macro- and microplastics from organic substances and foreign impurities, laboratory experiments were conducted on model media and contaminated plastic particles found in the landfill samples. Methodologies focused on the "soft" removal of organic substances through the use of oxidants without damaging the plastics were tested using a water-bath therapeutic treatment. The method employing a 30% H_2O_2 and 0.05 M $FeSO_4$ solution, with pretreatment with H_2O (at 60 °C) and thermostating in a water bath (at 50 °C), demonstrated the highest efficiency for purifying plastics from organic impurities. The experiments established parameters, such as the mass ratio of contaminated plastics to oxidizer solutions, oxidation duration, mixing conditions, and temperature exposure interval. The cleaning efficiency was evaluated by observing the surface cleanliness of plastics under a microscope and through weight measurements. The laboratory experiments yielded quantitative data on contamination sorption on plastic particles, ranging from 3.94% to 4.89%.

The analysis of soil samples taken from the landfills revealed the final containment of microplastic particles due to leachate and rainwater flow. Filtrates were obtained from the underlying soil samples using density separation based on a three-step extraction procedure and subsequent filtration of the supernatants. Centrifugation was employed to improve extraction and phase separation. The soil samples were also studied using dry-phase particle fractional separation. The particles were counted, classified, and measured for size using microscopic methods with photo recording. The content of the microplastic particles averaged 0.81 g^{-1}. The microplastic particles were classified according to their largest size: less than 5 mm (32.14%), 5–10 mm (50.00%), 10–20 mm (14.29%), and 20–50 mm (3.57%).

All these findings will be considered as input data for the future numerical modeling of microplastics' behavior in soil and water, which is planned as a continuation of this research project, the initial results of which have been described in this paper.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15162889/s1, Figure S1: Location of the 24 landfills of the Akmola region and associated population; Figure S2: Annual disposal of waste in each landfill in 2022 (data in t); Figure S3: Locations of sampling sites at the MSW landfill of Shchuchinsk; Figure S4: Location of Sampling Sites at Stepnogorsk Landfill; Table S1: Municipal Solid Waste production and recycling data in Kazakhstan (data in kt/year); Table S2: Component composition of municipal solid waste to landfills, 2020 (data in kt/year); Table S3: Location of landfills in Akmola region and socio-economic characteristics of the regions; Table S4: Statistical data on licensed landfills in the Akmola region.

Author Contributions: Conceptualization, N.S.S. and J.R.-I.; methodology, N.S.S.; software, J.R.-I. and M.-E.R.-C.; validation, N.S.S.; formal analysis, N.S.S. and J.R.-I.; investigation, N.S.S. and J.R.-I.; resources, N.S.S. and J.R.-I.; data curation, N.S.S., S.E.U., A.Z.A. and K.M.M.; writing—original draft preparation, N.S.S. and J.R.-I.; writing—review and editing, J.R.-I. and M.-E.R.-C.; visualization, J.R.-I. and M.-E.R.-C.; supervision, N.S.S. and J.R.-I.; project administration, N.S.S.; funding acquisition, N.S.S. and J.R.-I. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan through the research project entitled *"Health Risk Modelling Based on the Identification of Microplastics in Water Systems and the Reasoning About Actions to Manage the Water Resources Quality"* (Grant No. AP14869081).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lambert, S.; Wagner, M. Characterization of nanoplastics in the degradation of polystyrene. *Chemosphere* **2016**, *45*, 265–268. [CrossRef]
2. Hartmann, N.B.; Hüffer, T.; Thompson, R.C.; Hassellöv, M.; Verschoor, A.; Daugaard, A.E.; Rist, S.; Karlsson, T.; Brennholt, N.; Cole, M.; et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. *Environ. Sci. Technol.* **2019**, *53*, 1039–1047. [CrossRef]
3. Besseling, E.; Redondo-Hasselerharm, P.; Foekema, E.M.; Koelmans, A.A. Quantifying ecological risks of aquatic micro- and nanoplastic. *Crit. Rev. Environ. Sci. Technol.* **2019**, *49*, 32–80. [CrossRef]
4. Dai, Y.; Shi, J.; Zhang, N.; Pan, Z.; Xing, C.; Chen, X. Current research trends on microplastics pollution and impacts on agro-ecosystems: A short review. *Sep. Sci. Technol.* **2016**, *57*, 656–669. [CrossRef]
5. Priya, A.K.; Jalil, A.A.; Dutta, K.; Rajendran, S.; Vasseghian, Y.; Qin, J.; Soto-Moscoso, M. Microplastics in the environment: Recent developments in characteristic, occurrence, identification and ecological risk. *Chemosphere* **2022**, *298*, 134161. [CrossRef]
6. Chia, R.; Lee, J.Y.; Jang, J.; Cha, J. Errors and recommended practices that should be identified to reduce suspected concentrations of microplastics in soil and groundwater: A review. *Environ. Technol. Innov.* **2022**, *28*, 102933. [CrossRef]
7. Jorquera, A.; Castillo, C.; Murillo, V.; Araya, J.; Pinochet, J.; Narváez, D.; Pantoja-Gutiérrez, S.; Urbina, M.A. Physical and anthropogenic drivers shaping the spatial distribution of microplastics in the marine sediments of Chilean fjords. *Sci. Total Environ.* **2022**, *814*, 152506. [CrossRef] [PubMed]
8. Sridhar, A.; Kannan, D.; Kapoor, A.; Prabhakar, S. Extraction and detection methods of microplastics in food and marine systems: A critical review. *Chemosphere* **2022**, *286*, 131653. [CrossRef] [PubMed]
9. Campanale, C.; Galafassi, S.; Savino, I.; Massarelli, C.; Ancona, V.; Volta, P.; Uricchio, V.F. Microplastics pollution in the terrestrial environments: Poorly known diffuse sources and implications for plants. *Sci. Total Environ.* **2022**, *805*, 150431. [CrossRef] [PubMed]
10. Sajjad, M.; Huang, Q.; Khan, S.; Khan, M.A.; Liu, Y.; Wang, J.; Lian, F.; Wang, Q.; Guo, G. Microplastics in the soil environment: A critical review. *Environ. Technol. Innov.* **2022**, *27*, 102408. [CrossRef]
11. Tian, L.; Jinjin, C.; Ji, R.; Ma, Y.; Yu, X. Microplastics in agricultural soils: Sources, effects, and their fate. *Curr. Opin. Environ. Sci. Health.* **2022**, *25*, 100311. [CrossRef]
12. Weber, C.J.; Opp, C. Spatial patterns of mesoplastics and coarse microplastics in floodplain soils as resulting from land use and fluvial processes. *Environ. Pollut.* **2020**, *267*, 115390. [CrossRef] [PubMed]
13. Choi, Y.R.; Kim, Y.N.; Yoon, J.H.; Dickinson, N.; Kim, K.H. Plastic contamination of forest, urban, and agricultural soils: A case study of Yeoju City in the Republic of Korea. *J. Soils Sediments* **2021**, *21*, 1962–1973. [CrossRef]
14. ASTM. *Standard Practice for Collection of Water Samples with High, Medium, or Low Suspended Solids for Identification and Quantification of Microplastic Particles and Fibers*; ASTM: West Conshohocken, PA, USA, 2020.
15. Fan, C.; Huang, Y.Z.; Lin, J.N.; Li, J. Microplastic quantification of nylon and polyethylene terephthalate by chromic acid wet oxidation and ultraviolet spectrometry. *Environ. Technol. Innov.* **2022**, *28*, 102683. [CrossRef]
16. Pérez, C.N.; Carré, F.; Hoarau-Belkhiri, A.; Joris, A.; Leonards, P.E.G.; Lamoree, M.H. Innovations in analytical methods to assess the occurrence of microplastics in soil. *J. Environ. Chem. Eng.* **2022**, *10*, 107421. [CrossRef]
17. Geye, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci. Adv.* **2017**, *3*, e1700782. [CrossRef]
18. Blair Crawford, C.; Quinn, B. *Microplastic Pollutants*, 1st ed.; Elsevier: Amsterdam, The Netherlands, 2016.
19. He, P.; Chen, L.; Shao, L.; Zhang, H.; Lü, F. Municipal solid waste (MSW) landfill: A source of microplastics? Evidence of microplastics in landfill leachate. *Water Res.* **2019**, *159*, 38–45. [CrossRef]
20. Su, Y.; Zhang, Z.; Wu, D.; Zhan, L.; Shi, H.; Xie, B. Occurrence of microplastics in landfill systems and their fate with landfill age. *Water Res.* **2019**, *164*, 114968. [CrossRef]
21. Alimi, O.S.; Budarz, J.F.; Hernández, L.M.; Tufenkji, N. Microplastics and Nanoplastics in Aquatic Environments: Aggregation, Deposition, and Enhanced Contaminant Transport. *Environ. Sci. Technol.* **2018**, *52*, 1704–1724. [CrossRef]
22. Klingelhöfer, D.; Braun, M.; Quarcoo, D.; Brüggmann, D.; Groneberg, D.A. Research landscape of a global environmental challenge: Microplastics. *Water Res.* **2020**, *170*, 115358. [CrossRef]
23. Golwala, H.; Zhang, X.; Iskander, S.M.; Smith, A.L. Solid waste: An overlooked source of microplastics to the environment. *Sci. Total Environ.* **2021**, *769*, 144581. [CrossRef] [PubMed]
24. Nizzetto, L.; Futter, M.; Langaas, S. Are Agricultural Soils Dumps for Microplastics of Urban Origin? *Environ. Sci. Technol.* **2016**, *50*, 10777–10779. [CrossRef] [PubMed]
25. Zhao, Y.; Lou, Z. *Pollution Control and Resource Recovery. Municipal Solid Wastes at Landfill*, 1st ed.; Elsevier: Amsterdam, The Netherlands, 2016.

26. Yang, G.; Zhang, G.; Wang, H. Current state of sludge production, management, treatment and disposal in China. *Water Res.* **2015**, *78*, 60–73. [CrossRef] [PubMed]
27. Carr, S.A.; Liu, J.; Tesoro, A.G. Transport and fate of microplastic particles in wastewater treatment plants. *Water Res.* **2016**, *91*, 174–182. [CrossRef]
28. Rillig, M.C. Microplastic in terrestrial ecosystems and the soil? *Environ. Sci. Technol.* **2012**, *46*, 6453–6454. [CrossRef]
29. Yadav, V.; Sherly, M.A.; Ranjan, P.; Tinoco, R.O.; Boldrin, A.; Damgaard, A.; Laurent, A. Framework for quantifying environmental losses of plastics from landfills. *Resour. Conserv. Recycl.* **2020**, *161*, 104914. [CrossRef]
30. Sui, Q.; Zhao, W.; Cao, X.; Lu, S.; Qiu, Z.; Gu, X.; Yu, G. Pharmaceuticals and personal care products in the leachates from a typical landfill reservoir of municipal solid waste in Shanghai, China: Occurrence and removal by a full-scale membrane bioreactor. *J. Hazard. Mater.* **2017**, *323*, 99–108. [CrossRef]
31. Pfohl, P.; Roth, C.; Meyer, L.; Heinemeyer, U.; Gruendling, T.; Lang, C.; Nestle, N.; Hofmann, T.; Wohlleben, W.; Jessl, S. Microplastic extraction protocols can impact the polymer structure. *Microplastics Nanoplastics* **2021**, *1*, 1–13. [CrossRef]
32. Yang, L.; Zhang, Y.; Kang, S.; Wang, Z.; Wu, C. Microplastics in soil: A review on methods, occurrence, sources, and potential risk. *Sci. Total Environ.* **2021**, *780*, 146546. [CrossRef]
33. Oni, B.A.; Sanni, S.E. Occurrence of Microplastics in Borehole Drinking Water and Sediments in Lagos, Nigeria. Environ. *Toxicol. Chem.* **2022**, *41*, 1721–1731. [CrossRef]
34. Chouchene, K.; Nacci, T.; Modugno, F.; Castelvetro, V.; Ksibi, M. Soil contamination by microplastics in relation to local agricultural development as revealed by FTIR, ICP-MS and pyrolysis-GC/MS. *Environ. Pollut.* **2022**, *303*, 119016. [CrossRef] [PubMed]
35. Thiele, C.J.; Hudson, M.D.; Russell, A.E. Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size. *Mar. Pollut. Bull.* **2019**, *142*, 384–393. [CrossRef]
36. Zhou, Q.; Zhang, H.; Fu, C.; Zhou, Y.; Dai, Z.; Li, Y.; Tu, C.; Luo, Y. The distribution and morphology of microplastics in coastal soils adjacent to the Bohai Sea and the Yellow Sea. *Geoderma* **2018**, *322*, 201–208. [CrossRef]
37. Bhatt, P.; Pathak, V.M.; Bagheri, A.R.; Bilal, M. Microplastic contaminants in the aqueous environment, fate, toxicity consequences, and remediation strategies. *Environ. Res.* **2021**, *200*, 111762. [CrossRef] [PubMed]
38. Huang, W.; Song, B.; Liang, J.; Niu, Q.; Zeng, G.; Shen, M.; Deng, J.; Luo, Y.; Wen, X.; Zhang, Y. Microplastics and associated contaminants in the aquatic environment: A review on their ecotoxicological effects, trophic transfer, and potential impacts to human health. *J. Hazard. Mater.* **2021**, *405*, 124187. [CrossRef]
39. Rochman, C.M.; Brookson, C.; Bikker, J.; Djuric, N.; Earn, A.; Bucci, K.; Athey, S.; Huntington, A.; McIlwraith, H.; Munno, K.; et al. Rethinking microplastics as a diverse contaminant suite. *Environ. Toxicol. Chem.* **2019**, *38*, 703–711. [CrossRef] [PubMed]
40. Fu, W.; Min, J.; Jiang, W.; Li, Y.; Zhang, W. Separation, characterization and identification of microplastics and nanoplastics in the environment. *Sci. Total Environ.* **2020**, *721*, 137561. [CrossRef] [PubMed]
41. He, D.; Luo, Y.; Lu, S.; Liu, M.; Song, Y.; Lei, L. Microplastics in soils: Analytical methods, pollution characteristics and ecological risks. *TrAC Trends Anal. Chem.* **2018**, *109*, 163–172. [CrossRef]
42. Zhang, Y.; Kang, S.; Allen, S.; Allen, D.; Gao, T.; Sillanpää, M. Atmospheric microplastics: A review on current status and perspectives. *Earth-Sci. Rev.* **2020**, *203*, 103118. [CrossRef]
43. Wang, W.; Wang, J. Investigation of microplastics in aquatic environments: An overview of the methods used, from field sampling to laboratory analysis. *TrAC Trends Anal. Chem.* **2018**, *108*, 195–202. [CrossRef]
44. Wang, W.; Ge, J.; Yu, X.; Li, H. Environmental fate and impacts of microplastics in soil ecosystems: Progress and perspective. *Sci. Total Environ.* **2020**, *708*, 134841. [CrossRef] [PubMed]
45. Bureau of National Statistics. Environmental Indicators. In *Waste*. Available online: https://old.stat.gov.kz/for_users/ecologic_indicators/ecologic_indicator/waste_generation (accessed on 31 July 2023).
46. Environmental Protection in the Republic of Kazakhstan. *Statistical Compendium, 2016-2020-Nur-Sultan: Agency for Strategic Planning and Reforms of the Republic of Kazakhstan*; Department of Production and Environment Statistics: Astana, Republic of Kazakhstan, 2021; 245p.
47. Sarbassov, Y.; Sagalova, T.; Tursunov, O.; Venetis, C.; Xenarios, S.; Inglezakis, V. Survey on Household Solid Waste Sorting at Source in Developing Economies: A Case Study of Nur-Sultan City in Kazakhstan. *Sustainability* **2019**, *11*, 6496. [CrossRef]
48. Abylkhani, B.; Aiymbetov, B.; Yagofarova, A.; Tokmurzin, D.; Venetis, C.; Poulopoulos, S.; Sarbassov, Y.; Inglezakis, V.J. Seasonal characterization of municipal solid waste from Astana city, Kazakhstan; composition and thermal properties of combustible fraction. *Waste Manag. Res.* **2019**, *37*, 1271–1281. [CrossRef]
49. Abylkhani, B.; Guney, M.; Aiymbetov, B. Detailed municipal solid waste composition analysis for Nur-Sultan City, Kazakhstan with implications for sustainable waste management in Central Asia. *Environ. Sci. Pollut.* **2021**, *28*, 24406–24418. [CrossRef]
50. Calero, M.; Martín-Lara, M.; Godoy, V.; Quesada, L.; Martínez, D.; Peula, F.; Soto, J. Characterization of plastic materials present in municipal solid waste: Preliminary study for their mechanical recycling. *Detritus* **2018**, *1*, 104–112. [CrossRef]
51. Mondelli, G.; Juárez, M.B.; Jacinto, C. Geo-environmental and geotechnical characterization of municipal solid waste from the selective collection in São Paulo city, Brazil. *Environ. Sci. Pollut.* **2022**, *29*, 19898–19912. [CrossRef]
52. Order Acting Minister of Health of the Republic of Kazakhstan, December 25, 2020 No. KR DSM-331/2020. Available online: https://adilet.zan.kz/rus/docs/V2000021934 (accessed on 31 July 2023).

53. Strady, E.; Dang, T.H.; Dao, T.D.; Dinh, H.N.; Do, T.T.D.; Duong, T.N.; Duong, T.T.; Hoang, D.A.; Kieu-Le, T.C.; Le, T.P.Q.; et al. Baseline assessment of microplastic concentrations in marine and freshwater environments of a developing Southeast Asian country Vietnam. *Mar. Pollut. Bull.* **2020**, *162*, 111870. [CrossRef]
54. Li, Z.; Wang, J.; Gao, X.; Du, J.; Sui, H.; Wu, J.; Zhong, Y.; Liang, B.; Huang, Y.; Ye, R.; et al. Investigation of Microplastics ($\geq$10 μm) in Meconium by Fourier Transform Infrared Microspectroscopy. *Toxics* **2023**, *11*, 310. [CrossRef]
55. Matsuguma, Y.; Takada, H.; Kumata, H.; Kanke, H.; Sakurai, S.; Suzuki, T.; Itoh, M.; Okazaki, Y.; Boonyatumanond, R.; Zakaria, M.P.; et al. Microplastics in sediment cores from Asia and Africa as indicators of temporal trends in plastic pollution. *Arch. Environ. Contam. Toxicol.* **2017**, *73*, 230–239. [CrossRef]
56. Reineccius, J.; Bresien, J.; Waniek, J.J. Separation of microplastics from mass-limited samples by an effective adsorption technique. *Sci. Tot. Environ.* **2021**, *788*, 147881. [CrossRef]
57. Zhang, S.; Wang, J.; Liu, X.; Qu, F.; Wang, X.; Wang, X.; Li, Y.; Sun, Y. Microplastics in the environment: A review of analytical methods, distribution, and biological effects. *TrAC Trends. Anal. Chem.* **2019**, *111*, 62–72. [CrossRef]
58. Lin, L.; Zuo, L.Z.; Peng, J.P.; Cai, L.Q.; Fok, L.; Yan, Y.; Li, H.X.; Xu, X.R. Occurrence and distribution of microplastics in an urban river: A case study in the Pearl River along Guangzhou City, China. *Sci. Total Environ.* **2018**, *644*, 375–381. [CrossRef] [PubMed]
59. Budimir, S.; Setälä, O.; Lehtiniemi, M. Effective and easy to use extraction method shows low numbers of microplastics in offshore planktivorous fish from the northern Baltic Sea. *Mar. Pollut. Bull.* **2018**, *127*, 586–592. [CrossRef] [PubMed]
60. Olsen, L.M.B.; Knutsen, H.; Mahat, S.; Wade, E.J.; Arp, H.P.H. Facilitating microplastic quantification through the introduction of a cellulose dissolution step prior to oxidation: Proof-of-concept and demonstration using diverse samples from the inner Oslofjord, Norway. *Mar. Environ. Res.* **2020**, *161*, 105080. [CrossRef] [PubMed]
61. Prata, J.C.; da Costa, J.P.; Duarte, A.C.; Rocha-Santos, T. Methods for sampling and detection of microplastics in water and sediment: A critical review. *TrAC Trends Anal. Chem.* **2019**, *110*, 150–159. [CrossRef]
62. Boyle, K.; Örmeci, B. Microplastics and Nanoplastics in the Freshwater and Terrestrial Environment: A Review. *Water* **2020**, *12*, 2633. [CrossRef]
63. Hurley, R.R.; Lusher, A.L.; Olsen, M.; Nizzetto, L. Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices. *Environ. Sci. Technol.* **2018**, *52*, 7409–7417. [CrossRef]
64. Rodríguez-Bruceta, P.A.; Pérez-Rodríguez, A.; Velázquez-Infante, J. Proposal of a procedure for the recycling of high-density polyethylene. *Rev. Cuba. Química* **2014**, *27*, 32–54.
65. Kratofil, L.; Hrnjak, Z.; Katančic, Z. Plastics and priority during the recycling. In *Handbook of Research on Advancements in Environmental Engineering*; Gaurina-Medjimurec, N., Ed.; IGI Global: Hershey, PA, USA, 2014; pp. 257–284.
66. *EN ISO 4611:1999*; Plastics—Determination of the Effects of Exposure to Damp Heat, Water Spray and Salt Mist. ISO: Geneva, Switzerland, 1999.
67. *GOST 28268–89*; Soils. Sampling. State Committee of Standards of the Council of Ministers of the USSR: Moscow, Russia, 1989.
68. Zobkov, M.B.; Esyukova, E.E. Microplastics in the marine environment: A review of methods for sampling, preparation and analysis of water samples, bottom sediments and coastal sediments. *Oceanology* **2018**, *1*, 53.
69. Zettler, E.R.; Mincer, T.J.; Amaral-Zettler, L.A. Life in the "plastisphere": Microbial communities on plastic marine debris. *Environ. Sci. Technol.* **2013**, *47*, 7137–7146. [CrossRef]
70. Romera-Castillo, C.; Pinto, M.; Langer, T.M.; Álvarez-Salgado, X.A.; Herndl, G.J. Dissolved organic carbon leaching from plastics stimulates microbial activity in the ocean. *Nat. Commun.* **2018**, *9*, 1430. [CrossRef] [PubMed]
71. Zhang, S.; Yang, X.; Gertsen, H.; Peters, P.; Salánki, T.; Geissen, V. A simple method for the extraction and identification of light density microplastics from soil. *Sci. Total Environ.* **2018**, *616–617*, 1056–1065. [CrossRef] [PubMed]
72. Corradini, F.; Meza, P.; Eguiluz, R.; Casado, F.; Huerta-Lwanga, E.; Geissen, V. Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal. *Sci. Total Environ.* **2019**, *671*, 411–420. [CrossRef] [PubMed]

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Microplastic in the Snow on Sledding Hills in Green Areas of Krakow

Jarosław Lasota [1], Wojciech Piaszczyk [1,*], Sylwester Tabor [2] and Ewa Błońska [1]

[1] Department of Ecology and Silviculture, Faculty of Forestry, University of Agriculture in Krakow, 29 Listopada 46 Str., 31-425 Kraków, Poland
[2] Department of Machinery Exploitation, Ergonomics and Production Processes, University of Agriculture in Krakow, Balicka 116 B Str., 31-149 Krakow, Poland
* Correspondence: wojciech.piaszczyk@urk.edu.pl

Abstract: Every year we are more and more exposed to the negative impact of microplastic. Our research aimed to determine the amount of microplastic in the snow on sledding hills in green areas of Krakow. The sledding hills in winter are very intensively used by children and it is very important to monitor the condition of these places in terms of microplastic contamination. In our research, we assessed whether children playing on sledding hills may be exposed to microplastic. Our research covered 10 sledding hills of various sizes located in the green areas of Krakow. Our research has confirmed the presence of significant amounts of microplastics in snow collected on sledding hills. Three times as much microplastic was found in the snow on the higher hills (2.78 mg/L) compared to the lower sledding hills (0.96 mg/L). In the snow collected on sledding hills from the green areas of Krakow, a large diversity of microplastic in terms of type, size, color, and shape was noted. The dominant type of microplastic found during the research was polypropylene (PP), polyurethane (PU), hydrocarbon resin (HCR), and polyester (PES). The share of two microplastic fractions of 1.1–2.0 mm and 2.1–3.0 mm accounted for over 50% of the whole amount. After melting the snow, microplastic goes to the soil surface, which can lead to changes in the properties of the soil, and due to its strong hydrophobicity, it will play an important role in the transport of toxic compounds, e.g., polycyclic aromatic hydrocarbons (PAHs). Our research suggests limiting the use of plastic sleds and replacing them with wooden sleds, which will not be a source of pollution for urban green spaces used by residents regardless of the season.

Keywords: children's playground; FTIR; land uses; microplastic; urban areas

Citation: Lasota, J.; Piaszczyk, W.; Tabor, S.; Błońska, E. Microplastic in the Snow on Sledding Hills in Green Areas of Krakow. *Sustainability* **2023**, *15*, 12995. https://doi.org/10.3390/su151712995

Academic Editors: Graeme Moad, Grigorios L. Kyriakopoulos, Antonis A. Zorpas, María Rocío Rodríguez Barroso and Vassilis J. Inglezakis

Received: 14 June 2023
Revised: 10 August 2023
Accepted: 28 August 2023
Published: 29 August 2023

1. Introduction

Plastic pollution is a global problem in terrestrial and aquatic ecosystems and enters the environment through landfills, atmospheric deposition, sewage treatment, and agricultural, urban, and industrial runoff [1–4]. Globally, the annual production of plastic is approximately 322 million tons and, despite the increase in plastic recycling, most of the plastic waste ends up in the environment [5]. Microplastics are plastic particles smaller than 5 mm often derived from the fragmentation of meso and macroplastics [6]. Under the influence of anthropogenic or environmental factors, large pieces of plastic are fragmented, becoming a source of microplastic in the environment [7]. In most studies conducted so far, the dominance of plastic particles of smaller fractions was indicated [4]. The most common microplastics detected in stormwater were rubber and asphalt particles from road surfaces, followed by textile fibers, films, fragments, and paint particles [8]. Most plastic garbage is generated on land, making soil an important long-term sink for microplastics [9]. About 79% of all plastic waste produced in the years 1950–2015 ended up in the soil and is the source of microplastic deposited in the soil [10,11]. Microplastic is released into the environment as a result of the deterioration of discarded plastic products through physical, chemical, and biological processes [12]. In the case of microplastic pollution,

depending on its type and size, different environmental impacts were noted. Microplastics significantly reduce bacterial diversity, change pH, and the content of some nutrients in the soil, but it also has a significant negative impact on the survival, growth, and reproduction of soil fauna [11]. Microplastic pollution regulates soil bacterial communities, promoting the growth of Ascomycota fungi and inhibiting the growth of Basidiomycota [13]. Microplastic pollutes soils and serves as a vector for other pollutants, which can also cause ecotoxicological effects on the soil ecosystems [14]. Microplastic can affect the formation of physicochemical and microbiological properties as well as soil fauna [15–17]. Microplastic can also be the carrier of hydrophobic organic pollutants, such as polychlorinated biphenyls, polycyclic organochlorine pesticides, and aromatic hydrocarbons, as well as heavy metals such as nickel, zinc, cadmium, and lead [5]. As a result of the degradation and fragmentation of polymeric materials, so-called secondary microplastics are formed. There is a reduction in the molecular weight of the polymer, and the released bonds are susceptible to microbiological degradation that occurs in aerobic and anaerobic environments, and decomposition to CO_2, H_2O, N_2, H_2, CH_4, and mineral salts can be complete or partial [18]. On the other hand, primary microplastics are polymer powders and micro granulates present, among others, in cosmetics and cleaning products. The European Chemicals Agency ECHA [19] published a proposal to limit the use of polymer plastics in cosmetic products. The document contains a list of 19 polymers and currently known cosmetics companies aware of the risks, out of concern for the good of the consumer, have eliminated the polymers on this list from the composition of their products [20].

The problem of plastic contamination has been described in variously managed areas [21–23]. Corradini et al. [24] identified microplastic in soils under four different land use systems with different management intensities (croplands, pastures, rangelands, and natural grasslands). Some studies show a clear relationship between the increasing abundance of microplastic and increasing levels of urbanization and industrialization [25]. Urban areas are heavily polluted with microplastic as a result of the high concentration of people [26]. Green spaces in urban areas are considered natural filters of pollution in cities due to the retention capacity of soil and vegetation [27]. Urban green spaces, such as parks, gardens, and squares, have several potential benefits for city dwellers and their use can improve physical and mental health through recreation and reduce anxiety and stress [28]. Therefore, the study of green areas is important in the context of risk assessment.

Krakow is the second largest city in Poland in terms of the number of inhabitants and area. The area of the city is 327 km^2, and its length from north to south is 18 km and from east to west is 31 km. Green areas in Krakow have arranged areas with technical infrastructure and buildings functionally related to them, covered with vegetation, and performing public functions. The area of public green areas with a recreational function per inhabitant is on average approximately 8.3 m^2/person [29]. Our research aimed to determine the abundance of microplastic in the snow on sledding hills in green areas of Krakow. The sledding hills in winter are very intensively used by children and it is very important to monitor the condition of these places in terms of microplastic contamination. In our research, we assessed whether sledding hills pose a risk for children using them, and whether children playing on sledding hills may be exposed to microplastic. Our research covered 10 sledding hills of various sizes located in the green areas of Krakow. There are no studies on microplastic in big cities in Poland such as Krakow. Therefore, this study aims to clarify the following questions: (1) How much microplastic ends up in the snow in different parts of sledding hills depending on their size?; (2) What types of microplastic are found in the sledding hills area?; (3) What amounts of microplastic get into the soil environment every year as a result of using sledding hills?

2. Materials and Methods

2.1. Study Area and Snow Sampling

The research was carried out in the green areas of Krakow, southern Poland (50°03′41″ N; 19°56′18″ E). The Municipal Greenery Authority in Krakow has prepared a map with the

location of the sledding hills for children. There are 43 sledding hills of various sizes in Krakow. Most of them are located in parks and each of them is properly secured against road traffic with fencing, while others are located deep in the park away from roads and cars. The research covered 10 sledding hills made available to children during the winter (Figure 1). The sledding hills covered by the research varied in size. We divided the sledding hills into two groups, the first group was characterized by the slope length of sledding hills below 10 m, and the second group had the slope length of sledding hills exceeding 10 m. Five sledding hills represented low sledding hills (G2, G3, G8, G9, and G10) and five were high sledding hills (G1, G4, G5, G6, and G7). Each sledding hill has been divided into three parts (upper, middle, and lower). Snow samples from ten locations were taken from each section for further testing. Ten sub-samples were used to prepare an aggregate sample. A 1 L snow sample was taken from each point using a metal sampler. Ten sub-samples of snow from each part of the sledding hills were combined into one and the microplastics were isolated after being transported to the lab. In total, laboratory analyses included 30 snow samples (3 parts of sledding hills × 10 sledding hills = 30 snow samples). Snow samples were collected in December 2022.

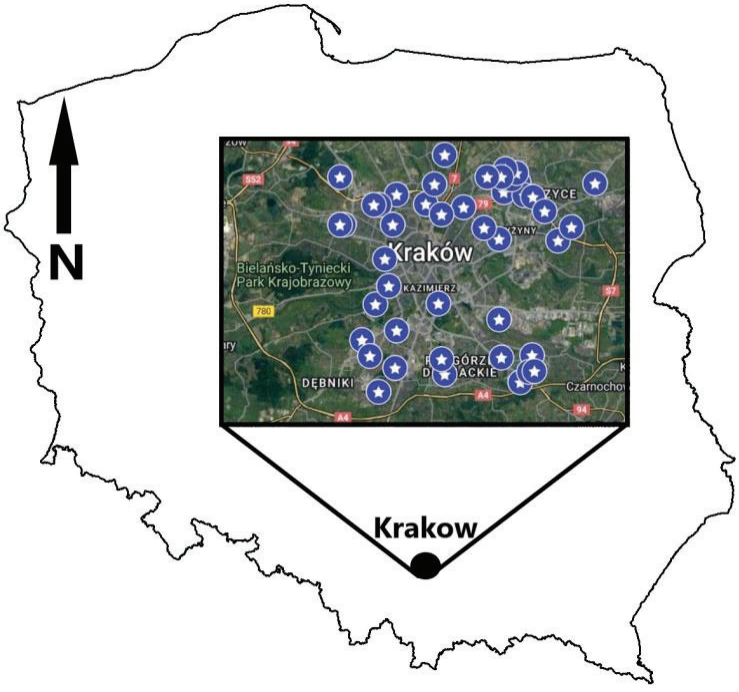

Figure 1. Location of sledding hills (blue star) in Krakow (source: Municipal Greenery Authority in Krakow).

2.2. Laboratory Analysis

Upon arrival at the laboratory, the snow samples were melted and filtered through filters (0.45 µm glass microfiber filters). Snow samples were placed in glass beakers and melted at room temperature. Plastic was extracted from the filters and transferred to a Petri dish. Visual analysis and sorting were performed. Microplastics were defined as particles made of synthetic polymers, smaller than 5 mm. Separated plastic samples were subjected to spectrophotometry analysis. Qualitative identification of the remains was obtained using an FTIR microscope Nicolet iN10 (ThermoFisher Scientific Inc., Waltham, MA, USA) with a cooling detector for sample mapping. The equipment used allows the

detection of microplastic particles from 0.01 mm. Infrared spectroscopy was performed in the reflectance mode. The collected spectra were analyzed using Omnic Spectra software with its database. To reduce the possible error due to cross-contamination, the microplastic abundance of the blank was used to correct all the results. Before starting the analysis in the laboratory, 5 Petri dishes with filter paper on which microplastic particles from the air could settle during the analysis were prepared. Blanks thus prepared were last analyzed on an FTIR microscope. Microplastic particles were classified by type, color, shape, and size. In the case of type and color, the percentage by weight was specified. In the case of size and shape, the percentage was determined by count. At the time of analysis, the library included reference spectra for Polyurethane (PU), Polyethylene terephthalate (PET), Polyester (PES), Polyvinyl butyral (PVB), Thermoplastic elastomer (TPE), Polyvinyl chloride (PCV), Polypropylene (PP), Polystyrene (PS), Hydrocarbon resin (HCR), Phenoxy resin (PHR), and Poly (isobutene isoprene) (PIBP).

2.3. Statistical Analysis

The principal component analysis (PCA) method was used to evaluate the relationships between analyzed variables. A general linear model (GLM) was used to investigate the effect of the sledding hills' size and location on sledding hills on the microplastic content. The Shapiro–Wilk test was used to assess normality, and Levene's test was used to check the homogeneity of variances. The Kruskal–Wallis test was used to assess the differences between the average values of microplastic amount between different locations on sledding hills. The U Mann–Whitney test was used to assess the differences between the average values of microplastic amount between different sizes of sledding hills. Statistical analyses were performed in the statistical programs R (R Core Team 2020), and R Studio (R Studio Team 2020).

3. Results

In the snow collected on sledding hills from the green areas of Krakow, a high content of microplastic and a large diversity of microplastics in terms of type, size, color, and shape were noted. The dominant type of microplastic noted during the research was polypropylene (PP), polyurethane (PU), hydrocarbon resin (HCR), and polyester (PES). In the case of one sledding hill (G1), a high proportion of urethane alkyd was noted (Figure 2). A small amount of phenoxy resin (PHR), poly(isobutene isoprene) (PIBP), and polystyrene (PS) was found in the samples of the tested snow (Figure 2). In the snow collected from the higher sledding hills, polypropylene, polyethylene terephthalate, and thermoplastic elastomer (TPE) appeared more often compared to the lower sledding hills. On the other hand, in the snow from the lower sledding hills, polyurethane was more often marked (Figure 2).

The microplastics determined in the snow samples varied in size (Figure 3). The highest content was recorded in the case of microplates of 1.1–2.0 mm and 2.1–3.0 mm. The share of these two microplastic fractions accounted for over 50% of all. The smallest share was recorded for microplastics sized 3.1–4.0 mm. This microplastic fraction was more present in the snow collected on the upper sledding hills (G1 and G4–G7). The highest-sized microplastics (4.1–5.0 mm) were more common in the snow from the higher sledding hills, while it was absent from the snow from the two low sledding hills (G8–G9) (Figure 3).

In snow samples collected from sledding hills, four types of microplastic shapes were noted, i.e., fiber, fiber ball, flake, and fragment (Figure 4). Fragments had the largest share, often their share accounted for over 75% of all microplastic shapes. Only the snow from the three sledding hills had less than 50% microplastic in the form of fragments. Flake-shaped microplastics had a significant share. Fiber and fiber balls were not recorded on all the study plots. Fiber and fiber balls were not recorded in the snow of the lower sledding hills (G2–G3 and G8–G10) (Figure 4).

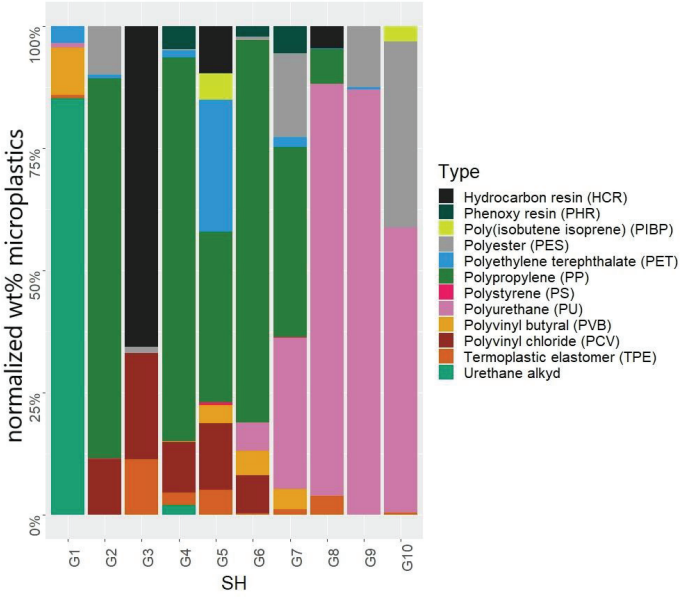

Figure 2. Microplastic particles (MCs) characterization by type of component in snow on different sledding hills (SH) (the percentage by weight was specified). The higher the percentage, the more of a particular microplastic compared to the others in the collected samples.

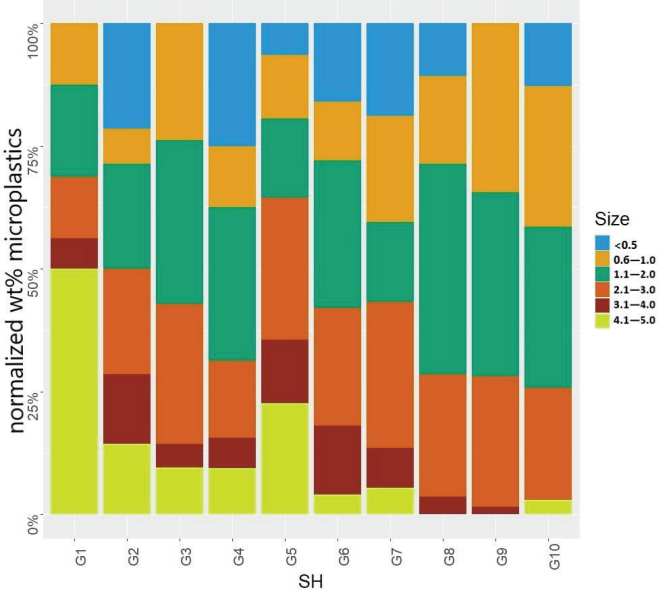

Figure 3. Distribution of microplastic particles (MCs) by size (mm) in snow on different sledding hills (SH) (the percentage by count was specified). The higher the percentage, the more of a particular microplastic compared to the others in the collected samples.

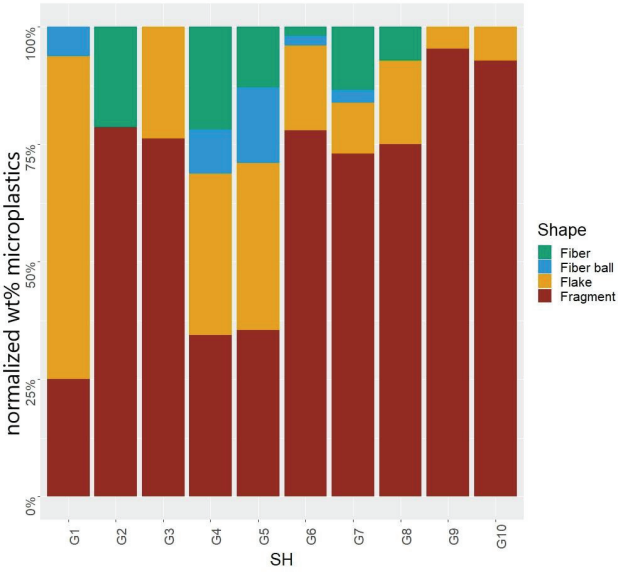

Figure 4. Distribution of microplastic particles (MCs) by shape in snow on different sledding hills (SH) (the percentage by count was specified). The higher the percentage, the more of a particular microplastic compared to the others in the collected samples.

The presence of microplastics in ten colors was determined in snow samples collected from sledding hills (Figures 5 and 6). The largest share was recorded for black microplastics. Blue, red, pink, and green microplastics also had a significant share. In the snow samples covered by the analyses, a small share of plastic in brown, violet, and white colors was noted (Figure 5).

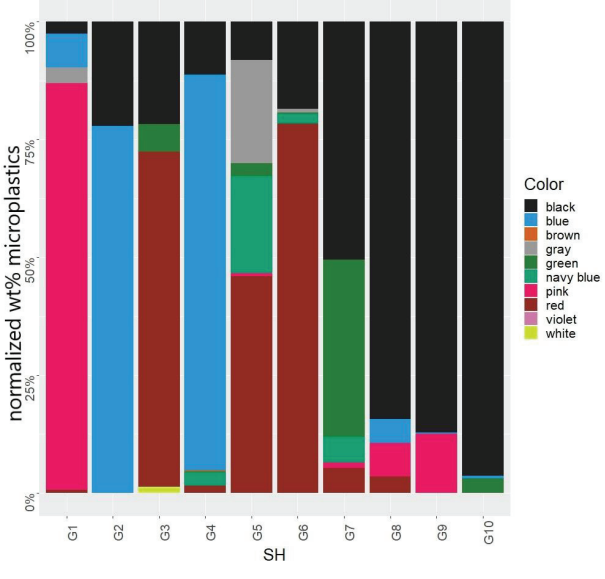

Figure 5. Distribution of microplastic particles (MCs) by color in snow on different sledding hills (SH) (the percentage by weight was specified). The higher the percentage, the more of a particular microplastic compared to the others in the collected samples.

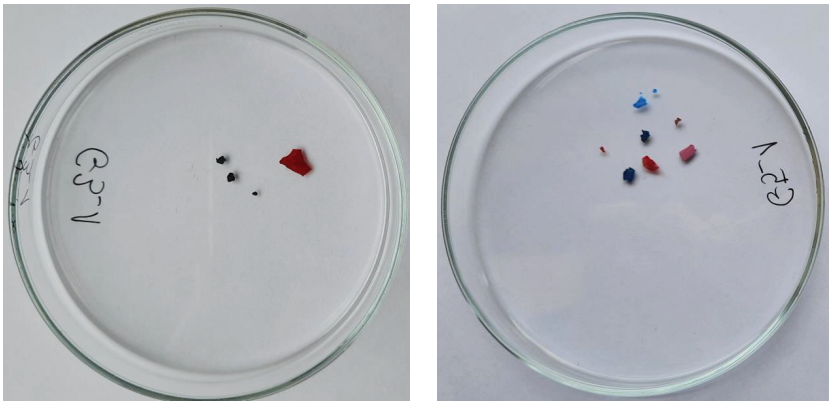

Figure 6. Examples of various microplastic particle types recorded in the snow on different sledding hills.

The conducted GLM analysis confirmed the importance of the size of sledding hills and the location of sledding hills in shaping the amount of microplastics (Table 1).

Table 1. GLM analysis for the microplastic weight (MCs weight mg/L) depends on the size of the sledding hill and the location of the sledding hill.

	MCs Weight	
	F	***p***
Size of sledding hill (S)	5.8367	*0.0236*
Location on sledding hill (L)	7.9621	*0.0022*
S × L	4.4017	*0.0235*

Significance effects ($p < 0.05$) are shown in italic.

In addition, the GLM analysis indicated an interactive effect of the size of sledding hills and the location of sledding hills in shaping the amount of microplastic. Within the examined sledding hills, the content of microplastics in snow varied from 2.8 mg/L to 61.0 mg/L (Figure 7). The median weight of microplastic in the snow samples from the upper sledding hills was three times higher compared to the lower sledding hills (2.78 mg/L and 0.96 mg/L, respectively) (Figure 8). The differences in the weight of the microplastics in the snow samples from the upper and lower sledding hills were not statistically significant. Statistically significant differences in the amount of microplastics were noted between the sledding hill locations. Lower locations (bottom) were characterized by a significantly higher content of microplastics compared to the middle and upper lower parts of the sledding hills. In the lower part of the sledding hill, the median content of microplastics was 20 times higher compared to the lowest part (Figure 8). The median microplastic content in the upper sledding hill was 0.50 mg/L, in the middle was 1.27 mg/L, and the lower 9.33 mg/L (Figure 8).

The performed PCA analysis confirmed the relationship between the studied characteristics of microplastic and the size of the sledding hill (Supplementary Figure S1). Two main factors contributed to the observed variance (51.6%): factor 1 accounts for 30.2% of the variance, while factor 2 explains 21.4% of the variance. Factor 1 is related to the size of the microplastic, while factor 2 is related to the type of microplastic. In addition, the PCA analysis confirmed the separation of high and low sledding hills concerning the type and size of the microplastic (Supplementary Figure S1). The lower hills were characterized by a higher share of PES and PU and a share of microplastics of 1.1–2.0 mm and 0.6–1.0 mm. Higher sledding hills were characterized by the highest mass of microplastics (MCs weight) and the share of microplastics with larger sizes (Supplementary Figure S1).

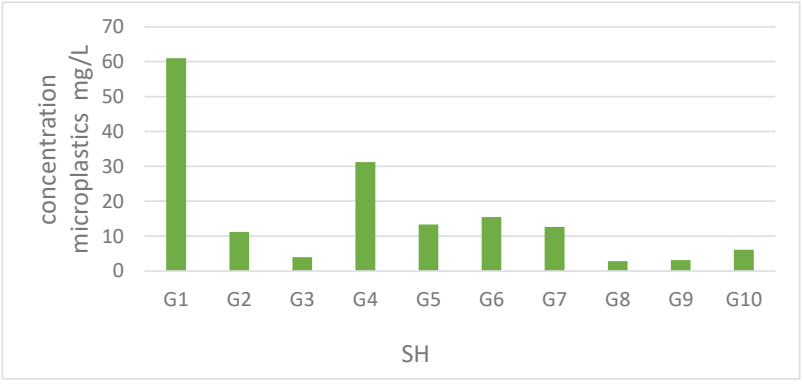

Figure 7. The content of microplastics (mg/L) in snow depends on the sledding hill.

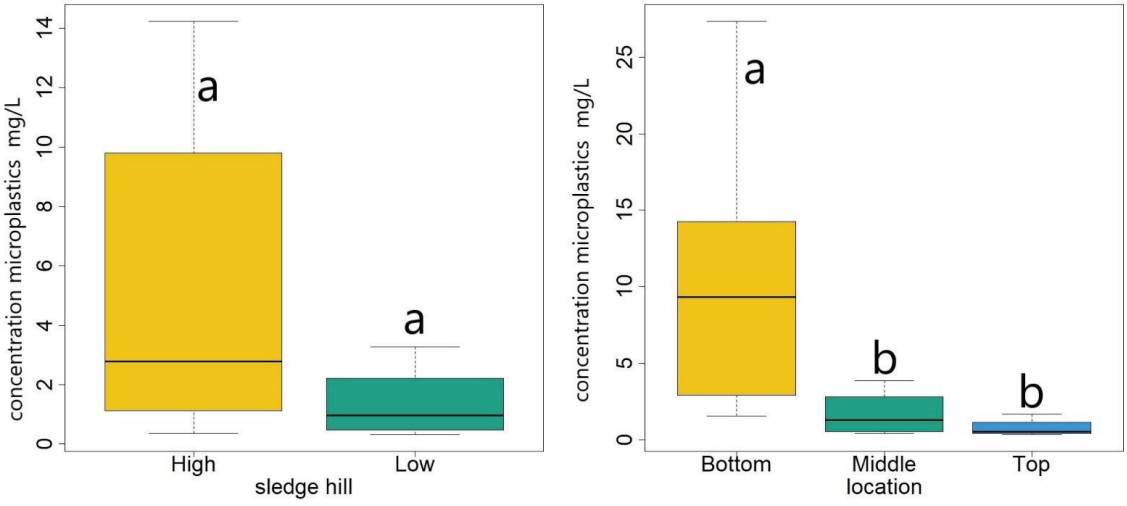

Figure 8. The content of microplastics (mg/L) in snow depends on the size of the sledding hill (high and low) and the location on the sledding hill (bottom, middle, top); colors indicate different variants of different sledding hill and different location on sledding hill, letters (a, b) mean significant differences between types of sledding hill and between different location on the sledding hill.

4. Discussion

In Krakow, the Municipal Greens Authority has prepared a map of 44 sledding hills that can be used during the winter, and 10 of them were tested for microplastic contamination. Our research shows significant contamination of the sledding hills in the green areas of Krakow with microplastics. Our research indicates the presence of a significant amount of microplastics that vary in type, size, color, and shape. Due to the high anthropogenic impact, the urban environment is considered to be one of the main sources of microplastic [25,26,30]. Microplastics have been commonly detected in the urban atmosphere, dust or soil, and urban rivers and are mainly produced in tire wear, landfill and wastewater treatment, and industrial activities [3,31]. Our study shows significant microplastic contamination of green areas as a result of using plastic sleds, shoes, and clothes on sledding hills. It should be emphasized that this microplastic, after melting the snow, ends up in various components of the environment. Microplastics migrate and transform in many urban environments through physical and biochemical factors [5,32]. Due to their lightweight and

low density, microplastics can easily float and transform between different environmental matrices in urban ecosystems. Through the runoff of rainwater, microplastics can end up in urban rivers, and by wind be transported to other urban ground surfaces. Microplastics from sledding hills and snow pose a potential health risk for urban residents. Microplastic pollutants were detected in all environmental matrices [33]; therefore, on the examined sledding hills they can be carried with dust or come from road pollution. Microplastics entering the soil environment are carried through food chains and food webs, which pose potential threats to human health [34]. The tissue accumulation of microplastics may cause a variety of inflammations that affect gene expression and cause cell lesions and maybe even cancer [35].

The dominant types of microplastics noted during our study were polypropylene, polyurethane, hydrocarbon resin, and polyester. According to the United Nations Environmental Program report, of the 388.2 million tons of plastics produced, 16% are polypropylene [36]. PP are dominant microplastic types found on sand and leaves in playgrounds, while playgrounds contain more microplastics than other areas in urban parks [37]. The natural aging process significantly changes the physicochemical properties of PP and enhances its sorption capacity, as a result of which PP presents a more vital ecological risk [38]. Microplastics can be vectors for transporting heavy metals in soils and increase the bioavailability of heavy metals posing negative biological effects [39]. According to Cao et al. [40], PP microplastics increased the concentration of bioavailable Cd in soils through decreasing soil retention. Microplastics in the form of biodegradable polyurethanes are prone to accumulation of PAHs from the soil and concentrations of PAHs in biodegradable polyurethanes were 70 times higher than in soil [41]. The high polyurethane contents noted in our studies confirm the possibility of higher contamination risk because the flexibility of the polyurethane polymeric network could be the main driving factor for the sorption. HDPE was detected in previous soil studies [34], but no such microplastic has been detected in our studies.

The microplastic marked in the sledding hills snow varied in size. The share of two microplastic fractions of 1.1–2.0 mm and 2.1–3.0 mm accounted for over 50% of all amounts. Microplastics sized less than 1.0 mm showed a significant share, often over 25%. Previous studies indicate that most or all of the microplastic particles extracted from different environments were below 1000 μm [42,43]. In the study of Leitão et al. [26], the average size of a microplastic was 116 μm; more than 80% of the particles in the general urban area sample measured less than 250 μm. According to Zhang et al. [44], the small-size microplastics favored the increase of pH, water content, organic matter, and adsorption capacities with Cd in the paddy soil compared with the large-size microplastics. The small-sized microplastics are more harmful to organisms because they have a larger surface area to absorb toxic chemicals [45]. Our research shows a relationship between the size of the microplastic and the size of the sledding hill. On the higher sledding hills, we recorded a greater share of microplastics of the largest sizes, i.e., 3.1–4.0 mm and 4.1–5.0 mm. Higher hills give the possibility of faster descents, stronger hits, and more friction, which leads to larger fragments of the plastic sled breaking off.

Microplastic shapes are generally classified as pellet/spherule, fragment/sheet, foam, fiber/line, and film [46]. In our study area, fragments had the largest share, often their share accounted for over 75% of all microplastic shapes. As a result of sliding down on plastic sleds, mechanical abrasion occurs and microplastics in the form of fragments are released into the environment. In the future, microplastic fragments will be fragmented as a result of ultraviolet radiation and biodegradation. A weathering experiment conducted in the laboratory indicates that 12 months of exposure to UV radiation and 2 months of mechanical abrasion of PP can produce 6084 $\pm$ 1061 particles [47]. In addition to the variation in the shape of the microplastic, we noted a large variation in the color of the microplastic. In our research, we isolated microplastics in 10 colors. This is probably the effect of using sledding hills, especially by children, for whom colorful toys and clothes are produced.

The conducted GLM analysis indicated the importance of the size of the sledding hills and the location on the sledding hills in shaping the amount of microplastics. A total of 3 times more microplastic was recorded on the higher hills compared to the lower hills. Higher sledding hills are more likely to be chosen by children and have higher speed descents, which result in more microplastic. A statistically significantly higher content of microplastic was recorded in the snow in the lower part of the sledding hills, where the sled reached the highest speed and braking occurred at the same time. By presenting the number of microplastics on sledding hills and their variation in type and size, we show that plastic snow slide devices and other products used for downhill can contribute to elevated concentrations of microplastic. It is justified to promote sliding equipment made of natural materials, such as wood. It is really important to consider switching from plastic gear to wooden gear (as long as they are not painted or varnished). Wood has long been widely used as a construction material due to its low cost and high strength, and is increasingly being seen as a viable alternative to plastic. Microplastics in urban green spaces from sledding hills can have negative effects on the soil environment. In the long term, microplastic pollution can have a potentially significant impact on the biodiversity of soil systems. Microplastic contamination in the soil strongly affects soil biota, and it particularly reduces the number of soil mesofauna and changes the structure of the microorganisms [48,49].

5. Conclusions

Our research shows significant microplastic contamination in the sledding hills located in the green areas of Krakow. Our analyses have confirmed that significant amounts of microplastics of various types, sizes, shapes, and colors get into the snow as a result of winter activity, especially by children. The higher sledding hills had a higher content of microplastics compared to the lower sledding hills. Our research suggests promoting children's use of wooden sleds and avoiding plastic products. The occurrence of microplastics in soil is a cause for concern. Microplastics on sledding hills can break down with exposure to sunlight or high temperatures and pose a serious threat to humans, animals, and plants. It is important to conduct further research on microplastic contamination of urban green spaces that are intensively used by residents.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su151712995/s1. Figure S1. Projection of variables on the plane of the first and second PCA factors (Polyurethane (PU), Polyethylene terephthalate (PET), Polyester (PES), Polyvinyl butyral (PVB), Termoplastic elastomer (TPE), Polyvinyl chloride (PCV), Polypropylene (PP), Polystyrene (PS), Hydrocarbon resin (HCR), Phenoxy resin (PHR), Poly(isobutene isoprene) (PIBP); High SH—high sledding hill, Low SH—low sledding hill).

Author Contributions: Conceptualization, J.L., W.P. and E.B.; Methodology, E.B.; Formal analysis, J.L. and E.B.; Investigation, J.L., W.P. and E.B.; Data curation, J.L. and E.B.; Writing—original draft, J.L., S.T. and E.B.; Writing—review & editing, W.P.; Visualization, E.B.; Funding acquisition, S.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry of Education and Science of the Republic of Poland (SUB/040012/D019).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This work was supported by the Ministry of Education and Science of the Republic of Poland (SUB/040012/D019).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Akdogan, Z.; Guven, B. Microplastics in the environment: A critical review of current understanding and identification of future research needs. *Environ. Pollut.* **2019**, *254*, 113011. [CrossRef]
2. Escalona-Segura, G.; Borges-Ramírez, M.M.; Estrella-Canul, V.; Rendón-von Osten, J. A methodology for the sampling and identification of microplastics in bird nests. *Green Anal. Chem.* **2022**, *3*, 100045. [CrossRef]
3. Parker, B.; Britton, J.R.; Pabortsava, K.; Barrow, M.; Green, I.D.; Almela, V.D.; Andreou, D. Distinct microplastic patterns in the sediment and biota of an urban stream. *Sci. Total Environ.* **2022**, *838*, 156477. [CrossRef] [PubMed]
4. Österlund, H.; Blecken, G.; Lange, K.; Marsalek, J.; Gopinath, K.; Viklander, M. Microplastics in urban catchments: Review of sources, pathways, and entry into stormwater. *Sci. Total Environ.* **2023**, *858*, 159781. [CrossRef] [PubMed]
5. Zhang, B.; Yang, X.; Chen, L.; Chao, J.; Teng, J.; Wang, Q. Microplastics in soils: A review of possible sources, analytical methods and ecological impacts. *J. Chem. Technol. Biotechnol.* **2019**, *95*, 2052–2068. [CrossRef]
6. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. Microplastics as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* **2011**, *62*, 2588–2597. [CrossRef]
7. Hidalgo-Ruz, V.; Gutow, L.; Thompson, R.C.; Thiel, M. Microplastics in the Marine Environment: A Review of the Methods Used for Identification and Quantification. *Environ. Sci. Technol.* **2012**, *46*, 3060–3075. [CrossRef]
8. Vijayan, A.; Österlund, H.; Magnusson, K.; Marsalek, J.; Viklander, M. Microplastics (MPs) in urban roadside snowbanks: Quantities, size fractions and dynamics of release. *Sci. Total Environ.* **2022**, *851*, 158306. [CrossRef] [PubMed]
9. Rillig, M.C.; Lehmann, A. Microplastic in terrestrial ecosystems research shifts from ecotoxicology to ecosystem effects and Earth system feedbacks. *Science* **2020**, *368*, 1430–1431. [CrossRef]
10. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci. Adv.* **2017**, *3*, e1700782. [CrossRef]
11. Wan, L.; Cheng, H.; Liu, Y.; Shen, Y.; Liu, G.; Su, X. Global meta-analysis reveals differential effects of microplastics on soil ecosystem. *Sci. Total Environ.* **2023**, *867*, 161403. [CrossRef] [PubMed]
12. Koutnik, V.S.; Leonard, J.; Alkidim, S.; DePrima, F.J.; Ravi, S.; Hoek, E.M.; Mohanty, S.K. Distribution of microplastics in soil and freshwater environments: Global analysis and framework for transport modeling. *Environ. Pollut.* **2021**, *274*, 116552. [CrossRef] [PubMed]
13. Luo, Y.; Wang, L.; Cao, T.; Chen, J.; Lv, M.; Wei, S.; Lu, S.; Tian, X. Microplastics are transferred by soil fauna and regulate soil function as material carriers. *Sci. Total Environ.* **2023**, *857*, 159690. [CrossRef]
14. De Souza Machado, A.A.; Kloas, W.; Zarfl, C.; Hempel, S.; Rillig, M.C. Microplastics as an emerging threat to terrestrial ecosystems. *Glob. Chang. Biol.* **2018**, *24*, 1405–1416. [CrossRef]
15. Rillig, M.C.; Ziersch, L.; Hempel, S. Microplastic transport in soil by earthworms. *Sci. Rep.* **2017**, *7*, 1362. [CrossRef]
16. Rillig, M.C. Microplastic Disguising As Soil Carbon Storage. *Environ. Sci. Technol.* **2018**, *52*, 6079–6080. [CrossRef]
17. de Souza Machado, A.A.; Lau, C.W.; Kloas, W.; Bergmann, J.; Bachelier, J.B.; Faltin, E.; Becker, R.; Görlich, A.S.; Rillig, M.C. Microplastics Can Change Soil Properties and Affect Plant Performance. *Environ. Sci. Technol.* **2019**, *53*, 6044–6052. [CrossRef]
18. Grima, S.; Bellon-Maurel, V.; Feuilloley, P.; Silvestre, F. Aerobic Biodegradation of Polymers in Solid-State Conditions: A Review of Environmental and Physicochemical Parameter Settings in Laboratory Simulations. *J. Polym. Environ.* **2000**, *8*, 183–195. [CrossRef]
19. Europeans Chemicals Agency (ECHA). Annex XV Restriction Report. Proposal for a Restriction. Version 1.2. 2019. Available online: https://echa.europa.eu/documents/10162/05bd96e3-b969-0a7c-c6d0-44118289372 (accessed on 16 June 2021).
20. Nielsen, M.B.; Clausen, L.P.W.; Cronin, R.; Hansen, S.F.; Oturai, N.G.; Syberg, K. Unfolding the science behind policy initiatives targeting plastic pollution. *Microplastics Nanoplastics* **2023**, *3*, 3. [CrossRef] [PubMed]
21. Thushari, G.G.N.; Senevirathna, J.D.M.; Yakupitiyage, A.; Chavanich, S. Effects of microplastics on sessile invertebrates in the eastern coast of Thailand: An approach to coastal zone conservation. *Mar. Pollut. Bull.* **2017**, *124*, 349–355. [CrossRef]
22. Bläsing, M.; Amelung, W. Plastics in soil: Analytical methods and possible sources. *Sci. Total Environ.* **2018**, *612*, 422–435. [CrossRef]
23. Forster, N.A.; Tighe, M.K.; Wilson, S.C. Microplastics in soils of wilderness areas: What is the significance of outdoor clothing and footwear? *Geoderma* **2020**, *378*, 114612. [CrossRef]
24. Corradini, F.; Casado, F.; Leiva, V.; Huerta-Lwanga, E.; Geissen, V. Microplastics occurrence and frequency in soils under different land uses on a regional scale. *Sci. Total Environ.* **2021**, *752*, 141917. [CrossRef]
25. Kunz, A.; Schneider, F.; Anthony, N.; Lin, H.-T. Microplastics in rivers along an urban-rural gradient in an urban agglomeration: Correlation with land use, potential sources and pathways. *Environ. Pollut.* **2023**, *321*, 121096. [CrossRef]
26. Leitão, I.; van Schaik, L.; Ferreira, A.; Alexandre, N.; Geissen, V. The spatial distribution of microplastics in topsoils of an urban environment—Coimbra city case-study. *Environ. Res.* **2023**, *218*, 114961. [CrossRef]
27. Diener, A.; Mudu, P. How can vegetation protect us from air pollution? A critical review on green spaces' mitigation abilities for air-borne particles from a public health perspective—With implications for urban planning. *Sci. Total Environ.* **2021**, *796*, 148605. [CrossRef] [PubMed]
28. Lopez, B.; Kennedy, C.; Field, C.; McPhearson, T. Who benefits from urban green spaces during times of crisis? Perception and use of urban green spaces in New York City during the COVID-19 pandemic. *Urban For. Urban Green.* **2021**, *65*, 127354. [CrossRef] [PubMed]
29. Rackiewicz, I. *Program Ochrony śRodowiska Dla Miasta Krakowana Lata 2012–2015 Z Uwzględnieniem Zadań Zrealizowanych W 2011 Roku Oraz Perspektywą NA Lata 2016–2019*; Rada Miasta Krakowa: Kraków, Poland, 2012.

30. Samandra, S.; Singh, J.; Plaisted, K.; Mescall, O.J.; Symons, B.; Xie, S.; Ellis, A.V.; Clarke, B.O. Quantifying environmental emissions of microplastics from urban rivers in Melbourne, Australia. *Mar. Pollut. Bull.* **2023**, *189*, 114709. [CrossRef] [PubMed]
31. Mercy, F.T.; Alam, A.K.M.R.; Akbor, M.A. Abundance and characreristics of microplastics in major urban lakes of Dhaka, Bangladesh. *Heliyon* **2023**, *9*, e14587. [CrossRef]
32. Cai, Y.; Li, C.; Zhao, Y. A review of the migration and transformation of micro plastics in inland water systems. *Int. J. Environ. Res. Public Health* **2022**, *19*, 148. [CrossRef]
33. O'Brien, S.; Rauert, C.; Ribeiro, F.; Okoffo, E.D.; Burrows, S.D.; O'Brien, J.W.; Wang, X.; Wright, S.L.; Thomas, K.V. There's something in the air: A review of sources, prevalence and behaviour of microplastics in the atmosphere. *Sci. Total Environ.* **2023**, *874*, 162193. [CrossRef]
34. Li, Z.; Yang, Y.; Chen, X.; He, Y.; Bolan, N.; Rinklebe, J.; Lam, S.S.; Peng, W.; Sonne, C. A discussion of microplastics in soil and risks for ecosystems and food chains. *Chemosphere* **2023**, *313*, 137637. [CrossRef] [PubMed]
35. Peixoto, D.; Pinheiro, C.; Amorim, J.; Oliva-Teles, L.; Guilhermino, L.; Vieira, M.N. Microplastic pollution in commercial selt for human consuption: A review. *Estuar. Coast Shelf Sci.* **2019**, *219*, 161–168. [CrossRef]
36. Ryberg, M.W.; Hauschild, M.Z.; Wang, F.; Averous-Monnery, S.; Laurent, A. Global environmental losses of plastics across their value chains. *Resour. Conserv. Recycl.* **2019**, *151*, 104459. [CrossRef]
37. Koutnik, V.S.; Leonard, J.; El Rassi, L.A.; Choy, M.M.; Brar, J.; Glasman, J.B.; Cowger, W.; Mohanty, S.K. Children's playgrounds contain more microplastics than other areas in urban parks. *Sci. Total Environ.* **2023**, *854*, 158866. [CrossRef]
38. He, S.; Sun, S.; Xue, H.; Kang, C.; Yu, S. Polypropylene microplastics aging under natural conditions in winter and summer and its effects on the sorption and desorption of nonylphenol. *Environ. Res.* **2023**, *225*, 115615. [CrossRef]
39. Huang, C.; Ge, Y.; Yue, S.; Zhao, L.; Qiao, Y. Microplastics aggravate the joint toxicity to earthworm Eisenia fetida with cadmium by altering its availability. *Sci. Total Environ.* **2021**, *753*, 142042. [CrossRef]
40. Cao, Y.; Ma, X.; Chen, N.; Chen, T.; Zhao, M.; Li, H.; Song, Y.; Zhou, J.; Yang, J. Polypropylene microplastics affect the distribution and bioavailability of cadmium by changing soil components during soil aging. *J. Hazard. Mater.* **2023**, *443*, 130079. [CrossRef]
41. Černá, T.; Pražanová, K.; Beneš, H.; Titor, J.; Klubalová, K.; Filipová, A.; Kluson, P.; Cajthaml, T. Polycyclic aromatic hydrocarbon accumulation in aged and unaged polyurethane microplastics in contaminated soil. *Sci. Total Environ.* **2021**, *770*, 145254. [CrossRef]
42. Rezaei, M.; Riksen, M.J.; Sirjani, E.; Sameni, A.; Geissen, V. Wind erosion as a driver for transport of light density microplastics. *Sci. Total Environ.* **2019**, *669*, 273–281. [CrossRef]
43. Li, B.; Wan, H.; Cai, Y.; Peng, J.; Li, B.; Jia, Q.; Yuan, X.; Wang, Y.; Zhang, P.; Hong, B.; et al. Human activities affect the multidecadal microplastic deposition records in a subtropical urban lake, China. *Sci. Total Environ.* **2022**, *820*, 153187. [CrossRef] [PubMed]
44. Zhang, Q.; Guo, W.; Wang, B.; Feng, Y.; Han, L.; Zhang, C.; Xie, H.; Liu, X.; Feng, Y. Influences of microplastics types and size on soil properties and cadmium adsorption in paddy soil after one rice season. *Resour. Environ. Sustain.* **2023**, *11*, 1000102. [CrossRef]
45. Xu, C.; Zhang, B.; Gu, C.; Shen, C.; Yin, S.; Aamir, M.; Li, F. Are we underestimating the sources of microplastic pollution in terrestrial environment? *J. Hazard. Mater.* **2020**, *400*, 123228. [CrossRef]
46. Yang, L.; Zhang, Y.; Kang, S.; Wang, Z.; Wu, C. Microplastics in soil: A review on methods, occurrence, sources, and potential risk. *Sci. Total Environ.* **2021**, *780*, 146546. [CrossRef] [PubMed]
47. Zhou, Y.; Wang, J.; Zou, M.; Jia, Z.; Zhou, S.; Li, Y. Microplastics in soils: A review of methods, occurrence, fate, transport, ecological and environmental risks. *Sci. Total Environ.* **2020**, *748*, 141368. [CrossRef] [PubMed]
48. Rillig, M.C.; de Souza Machado, A.A.; Lehmann, A.; Klümper, U. Evolutionary implications of microplastics for soil biota. *Environ. Chem.* **2018**, *16*, 3–7. [CrossRef]
49. Lin, D.; Yang, G.; Dou, P.; Qian, S.; Zhao, L.; Yang, Y.; Fanin, N. Microplastics negatively affect soil fauna but stimulate microbial activity: Insights from a field-based microplastic addition experiment. *Proc. R. Soc. B Boil. Sci.* **2020**, *287*, 20201268. [CrossRef]

water

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Article

Spatial–Temporal Distribution and Ecological Risk Assessment of Microplastics in the Shiwuli River

Lei Hong [1,2,3,4], Xiangwu Meng [1], Teng Bao [1,2], Bin Liu [1], Qun Wang [1], Jie Jin [2] and Ke Wu [2,3,*]

[1] School of Biology, Food and Environment, Hefei University, Hefei 230601, China; honglei@hfuu.edu.cn (L.H.)
[2] Hefei Institute of Environmental Engineering, Hefei University, Hefei 230601, China
[3] Collaborative Innovation Center for Environmental Pollution Control and Ecological Restoration of Anhui Province, Hefei 230601, China
[4] Anhui Key Laboratory of Sewage Purification and Ecorestoration Materials, Hefei 230001, China
* Correspondence: wuke@hfuu.edu.cn

Abstract: This study aimed to investigate the distribution of microplastics (MPs) within the Shiwuli River in Hefei, a Chinese inland city. Water and sediment samples were collected during flood season (from May to September) and non-flood season (from October to April) at 10 representative points along the truck stream. The electron microscope, the laser direct infrared chemical imaging system (LDIR), and the scanning electron microscope (SEM) were used to observe and quantify the colour and shape of the MPs, to identify the number, size, and polymer composition of the MPs, and to observe the microstructures of typical MP particles, respectively. The polymer risk index (RI) model and the pollution load index (PLI) model were used to assess the polymer-related risks and the overall extent of MP pollution in the river, respectively. Analysis of MP abundance for different sampling points showed that the water of Shiwuli River had an average abundance of MPs of 8.4 ± 2.5 particles/L during the flood season and 5.8 ± 1.7 particles/L during the non-flood season; the sediment had an average abundance of MPs of 78.9 ± 8.3 particles/kg during the flood season and 63.9 ± 7.1 particles/kg during the non-flood season. The abundance of MPs of different points was investigated. Result show that the more abundances of MPs were found at confluences with tributaries (S4, S5, and S6), where they are also close to the residential and industrial development, while lower values were found in agricultural areas (S8) and wetland ecological regions (S9 and S10). In water, the maximum appeared at S5 with 21.7 ± 4.6 particles/L during the flood season and 15.9 ± 4.2 particles/L during the non-flood season, respectively; the minimum appeared at S9 with 1.8 ± 1.0 particles/L during the flood season and 2.2 ± 0.4 particles/L during the non-flood season, respectively. In sediment, the maximum appeared at S5 with 174.1 ± 10.1 particles/kg during the flood season and 143.6 ± 10.4 particles/kg during the non-flood season, respectively; the minimum appeared at S8 with 10.3 ± 2.8 particles/kg during the flood season and at S9 with 12.1 ± 3.2 particles/kg during the non-flood season, respectively. MP characteristics were also studied. Results show that the MPs mainly exhibited a fibroid morphology (27.90–34%), and red-coloured particles (19.10%) within the smaller size less than 500 μm (38.60%) were more prevalent. Additionally, the result of LDIR scanning shows that a total of eleven types of MP polymers were found in the river water and sediment, including acrylates (ACR), chlorinated polyethylene (CPE), ethylene vinyl acetate (EVA), polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyurethane (PU), polyvinylchloride (PVC), polyamide (PA), and silicon. The most common particle was PE (19.3–21.6%). Furthermore, the environmental risk assessment demonstrated that the PS polymer posed a Level-III risk in the water samples and a Level-II risk in the sediment samples from the Shiwuli River. The remaining polymer types exhibited Level-I risk. The PLI_{zone} value for water was 2.24 during the flood season, indicating heavy pollution, and 1.66 during the non-flood season, indicating moderate pollution. Similarly, the PLI_{zone} value for sediments was 2.34 during the flood season and 1.91 during the non-flood season, both suggesting a heavy pollution. These findings highlight the potential risk posed by MP pollution in the Shiwuli River to the quality of drinking water sources in Chaohu Lake in Hefei. They provide valuable insights into management, pollution control, and integrated management strategies pertaining to MPs in urban inland rivers in Hefei.

Citation: Hong, L.; Meng, X.; Bao, T.; Liu, B.; Wang, Q.; Jin, J.; Wu, K. Spatial–Temporal Distribution and Ecological Risk Assessment of Microplastics in the Shiwuli River. *Water* **2023**, *15*, 2330. https://doi.org/10.3390/w15132330

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

Received: 21 May 2023
Revised: 18 June 2023
Accepted: 19 June 2023
Published: 23 June 2023

Keywords: microplastics; spatial–temporal distribution; ecological risk assessment

1. Introduction

Microplastics (MPs), defined as plastic particles smaller than 5 mm in size, represent persistent organic pollutants and emerging contaminants [1,2] that exhibit resistance to degradation and are ubiquitously present in various environmental compartments, including surface water, sediment, atmosphere, and soil, thereby posing significant ecological risks [3]. In general, MPs can be derived from both industrially produced primary plastic particles and secondary plastic particles generated through the fragmentation of large plastic debris commonly used in daily production and consumption [4]. MPs possess distinctive surface characteristics and display a phenomenon known as the Trojan horse effect [5]. This effect enables them to act as carriers for the adsorption of heavy metals, antibiotics, and other pollutants in the environment, facilitating their migration. Moreover, MPs can be ingested by aquatic organisms and mammals [6–8]; subsequently causing bioaccumulation through the food chain and potentially impacting human health [9–11]. These issues became widely concerning around the world.

Recent research predominantly concentrated on elucidating MP transport mechanisms within marine environments [12–14], assessing their consequential toxicological impacts on various organisms [15,16], and investigating their distribution in freshwater lakes and rivers [17–19]. Drabinski et al. explored the abundance and spatial dispersion of MPs in three distinct freshwater rivers situated in Rio de Janeiro, Brazil [20]. Similarly, Pradit et al. examined the quantification of MP particles in the U-Taphao River located in southern Thailand, investigating temporal variations across different time intervals [21]. Furthermore, Lin et al. explored the abundance and distribution patterns of MPs in the Pearl River, China [22]. These studies collectively shed light on the natural migratory pathways of MPs originating from urban industrial processes and residential activities as they traverse riverine ecosystems.

Various methodologies and models were developed to assess the risk associated with MP pollution in aquatic ecosystems. For instance, Kim et al. employed a species sensitivity distribution (SSD) method to evaluate the ecological risk of MP pollution by determining the sensitivity of soil biota to microplastics [23]. Similarly, Zhong et al. utilized a pollution load index (PLI) model to determine the level of risk posed by MPs in Dongshan Bay, China [24]. Furthermore, Ranjani et al. applied the PLI model to evaluate the presence of MPs in sediments from the east and west coasts of India [25]. In a separate study, Kai et al. utilized a polymer risk index (RI) model to assess the extent of MP pollution in Chagan Lake and Xianghai Lake, China [26]. By employing these diverse models, researchers paved the way for conducting comprehensive ecological risk assessments pertaining to MP pollution in water basin environments. These assessments, in turn, provide valuable insight into effective preventative measures and controls against MP pollution.

The Shiwuli River, located within Hefei, serves as a primary tributary of Chaohu Lake. Chaohu Lake is a significant freshwater lake in China with a water area of 769.6 km^2 and is recognized as a national drinking water source. This river connects Chaohu Lake with the urban area of Hefei, the capital city of Anhui Province, with a population of 9,634,000. The drainage basin of the Shiwuli River encompasses diverse functional zones, including administrative and commercial areas, industrial and agricultural production areas, residential living areas, as well as ecological landscape and wetland ecological protection areas. The river faces substantial risks from both point source and non-point source pollution, which act as major pathways for the enrichment and migration of MPs and other pollutants into Chaohu Lake. Currently, there is limited research focusing on the characteristics and ecological risk assessment of MPs in urban rivers situated inland, especially those that flow into drinking water source lakes. Additionally, no published studies investigated the ecological risk assessment of MPs in Chaohu Lake and its tributaries. Herein, this

study aims to investigate the spatial and temporal distribution of MPs in both river water and sediment within the Shiwuli River. Furthermore, it aims to quantitatively assess the potential ecological risks and overall pollution load using two models: the pollution load index (PLI) and the polymer risk index (RI). The ultimate goal is to provide a scientific foundation for comprehensive pollution assessment and source control management of MPs in the Chaohu Lake basin.

2. Materials and Experimental Methods

2.1. Profile of the Sampling Points

The Shiwuli River Basin is located at 31.727°–31.835° N, 117.198°–117.377° E, and covers an area of 111.25 km^2; (Figure 1). The population in the basin is currently approximately 576,500. The precipitation during the flood season accounts for 60.5% of the total precipitation. The main stream of the Shiwuli River is 24.74 km long, the average slope of the watercourse is only 0.72‰, and the water surface ratio is only 4.4%. At present, the water resource utilization pattern is watercourse ecological water. There is no stable clean water source at the upper reaches and water supplementation mainly comes from natural precipitation, tailwater discharged from sewage treatment plants, and overflows from Swan Lake. The water volume cannot meet the basic ecological flow requirements. At present, point source pollution in the river comes mainly from the discharges of urban sewage plants and the effluent from industrial plants. Non-point sources are mainly urban surface runoff, rural domestic wastewater, and agricultural planting and cultivation. Many detrimental environmental factors have an impact on the basin, including incomplete rain pollution distributaries, a sharp increase in non-point source pollution during the flood season, and watercourse cut-off during the dry season, among others [27].

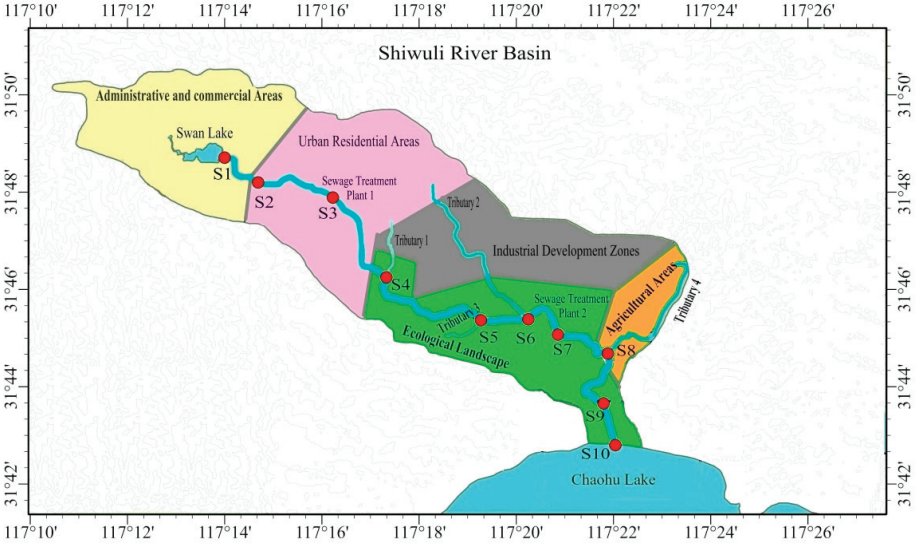

Figure 1. Sampling points and functional areas in Shiwuli River Basin. The sampling points selected along the river are labelled as S1–S10.

2.2. Sample Methods

According to spatial factors, such as the different urban functional areas that the Shiwuli River runs through, the type of outlets and the confluence of the tributaries, a total of 10 sampling points were chosen. Specifically, one sampling point was at the starting and one at the ending sections of the river, respectively, four points were at the intersection of the tributaries, two points were at outlet areas of sewage treatment plants, and three points

were at typical functional regions (Figure 1 and Table 1). Sampling was performed during the flood season (June 2022) and the non-flood season (November 2022). Samples of both water and sediment were obtained at each sampling point. A total of 2 L water samples were collected from each sampling point at three different depths (0–20 cm, 40–80 cm, and 100–150 cm) and uniformly mixed; 1.5 kg of sediments was collected at three different locations from each sampling point and uniformly mixed.

Table 1. Description of sampling points.

Sampling Points	Locations	Characteristics
S1	Administrative and commercial areas	Starting section, estuary of Swan Lake
S2	Urban residential areas	Beside main streets of urban traffic, outlet below the Overpass of Jinzhai Road
S3	Urban residential areas	Outlet of the Hudaying Sewage Treatment Plant
S4	Ecological landscape areas	Intersection with the tributary Xingfu Channel, next to the industrial development zone
S5	Ecological landscape areas	Intersection with the tributary Wangniangou, the planned large ecological park area
S6	Ecological landscape areas	Intersection with the tributary Xuxiaohe, the planned large ecological park area
S7	Ecological landscape areas	Outlet of the Shiwulihe Sewage Treatment Plant
S8	Agricultural areas	Intersection with the tributary Xuxi River, agricultural planting and aquaculture
S9	Wetland ecological protection areas	Large water area
S10	Wetland ecological protection areas	Terminal section, estuary of Chaohu Lake

2.3. Experimental Scheme

2.3.1. Microplastics Separation and Extraction

The treatment of the samples was carried out according to the method of Meng et al. [28]. For water samples, stainless steel sieves with different pore sizes (1 mm, 5 mm, 15 μm, and 25 μm) were piled up one by one for coarse filtering. All the above sieves were rinsed by deionized water and filtered again using a filter membrane (LONGJIN, PTFE, aperture 5 μm, diam 50 mm, Nantong, China). Then, the filter membrane was placed in an open beaker containing 30% H2O2 solution for digestion and stirred by a magnetic stirrer (HUXI, HMS-203D, Shanghai, China) for 72 h. The temperature was 60–65 °C and the rotating speed was 550 rpm. The digested mixture was extracted for a second time and the wet filter membrane was placed in a petri dish, which was then transferred into an oven for dehydration for 1 h at 100–105 °C. The dried filter membrane was transferred into a saturated NaCl solution for density floating. Meanwhile, oscillation was carried out and the solution was left static for 24 h. The supernate was extracted and the residues in the beaker were poured into the saturated NaCl solution again for density floating. This process was repeated three times and three pieces of filter membrane were obtained. For the sediment samples, they were paved onto a petri dish and dried in an oven at 105 °C for 14 h. The subsequent digestion steps were the same as described above for the water samples.

2.3.2. Observation and Identification

The electron microscope (AOSVI, HK830–5870, China) was used to observe and quantify the colour and shape of the MPs; the laser direct infrared chemical imaging system (LDIR, Agilent 8700, Santa Clara, CA, USA) was used to scan all particles from the filter membranes and the Agilent Clarity software (version 1.1.2) in this system analyzed the MPs in each sample automatically and individually combined with its own spectral library (Microplastic starter 1.0) to obtain all polymer types with a matching degree greater than 0.80, as well as the number and size of particles in each type [29]. In addition, the microstructures of different types of typical MP particles were observed using a scanning

electron microscope (SEM, Hitachi S4800, Tokyo, Japan). All of the above were carried out in a closed and dust-free environment.

Quantification of the characteristics and components of the MPs was conducted with reference to the quantitative analysis method of Pivokonsky et al. [30].

$$N_m = \frac{\sum_{i=0}^{5} * N_i * S_m}{5 S_f} \quad (1)$$

N_i is the number of MPs on each quadrate (particles/L, particles/kg), $S_m \approx 9.26$ cm^2 is the contact area of impurities on a single high reflector, and $S_f = 0.84$ cm^2 is the area of a single s quadrate. The length of a quadrate is 12.5 mm and its width is 6.72 mm.

2.4. Potential Ecological Risk Assessment

The polymer risk index (RI) model was used to assess the polymer-related risks of MPs pollution in the river. The ecological risk index H of MP polymers was calculated as follows:

$$H = \sum P_n \times S_n \quad (2)$$

P_n is the proportion of different MP types in the samples from each point, and S_n is the risk scores of the different types of MP polymers [31].

The pollution load index (PLI) model was used to assess the overall extent of MP pollution in the river. The abundances of MPs at the regional sample points were used as the major indices according to the PLI model, which was proposed by Tolminson et al. [32]. The assessment model was defined as follows:

$$CF_i = C_i / C_{oi} \quad (3)$$

CF_i was defined as the ratio of the abundance of MPs (C_i) at each sampling point to the minimum abundance of MPs (C_{oi}) at each sampling point.

$$PLI_i = \sqrt{CF_i} \quad (4)$$

$$PLI_{zone} = \sqrt[n]{PLI_1 \times PLI_2 \times \ldots \times PLI_n} \quad (5)$$

PLI_i is the pollution load index of MPs for a single sample, while PLI_{zone} represents the pollution load index of MPs for the river.

The risk level classification for the two models is shown in Table 2.

Table 2. Risk level classification for the two models [31,32].

Model (Indexes)	Risk Category			
	I (Very Low Hazard)	**II (Low Hazard)**	**III (Medium Hazard)**	**IV (High Hazard)**
RI (H value)	<10	10~100	100~1000	>1000
PLI (PLI_{zone} value)	<10	10~20	20~30	>30

2.5. Data Analysis

Microsoft Excel 2019 was used for data pre-processing and SPSS 26.0 (IBM Co. Ltd., Armonk, NY, USA) for correlation analysis. Origin 2018 (Origin Lab., Farmington, ME, USA) and Microsoft Visio 2016 were used for data analysis and graph plotting.

3. Results and Discussion

3.1. Spatial–Temporal Distribution of Microplastics

3.1.1. Abundance Distribution

Figure 2 shows the result of MPs in water and sediment samples from the 10 points (S1–S10). The abundances of MPs in water and sediment were described by the number of MP particles/L and the number of MP particles/kg dry weight, respectively.

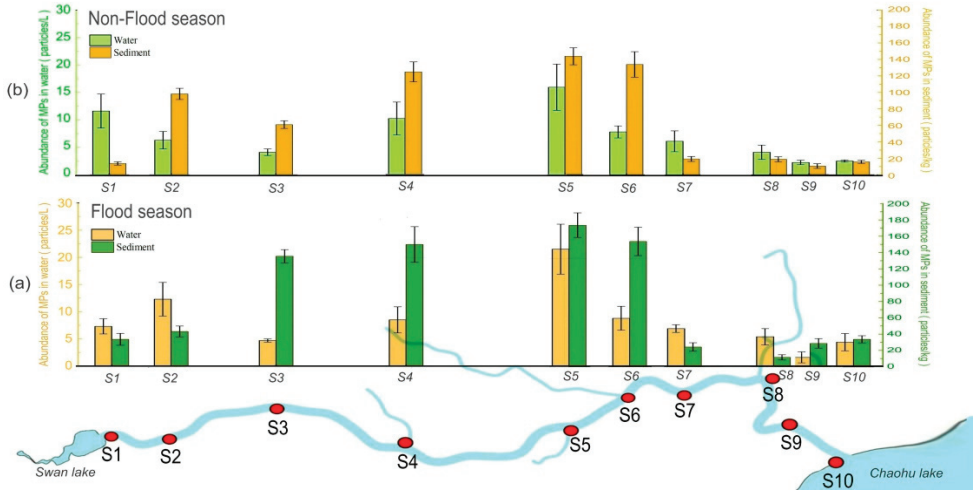

Figure 2. Distribution of microplastics abundance at sampling points along the Shiwuli River. The sampling points selected along the river were labelled as S1–S10. (**a**) Flood season; (**b**) Non-flood season.

In water, the average MPs abundance during the flood season was 8.4 ± 2.5 particles/L. The maximum was at S5 (21.7 ± 4.6 particles/L), followed by S2 (12.5 ± 3.1 particles/L), and the minimum was at S9 (1.8 ± 1.0 particles/L). The average MPs abundance during the non-flood season was 5.8 ± 1.7 particles/L. The maximum also was observed at S5 (15.9 ± 4.2 particles/L), followed by S1 (11.6 ± 3.1 particles/L), and the minimum also was at S9 (2.2 ± 0.4 particles/L). In sediments, the average MPs abundance was 78.9 ± 8.3 particles/kg during the flood season. The maximum was at S5 (174.1 ± 10.1 particles/kg) and the minimum was at S8 (10.3 ± 2.8 particles/kg). The average MPs abundance was 63.9 ± 7.1 particles/kg during the non-flood season. The maximum also was at S5 (143.6 ± 10.4 particles/kg) and the minimum was at S9 (12.1 ± 3.2 particles/kg).

When combining the statistical results for the water and sediment samples, it can be seen that the abundance of MPs was generally higher during the flood season (Figure 2a) compared to that during the non-flood season (Figure 2b).

The spatial variation laws of MP abundances in water and sediment were generally consistent and showed a trend of first increasing and then decreasing from upstream to downstream of the river [33]. The MPs were mainly concentrated in the middle and lower reaches of the river, influenced by the flow of the water [34]. The abundance of MPs in the sediment samples was higher than that in the water samples. This is mainly due to the fact that the water is poorly flowing in the middle reaches and stays stagnant for a long period of time [28]. MPs in the middle reaches can generate an absorption effect and can be wrapped by other substances, leading to their precipitation and accumulation over the years. Research showed that MPs can be easily wrapped in gravel [35]. When the water volume cannot meet the basic ecological flow requirement, MPs with a high density easily precipitate in water, while MPs with a low density may be adsorbed by algae, thus further increasing the ecological pollution load [14].

The abundances of MPs from S4, S5, and S6 were higher than those of the other points. Although these three points are located in the ecological landscape zone, they are all near residential living areas or industrial development areas. MPs produced by household waste and industrial waste are easily carried into rivers by surface rainfall runoff [18,36]. Sewage treatment approaches can intercept MPs to some extent. Therefore, the abundances of MPs in water at S3 and S7 were relatively low due to the influence of sewage treatment plant supplementation into the river. Chen et al. demonstrated that wetland environments have a significant effect on the removal efficiency of MPs. [37]. Due to the presence of wetlands, the water and mudflat areas at S9 are relatively large, providing better conditions for the natural degradation of MPs; this accounts for the low abundance of MPs at this point [38].

3.1.2. Shape Characteristics

According to previous studies, the shapes of MPs observed under the microscope were divided into the following categories: particles, membranes, fragments, and fibers. The shapes of the MPs observed via microscope are shown in Figure 3.

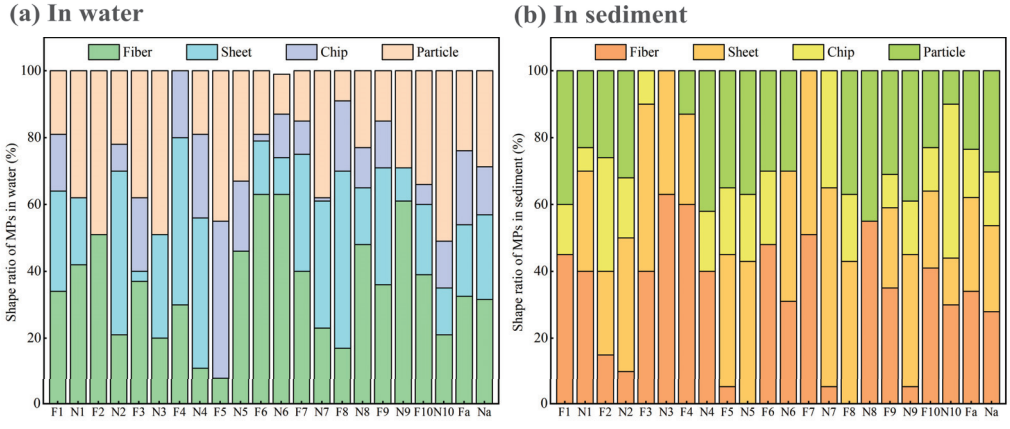

Figure 3. Shape characteristics of microplastics. "F1, F2 . . . F10" represent the proportion of MPs from the relevant sampling points (S1, S2 . . . S10) during the flood season; "N1, N2 . . . N10" represent the proportion of MPs from the relevant sampling points (S1, S2 . . . S10) during the non-flood season; "Fa" represents the average proportion of MPs across all sampling points during the flood season and "Na" represents the average proportion of MPs across all sampling points during the non-flood season. (**a**) in Water; (**b**) in sediment.

The proportion of fibrous MPs was the highest (27.90–34%) and the proportion of membrane MPs was the lowest (14.40–22.20%). The proportions of the MP shapes were relatively uniformly distributed, which is consistent with a study of the Wuhe River Basin in Poyang Lake and a study of the headwaters of Yangtze River [39]. Fibrous MPs can be attributed, to some extent, to sewage discharge from laundries [40]. Studies indicated that an average of more than 1900 fibers were produced by the cleaning of one cloth and more than 700,000 fibers were produced by a machine washing of 6 kg of acrylic clothes [41]. Li et al. reported that the removal rate of fibrous MPs by sewage treatment plants can reach 93.9%, but some fibers may still enter rivers after processing [42]. Fibrous plastics, which are also present in high proportions in air, can be precipitated directly with rainwater or enter into rivers via surface runoff, especially during flood seasons, dramatically increasing the concentration of fibrous MPs in a short period of time [43]. It is essential to note that the proportion of membrane MPs produced by agricultural planting or plastic packaging is not high. No anomalies in membrane MPs were detected at S8, which is located within an agricultural production area. This might indicate that recent measures to strengthen control over local agricultural plastic pollutants were relatively effective.

3.1.3. Size Characteristics of MPs

The size of the MPs, as scanned by LDIR, was divided into four categories: 0–500 μm, 500–1000 μm, 1000–2500 μm, and 2500–5000 μm. The size analysis of MPs is presented in Figure 4.

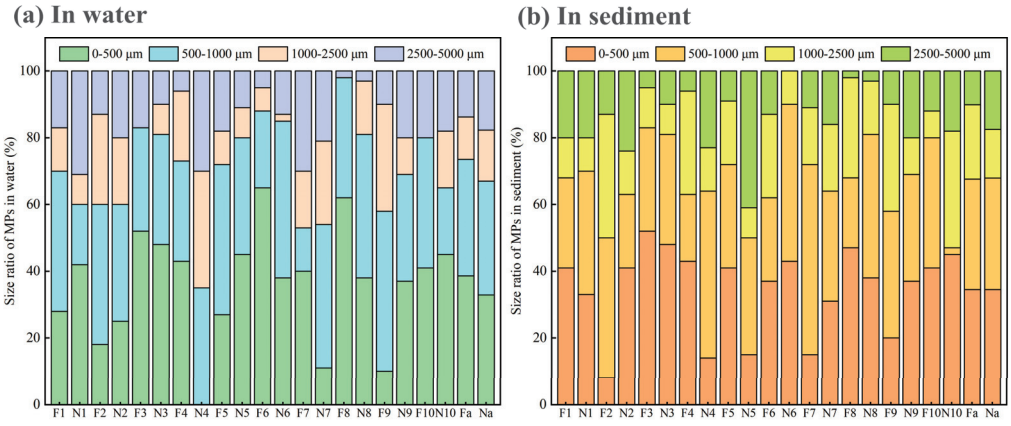

Figure 4. Size of microplastics determined by LDIR. "F1, F2 . . . F10" represent the proportion of MPs from the relevant sampling points (S1, S2 . . . S10) during the flood season; "N1, N2 . . . N10" represent the proportion of MPs from the relevant sampling points (S1, S2 . . . S10) during the non-flood season; "Fa" represents the average proportion of MPs across all sampling points during the flood season and "Na" represents the average proportion of MPs across all sampling points during the non-flood season. (**a**) in Water; (**b**) in sediment.

In water, the abundance of MPs sized 0–500 μm was the highest (38.60%) and the abundance of MPs sized 1000–2500 μm was the lowest (12.70%) during the flood season. During the non-flood season, the abundance of 500–1000 μm MPs was the highest (34.10%) and the abundance of 1000–2000 μm MPs was the lowest (15.30%). In sediments, the abundance of 0–500 μm MPs was the highest and the abundance of 2500–5000 μm MPs was the lowest (10.10%) during the flood season. During the non-flood season, the abundance of 0–500 μm MPs was the highest and the abundance of 2500–5000 μm MPs was the lowest (17.50%). There were equivalent proportions of MPs sized 0–500 μm and 500–1000 μm during the flood season and non-flood season. The statistical analysis revealed that the proportion of MPs sized 0–1000 μm was significantly higher (67–73.5%) when compared with those sized 1000–5000 μm, indicating that small-sized MPs were predominant in the Shiwuli River.

This is consistent with previous research results [44]. The volume of plastics decreases continuously in the natural environment due to secondary weathering, erosion, wearing, and degradation [45]. In the study by Murphy et al. [46], larger MPs were found to more easily coagulate and precipitate during sewage processing, resulting in a higher proportion of small-sized MPs being discharged into the river. Thus, the proportion of small-sized MPs that are finally discharged into rivers is relatively high. Further, MPs < 1 mm in size are easily making them attractive to aquatic organisms for ingestion and entering into the food chain, posing greater pollution threats [9,47]. There were more large MPs in the range of 1000–2000 μm and 2500–5000 μm during the non-flood season than during the flood season. It was reported that the size reduction of plastics in water bodies due to the hydraulic effect is negatively related to the stability of the water environment [48].

3.1.4. Colour Characteristics

The colours of MPs observed by microscope can generally be divided into seven types based on the colours white, red, green, blue, black, yellow, and hyaline in Figure 5.

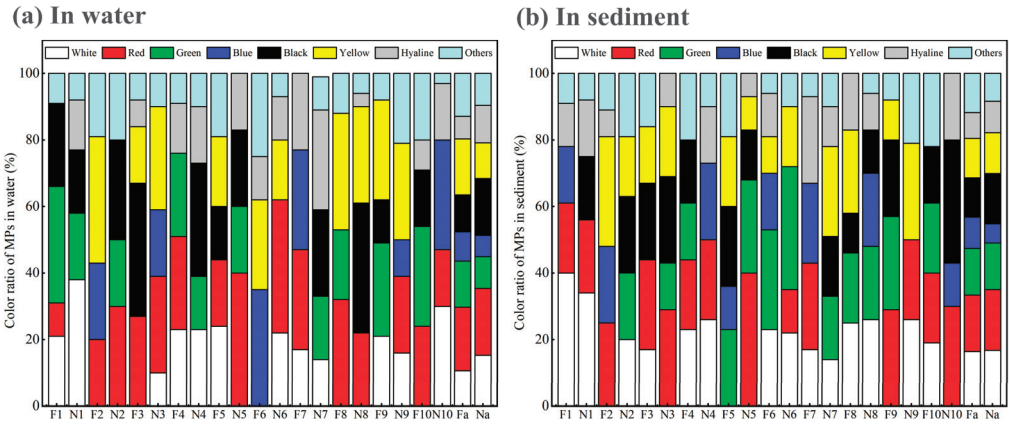

Figure 5. Colour characteristics of microplastics. "F1, F2 . . . F10" represent the proportion of MPs from the relevant sampling points (S1, S2 . . . S10) during the flood season; "N1, N2 . . . N10" represent the proportion of MPs from the relevant sampling points (S1, S2 . . . S10) during the non-flood season; "Fa" represents the average proportion of MPs across all sampling points during the flood season and "Na" represents the average proportion of MPs across all sampling points during the non-flood season. (**a**) in Water; (**b**) in sediment.

In water, the proportion of red MPs was the highest (19.10%) and the hyaline MPs was the lowest (6.80%) during the flood season. The proportion of red MPs also was the highest (20.10%) and the proportion of blue MPs was the lowest (6.40%) during the non-flood season. In sediments, the proportion of red MPs was the highest (17.00%) and the proportion of hyaline MPs was the lowest during the flood season. The proportion of red MPs was the highest (18.20%) and the proportion of blue MPs was the lowest (5.80%) during the non-flood season. The colours of the MPs indicated that the MPs were derived from extensive sources. The proportions of MPs of each colour were relatively uniform; however, the proportion of red MPs was the highest. The proportion of black MPs during the non-flood season was significantly higher than during the flood season. This might be because the MPs can absorb more pollutants and for longer in static water during the non-flood season, resulting in physical and chemical changes, which lead to discolouration [48,49]. The morphological observations revealed that the red MPs were mainly fibrous, the white and blue MPs were mainly particles, the green MPs were mainly fragments, and the yellow and hyaline MPs were mainly membranes.

Generally speaking, the colour distribution of MPs in the Shiwuli River Basin was relatively stable at the temporal scale, but there were differences in the spatial distributions. This situation is similar to the MP distribution in the Xiangxi River Basin [50]. The production and the consumption processes often involve colour modulation so as to improve attraction. Colourful MPs may fade over time and with external stress. Hence, the proportion of hyaline MPs in the sediment samples was significantly higher than in the water samples and the proportion of hyaline MPs during the non-flood season was much higher than during the flood season. In the fading process, common heavy metals in pigments might be released into the water, resulting in heavier pollution [51]. Moreover, it was demonstrated that small-sized colourful MPs are more easily attracted by aquatic animals and enter the human biological chain after being eaten, thus posing health risks [49,52].

3.1.5. Composition Characteristics

All particles in the samples from the Shiwuli River were scanned by LDIR, and after automatic comparison with the absorption peak profile of each MP polymer in the spectral library, a total of eleven MP polymers, such as acrylates (ACR), chlorinated polyethylene (CPE), ethylene vinyl acetate (EVA), polyethylene (PE), polyethylene terephthalate

(PET), polypropylene (PP), polystyrene (PS), polyurethane (PU), polyvinylchloride (PVC), polyamide (PA), and silicon, with matching degrees greater than 0.80, were identified. Six common types of polymer, PE, PP, PET, PA, PS, and PVC, as well as others, were selected for the proportional analysis. From Figure 6a, it can be seen that polyethylene (PE, 21.60%) dominates in water during the flood season, followed by polystyrene (PS, 15.60%). During the non-flood season, the proportion of PE (19.30%) was the highest, followed by the proportions of polypropylene (PP, 16.80%) and polyvinyl chloride (PVC, 15.50%). In sediments (Figure 6b), the proportion of PP (22.70%) was the highest during the flood season, followed by the proportions of PE (16.50%) and PVC (15.40%). The proportion of PE (23.20%) was the highest during the non-flood season, followed by PP (21.80%) and polyethylene terephthalate (PET, 18.10%). Generally speaking, the proportions of PE, PP, and PVC were relatively high, with the proportion of PE being the highest. This is consistent with other studies [34,53]. Examples of the qualitative results for the different types of polymer particles are shown in Figure 7.

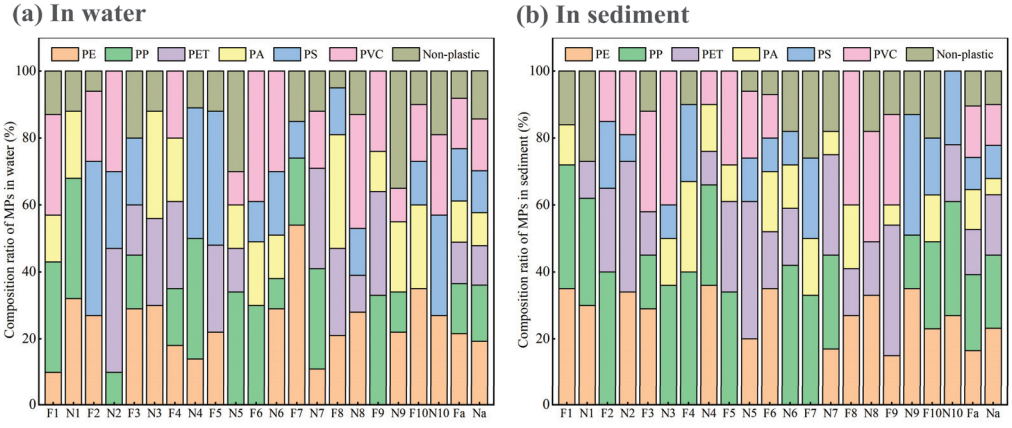

Figure 6. Composition characteristics of microplastics. "F1, F2 . . . F10" represent the proportion of MPs from the relevant sampling points (S1, S2 . . . S10) during the flood season; "N1, N2 . . . N10" represent the proportion of MPs from the relevant sampling points (S1, S2 . . . S10) during the non-flood season; "Fa" represents the average proportion of MPs across all sampling points during the flood season and "Na" represents the average proportion of MPs across all sampling points during the non-flood season. (**a**) in Water; (**b**) in sediment.

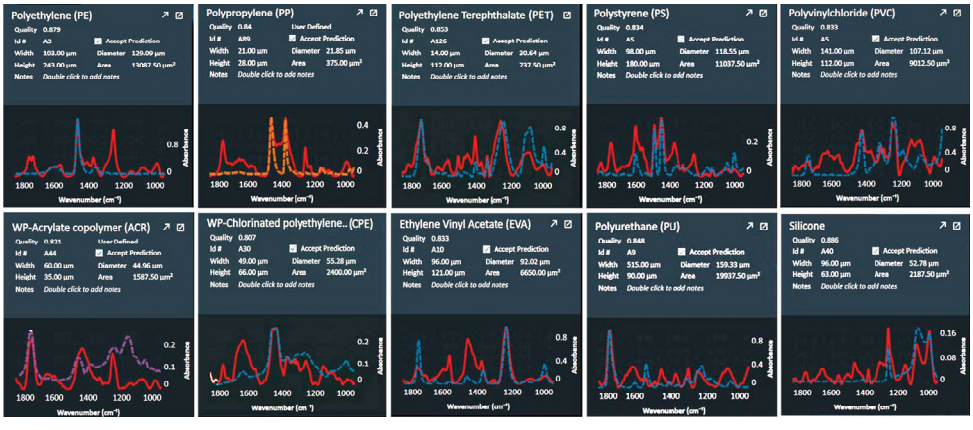

Figure 7. Curves of different particle types identified by LDIR (matching degree > 0.80).

Figure 8 shows the microstructure of common polymers scanned by SEM. The PEs were fragmented in shape (Figure 8a) with porous and rough surfaces. The PPs exhibited a membrane appearance (Figure 8b) with obvious fracture traces. The PETs were fragmented (Figure 8c) with rough cracks on the surface. The PAs were fibrous shapes (Figure 8d) with microfiber expenses on the surface. The PSs were particles (Figure 8e). The PVCs were fragments (Figure 8f) with obvious wearing. The detection rate in sediment samples was relatively high for PVC with a density of 1.38 g/cm^3;. The proportions of PP and PE, with low densities, were higher in the sediment samples, thus verifying their ability to adsorb and accumulate other pollutants [54]. This will lead to increases in the densities of PP and PE, resulting in their precipitation [55].

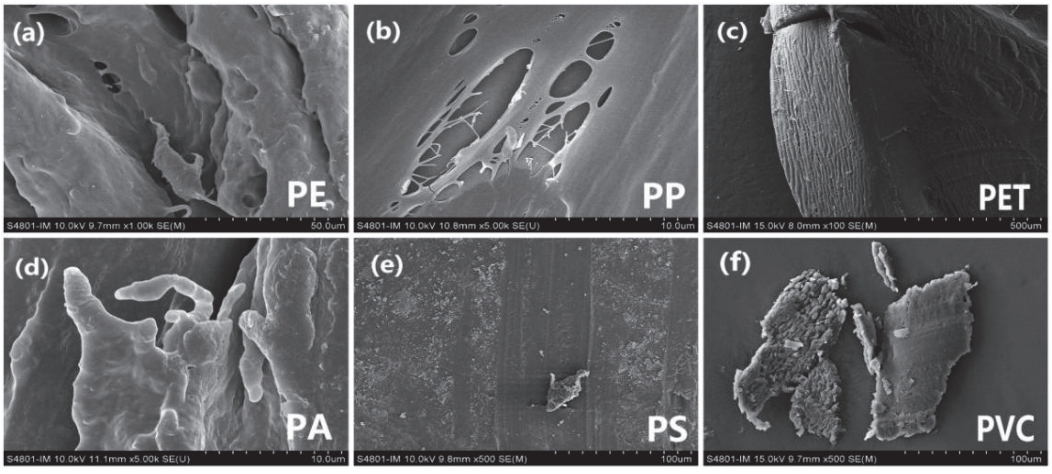

Figure 8. SEM images of common polymers. (**a**) PE; (**b**) PP; (**c**) PET; (**d**) PA; (**e**) PS; (**f**) PVC.

3.2. Pollution Risk Assessment of Microplastics

In the Shiwuli River, the risk index *H* and the pollution load index PLI_{zone} were calculated. The results are shown in Table 3.

Table 3. Risk assessment of microplastics in the Shiwuli River.

	PE		**PP**		**PET**		**PA**		**PS**		**PVC**	
S_n [31] (hazard score, highest level)	11		1		30		50		871		30	
Seasons (f.: flood; n.-f.: non-flood)	f.	n.-f.	f.	n.-f.	f.	n.-f.	f.	n.-f.	f.	n.-f.	f.	n.-f.
P_n (%)												
Water	21.6	19.3	14.9	16.8	12.4	11.7	12.3	9.9	15.6	12.5	15.1	15.5
Sediments	16.5	23.2	22.7	21.8	13.5	18.1	11.9	4.8	9.6	9.9	15.4	12.2
H value												
Water	2.38	2.12	0.15	0.17	3.72	3.51	6.15	4.95	135.88	108.88	4.53	4.65
Sediments	1.82	2.55	0.23	0.22	4.05	5.43	5.95	2.40	83.62	86.23	4.62	3.66
Risk level												
Water	I	I	I	I	I	I	I	I	III	III	I	I
Sediments	I	I	I	I	I	I	I	I	II	II	I	I
PLI_{zone}												
Water	PLI_{zone} (f.) value is 2.24 (heavy pollution); PLI_{zone} (n.-f.) value is 1.66 (moderate pollution)											
Sediments	PLI_{zone} (f.) value is 2.34 (heavy pollution); PLI_{zone} (n.-f.) value is 1.91 (heavy pollution) [32]											

The risks of PE, PP, PET, PA, and PVC in the water were classified as Level-I and the risk of PS was classified as Level-III. During the flood season, the PLI_{zone} of MPs was

2.24 in water and 2.34 in sediments, both reflecting heavy pollution. During the non-flood season, the PLI_{zone} of MPs was 1.66 in water and 1.91 in sediments, reflecting moderate pollution and heavy pollution, respectively. The risk index of PS indicated a higher risk degree in water than in sediments, whereas the PLI_{zone} indicated that the pollution load in the sediments was higher. This is mainly attributed to differences in the reference indices between the two assessment models. The RI model uses the chemical toxicity of various polymer types as the major index, without consideration of the influences of the abundances of MPs. The PLI model uses the abundances of MPs as the major reference [31,32].

4. Conclusions

The spatial and temporal distribution characteristics of MPs in water and sediment samples from 10 representative points along the Shiwuli River in Hefei during both flood and non-flood seasons were studied. Additionally, an assessment of the ecological risks associated with MP pollution was conducted.

There was a difference in particle abundance among ten points. Results show that the water of the Shiwuli River had an average abundance of MPs of 8.4 ± 2.5 particles/L during the flood season and 5.8 ± 1.7 particles/L during the non-flood season; the sediment had an average abundance of MPs of 78.9 ± 8.3 particles/kg during the flood season and 63.9 ± 7.1 particles/kg during the non-flood season. The more abundance of MPs exhibited at confluences with tributaries (S4, S5, and S6) were close to the residential and industrial development, while lower values were identified in agricultural areas (S8) and wetland ecological regions (S9 and S10). In water, the maximum appeared at S5 with 21.7 ± 4.6 particles/L during the flood season and 15.9 ± 4.2 particles/L during the non-flood season, respectively; the minimum appeared at S9 with 1.8 ± 1.0 particles/L during the flood season and 2.2 ± 0.4 particles/L during the non-flood season, respectively. In sediment, the maximum appeared at S5 with 174.1 ± 10.1 particles/kg during the flood season and 143.6 ± 10.4 particles/kg during the non-flood season, respectively; the minimum appeared at S8 with 10.3 ± 2.8 particles/kg during the flood season and at S9 with 12.1 ± 3.2 particles/kg during the non-flood season, respectively. Analysis of MP characteristics showed that the MPs mainly exhibited a fibroid morphology (27.90–34%), likely originating from laundry activities and packaging breakage, and red-coloured particles (19.10%) within the smaller size less than 500 μm (38.60%) were more prevalent, making them attractive to aquatic organisms for ingestion. Additionally, results of MP identification show that a total of 11 types of polymers were found in the river water and sediment by using LDIR. According to matching analysis, these particles were identified as ACR, CPE, EVA, PE, PET, PP, PS, PU, PVC, PA, and silicone. Among them, PE emerged as the most prevalent polymer type (19.3−21.6%) due to its widespread use in daily life and relatively low cost.

The ecological risk assessment of different types of MP polymers revealed that during both flood and non-flood seasons, the risk levels of PS were classified as III in water and II in sediments in the Shiwuli River. However, for all other polymer types, the risk levels were categorized as I. The overall assessment of the MP pollution load of the Shiwuli River showed that during the flood season, the PLI_{zone} value for water was 2.24, indicating heavy pollution, while during the non-flood season, it was 1.66, indicating moderate pollution. In the case of sediments, the PLI_{zone} value was 2.34 during the flood season and 1.91 during the non-flood season, both indicating heavy pollution. These findings suggest that the MP pollution in the Shiwuli River poses a significant risk to the drinking water source of Chaohu Lake. This study aligns closely with other similar studies, as shown in Table 4.

Table 4. Comparison of the results of this study with other studies.

Research Object	Country	Abundance	Assessment Models	Results of Assessment	References
Coast of India	India	12.22–439 items/kg in sediment	Pollution load index (PLI)	*PLI* of west coast of India: 3.03–15.5 (heavy pollution) *PLI* of east coast of India: 1–6.14 (moderate to heavy pollution)	[21]
Chagan lake and Xianghai lake	China	Chagan Lake: 3.61 ± 2.23 particles/L; Xianghai lake: 0.29 ± 0.11 particles/L	Risk index (RI)	Levels-III (heavy pollution) in Chagan Lake and Xianghai Lake	[22]
Manas River Basin	China	17 ± 4 items/L (April) 14 ± 2 items/L (July)	Risk index (RI) Pollution load index (PLI)	Most of the study areas: Level-III (heavy pollution) All the sampling sites: slightly polluted	[56]
Moheshkhali channel of Bangladesh	Bangladesh	Sediment: 138.33 items/m^2 Water: ~0.1 items/m^3	Pollution load index (PLI)	$PLI_{sediments}$: 2.51 (heavy pollution) $PLI_{surface\ water}$: 1.67 (moderate pollution)	[57]
Shiwuli River (this study)	China	Water: Flood season (f.): 8.4 ± 2.5 particles/L Non-flood season (n.-f.): 5.8 ± 1.7 particles/L; Sediment: Flood season (f.): 78.9 ± 8.3 particles/kg Non-flood season (n.-f.): 63.9 ± 7.1 particles/kg.	Risk index (RI) Pollution load index (PLI)	PS: Level-III in water and Level-II in sediments; Other polymers: Level-I Water: PLI_{zone} (f.): 2.24 (heavy pollution); PLI_{zone} (n.-f.): 1.66 (moderate pollution) Sediments: PLI_{zone} (f.): 2.34 (heavy pollution); PLI_{zone} (n.-fl.): 1.91 (heavy pollution)	-

The findings of this study indicate that further research into the degradation of MPs in several urban inland rivers that discharge into lakes that are the sources of drinking water is recommended. Emphasis should be placed on enhancing non-point source pollution control measures in urban river basins, including the implementation of infrastructure improvements, such as rainwater and sewage separation systems. These measures can effectively impede land-based MP entry into these rivers. Additionally, research should be conducted on effective engineering techniques for removing MPs, particularly those of smaller particle sizes, during sewage treatment processes. Urban inland rivers should be equipped with comprehensive water management systems to ensure ecological basic flow, and the natural degradation of MPs should be promoted through ecological methods, as is the case with constructed wetlands. From a regulatory standpoint, it is necessary to establish MPs risk assessment standards and conduct regular assessments of MP pollution in key rivers and lakes.

Author Contributions: Conceptualization, L.H. and T.B.; data curation, X.M. and Q.W.; investigation, X.M.; methodology, L.H. and B.L.; project administration, K.W.; resources, X.M. and Q.W.; supervision, K.W.; validation, L.H. and J.J.; writing—original draft, L.H.; writing—review and editing, T.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by "Anhui innovation team for municipal solid waste treatment", "Anhui Key Laboratory of Sewage Purification and Eco-restoration Materials (YJ20230301-2)" and "National Special Item on Water Resource and Environment (2017ZX07603-003)".

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Thompson, R.; Olsen, Y.; Mitchell, R.; Davis, A.; Russell, A. Supporting online material, lost at sea, where is all the plastic, material and methods. *Science* **2004**, *304*, 838. [CrossRef]
2. Zhao, Q.; Zhao, X.; Cao, J. 7-Advanced Nanomaterials for Degrading Persistent Organic Pollutants. In *Advanced Nanomaterials for Pollutant Sensing and Environmental Catalysis*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 249–305.
3. Alimi, O.S.; Farner Budarz, J.; Hernandez, L.M.; Tufenkji, N. Microplastics and nanoplastics in aquatic environments, aggregation, deposition, and enhanced contaminant transport. *Environ. Sci. Technol.* **2018**, *52*, 1704–1724. [CrossRef]
4. Hwang, J.; Choi, D.; Han, S.; Jung, S.; Choi, J.; Hong, J. Potential toxicity of polystyrene microplastic particles. *Sci Rep.* **2020**, *10*, 7391. [CrossRef] [PubMed]
5. Wang, J.; Liu, X.; Li, Y.; Powell, T.; Wang, X.; Wang, G.; Zhang, P. Microplastics as contaminants in the soil environment, a mini-review. *Sci. Total Environ.* **2019**, *691*, 848–857. [CrossRef]
6. Ahmed, R.; Hamid, A.K.; Krebsbach, S.A.; He, J.; Wang, D. Critical review of microplastics removal from the environment. *Chemosphere* **2022**, *293*, 133557. [CrossRef]
7. Li, J.; Zhang, K.N.; Zhang, H. Adsorption of antibiotics on microplastics. *Environ. Pollut.* **2018**, *237*, 460–467. [CrossRef]
8. Endo, S.; Takizawa, R.; Okuda, K.; Kanehiro, H.; Ogi, H.; Yamashita, R.; Date, T. Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets, variability among individual particles and regional differences. *Mar. Pollut. Bull.* **2005**, *50*, 1103–1114. [CrossRef] [PubMed]
9. Zhou, Q.; Tu, C.; Fu, C.; Li, Y.; Luo, Y. Characteristics and distribution of microplastics in the coastal mangrove sediments of China. *Sci. Total Environ.* **2019**, *703*, 134807. [CrossRef] [PubMed]
10. Li, J.; Liu, H.; Chen, J.P. Microplastics in freshwater systems, a review on occurrence, environmental effects, and methods for microplastics detection. *Water Res.* **2018**, *137*, 362–374. [CrossRef]
11. Lin, Z.; Jin, T.; Zou, T.; Xu, L.; Xi, B.; Xu, D.; He, J.; Xiong, L.; Tang, C.; Peng, J.; et al. Current progress on plastic/microplastic degradation, Fact influences and mechanism. *Environ. Pollut.* **2022**, *304*, 119159. [CrossRef]
12. Zhang, W.; Zhang, S.; Wang, J.; Wang, Y.; Mu, J.; Wang, P.; Lin, X.; Ma, D. Microplastic pollution in the surface waters of the Bohai Sea, China. *Environ. Pollut.* **2017**, *231*, 541–548. [CrossRef] [PubMed]
13. Li, Y.; Wolanski, E.; Dai, Z.; Lambrechts, J.; Tang, C.; Zhang, H. Trapping of plastics in semi-enclosed seas, insights from the Bohai Sea, China. *Mar Pollut. Bull.* **2018**, *137*, 509–517. [CrossRef]
14. Pan, Z.; Guo, H.; Chen, H.; Wang, S.; Sun, X.; Zou, Q.; Zhang, Y.; Lin, H.; Cai, S.; Huang, J. Microplastics in the northwestern Pacific, abundance, distribution, and characteristics. *Sci. Total Environ.* **2018**, *650*, 1913–1922. [CrossRef] [PubMed]
15. Miloloža, M.; Bule, K.; Prevarić, V.; Cvetnić, M.; Ukić, Š.; Bolanča, T.; Kučić Grgić, D. Assessment of the influence of size and concentration on the ecotoxicity of microplastics to microalgae scenedesmus sp., Bacterium Pseudomonas putida and Yeast Saccharomyces cerevisiae. *Polymers* **2022**, *14*, 1246. [CrossRef] [PubMed]
16. Khan, S.; Naushad, M.; Lima, E.C.; Zhang, S.; Shaheen, S.M.; Rinklebe, J. Global soil pollution by toxic elements: Current status and future perspectives on the risk assessment and remediation strategies—A review. *J. Hazard. Mater.* **2021**, *417*, 126039. [CrossRef]
17. Schell, T.; Hurley, R.; Nizzetto, L.; Rico, A.; Vighi, M. Spatio-temporal distribution of microplastics in a Mediterranean river catchment, the importance of wastewater as an environmental pathway. *J. Hazard. Mat.* **2021**, *420*, 126481. [CrossRef] [PubMed]
18. Li, J.; Ouyang, Z.; Liu, P.; Zhao, X.; Wu, R.; Zhang, C.; Lin, C.; Li, Y.; Guo, X. Distribution and characteristics of microplastics in the basin of Chishui River in Renhuai, China. *Sci. Total Environ.* **2021**, *806*, 145591. [CrossRef]
19. Soltani, N.; Keshavarzi, B.; Moore, F.; Busquets, R.; Nematollahi, M.J.; Javid, R.; Gobert, S. Effect of land use on microplastic pollution in a major boundary waterway, the Arvand River. *Sci. Total Environ.* **2022**, *830*, 154728. [CrossRef]
20. Drabinski, T.L.; de Carvalho, D.G.; Gaylarde, C.C.; Lourenço, M.F.P.; Machado, W.T.V.; da Fonseca, E.M.; da Silva, A.L.C.; Baptista Neto, J.A. Microplastics in freshwater fiver in Rio de Janeiro and its role as a source of microplastic pollution in Guanabara Bay, SE Brazil. *Micro* **2023**, *3*, 208–223. [CrossRef]
21. Pradit, S.; Noppradit, P.; Sengloyluan, K.; Suwanno, P.; Tanrattanakul, V.; Sornplang, K.; Nuthammachot, N.; Jitkaew, P.; Nitiratsuwan, T. Occurrence of microplastics in river water in southern Thailand. *Mar. Sci. Eng.* **2023**, *11*, 90. [CrossRef]
22. Lin, L.; Zuo, L.; Peng, J.; Cai, L.; Fok, L.; Yan, Y.; Li, H.; Xu, X. Occurrence and distribution of microplastics in an urban river: A case study in the Pearl River along Guangzhou city, China. *Sci. Total Environ.* **2018**, *644*, 375–381. [CrossRef] [PubMed]
23. Kim, D.; Kim, H.; An, Y.J. Species sensitivity distributions of micro- and nanoplastics in soil based on particle characteristics. *J. Hazard. Mater.* **2023**, *452*, 131229. [CrossRef] [PubMed]
24. Pan, Z.; Liu, Q.; Jiang, R.; Li, W.; Sun, X.; Lin, H.; Jiang, S.; Huang, H. Microplastic pollution and ecological risk assessment in an estuarine environment: The Dongshan Bay of China. *Chemosphere* **2021**, *262*, 127876. [CrossRef]
25. Ranjani, M.; Veerasingam, S.; Venkatachalapathy, R.; Mugilarasan, M.; Andrei, B.; Mukhanov, V.; Vethamony, P. Assessment of potential ecological risk of microplastics in the coastal sediments of India: A meta-analysis. *Mar. Pollut. Bull.* **2021**, *163*, 111969. [CrossRef]

26. Yin, K.; Wang, D.; Zhao, H.; Wang, Y.; Guo, M.; Liu, Y.; Li, B.; Xing, M. Microplastics pollution and risk assessment in water bodies of two nature reserves in Jilin province: Correlation analysis with the degree of human activity. *Sci. Total Environ.* **2021**, *799*, 149390. [CrossRef]
27. Tan, Y.; Dai, J.; Wu, X.; Wu, S.; Zhang, J. Characteristics, occurrence and fate of non-point source microplastic pollution in aquatic environments. *J. Clean Prod.* **2022**, *341*, 130766. [CrossRef]
28. Meng, X.; Bao, T.; Hong, L.; Wu, K. Occurrence Characterization and Contamination Risk Evaluation of Microplastics in Hefei's Urban Wastewater Treatment Plant. *Water* **2023**, *15*, 686. [CrossRef]
29. Hildebrandt, L.; Gareb, F.; Zimmermann, T.; Klein, O.; Kerstan, A.; Emeis, K.C.; Pröfrock, D. Spatial distribution of microplastics in the tropical Indian Ocean based on laser direct infrared imaging and microwave-assisted matrix digestion. *Environ. Pollut.* **2022**, *307*, 119547. [CrossRef]
30. Pivokonsky, M.; Cermakova, L.; Novotna, K.; Peer, P.; Cajthaml, T.; Janda, V. Occurrence of microplastics in raw and treated drinking water. *Sci. Total Environ.* **2018**, *643*, 1644–1651. [CrossRef]
31. Lithner, D.; Larsson, A.; Dave, G. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. *Sci. Total Environ.* **2011**, *409*, 3309–3324. [CrossRef]
32. Tomlinson, D.L.; Wilson, J.G.; Harris, C.R.; Jeffrey, D.W. Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. *Helgolnder Meeresunters.* **1980**, *33*, 566–575. [CrossRef]
33. Di, M.; Wang, J. Microplastics in surface waters and sediments of the Three Gorges Reservoir, China. *Sci. Total Environ.* **2018**, *616–617*, 1620–1627. [CrossRef]
34. Fan, J.; Zou, L.; Duan, T.; Qin, L.; Qi, Z.; Sun, J. Occurrence and distribution of microplastics in surface water and sediments in china's inland water systems, a critical review. *J. Clean Prod.* **2022**, *331*, 129968. [CrossRef]
35. Santana, M.F.M.; Ascer, L.G.; Custodio, M.R.; Moreira, F.T.; Turra, A. Microplastic contamination in natural mussel beds from a brazilian urbanized coastal region, rapid evaluation through bioassessment. *Mar. Pollut. Bull.* **2016**, *106*, 183–189. [CrossRef]
36. Bai, M.; Zhu, L.; An, L.; Peng, G.; Li, D. Estimation and prediction of plastic waste annual input into the sea from china. *Acta Oceanol. Sin.* **2018**, *37*, 26–39. [CrossRef]
37. Chen, Y.; Li, T.; Hu, H.; Ao, H.; Wu, C. Transport and fate of microplastics in constructed wetlands: A microcosm study. *J. Hazard. Mater.* **2021**, *415*, 125615. [CrossRef] [PubMed]
38. Helcoski, R.; Yonkos, L.T.; Sanchez, A.; Baldwin, A.H. Wetland soil microplastics are negatively related to vegetation cover and stem density. *Environ. Pollut.* **2020**, *256*, 113391. [CrossRef] [PubMed]
39. Zhao, S.; Zhu, L.; Wang, T.; Li, D. Suspended microplastics in the surface water of the yangtze estuary system, china, first observations on occurrence, distribution. *Mar. Pollut. Bull.* **2014**, *86*, 562–568. [CrossRef] [PubMed]
40. Liu, X.; Yuan, W.; Di, M.; Li, Z.; Wang, J. Transfer and fate of microplastics during the conventional activated sludge process in one wastewater treatment plant of China. *Chem. Eng. J.* **2019**, *362*, 176–182. [CrossRef]
41. He, Z.; Yang, W.; Ren, Y.; Jin, H.; Wang, A. Occurrence, effect, and fate of residual microplastics in anaerobic digestion of waste activated sludge: A state-of-the-art review. *Bioresour. Technol.* **2021**, *331*, 125035. [CrossRef]
42. Li, X.; Chen, L.; Mei, Q.; Dong, B.; Dai, X.; Ding, G.; Zeng, E.Y. Microplastics in sewage sludge from the wastewater treatment plants in China. *Water Res.* **2018**, *142*, 75–85. [CrossRef]
43. Gasperi, J.; Gromaire, M.C.; Kafi, M.; Moilleron, R.; Chebbo, G. Contributions of wastewater, runoff and sewer depositerosion to wet weather pollutant loads in combined sewersystems. *Water Res.* **2010**, *44*, 5875–5886. [CrossRef]
44. Claessens, M.; Clerck, K.D.; Landuyt, L.V.; Meester, S.D.; Janssen, C.R. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Mar. Pollut. Bull.* **2011**, *62*, 2199–2204. [CrossRef] [PubMed]
45. Siegel, H.; Fischer, F.; Lenz, R.; Fischer, D.; Labrenz, M. Identification and quantification of microplastic particles in drinking water treatment sludge as an integrative approach to determine microplastic abundance in a freshwater river. *Environ. Pollut.* **2021**, *286*, 117524. [CrossRef] [PubMed]
46. Murphy, F.; Ewins, C.; Carbonnier, F.; Quinn, B. Wastewatertreatment works (WwTW) as a source of microplastics in theaquatic environment. *Environ. Sci. Technol.* **2016**, *50*, 5800–5808. [CrossRef] [PubMed]
47. Senathirajah, K.; Attwood, S.; Bhagwat, G.; Carbery, M.; Wilson, S.; Palanisami, T. Estimation of the mass of microplastics ingested—A pivotal first step towards human health risk assessment. *J. Hazard. Mater.* **2021**, *404 Pt B*, 124004. [CrossRef]
48. Filgueiras, A.V.; Gago, J.; Campillo, J.A.; León, V.M. Microplastic distribution in surface sediments along the spanish mediterranean continental shelf. *Environ. Sci. Pollut. Res.* **2019**, *26*, 21264–21273. [CrossRef]
49. Vibhatabandhu, P.; Srithongouthai, S. Influence of seasonal variations on the distribution characteristics of microplastics in the surface water of the Inner Gulf of Thailand. *Mar. Pollut. Bull.* **2022**, *180*, 113747. [CrossRef]
50. Liu, R.; Miao, Y.; Wang, Q.; Jiao, L.; Wang, Y.; Li, L.; Cao, L. Effectively and efficiency of best management practices based on a survey and SWAPP model of the Xiangxi River Basin. *Water.* **2021**, *13*, 985. [CrossRef]
51. Abdallah, M.A.M.; Badr-ElDin, A.M. Ecological risk assessment of surficial sediment by heavy metals from a submerged archaeology harbor, south Mediterranean Sea, Egypt. *Acta Geochim.* **2020**, *39*, 226–235. [CrossRef]
52. Bertoldi, C.; Lara, L.Z.; Mizushima, F.; Martins, F.; Fernandes, A.N. First evidence of microplastic contamination in the freshwater of Lake Guaíba, Porto Alegre, Brazil. *Sci. Total Environ.* **2020**, *759*, 143503. [CrossRef] [PubMed]
53. Liu, D.; Zheng, Y.; Chen, L.; Wen, D. Prevalence of small-sized microplastics in coastal sediments detected by multipoint confocal micro-raman spectrum scanning. *Sci. Total Environ.* **2022**, *831*, 154741. [CrossRef]

54. Bolívar-Subirats, G.; Rivetti, C.; Cortina-Puig, M.; Barata, C.; Lacorte, S. Occurrence, toxicity and risk assessment of plastic additives in Besos River, spain. *Chemosphere* **2020**, *263*, 128022. [CrossRef] [PubMed]
55. Joo, S.; Liang, Y.; Kim, M.; Byun, J.; Choi, H. Microplastics with adsorbed contaminants: Mechanisms and treatment. *Environ. Chall.* **2021**, *3*, 100042. [CrossRef]
56. Wang, G.; Lu, J.; Li, W.; Ning, J.; Zhou, L.; Tong, Y.; Liu, Z.; Zhou, H.; Xiayihazi, N. Seasonal variation and risk assessment of microplastics in surface water of the Manas River Basin, China. *Ecotox. Environ. Safe* **2021**, *208*, 111477. [CrossRef] [PubMed]
57. Nahian, S.; Rakib, M.R.; Kumar, R.; Haider, S.; Sharma, P.; Idris, A.M. Distribution, characteristics, and risk assessments analysis of microplastics in shore sediments and surface water of Moheshkhali channel of bay of Bengal, Bangladesh. *Sci. Total Environ.* **2023**, *855*, 158892. [CrossRef] [PubMed]

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Assessing the Occurrence and Distribution of Microplastics in Surface Freshwater and Wastewaters of Latvia and Lithuania

Reza Pashaei [1,*], Viktorija Sabaliauskaitė [1], Sergej Suzdalev [1], Arūnas Balčiūnas [1], Ieva Putna-Nimane [2], Robert M. Rees [3] and Reda Dzingelevičienė [4]

[1] Marine Research Institute, Klaipeda University, 92294 Klaipeda, Lithuania
[2] Latvian Institute of Aquatic Ecology, 1007 Riga, Latvia
[3] Scotland's Rural College (SRUC), West Mains Road, Edinburgh EH9 3JG, UK
[4] Faculty of Health Sciences, Marine Research Institute, Klaipeda University, 92294 Klaipeda, Lithuania
* Correspondence: reza.pashaei@ku.lt

Abstract: Microplastic concentrations in surface water and wastewater collected from Daugavpils and Liepaja cities in Latvia, as well as Klaipeda and Siauliai cities in Lithuania, were measured in July and December 2021. Using optical microscopy, polymer composition was characterized using micro-Raman spectroscopy. The average abundance of microplastics in surface water and wastewater samples was 16.63 ± 20.29 particles/L. The dominant shape group of microplastics in water was fiber, with dominant colors found to be blue (61%), black (36%), and red (3%) in Latvia. Similar distribution in Lithuania was found, i.e., fiber (95%) and fragments (5%) with dominant colors, such as blue (53%), black (30%), red (9%), yellow (5%), and transparent (3%). The micro-Raman spectroscopy spectra of visible microplastics were identified to be polyethylene terephthalate (33%) and polyvinyl chloride (33%), nylon (12%), polyester (PS) (11%), and high-density polyethylene (11%). In the study area, municipal and hospital wastewater from catchment areas were the main reasons for the contamination of microplastics in the surface water and wastewater of Latvia and Lithuania. It is possible to reduce pollution loads by implementing measures such as raising awareness, installing more high-tech wastewater treatment plants, and reducing plastic use.

Keywords: microplastics; occurrence; distribution; surface water; wastewaters

Citation: Pashaei, R.; Sabaliauskaitė, V.; Suzdalev, S.; Balčiūnas, A.; Putna-Nimane, I.; Rees, R.M.; Dzingelevičienė, R. Assessing the Occurrence and Distribution of Microplastics in Surface Freshwater and Wastewaters of Latvia and Lithuania. *Toxics* **2023**, *11*, 292. https://doi.org/10.3390/toxics11040292

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas, María Rocío Rodríguez Barroso and Dayong Wang

Received: 8 February 2023
Revised: 11 March 2023
Accepted: 22 March 2023
Published: 23 March 2023

1. Introduction

The emergence of plastic as a major component of modern life began in the 1950s and has grown exponentially ever since. Plastic production and consumption are estimated to have reached 368 million tons in 2019, representing an increase of over 200% from the pre-1950s era [1]. As a result, plastic has become an omnipresent component of our lives and is found in nearly every household and commercial space. Such widespread use and disposal of plastics have raised significant environmental concerns, such as plastic pollution, which have become global issues in recent years. Plastics are often preferred to other materials due to their durability, malleability, low cost, versatility, and impermeability. However, concerns are increasingly being raised about the persistence and accumulation of plastics in the environment [2,3]. An increase in environmental concentrations of plastics is expected due to increased demand and production and a lack of adequate waste processing capacity [4]. Microplastic (MPs) in the aquatic environment originate from intentional and unintentional losses of plastics and MPs, such as littering, loss of fishing gear, loss of granules used for manufacture, and release in wastewater effluents [5–7]. In order to be more effective at removing MPs from the environment, advanced techniques must be developed for wastewater treatment plants (WWTPs) [8]. Several studies have been conducted on microplastic (MP) pollution in the Baltic Sea and adjacent countries, showing high rates of MP pollution [9–19].

The Baltic Sea is one of the world's largest brackish water bodies and includes the Kattegat, which is home to 6065 species overall, including 1700 phytoplankton, 442 phytobenthos, 1199 zooplankton, 569 meiozoobenthos, 1476 macrozoobenthos, 380 vertebrate parasites, 200 fish, three seal species, and 83 bird species [20]. This richness in species diversity highlights the sensitivity of the environment to pollution and the need for more knowledge and understanding of the prevalence and behavior of MPs in the region.

2. Materials and Methods

2.1. Sample Collection and Sample Preparation

In Latvia and Lithuania, a comprehensive sampling campaign was conducted in July and December 2021 to evaluate the water quality in various locations within each city. In Daugavpils and Liepaja (Latvia) and Klaipeda and Siauliai (Lithuania), a total of 32 water samples were collected from four different sites in each city, including influent, effluent, upstream, and downstream locations (Lithuania). Before and after treatment, influent (n = 8) and effluent (n = 8) samples were collected at each sampling site. Following wastewater treatment, 16 samples of background surface water from upstream (n = 8) and downstream (n = 8) points were collected at a distance of 500 m from the discharge point. Notably, the upstream and downstream samples (SLV3/WLV3, SLV4/WLV4) of Liepaja were collected in the Baltic Sea, above the wastewater discharge point, where brackish water is present. Indeed, four samples were collected from each city in July and December. Figure 1 and Table S1 provide additional information on sampling locations and methods.

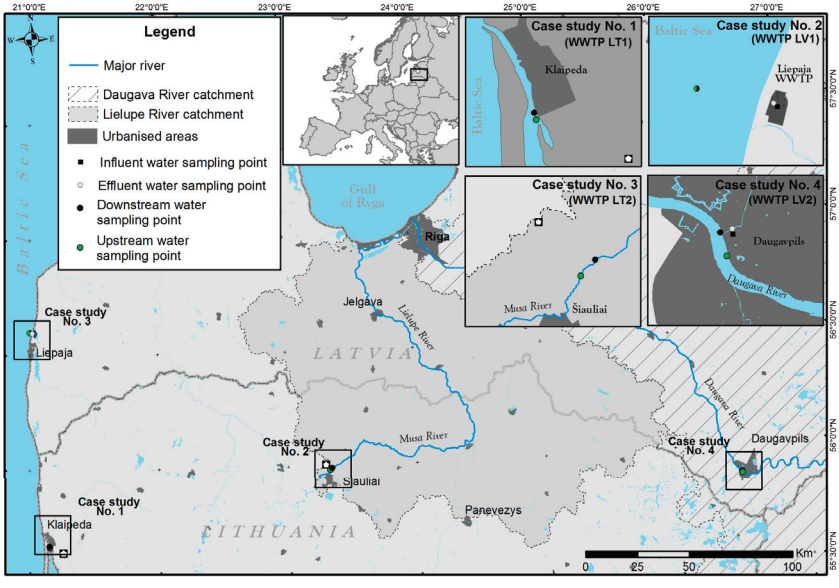

Figure 1. Sampling stations of Latvia and Lithuania.

2.2. Sample Preparation

In July and December 2021, 200 mL samples were collected from the influent, the effluent, the upstream, and the downstream. The pH, temperature, and dissolved oxygen of the samples were measured at the time of collection using the Multi 9630 IDS, WTW, ODO200, EcoSense, and ODO200, EcoSense instruments, respectively (Table S1). The water samples were stored at −20 °C in appropriate storage containers that were clean, dry, and properly labeled with the date and location of the sample. After that, the samples were removed from storage and allowed to come to room temperature. 20 mL of hydrogen peroxide solution 30% (Merck, Germany) was added to each sample bottle and shaken for

2 h at room temperature. The samples were filtered through a pre-weighed 0.45 μm, 47 mm white-gridded mixed cellulose-ester membrane filter (Frisenette, Denmark).

2.3. Laboratory Analysis

2.3.1. Optical Microscopy Analysis of the Microplastics

Analysis via optical microscopy is a common technique for examining MPs in water samples. However, it may not always be possible to differentiate plastic particles from other organic or inorganic materials based solely on their morphological characteristics. To confirm the identity of the particles, additional analytical methods, such as micro-Raman spectroscopy, are required. In our study, we analyzed the quantity, color, shape, and size of the suspected MPs using optical microscopy as the initial step in plastic screening for all samples. Using optical microscopy, small fragments of shells and other materials may be misidentified as MPs. We used micro-Raman spectroscopy to detect and confirm the presence of MP particles in the water samples in order to address this issue. We utilized a Nikon SMZ800N stereo microscope with a camera and an Olympus KL 1500 LCD microscope to directly examine filter samples obtained in situ. The particle sizes were classified into four categories: <0.25 mm, 0.25–0.5 mm, 0.5–1 mm, and 1–5 mm.

Optical microscopy is a reliable technique for detecting and analyzing MPs because it permits the visualization and characterization of the particles at relatively high magnification. Notably, optical microscopy has limitations when it comes to the detection and analysis of MPs. Optical microscopy is only able to detect MPs that are large enough to be resolved by the objective lens. This means that optical microscopy may not be able to detect MPs that are smaller than 50 μm in size. Optical microscopy can only detect transparent or semi-transparent MPs due to the technique's reliance on light transmission through the sample. MPs that are opaque, such as those that are black or extremely dark in color, may not be detectable with optical microscopy. In addition, certain types of MPs, such as those composed of particular plastics or coated with particular substances, may be difficult to visualize using optical microscopy due to the way in which they scatter light. Overall, while optical microscopy can be a useful tool for detecting and analyzing MPs, it is important to consider its limitations and to use other techniques, such as Raman spectroscopy, in conjunction with optical microscopy in order to obtain a complete understanding of the MPs in a sample.

2.3.2. Micro-Raman Spectroscopy Analysis of the Microplastics

Visual observation can detect millimeter-sized plastic particles, but identifying the specific type of plastic requires advanced spectroscopic techniques, such as Fourier transform infrared spectroscopy, micro-Raman spectroscopy, optical microscopy, and scanning electron microscopy in combination with energy dispersive X-ray spectroscopy [21,22]. These techniques enable a detailed analysis of the chemical composition and structural properties of MPs, allowing for a greater comprehension of their environmental impact. Micro-Raman is used to determine the type of polymer and to specify the particle size and distribution of MP particles [23]. Specifically, the Raman technique is gaining ground rapidly in the analysis of MPs due to its higher spatial resolution (1 μm), broader spectral coverage, greater sensitivity to non-polar functional groups, lower water interference, and narrower spectral bands [24]. In addition, micro-Raman is an indispensable tool for the analysis of very small MPs (<20 μm) [25]. The micro-Raman technique is a powerful analytical tool that can be used to identify and characterize materials. It is a non-destructive technique that can be used to analyze a wide range of materials, including polymers, metals, semiconductors, and biological samples. Micro-Raman spectroscopy is an effective and efficient technique for detecting and identifying MPs in water samples. It is a non-destructive, sensitive, fast, inexpensive, and safe technique that can be used in a variety of settings. The polymeric composition of a selection of MPs from different locations and of different sizes and shapes were determined using a micro-Raman spectrometer (LabRAM HR, Horiba, Japan) with a laser of 785 nm, a Raman shift of 400–1800 cm^{-1} and acquisition times

between 20 and 30 s. We used a microscope, needle, and tweezers to transfer MPs onto a conductive copper adhesive. To identify the polymers present, we utilized a tool called Open Specy (https://openanalysis.org/openspecy/) (accessed on 15 December 2022). This online, open-access database was developed by Cowger et al. [26] and enables users to compare spectra for material identification.

2.3.3. Quality Assurance and Quality Control

A plastic avoidance procedure was taken prior to sampling, as all sampling containers and tools were washed with water, previously filtered through a white-gridded mixed cellulose ester membrane filter with a diameter of 47 mm and pore size of 0.45-µm (Frisenette, Denmark) and sealed. A blank sample procedure was developed to estimate the amount of contamination caused by the experiment to prevent sampling and analysis errors. It was confirmed that the blank samples were free of MP pollution. In addition, we used 100% cotton clothing and glass laboratory materials, wrapped materials immediately after treatments, and rinsed and cleaned all instruments prior to conducting laboratory analysis in the laboratory. To ensure that no microparticles remained in the solutions, all chemical solutions were filtered with sterile filter paper. A hydrogen peroxide test was provided for sample preparation [27]. In addition, SPSS Statistics 23.0 software was used for data analysis. The Kruskal-Wallis test was applied to the data on MPs occurrence and distribution in surface freshwater and wastewaters of Latvia and Lithuania to determine if there were statistically significant differences between four groups: summer samples in Latvia (SLV), winter samples in Latvia (WLV), summer samples in Lithuania (SLT), and winter samples in Lithuania (WLT) (WLT). At a confidence level of 95%, the test statistic value of 0.466 indicated that there was no significant difference in the median levels of MPs between the groups.

3. Results

3.1. Optical Microscopy Results

A comprehensive analysis of the presence and abundance of MPs in various water samples was conducted. As detailed in Tables S2 and S3, 103 particles were detected and recorded in total (Tables S2 and S3). It was determined that the average MP particle concentration in the influent was 5.00 ± 5.35 particles/L; in the effluent, it was 28.33 ± 23.17 particles/L, upstream it was 24.29 ± 31.55 particles/L, and downstream it was 15.00 ± 10.00 particles/L. It has been determined that the average concentration of MP particles in the cities of Liepaja, Daugavpils, Klaipeda, and Siauliai varies seasonally. In the summer, the MP particle concentration in Liepaja is 12.5 ± 11.90 particles/L; in Daugavpils, it is 28.75 ± 22.87 particles/L; in Klaipeda, it is 13.75 ± 11.09 particles/L, and in Siauliai, it is 27.5 ± 19.36 particles/L. In the winter, the MP particle concentration in Liepaja is 0.5 ± 3.54 particles/L; in Daugavpils, it is 0.25 ± 2.50 particles/L; in Klaipeda, it is 26.25 ± 42.70 particles/L, and in Siauliai, it is 11.25 ± 7.50 particles/L.

The average concentration of MPs ©n effluent samples is significantly higher than in influent samples, according to this study. The average concentration of MP particles was determined to be 28.33 ± 23.17 particles/L in the effluent and 5.00 ± 5.35 particles/L in the influent. Multiple factors may contribute to the increased concentration of MPs in effluent samples. One factor is that the water treatment process is ineffective at removing MPs. A source of MPs may also exist within the treatment facility, such as the breakdown of larger plastic items or the release of microfibers from textiles during laundering. In addition, the accumulation of MPs within the treatment facility over time may also have contributed to the increased concentration of MPs in effluent samples. This finding has significant implications for the management and treatment of wastewater, as it suggests that current treatment methods may not be fully effective at removing or reducing the abundance of MPs.

In 2021, samples from Lithuania fell within the smallest and biggest size category, with 11% and 47% of the samples averaging <0.25 mm and 1–5 mm, respectively. In Latvia

and Lithuania, most samples were less than 1–5 mm in size. Moreover, the shape of MPs was evaluated. Fiber-shaped particles made up 95% of the samples from Lithuania and 5% of fragment shapes. Similarly, Latvia samples were primarily composed of fiber-shaped particles. Most of the particles found in Latvian samples were blue and black (61% and 36%) (Figure S2).

3.2. Micro-Raman Spectroscopy Results

A micro-Raman spectroscopy analysis was conducted on nine samples, representing 28% of the total sample population. These results provide important evidence for further investigation into the structure and composition of these samples.

Figure 2 depicts the micro-Raman spectra of HDPE MPs in Klaipeda's upstream water, wastewater influent, wastewater effluent, and downstream water samples. The characteristic peaks at 1002 cm^{-1}, 1160 cm^{-1}, and 1450 cm^{-1} in the micro-Raman spectra of HDPE MPs are attributed to the CH_2 bending mode, CH_2 wagging mode, and CH_2 rocking mode, respectively. The peak at 1694 cm^{-1} is due to the C = O stretching mode, which likely originates from an adhesive or coating material on the MP's surface. The similar intensities and peak positions of the spectra in panels (A) and (B) indicate that the concentration and composition of HDPE MPs in upstream and influent water are comparable. However, the spectra in panels (C) and (D) have lower intensities and peak shifts than those in panels (A) and (B), indicating that the concentration and composition of HDPE MPs in the upstream and wastewater influent are different from those in the effluent and downstream. This figure indicates that HDPE MPs are present in Klaipeda's surface freshwater and wastewater and that their concentration and composition are influenced by wastewater treatment processes.

The results of the micro-Raman analysis of selected MPs indicated that there were five types of polymers. The most commonly encountered polymers among the fibers analyzed were polyethylene terephthalate (PET) (33%) and polyvinyl chloride (PVC) (33%), with lower percentages of nylon (NL) (12%), polyester (PS) (11%), and HDPE (11%) also detected (Figure S1). For instance, Figure 2 compares the micro-Raman spectra of HDPE MPs in surface freshwater and wastewater in Klaipeda, Lithuania. These comparisons shed light on the occurrence and distribution of HDPE MPs in surface freshwater and wastewater. Spectra from four distinct locations were analyzed and compared: upstream, wastewater influent, wastewater effluent, and downstream. The results indicate that all spectra exhibit an HDPE-specific peak. However, the intensity of the HDPE peak differs between spectra, indicating that the concentration of HDPE MPs in the various locations differs. The downstream and effluent spectrum has the lowest intensity, whereas the upstream and influent spectrum has greater intensity, indicating a greater concentration of HDPE MPs in the wastewater. Overall, the comparisons of micro-Raman spectra shown in Figure 2 demonstrate the widespread presence of HDPE MPs in surface freshwater and wastewater in Klaipeda. In addition, the results suggest that wastewater treatment processes may not be able to completely remove HDPE MPs from wastewater before it is discharged into the environment. Most of the particles analyzed via micro-Raman analysis were white (45%) or blue (33%). Black and red had the lowest rate among colors, with 11% each. This suggests that WWTPs may not be removing all pollutant particulates from the water, which could lead to adverse environmental impacts. In order to improve water quality, it is important to identify the sources of these pollutants and to implement measures that will reduce their presence in the water. The results of the micro-Raman analysis revealed that the vast majority of pollutants were between 1–5 mm (33%) and 0.5–1 mm (33%). A significantly smaller percentage of pollutants were between 0.25–0.5 mm (22%), while an even smaller percentage was below 0.25 mm (12%). These results suggest that there are a variety of pollutant sizes present in the surface water, which could have considerable impacts on water quality and the environment. Moreover, the shape of MPs was evaluated, and all of the particles were fiber-shaped. This is an indicator of the potential sources of the MPs in materials, such as synthetic textiles, which have been identified as potential sources of

these contaminants. Additionally, the evaluation of MPs allowed for a comparison between the cities regarding their wastewater treatment efficiency. The results from this comparison will be used to guide further research into wastewater treatment and its potential effects on water quality.

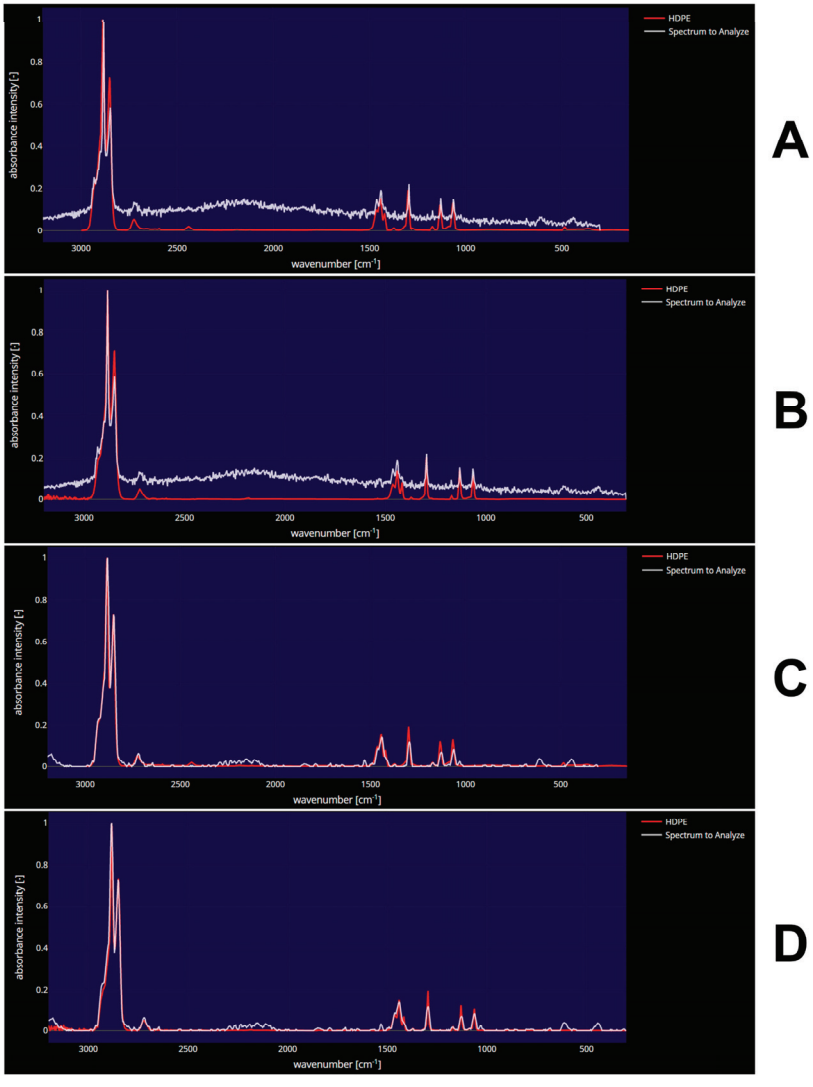

Figure 2. High-Density Polyethylene (HDPE) micro-Raman spectrum comparisons: (**A**) upstream in Klaipeda; (**B**) wastewater influent in Klaipeda; (**C**) wastewater effluent in Klaipeda; and (**D**) downstream in Klaipeda.

4. Discussion

4.1. Study Limitations

This study has several limitations that should be noted. First, there were relatively few samples collected, which might limit the generalizability of the findings. In addition, the study concludes that municipal wastewater from catchment areas was the primary source of MP contamination in the surface water and wastewater of Latvia and Lithuania and

suggests implementing pollution-reduction strategies, such as raising awareness, installing more high-tech WWTPs, and reducing plastic use. However, the study has a limited sample size and limited sampling periods, and the results may not be representative of other regions or seasons.

4.2. Microplastic Comparison in Latvia, Lithuania, and Other Aquatic Environments

In Latvia and Lithuania, there has been limited research on the levels of MPs in surface water and wastewater. It is important to study these levels in order to better understand the occurrence and distribution of MPs in these countries and to identify potential sources of contamination. By comparing the concentrations of MPs in Latvian and Lithuanian surface water and wastewater to those in other countries, researchers can gain a better understanding of the global distribution of MPs and the potential impacts they may have on aquatic ecosystems (Table 1).

The detection of MPs In aquatic environments, such as surface water and wastewater, has been extensively documented in the scientific literature. For instance, MPs have been detected in aquatic environments, such as brackish water [12], surface water [9], wastewater influent [28], and wastewater effluent [29]. This study is the first to report the occurrence of MPs in surface water and wastewater in Latvia and Lithuania, where they were found to have various shapes, colors, and sizes and to be composed of a range of polymers. Surface water and wastewater contain polymers with toxic and carcinogenic properties that may originate from industrial discharges, agricultural runoff, and sewage. Certain plastics, such as PVC and PET, and synthetic rubbers, such as neoprene, are examples of toxic polymers that can be found in surface waters and wastewater. Here, MPs were identified as having several shapes (fibers and fragments), various colors (transparent, yellow, red, blue, and black), and sizes (<0.25, 0.25–0.5, 0.5–1, and 1–5). Additionally, PET, PVC, NL, and PS polymers were determined in the form of a variety of polymers in various colors, forms, and sizes, demonstrating the diversity of MPs sources. The diversity of MPs sources observed in this study suggests that they may be coming from a variety of sources, including ships, wind, urban wastewater, and hospital wastewater. It can be said that MPs found in surface water and wastewater samples are likely to have originated from ships [30], wind [31], urban wastewater [32], and hospital wastewater [33].

The number of MPs identified in 32 samples of influent, effluent, upstream, and downstream ranged from zero (not detected) in two samples to 11 in samples SLT6 and WLT3. Besides, RA analysis showed different polymers, namely PET, PVC, NL, and PS, in 9 samples. There is a lack of information about the concentration of MPs in Latvia and Lithuania, making comparing this value with literature concentrations of MPs in surface water and wastewater problematic. For instance, one study on MPs in Lithuania detected 2982 $\pm$ 54 particles/L in the influent and 1244 $\pm$ 21 particles/L in the effluent [34]. In another study, [9] found 4430 particles/L in the Gulf of Riga.

Table 1. The abundance of MPs found in surface water and wastewater from different locations in the world, with the most abundant morphology, color, and chemical composition.

Location	Abundance (Particles/L)	Shape	Size	Color	Polymer	Reference
Latvia	3.50 ± 2.38 [1]	Fiber	<0.25 mm 0.5–1 mm 1–5 mm	Red Blue Black	PET PVC NL PS	This study
Lithuania	7.50 ± 6.45 [1]	Fiber	<0.25 mm 0.25–0.5 mm 0.5–1 mm 1–5 mm	Red Blue Black	PET PVC NL PS	This study

Table 1. *Cont.*

Location	Abundance (Particles/L)	Shape	Size	Color	Polymer	Reference
China	10.5 ± 2.5 [1]	Fragments	0.01–0.1 mm 0.1–1 mm 1–5 mm	Transparent White Blue Black Yellow	PE PS PP PVC	[35]
China	654 [1]	Fibers Fragments	50–100 µm 100–200 µm 200–500 µm 500–5000 µm	-	-	[36]
Portugal	231 [1]	Fragments Spherule Fibers	-	Black Blue Brown White	-	[37]
Iran	0.027 ± 0.042 [1]	Spherule Fibers	0.05–0.5 mm 0.5–1 mm 1–2.5 mm 2.5–5 mm >5 mm	Red	-	[38]
Lithuania	33.75 ± 40.08 [2]	Fiber Fragment	<0.25 mm 0.25–0.5 mm 0.5–1 mm 1–5 mm	Transparent Yellow Red Blue Black	PET PVC NL PS	This study
Latvia	11.67 ± 12.58 [2]	Fiber	<0.25 mm 0.25–0.5 mm 0.5–1 mm 1–5 mm	Blue Black	PET PVC NL PS	This study
Lithuania	2982 ± 54 [2]	Fiber Fragment Pellet	20–50 µm 50–100 µm 100–200 µm 200–500 µm 500–1000 µm	Black White Transparent Brown Yellow Blue Other	PET PS PP	[34]
Lithuania	1244 ± 21 [2]	Fiber Fragment Pellet	20–50 µm 50–100 µm 100–200 µm 200–500 µm 500–1000 µm	Black White Transparent Brown Yellow Blue Other	PET PS PP	[34]

[1] Surface water. [2] Wastewater. CE—Cellulose, ET—Ethylene, EVA—Poly (Ethylene Co Vinyl Acetate), HDPE—High—density polyethylene, NL—Nylon, PAA—Poly (Acrylic Acid), PE—Polyethylene, PEL—Poly (ether- urethane), PET—Polyethylene terephthalate, PP—Polypropylene, PS—Polystyrene.

4.3. Challenges and Potential Solutions for Removing Microplastics from Wastewater

Several filtration systems can be used to remove MPs from wastewater. These systems typically use physical or chemical processes to capture and remove MPs from the water. One type of filtration system that is commonly used to remove MPs from wastewater is a microfiltration system [39]. Another type of filtration system that can be used to remove MPs from wastewater is ozonation [23]. Other types of filtration systems that can be used to remove MPs from wastewater include gravity filters [40], sand filters [40], ultraviolet radiation [40], chlorination [40], advanced oxidation processes [41], and activated carbon

filters [40]. Because MPs are so small and can easily pass through a variety of filters, there is no known filtering system that can remove 100% of MPs from wastewater. Efforts are ongoing to develop more effective methods for removing MPs from water, but it is currently impossible to eliminate them entirely from wastewater.

4.4. Untested Hypotheses and Potential Avenues for Future Research

There are several hypotheses that remain untested and potential avenues for future research that have been identified in this study. The first step is to identify the sources of MPs in Latvian and Lithuanian surface freshwater and wastewater in order to develop effective mitigation strategies. Secondly, further research is needed to determine how MPs are transported in freshwater systems. The transport of MPs can be affected by factors such as water flow rate, temperature, and sedimentation. Thirdly, it is unclear what effect MPs will have on freshwater ecosystems. A comprehensive assessment of MPs' effects on aquatic organisms and the environment is essential. Fourthly, to comprehend the long-term consequences of MP pollution, it is necessary to investigate the fate and persistence of MPs in freshwater systems. Lastly, it is necessary to investigate the occurrence and distribution of MPs in groundwater systems in order to identify potential sources and pathways of MPs and their potential environmental impacts.

5. Conclusions

This study presents the first assessment of the abundance and physical and chemical characteristics of MP litter from 16 sampling points located on the influent, effluent, upstream, and downstream locations in Latvia and Lithuania on the Baltic Sea. For this study, optical microscopy and micro-Raman spectroscopy methods were chosen to analyze samples because optical microscopy can observe color, shape, and size, while micro-Raman can identify polymer types and identify small MPs with a size of 20 microns; the combination of optical microscopy and micro-Raman spectroscopy allows accurate quantification of MPs and polymeric recognition. Finally, MPs detected in surface water and wastewater were mainly polymeric structures with various shapes, sizes, and colors. The nature of the different MPs indicates that the majority were secondary in nature, probably originating from rivers near the sampling stations, which receive municipal and industrial wastewater. As a result of the chemical characterization, polymers were identified as being very common in household waste, demonstrating the source of MP pollution. In addition, surface water and wastewaters contain polymers with toxic and carcinogenic properties. Having these data to evaluate the pollution caused by MPs on a local scale is fundamentally essential. As well as identifying the primary sources of pollution, they will serve as a basis for identifying possible accumulation hotspots.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/toxics11040292/s1, Figure S1: Types of polymers detected by RS in 9 water samples in Latvia and Lithuania.; Figure S2: Images of MPs observed in Latvia and Lithuania; (A) black fibers with a length of 1.1 mm in Klaipeda upstream, (B) black fibers with a length of 0.7 mm in Klaipeda effluent, (C) black fibers with a length of 0.6 mm in Siauliai influent, (D) black fibers with a length of 1.06 mm and 1.08 mm in Siauliai effluent, (E) black fibers with a length of 1.09 mm in Liepaja upstream, (F) black fibers with a length of 0.9 mm in Liepaja downstream, (G) black fibers with a length of 2.06 mm and 0.6 mm in Daugavpils influent, (H) black fibers with a length of 1.08 mm and 0.6 mm in Daugavpils effluent.; Table S1: Sampling location and physicochemical properties of influent, effluent, upstream and downstream in Latvia and Lithuania.; Table S2: Size, shape, and color of MP particles in surface water and wastewater of Latvia.; Table S3: Size, shape, and color of MP particles in surface water and wastewater of Lithuania.

Author Contributions: R.P.: Sample collection, Conceived and designed the study, conducted laboratory work, analyzed the data, and write the manuscript. V.S.: Conducted laboratory work. S.S.: Sample collection and Conducted laboratory work. A.B.: Conducted laboratory work and reviewed the manuscript. I.P.-N.: Sample collection and Conducted laboratory work. R.M.R.: Conceived and designed the study and reviewed the manuscript. R.D.: Sample collection, Conducted laboratory work, and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The work received minor financial support from the project "The development of the methodological guidelines for marine litter monitoring", which is implemented under the "Marine litter monitoring" initiative and public tender call of the Environment Protection Agency under the Ministry of Environment of Lithuania (2021–2023). This project has received funding from the European Regional Development Fund (project No 13.1.1-LMT-K-718-05-0014) under a grant agreement with the Research Council of Lithuania (LMTLT), and it was funded as part of the European Union's measure in response to the COVID-19 pandemic. Interreg V-A Latvia-Lithuania Programme 2014–2020 project LLI-527 "Pharmaceuticals in wastewaters-levels, impacts and reduction" (MEDWwater).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The authors confirm that all data supporting the findings of this study are available from the corresponding author upon request.

Acknowledgments: This research was supported by the Doctorate Study Program in Ecology and Environmental Sciences, Marine Research Institute of Klaipeda University.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sulistyowati, L.; Riani, E.; Cordova, M.R. The occurrence and abundance of microplastics in surface water of the midstream and downstream of the Cisadane River, Indonesia. *Chemosphere* **2022**, *291*, 133071. [CrossRef] [PubMed]
2. Galgani, L.; Loiselle, S.A. Plastic pollution impacts on marine carbon biogeochemistry. *Environ. Pollut.* **2021**, *268*, 115598. [CrossRef] [PubMed]
3. Hale, R.C.; Seeley, M.E.; La Guardia, M.J.; Mai, L.; Zeng, E.Y. A Global Perspective on Microplastics. *J. Geophys. Rese. Oceans* **2020**, *125*, e2018JC014719. [CrossRef]
4. Moyo, M.; Falayi, T. Generation and management of microplastic waste. In *Impact of Plastic Waste on the Marine Biota*; Springer: Singapore, 2022; pp. 3–20.
5. Andrady, A.L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62*, 1596–1605. [CrossRef] [PubMed]
6. Lusher, A.L.; Mchugh, M.; Thompson, R.C. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Mar. Pollut. Bull.* **2013**, *67*, 94–99. [CrossRef] [PubMed]
7. Pashaei, R.; Dzingelevičienė, R.; Abbasi, S.; Szultka-Młyńska, M.; Buszewski, B. Determination of the pharmaceuticals–nano/microplastics in aquatic systems by analytical and instrumental methods. *Environ. Monit. Assess.* **2022**, *194*, 93. [CrossRef]
8. Golgoli, M.; Khiadani, M.; Shafieian, A.; Sen, T.K.; Hartanto, Y.; Johns, M.L.; Zargar, M. Microplastics fouling and interaction with polymeric membranes: A review. *Chemosphere* **2021**, *283*, 131185. [CrossRef]
9. Aigars, J.; Barone, M.; Suhareva, N.; Putna-Nimane, I.; Dimante-Deimantovica, I. Occurrence and spatial distribution of microplastics in the surface waters of the Baltic Sea and the Gulf of Riga. *Mar. Pollut. Bull.* **2021**, *172*, 112860. [CrossRef]
10. Beer, S.; Garm, A.; Huwer, B.; Dierking, J.; Nielsen, T.G. No increase in marine microplastic concentration over the last three decades–a case study from the Baltic Sea. *Sci. Total Environ.* **2018**, *621*, 1272–1279. [CrossRef]
11. Bollmann, U.E.; Simon, M.; Vollertsen, J.; Bester, K. Assessment of input of organic micropollutants and microplastics into the Baltic Sea by urban waters. *Mar. Pollut. Bull.* **2019**, *148*, 149–155. [CrossRef]
12. Ferrero, L.; Scibetta, L.; Markuszewski, P.; Mazurkiewicz, M.; Drozdowska, V.; Makuch, P.; Jutrzenka-Trzebiatowska, P.; Zaleska-Medynska, A.; Andò, S.; Saliu, F.; et al. Airborne and marine microplastics from an oceanographic survey at the Baltic Sea: An emerging role of air-sea interaction? *Sci. Total Environ.* **2022**, *824*, 153709. [CrossRef] [PubMed]
13. Halbach, M.; Vogel, M.; Tammen, J.K.; Rüdel, H.; Koschorreck, J.; Scholz-Böttcher, B.M. 30 years trends of microplastic pollution: Mass-quantitative analysis of archived mussel samples from the North and Baltic Seas. *Sci. Total Environ.* **2022**, *826*, 154179. [CrossRef] [PubMed]
14. Mishra, A.; Buhhalko, N.; Lind, K.; Lips, I.; Liblik, T.; Väli, G.; Lips, U. Spatiotemporal Variability of Microplastics in the Eastern Baltic Sea. *Front. Mar. Sci.* **2022**, *9*, 686. [CrossRef]

15. Narloch, I.; Gackowska, A.; Wejnerowska, G. Microplastic in the Baltic Sea: A review of distribution processes, sources, analysis methods and regulatory policies. *Environ. Pollut.* **2022**, *315*, 120453. [CrossRef] [PubMed]
16. Piehl, S.; Hauk, R.; Robbe, E.; Richter, B.; Kachholz, F.; Schilling, J.; Lenz, R.; Fischer, D.; Fischer, F.; Labrenz, M.; et al. Combined approaches to predict microplastic emissions within an urbanized estuary (Warnow, southwestern Baltic Sea). *Front. Environ. Sci.* **2021**, *9*, 616765. [CrossRef]
17. Schernewski, G.; Radtke, H.; Hauk, R.; Baresel, C.; Olshammar, M.; Osinski, R.; Oberbeckmann, S. Transport and behavior of microplastics emissions from urban sources in the Baltic Sea. *Front. Environ. Sci.* **2020**, *8*, 579361. [CrossRef]
18. Tamminga, M.; Hengstmann, E.; Fischer, E.K. Microplastic analysis in the South Funen Archipelago, Baltic Sea, implementing manta trawling and bulk sampling. *Mar. Pollut. Bull.* **2018**, *128*, 601–608. [CrossRef]
19. Wang, Z.M.; Wagner, J.; Ghosal, S.; Bedi, G.; Wall, S. SEM/EDS and optical microscopy analyses of microplastics in ocean trawl and fish guts. *Sci. Total Environ.* **2017**, *603*, 616–626. [CrossRef]
20. Ojaveer, H.; Jaanus, A.; MacKenzie, B.R.; Martin, G.; Olenin, S.; Radziejewska, T.; Telesh, I.; Zettler, M.L.; Zaiko, A. Status of biodiversity in the Baltic Sea. *PLoS ONE* **2010**, *5*, e12467. [CrossRef]
21. Jung, S.; Cho, S.H.; Kim, K.H.; Kwon, E.E. Progress in quantitative analysis of microplastics in the environment: A review. *Chem. Eng. J.* **2021**, *422*, 130154. [CrossRef]
22. Sierra, I.; Chialanza, M.R.; Faccio, R.; Carrizo, D.; Fornaro, L.; Pérez-Parada, A. Identification of microplastics in wastewater samples by means of polarized light optical microscopy. *Environ. Sci. Pollut. Res.* **2020**, *27*, 7409–7419. [CrossRef] [PubMed]
23. Pashaei, R.; Dzingelevičienė, R.; Bradauskaitė, A.; Lajevardipour, A.; Mlynska-Szultka, M.; Dzingelevičius, N.; Raugelė, S.; Razbadauskas, A.; Abbasi, S.; Rees, R.M.; et al. Pharmaceutical and Microplastic Pollution before and during the COVID-19 Pandemic in Surface Water, Wastewater, and Groundwater. *Water* **2022**, *14*, 3082. [CrossRef]
24. Käppler, A.; Fischer, D.; Oberbeckmann, S.; Schernewski, G.; Labrenz, M.; Eichhorn, K.J.; Voit, B. Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? *Anal. Bioanal. Chem.* **2016**, *408*, 8377–8391. [CrossRef] [PubMed]
25. Araujo, C.F.; Nolasco, M.M.; Ribeiro, A.M.P.; Ribeiro-Claro, P.J.A. Identification of microplastics using Raman spectroscopy: Latest developments and future prospects. *Water Res.* **2018**, *142*, 426–440. [CrossRef] [PubMed]
26. Cowger, W.; Steinmetz, Z.; Gray, A.; Munno, K.; Lynch, J.; Hapich, H.; Primpke, S.; De Frond, H.; Rochman, C.; Herodotou, O. Microplastic spectral classification needs an open source community: Open specy to the rescue! *Anal. Chem.* **2021**, *93*, 7543–7548. [CrossRef] [PubMed]
27. Löder, M.G.; Imhof, H.K.; Ladehoff, M.; Löschel, L.A.; Lorenz, C.; Mintenig, S.; Piehl, S.; Primpke, S.; Schrank, I.; Laforsch, C.; et al. Enzymatic purification of microplastics in environmental samples. *Environ. Sci. Technol.* **2017**, *51*, 14283–14292. [CrossRef]
28. Long, Z.; Pan, Z.; Wang, W.; Ren, J.; Yu, X.; Lin, L.; Lin, H.; Chen, H.; Jin, X. Microplastic abundance, characteristics, and removal in wastewater treatment plants in a coastal city of China. *Water Res.* **2019**, *155*, 255–265. [CrossRef]
29. Mintenig, S.M.; Int-Veen, I.; Löder, M.G.; Primpke, S.; Gerdts, G. Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. *Water Res.* **2017**, *108*, 365–372. [CrossRef]
30. Peeken, I.; Primpke, S.; Beyer, B.; Gütermann, J.; Katlein, C.; Krumpen, T.; Bergmann, M.; Hehemann, L.; Gerdts, G. Arctic sea ice is an important temporal sink and means of transport for microplastic. *Nat. Commun.* **2018**, *9*, 1505. [CrossRef]
31. Brahney, J.; Hallerud, M.; Heim, E.; Hahnenberger, M.; Sukumaran, S. Plastic rain in protected areas of the United States. *Science* **2020**, *368*, 1257–1260. [CrossRef]
32. Bayo, J.; Olmos, S.; López-Castellanos, J. Microplastics in an urban wastewater treatment plant: The influence of physicochemical parameters and environmental factors. *Chemosphere* **2020**, *238*, 124593. [CrossRef] [PubMed]
33. Kashfi, F.S.; Ramavandi, B.; Arfaeinia, H.; Mohammadi, A.; Saeedi, R.; De-la-Torre, G.E.; Dobaradaran, S. Occurrence and exposure assessment of microplastics in indoor dusts of buildings with different applications in Bushehr and Shiraz cities, Iran. *Sci. Total Environ.* **2022**, *829*, 154651. [CrossRef] [PubMed]
34. Uogintė, I.; Pleskytė, S.; Pauraitė, J.; Lujanienė, G. Seasonal variation and complex analysis of microplastic distribution in different WWTP treatment stages in Lithuania. *Environ. Monit. Assess.* **2022**, *194*, 829. [CrossRef] [PubMed]
35. Wu, P.; Tang, Y.; Dang, M.; Wang, S.; Jin, H.; Liu, Y.; Jing, H.; Zheng, C.; Yi, S.; Cai, Z. Spatial-temporal distribution of microplastics in surface water and sediments of Maozhou River within Guangdong-Hong Kong-Macao Greater Bay Area. *Sci. Total Environ.* **2020**, *717*, 135187. [CrossRef]
36. Han, M.; Niu, X.; Tang, M.; Zhang, B.T.; Wang, G.; Yue, W.; Kong, X.; Zhu, J. Distribution of microplastics in surface water of the lower Yellow River near estuary. *Sci. Total Environ.* **2020**, *707*, 135601. [CrossRef]
37. Prata, J.C.; Godoy, V.; da Costa, J.P.; Calero, M.; Martín-Lara, M.A.; Duarte, A.C.; Rocha-Santos, T. Microplastics and fibers from three areas under different anthropogenic pressures in Douro river. *Sci. Total Environ.* **2021**, *776*, 145999. [CrossRef]
38. Pashaei, R.; Loiselle, S.A.; Leone, G.; Tamasi, G.; Dzingelevičienė, R.; Kowalkowski, T.; Gholizadeh, M.; Consumi, M.; Abbasi, S.; Buszewski, B.; et al. Determination of nano and microplastic particles in hypersaline lakes by multiple methods. *Environ. Monit. Assess.* **2021**, *193*, 668. [CrossRef]
39. Pizzichetti, A.R.P.; Pablos, C.; Álvarez-Fernández, C.; Reynolds, K.; Stanley, S.; Marugán, J. Evaluation of membranes performance for microplastic removal in a simple and low-cost filtration system. *Case Stud. Chem. Environ. Eng.* **2021**, *3*, 100075. [CrossRef]

40. Tang, K.H.D.; Hadibarata, T. Microplastics removal through water treatment plants: Its feasibility, efficiency, future prospects and enhancement by proper waste management. *Environ. Chall.* **2021**, *5*, 100264. [CrossRef]
41. Shen, M.; Song, B.; Zhou, C.; Hu, T.; Zeng, G.; Zhang, Y. Advanced oxidation processes for the elimination of microplastics from aqueous systems: Assessment of efficiency, perspectives and limitations. *Sci. Total Environ.* **2022**, *842*, 156723. [CrossRef]

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High-Efficiency Microplastic Sampling Device Improved Using CFD Analysis

Seonghyeon Ju [1], Jongchan Yi [2], Junho Lee [1], Jiyoon Kim [1], Chaehwi Lim [1], Jihoon Lee [1], Kyungtae Kim [1] and Yeojoon Yoon [1,*]

[1] Department of Environmental and Energy Engineering, Yonsei University, Wonju 26493, Republic of Korea
[2] Water Environmental Safety Management Department, K-Water, Daejeon 34045, Republic of Korea
* Correspondence: yajoony@yonsei.ac.kr

Abstract: Since microplastics are considered harmful to the human body, studies on their samplings, pretreatments and analyses environmental media, such as water, are continuously being conducted. However, a standard sampling and pretreatment method must be established, particularly because microplastics of a few micrometers in size are easily affected by external contamination. In this study, a microplastic sampling device was designed and developed to obtain a high recovery rate of microplastics and prevent plastics contamination during all processes. For the evaluation of the developed device, microplastic reference materials were produced and used, and computational fluid dynamics (CFD) analysis was performed. This device has not only been applied to the relatively large previously studied microplastics (100 μm) but also to microplastics of approximately 20 μm that are vulnerable to contamination. A recovery rate of 94.2% was obtained using this device, and the particles were separated by filtration through a three-stage cassette. In conclusion, we propose a method to increase the accuracy and reproducibility of results for microplastic contamination in the environment. This method is able to consistently obtain and manage microplastics data, which are often difficult to compare using various existing methods.

Keywords: microplastics; sampling device; CFD analysis; recovery rate; size classification

Citation: Ju, S.; Yi, J.; Lee, J.; Kim, J.; Lim, C.; Lee, J.; Kim, K.; Yoon, Y. High-Efficiency Microplastic Sampling Device Improved Using CFD Analysis. *Sustainability* **2023**, *15*, 3907. https://doi.org/10.3390/su15053907

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

Received: 2 February 2023
Revised: 17 February 2023
Accepted: 17 February 2023
Published: 21 February 2023

1. Introduction

Microplastics are plastics with a diameter of less than 5 mm. They are classified as either primary and secondary microplastics, which are produced by human activities and environmental decomposition, respectively. Recently, microplastics have attracted much research attention because of their toxicity, bioaccumulation [1–4], and organic matter transportation [5]. Microplastics can cause contamination problems in all environmental media, such as fresh water, tap water, sea, air, and soil [6–10]. Secondary microplastics are microplastics produced by the decomposition of discharged plastics and pose a higher risk to the environment and life forms as their size decreases over time [11–14]. In particular, microplastics with sizes of a few microns are involved in bioaccumulation and organic matter accumulation [1,5,15]. However, research on microplastic sampling and analysis methods is being actively conducted, and ISO standardization is in progress (ISO/DIS 24187).

The most widely used devices for isolating plastic particles in the aqueous phase are the manta trawl, plankton net, and Van Dorn sampler [16]. However, there are disadvantages to using these samplers, and additional equipment, such as a boat, is required to transport large samplers during the research process. Additionally, a manta trawl cannot be used for microplastics of less than 50 μm in diameter, and it is difficult to control tool contamination [17–20]. To overcome these problems, in this study, small-sized microplastic samples were collected using the encapsulation and centrifuge methods [17,19]. The encapsulation method consumes less energy, easily controls pollution, and collects

microplastics of a few microns in diameter depending on the pore of the woven stainless filter. To improve the device and ensure efficiency, a CFD analysis was conducted to check the fluid flow inside the device, and to identify, predict, and correct the cause of sampling loss. This analysis can identify the cause of a problem by comparing it with the actual result and performing an improved complementary analysis. In addition, in the case of fouling that occurs in sample collection using a filter, studies are currently being conducted to increase the efficiency of sample collection by finding the cause of pressure decreases through CFD [21,22]. In this study, the cause of the pressure decrease that may occur during filter filtration was predicted using modeling and used to solve the problems of previous studies.

Fragments of microplastics present in devices, which are only a few microns in size, can affect the analysis results [23]. The microplastics present in a typical laboratory environment can affect microplastic samples with sizes of a few microns [24–26]. In another study of ours, POM fragments were found in environmental and falling blank samples during the sampling process, which used a polyoxymethylene (POM) cassette in the most commonly used low-volume air sampler. However, as a result of using a stainless filter and cassette, the POM cassette was no longer found in the atmospheric sample (Figure 1 and Table 1).

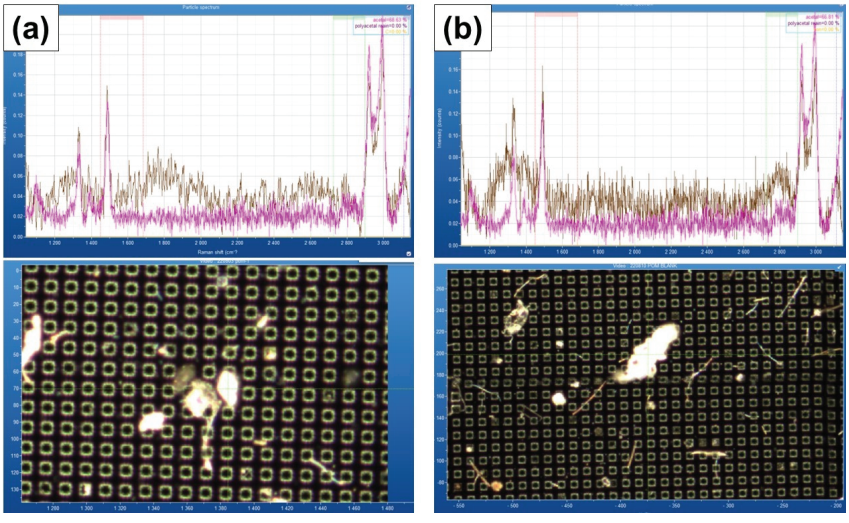

Figure 1. μ-Raman result of (**a**) air blank sample and (**b**) air sample for POM cassette.

Table 1. μ-Raman result for POM and STS cassettes.

Cassette Material	Sample	PET	PS	PVC	PE	PP	POM
		(#/m^3)					
POM	Passive sample	0	0.209	0	1.044	0.209	0.209
	Active sample	0.417	0.417	0	4.174	2.087	0.209
STS	Active sample	2.805	0.301	0	41.675	0	0

Although the need for contamination control has been frequently reported, the control method used in each study is different. For instance, one study used high-temperature treatment to remove plastic contamination [25]. Another study conducted by Hermsen et al. [27] found that "laboratory preparation" has the second lowest performance in the quality assessment of microplastics. The aim of this study was to develop a high-efficiency and low-cross contamination device to sample microplastics a few microns in sizes and separate them by sizes. A CFD analysis was then used to identify and improve the mechanical defects and sample losses in the developed device.

2. Materials and Methods

2.1. Reagents

A 35% hydrogen peroxide (H_2O_2) solution was purchased from Daejung Chemical & Metals Co., Ltd., Seoul, Republic of Korea. Ethyl alcohol (C_2H_6O, 99%) solution and zinc chloride ($ZnCl_2$, 98%) powder were purchased from DUKSAN Pure Chemicals, Inc., Seoul, Republic of Korea. The high-performance liquid chromatography (HPLC) equipment for analyzing field blank samples was purchased from Baker. Distilled, deionized (DI) water was used in all experiments. All reagents were first filtered using a Whatman's glass fiber filter (GF/F) and then filtered again using an STS filter to remove microplastics and glass fibers from the reagents. Before use, the GF/F and STS filters were washed with ethyl alcohol and oven-dried at 100 °C for 24 h.

2.2. Analytical Devices

The surfaces of the STS filter and plastic were analyzed using scanning electron microscopy (SEM; JSM-IT500). The blank sample was analyzed using μ-Raman spectroscopy (New XploRA Plus, HORIBA, Kyoto, Japan) and silicon filter (Si-filter). Micro-balance (BM-20, A&D, Tokyo, Japan) which has 0.001 mg resolution was used to measure the weight of the STS filter to estimate the recovery rate of the sampler and pretreatment process.

2.3. Sampling and Pretreatment Device Development

A device was developed to sample small-sized microplastics over 5 μm in environmental media. In addition, it was modified into three types according to the problems that occurred during the sampling process. The sampling device is based on a filtration method to allow for flexible filter pore size and easy filter replacement after contamination. Each device was manufactured according to the design depicted in Figure 2. All sampling devices consisted of a housing and cassette, and each cassette was watertight with a silicone O-ring or gasket. The cassettes could be easily stacked in multiple stages within the housing. In the case of conventional sampling and pretreatment devices, additional units (several individual housings) or processes for size classification were required. However, the developed sampling device requires only one compact housing and several optional cassettes for size classification. In this study, three-stage cassettes equipped with filters of different pores were used for size classification. The devices were made from an aluminum alloy with a low cost, easy machinability, light weight, and higher chemical resistance than conventional metals such as stainless steel. The device was modified and supplemented to increase the recovery rate of the microplastic sampler. Similar to other studies, by applying a stainless steel (STS) filter, it was possible to collect a large volume of samples and microplastics measuring several microns with the device at the user's discretion. In addition, the STS filter could withstand vacuum pump pressure and had a strong chemical resistance, maintaining filter pore during sampling and pretreatment.

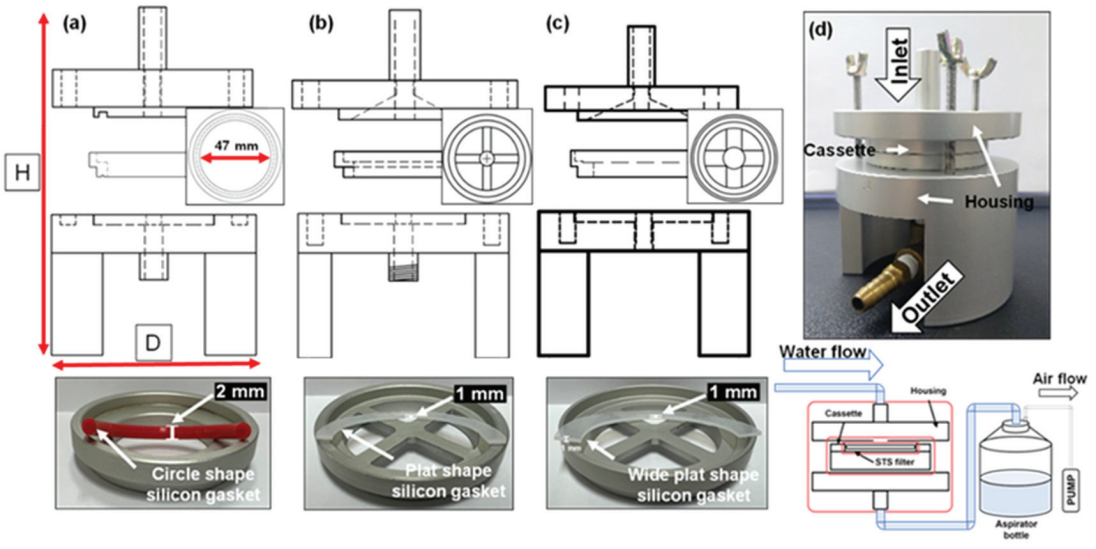

Figure 2. Sampling devices and cassette with silicon gasket: (**a**) Device 1, (**b**) Device 2, (**c**) and Device 3. (**d**) Assembly of the sampling device (D × H = 80 mm × 100 mm) and scheme of the sampling process.

2.4. Computational Fluid Dynamics (CFD) Analysis

The water flow of the sampling device was simulated using Flow-3D software (Flow Science, Inc., Santa Fe, NM, USA). The solver version was 12.0.3.02 lnx64 02/05/2021 HPC. More details regarding the software, hardware, and fluid properties are provided in Table S1. Before the CFD analysis, the flow velocity was studied in the following three cases:

- Case 1 (XX): Filtration without filters and microplastics.
- Case 2 (FX): Filtration with an STS filter inside the device without microplastics.
- Case 3 (FM): Filtration with an STS filter inside the device and 30 mg of microplastic solution dispersed in 30 mL of ethanol.

The amount of microplastics selected for the flow velocity experiment was set at 30 mg, which minimizes the influence of external factors such as air flow and vibration on the measured value of the ultra-microbalance. Since the device has a symmetrical structure, the simulation was carried out for only one quarter of the device, and the microplastic particles were excluded from the CFD analysis.

2.5. Reference Material Recovery of the Sampling Device

The recovery rate of the sampling device was confirmed by the difference in weight before and after the filtration of microplastics of 20 μm or less using an ultra-microbalance. A woven STS filter was used for filtration to prevent plastic contamination using a membrane filter, and the SEM image is shown in (Figure 3). The microplastic reference materials used were polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and polyethylene (PE). These five plastics are the most frequently found plastics in the environment [28].

The microplastics were prepared with multi-stage sieve shaking after ultrafine grinding at the Korea Testing & Research Institute (KTR). Two types of microplastic reference materials produced by Bundesanstalt fuer Materialforschung und -pruefung (BAM) in Germany, PS and PE, with diameters in the range of 10–300 μm, were used. The microplastics produced by KTR are diverse and several micrometers in size. These reference materials were divided into two size ranges. The first range included microplastics with diameters of dozens of microns, and the second range included microplastics with sizes of a few

hundred microns. The lower 10% size, middle size, and upper 90% size for the particle size of each range are shown in (Tables 2 and 3). The average particle size and the distribution of the microplastics were analyzed using a particle size analyzer (LA-350, HORIBA), and the shape of the particles was confirmed using a SEM analysis (Figure 2).

Table 2. Size distribution of the first range of microplastic reference materials.

Cumulative Diameter		d (10%)	d (50%)	d (90%)
Cumulative diameter (μm)	PE	11.0	18.0	29.7
	PET	9.8	15.8	25.0
	PS	9.0	14.1	21.5
	PP	10.0	16.4	29.3
	PVC	10.6	16.7	25.2

d (10%): 10% of the total particles are smaller than this size. d (50%): 50% of the total particles are smaller than this size. d (90%): 90% of the total particles are smaller than this size.

Table 3. Size distribution of the second range of microplastic reference materials.

Cumulative Diameter		d (10%)	d (50%)	d (90%)
Cumulative diameter (μm)	PE	102.1	203.3	331.1
	PET	108.4	165.4	235.5
	PS	70.4	140.1	218.0
	PP	120.1	170.0	253.7
	PVC	109.8	160.3	233.3

d (10%): 10% of the total particles are smaller than this size. d (50%): 50% of the total particles are smaller than this size. d (90%): 90% of the total particles are smaller than this size.

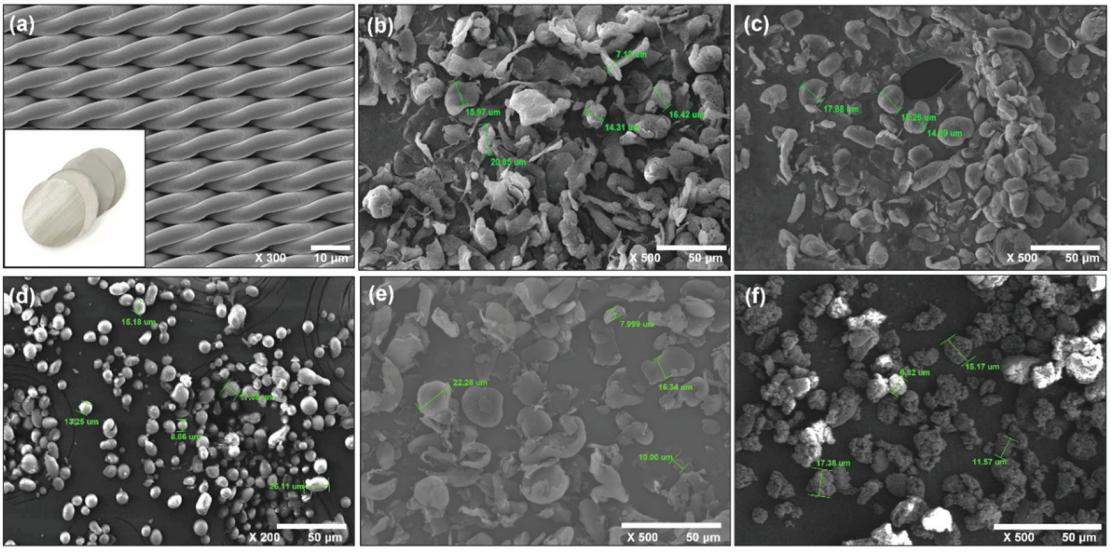

Figure 3. SEM image of (**a**) a STS filter, (**b**) polyethylene, (**c**) polyethylene terephthalate, (**d**) polystyrene, (**e**) polypropylene, and (**f**) polyvinyl chloride.

First, the filter was washed with an 99 % filtered ethanol solution and oven-dried at 90 °C for 1 h in a glass Petri dish. Microplastics were weighed and sonicated in 500 mL of DI water for 30 min. The DI water containing microplastics was filtered through a sampling

device that was set to the STS filter on the cassette. After filtration, the STS filter was oven-dried at 90 °C for 1 h and then weighed again. By comparing the injected plastic weight (w_{inj}), the difference in the weights of the STS filter before (Fw_{ini}) and after (Fw_{fin}) filtration was calculated to estimate the recovery rate of the device (Equation (1)).

$$Recovery\ rate\ (\%) = \frac{Fw_{fin} - Fw_{ini}}{w_{inj}} \times 100 \quad (1)$$

All procedures for the recovery experiment were conducted while covered with an aluminum foil to minimize contamination caused by exposure to air, and a cotton lab coat and neoprene powder-free gloves (Microflex®, Richmond, Australia) were used. All tools and dishes were washed with filtered ethanol and DI water. After filtration, all filters containing Petri dishes were sealed with a paraffin film. All pieces of the device were washed with filtered ethanol and DI water before use. The samples were covered with aluminum foil after oven drying. The device inlet and all sample passages had no contact with any filter or cassette. After using the device, all parts were removed, followed by washing with ethanol and drying in an oven.

The recovery from the pretreatment process was performed using an ultra-microbalance. The STS filter was weighed before and after each pretreatment process to estimate the recovery rate of the sampling device. Pretreatment involved conventional methods such as wet peroxide oxidation (WPO) and density separation with $ZnCl_2$ [29,30] (Figure 4).

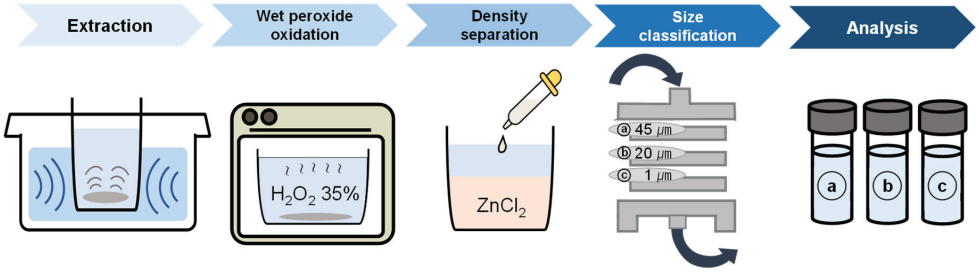

Figure 4. Pretreatment processes (WPO was performed at 80 °C for 3 h; Density separation was performed at 1.6 kg/L density; Size classification was performed with the sampling device equipped with three multi-stage filters, a: 45 μm, b: 20 μm, and c: 1 μm).

3. Result and Discussion

3.1. Sampling Device Modifications

First, the passage of samples cause by the occurrence of wrinkles in the existing filter was minimized, and the loss of samples due to circle-shaped silicon O-rings and low watertightness was reduced (Figures 5a and S2). Circular silicone O-rings were prone to microplastic attachment and loss. The second improvement changed the overall support cross and sealing shape of the cassette so that the filter was positioned inside the gasket. Therefore, the filter wrinkling phenomenon was further prevented as shown in Table 4. The height difference due to filter crumpling disappeared via improvements in the sampling device (Table 4, Figure S2), and watertightness was improved to prevent the sample from passing through the side of the filter; this was verified by the 3.3 size distribution. Furthermore, a support structure was added to the cassette to prevent the wrinkling of the filter and to divide the sampling sites for analysis (Figure 5b). Finally, the upper space of the device was increased by improving the flow inside the device to weaken turbulence and flow velocity (Figure 5c).

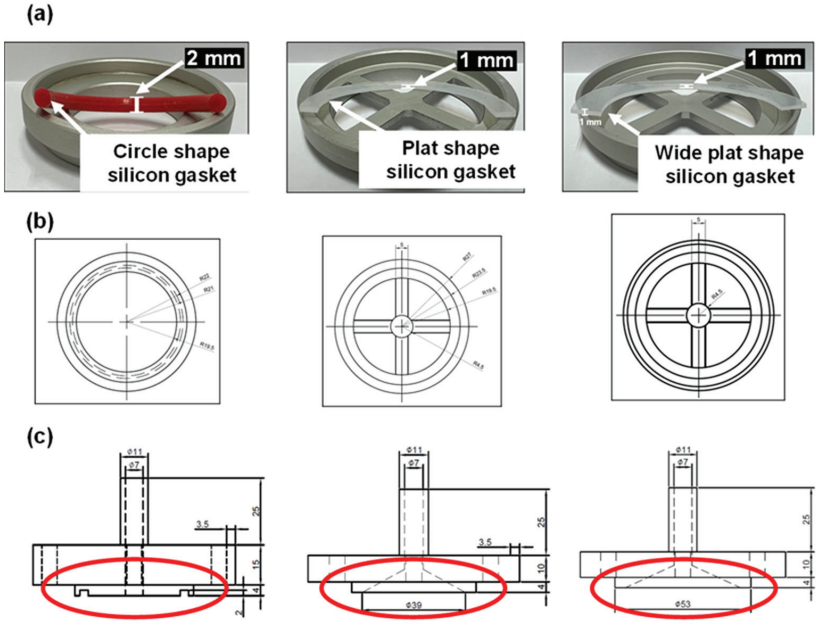

Figure 5. Device modifications: (**a**) sealing silicon shape and methods, (**b**) housing upper shape and space, (**c**) cassette cross support.

Table 4. Filter height change in STS filter after filtration. (Average and standard deviation value of four side of filter height).

	Device 1	Device 2	Device 3
Filter height (mm)	0.30 ± 0.14	0.13 ± 0.05	0.00 ± 0.00

Height reduction: Device 1 to 2 was **56.7%**, Device 2 to 3 was **100%**.

3.2. Changes in Flow Velocity and Turbulence in the Device

Figure 6 shows the average flow velocity (Superficial velocity to filtration direction; top to down) via—the sampling devices. Device 1 and 2 showed a relatively low flow velocity compared to Device 3 in the cases of XX and FX. This indicated that head loss in Device 3 was low compared to the other devices due to its structure. Device 3 had a relatively larger volume in the space between the cassette and the housing (Figures 2, 5 and 7). On the other hand, in Device 1, the water flow was obstructed due to the sudden expansion at the end of the inlet pipe, and the flow between the filter and the top of the housing was restricted due to the very narrow space between the cassette and the housing. As a result, the horizontal flow over the filter was restricted, and rapid vortices and turbulences were formed, intensifying the flow toward the center of the filter. CFD simulation results also supported this, as shown in Figure 8. In Device 1, unlike the other cases, strong turbulence formation could be seen at the outer edge of the central inlet pipe. The flow velocity distribution on the filter surface also showed strong fluctuations in Device 1 as shown in Figure 9.

This structural feature of Device 1 could lead to two poor outcomes as a sampling device. One outcome is that microplastics tend to be pushed out of the center of the filter and accumulate on the sides due to the concentrated central flow (Figure S2). Furthermore, the lateral flow with strong vortices forces the microplastics into contact with the housing walls O-ring and increases the risk of microplastic loss during the sampling process. Another outcome is the deformation of the filter caused by a centralized flow. The relatively unbalanced flow applies a strong vertical force to the center of the filter, which causes filter

deformation, as shown in Table 4. In addition, in the case of Device 1, such deformation may be strongly formed due to the absence of a support under the filter. If the filter is deformed in this way, it may make it difficult to handle and increase errors in the analysis.

Meanwhile, the reverse flow at the bottom of the filter was confirmed in the CFD simulation as shown in Figure 8d–f. This reverse flow, which appeared as a rapid change in flow in a narrow space, was believed to decrease the filtration rate and increase resistance, as shown in Figure 7. Devices 2 and 3 had similar structural characteristics, but Device 3 had a larger space above and below the filter and a wider filter support. This structural difference seemed to weaken the reverse flow from the bottom to the top of the filter, as shown in Figure 8e,f. As a result, the flow in the cassette was relatively stable in Device 3, and the filtration stability was relatively secure. This flow stability may affect high recovery during the sampling.

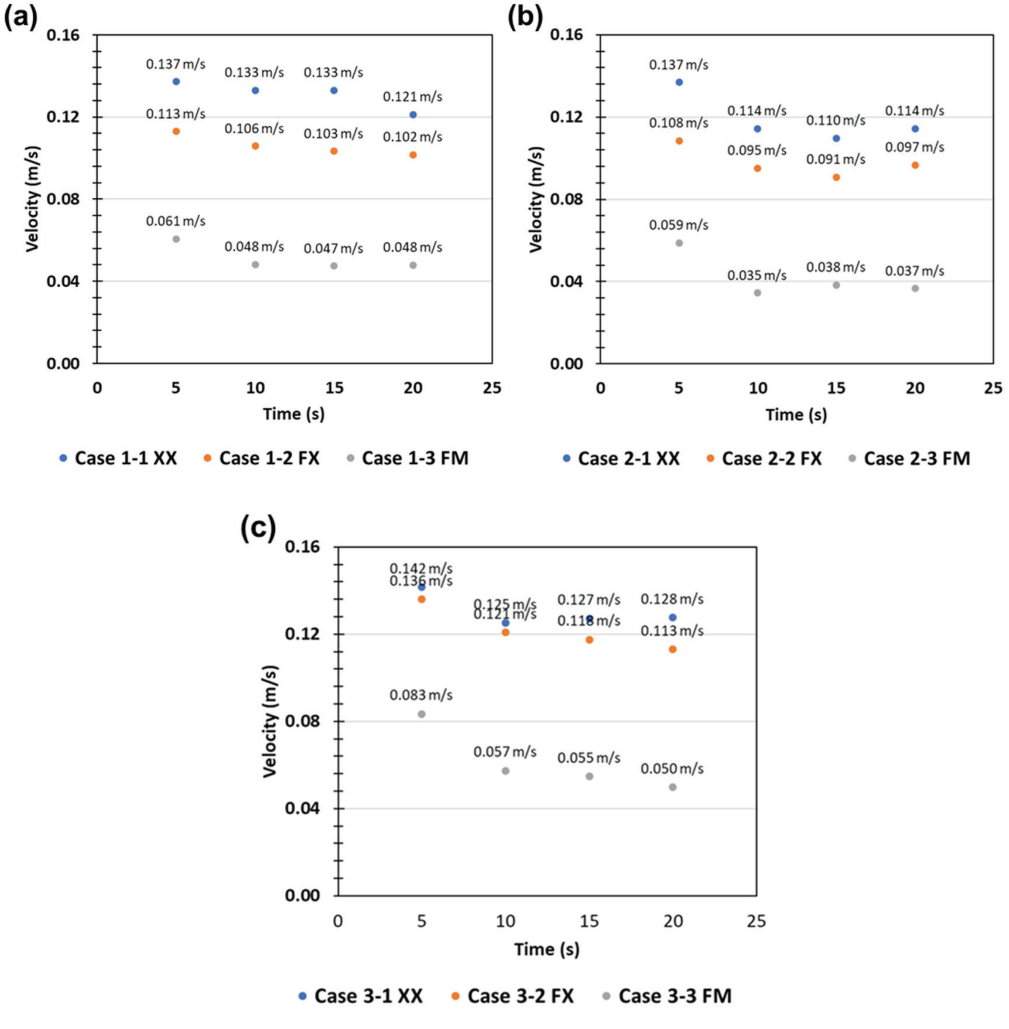

Figure 6. Average water flow velocity through sampling devices. (**a**) Device 1, (**b**) Device 2, and (**c**) Device 3. Symbols in this graph represent the case as follows—XX: without filter and microplastics, FX: equipped filter, FX: equipped filter with microplastic in water flow.

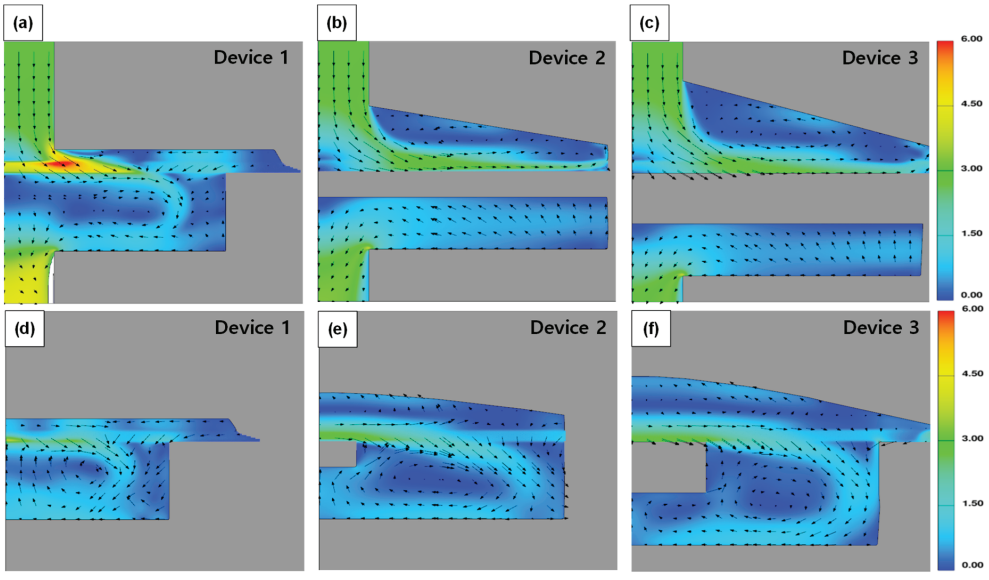

Figure 7. Velocity magnitude contour and vectors of sampling devices. (**a**–**c**) show cross-sectional view of the center of the devices. (**d**–**f**) show cross-sectional view at the half point from the center to edge.

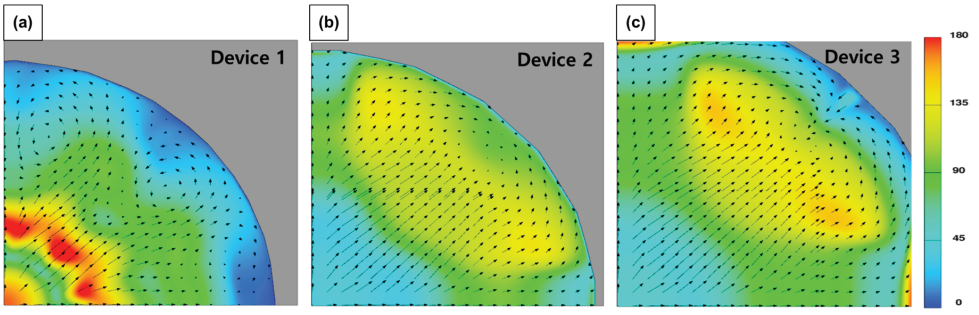

Figure 8. Turbulence intensity contour and vectors on the surface of the filter: (**a**) Device 1, (**b**) Device 2, and (**c**) Device 3.

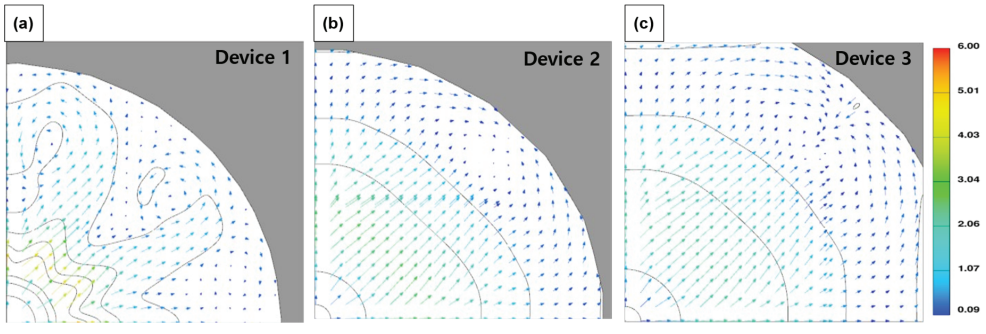

Figure 9. Velocity magnitude vectors on the surface of filter: (**a**) Device 1, (**b**) Device 2, and (**c**) Device 3.

3.3. Size Distribution of Filtered Reference Microplastics with Device

The size classification was performed by applying a three-stage filter to the sampling device, where the pores of the STS filter were 1, 20, and 45 μm in size. Three stages were applied to classify against the minimum particle size of the injected reference material. In the size distribution experiments, a mixture of the two size range reference materials was used. The average particle diameter and cumulative diameter of the reference material filtered through Device 3 are listed in (Tables 4, S5 and S6). Microplastics with an average target diameter could be sorted using multi-stage filtration. It is confirmed that the median particle size of microplastics present in the filtered filter increased according to the filter pores installed in Device 3 and was separated by filter pores. However, when the large amount of reference material was injected (over 30 mg), the small size of particles could not enter the next stage due to the fouling of the pores in the first stage. Therefore, the median particle size of some stages equipped with fouled filter, showed smaller than the installed filter pore size (Table 5). Microplastics of several microns present in the environment were difficult to analyze using microscopy and thermal analysis. Therefore, in this study, a relatively large amount of microplastics were used to evaluate the size classification and recovery rate of the device. Thus, fouling occurred in the size classification result. However, since the pretreated microplastic samples from the environments were present in a range of several micrograms [25,31–34], the fouling phenomenon of the filter was expected to be significantly reduced.

Table 5. Median (d 50%) value results of size distribution.

	3rd Stage	2nd Stage	1st Stage
STS filter pore size	5 μm	20 μm	45 μm
PE	19.2	28.3	61.0
PET	12.0	14.8	77.8
PS	14.9	23.9	105.9
PP	13.8	16.1	24.1
PVC	14.8	17.6	60.0

3.4. Microplastic Recovery Results of the Sampling Device

The final recovery rate of Device 3 was 94.2%, and the overall recovery rate of microplastics showed an increasing trend as the device improved (Figure 10). In the case of Device 3, a recovery rate of over 90% was confirmed for all plastics. A comparison of recovery rates found in previous studies that used the same encapsuled method is shown in Table 6. The developed device had the highest recovery rate. This rate increased when the loss in the device where the sample comes into contact with CFD was confirmed and improved.

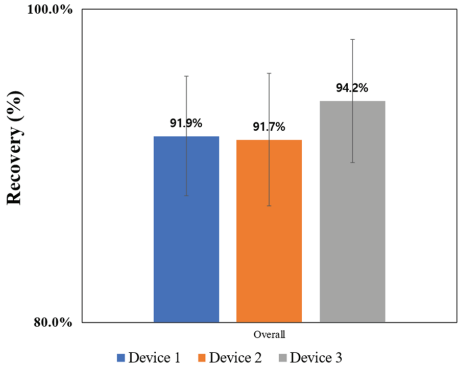

Figure 10. Overall microplastic recovery of the sampling devices.

Table 6. Recovery rate of encapsuled methods.

	Yuan et al. [35]	Harrold et al. [36]	This Study
Recovery rate (%)	80.2	88	94.2
Size range of references materials (μm)	125–150	5–296	5–30

Recovery rate experiment of pretreatment process was performed with Device 3 which has the highest recovery rate. The recovery rate of wet peroxide oxidation and density separation was at least 89.5% (Table 7). Thus, a high efficiency was confirmed even when sampling and pre-processing devices were used, as opposed to the experimental tools and equipment used in the previous study. Additionally, our proposed device showed less cross-contamination than was observed in other studies (Table 8). In the case of negative blank samples, 4–17 and 0–6 contaminating particles smaller than 20 μm appeared during the sampling and pretreatment processes, respectively.

Table 7. Recovery rate of pretreatment.

No.	Species	Recovery Rate of WPO (%)	Recovery Rate of Density Separation (%)
1	PP	91.4	98.8
2	PS	94.3	98.0
3	PET	99.5	89.5
4	PVC	97.0	99.7
5	PE	92.9	98.1

Table 8. Results regarding the blank sample subjected to the sampling device and pretreatment process.

No.	Concentration of MPs	Phase	Control	Method	Ref.
1	0.172 particles/L	Water	X	Encapsuled	[37]
2	91–141 particles	Procedure blank	X		[26]
3	* 5–9 particles	Air	O	Passive air	[38]
4	31 particles	Water	O	Manta net	[20]
5	** 4–7 particles	Air and water	O	Encapsuled	The current
6	** 0–6 particles	Pretreatment	O	Encapsuled	study

* Over 50 μm. ** Under 25 μm.

For the encapsuled method used in this study, the amount of sample taken per sample was smaller than other methods. This limitation was improved by replacing the filter between samples, but this could increase the probability of contamination due to increased external exposure of samples. As a result, relatively small size and small amount of plastic contamination still occurred. In addition, since pump power is still required during sampling, an electrical device or sample transfer process is essential. Applying high-temperature treatment and using negative-pressure laboratories, methods employed in other studies, will solve the problem of pollution [17].

The smaller the microplastics, the more easily they can enter the human body and the greater risk they pose [39]. However, a new and cost-effective filter has a minimum pore size of 1 μm, and it is difficult to analyze microplastics at a nanoscale using spectroscopic analysis or thermal analysis. Therefore, the development and improvement of a device for analyzing microplastics of 10 μm or less will be helpful for tracking and analyzing microplastics, provided a suitable filter is used.

4. Conclusions

We proposed a microplastic sampling device and pretreatment process that excludes plastic from the entire process. In this study, using a highly efficient encapsulation method of sampling, sample loss and external contamination were minimized, and a device capable of classification by size was developed. The device was evaluated by using five types of reference microplastics. While carrying out structural modifications to the device, a CFD analysis was performed to identify the shape and flow velocity of turbulence that may occur during sampling. It was confirmed that the efficiency, contamination, minimization of loss, and the flow of fluids were made more suitable for the sampling via a structural improvement in the device. In addition, the minimization of the loss of microplastics contained in a sample has been verified by an experiment of recovery rates depending on weight of the used filter. As a result of the modification, a recovery rate of over 94% was confirmed, which is higher than the previous studies. Therefore, it could be easily applied by separating microplastics from different environmental samples and collecting microplastic samples of desired size. In conclusion, the proposed device can overcome existing limitations caused by contaminants generated during the pretreatment process and can prepare for high-quality microplastic analysis with a high recovery rate.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15053907/s1, Table S1: CFD analysis conditions for the sampling devices; Table S2: Recovery results using Device 1 with reference material; Table S3: Recovery results using Device 2 with reference material; Table S4: Recovery results using Device 3 with reference material; Table S5: Size distribution 10% value size (μm); Table S6: Size distribution 90% value size (μm); Figure S1: SEM image of STS filter (a) before and (b) after filtration; Figure S2: Visual results after filtration using the STS filter in (a) Device 1, (b) Device 2, (c) Device 3.

Author Contributions: Conceptualization, Y.Y. and S.J.; methodology, S.J.; software, J.Y., J.L. (Jihoon Lee); validation, J.Y., J.L. (Junho Lee) and J.L. (Jihoon Lee); formal analysis, K.K.; resources, C.L.; data curation, K.K.; writing—original draft preparation, J.L. (Junho Lee) and S.J.; writing—review and editing, S.J., J.Y.; visualization, J.K.; supervision, Y.Y.; project administration, Y.Y.; funding acquisition, Y.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Korea Environment Industry & Technology Institute (KEITI) through the Measurement and Risk Assessment Program for Management of Microplastics Project funded by the Korea Ministry of Environment (MOE) (Grant Number 2020003110005).

Institutional Review Board Statement: Not Applicable.

Informed Consent Statement: Not Applicable.

Data Availability Statement: All data used in this study are public.

Acknowledgments: Microplastics reference materials were provided by Korea Testing & Research Institute (KTR).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ma, Y.; Huang, A.; Cao, S.; Sun, F.; Wang, L.; Guo, H.; Ji, R. Effects of nanoplastics and microplastics on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water. *Environ. Pollut.* **2016**, *219*, 166–173. [CrossRef] [PubMed]
2. Ziccardi, L.M.; Edgington, A.; Hentz, K.; Kulacki, K.J.; Kane Driscoll, S. Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: A state-of-the-science review. *Environ. Toxicol. Chem.* **2016**, *35*, 1667–1676. [CrossRef] [PubMed]
3. Dawson, A.; Huston, W.; Kawaguchi, S.; King, C.; Cropp, R.; Wild, S.; Eisenmann, P.; Townsend, K.; Bengtson Nash, S. Uptake and Depuration Kinetics Influence Microplastic Bioaccumulation and Toxicity in Antarctic Krill (Euphausia superba). *Environ. Sci. Technol.* **2018**, *52*, 3195–3201. [CrossRef] [PubMed]
4. Miller, M.E.; Hamann, M.; Kroon, F.J. Bioaccumulation and biomagnification of microplastics in marine organisms: A review and meta-analysis of current data. *PLoS ONE* **2020**, *15*, e0240792. [CrossRef]
5. Lohmann, R. Microplastics are not important for the cycling and bioaccumulation of organic pollutants in the oceans-but should microplastics be considered POPs themselves? *Integr. Environ. Assess Manag.* **2017**, *13*, 460–465. [CrossRef]

6. Zhang, Q.; Xu, E.G.; Li, J.; Chen, Q.; Ma, L.; Zeng, E.Y.; Shi, H. A Review of Microplastics in Table Salt, Drinking Water, and Air: Direct Human Exposure. *Environ. Sci. Technol.* **2020**, *54*, 3740–3751. [CrossRef] [PubMed]
7. Gasperi, J.; Wright, S.L.; Dris, R.; Collard, F.; Mandin, C.; Guerrouache, M.; Langlois, V.; Kelly, F.J.; Tassin, B. Microplastics in air: Are we breathing it in? *Curr. Opin. Environ. Sci. Health* **2018**, *1*, 1–5. [CrossRef]
8. Castañeda, R.A.; Avlijas, S.; Simard, M.A.; Ricciardi, A.; Smith, R. Microplastic pollution in St. Lawrence River sediments. *Can. J. Fish. Aquat. Sci.* **2014**, *71*, 1767–1771. [CrossRef]
9. Guo, J.J.; Huang, X.P.; Xiang, L.; Wang, Y.Z.; Li, Y.W.; Li, H.; Cai, Q.Y.; Mo, C.H.; Wong, M.H. Source, migration and toxicology of microplastics in soil. *Environ. Int.* **2020**, *137*, 105263. [CrossRef]
10. McCormick, A.; Hoellein, T.J.; Mason, S.A.; Schluep, J.; Kelly, J.J. Microplastic is an abundant and distinct microbial habitat in an urban river. *Environ. Sci. Technol.* **2014**, *48*, 11863–11871. [CrossRef]
11. Baldwin, A.K.; Spanjer, A.R.; Rosen, M.R.; Thom, T. Microplastics in Lake Mead National Recreation Area, USA: Occurrence and biological uptake. *PLoS ONE* **2020**, *15*, e0228896. [CrossRef] [PubMed]
12. Weinstein, J.E.; Crocker, B.K.; Gray, A.D. From macroplastic to microplastic: Degradation of high-density polyethylene, polypropylene, and polystyrene in a salt marsh habitat. *Environ. Toxicol. Chem.* **2016**, *35*, 1632–1640. [CrossRef] [PubMed]
13. El Hadri, H.; Gigault, J.; Maxit, B.; Grassl, B.; Reynaud, S. Nanoplastic from mechanically degraded primary and secondary microplastics for environmental assessments. *NanoImpact* **2020**, *17*, 100206. [CrossRef]
14. Wang, C.; Xian, Z.; Jin, X.; Liang, S.; Chen, Z.; Pan, B.; Wu, B.; Ok, Y.S.; Gu, C. Photo-aging of polyvinyl chloride microplastic in the presence of natural organic acids. *Water Res.* **2020**, *183*, 116082. [CrossRef] [PubMed]
15. Yang, H.; Xiong, H.; Mi, K.; Xue, W.; Wei, W.; Zhang, Y. Toxicity comparison of nano-sized and micron-sized microplastics to Goldfish Carassius auratus Larvae. *J. Hazard Mater.* **2020**, *388*, 122058. [CrossRef] [PubMed]
16. Filella, M. Questions of size and numbers in environmental research on microplastics: Methodological and conceptual aspects. *Environ. Chem.* **2015**, *12*, 5. [CrossRef]
17. Prata, J.C.; da Costa, J.P.; Duarte, A.C.; Rocha-Santos, T. Methods for sampling and detection of microplastics in water and sediment: A critical review. *TrAC Trends Anal. Chem.* **2019**, *110*, 150–159. [CrossRef]
18. Lenz, R.; Labrenz, M. Small Microplastic Sampling in Water: Development of an Encapsulated Filtration Device. *Water* **2018**, *10*, 1055. [CrossRef]
19. Wang, W.; Wang, J. Investigation of microplastics in aquatic environments: An overview of the methods used, from field sampling to laboratory analysis. *TrAC Trends Anal. Chem.* **2018**, *108*, 195–202. [CrossRef]
20. Tamminga, M.; Stoewer, S.C.; Fischer, E.K. On the representativeness of pump water samples versus manta sampling in microplastic analysis. *Environ. Pollut.* **2019**, *254 Pt A*, 112970. [CrossRef]
21. Mu, J.; Zhang, S.; Qu, L.; Jin, F.; Fang, C.; Ma, X.; Zhang, W.; Wang, J. Microplastics abundance and characteristics in surface waters from the Northwest Pacific, the Bering Sea, and the Chukchi Sea. *Mar. Pollut. Bull.* **2019**, *143*, 58–65. [CrossRef] [PubMed]
22. Liu, K.; Zhao, Y.; Jia, L.; Hao, R.; Fu, D. A novel CFD-based method for predicting pressure drop and dust cake distribution of ceramic filter during filtration process at macro-scale. *Powder Technol.* **2019**, *353*, 27–40. [CrossRef]
23. Wang, Q.; Liu, Y.; Huang, A.; Yan, W.; Gu, H.; Li, G. CFD investigation on influence of orifice geometry on micro-scale inclusion movement. *Powder Technol.* **2020**, *367*, 358–375. [CrossRef]
24. Gwinnett, C.; Miller, R.Z. Are we contaminating our samples? A preliminary study to investigate procedural contamination during field sampling and processing for microplastic and anthropogenic microparticles. *Mar. Pollut. Bull.* **2021**, *173 Pt B*, 113095. [CrossRef]
25. Woodall, L.C.; Gwinnett, C.; Packer, M.; Thompson, R.C.; Robinson, L.F.; Paterson, G.L. Using a forensic science approach to minimize environmental contamination and to identify microfibres in marine sediments. *Mar. Pollut. Bull.* **2015**, *95*, 40–46. [CrossRef]
26. Prata, J.C.; Reis, V.; da Costa, J.P.; Mouneyrac, C.; Duarte, A.C.; Rocha-Santos, T. Contamination issues as a challenge in quality control and quality assurance in microplastics analytics. *J. Hazard Mater.* **2021**, *403*, 123660. [CrossRef]
27. De Frond, H.; Thornton Hampton, L.; Kotar, S.; Gesulga, K.; Matuch, C.; Lao, W.; Weisberg, S.B.; Wong, C.S.; Rochman, C.M. Monitoring microplastics in drinking water: An interlaboratory study to inform effective methods for quantifying and characterizing microplastics. *Chemosphere* **2022**, *298*, 134282. [CrossRef] [PubMed]
28. Hermsen, E.; Mintenig, S.M.; Besseling, E.; Koelmans, A.A. Quality Criteria for the Analysis of Microplastic in Biota Samples: A Critical Review. *Environ. Sci. Technol.* **2018**, *52*, 10230–10240. [CrossRef]
29. Koelmans, A.A.; Mohamed Nor, N.H.; Hermsen, E.; Kooi, M.; Mintenig, S.M.; De France, J. Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. *Water Res.* **2019**, *155*, 410–422. [CrossRef]
30. Coppock, R.L.; Cole, M.; Lindeque, P.K.; Queiros, A.M.; Galloway, T.S. A small-scale, portable method for extracting microplastics from marine sediments. *Environ. Pollut.* **2017**, *230*, 829–837. [CrossRef]
31. Zhu, J.; Wang, C. Recent advances in the analysis methodologies for microplastics in aquatic organisms: Current knowledge and research challenges. *Anal. Methods* **2020**, *12*, 2944–2957. [CrossRef] [PubMed]
32. Plee, T.A.; Pomory, C.M. Microplastics in sandy environments in the Florida Keys and the panhandle of Florida, and the ingestion by sea cucumbers (Echinodermata: Holothuroidea) and sand dollars (Echinodermata: Echinoidea). *Mar. Pollut. Bull.* **2020**, *158*, 111437. [CrossRef] [PubMed]

33. Akanyange, S.N.; Lyu, X.; Zhao, X.; Li, X.; Zhang, Y.; Crittenden, J.C.; Anning, C.; Chen, T.; Jiang, T.; Zhao, H. Does microplastic really represent a threat? A review of the atmospheric contamination sources and potential impacts. *Sci. Total Environ.* **2021**, *777*, 146020. [CrossRef] [PubMed]
34. Li, J.; Liu, H.; Paul Chen, J. Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water Res.* **2018**, *137*, 362–374. [CrossRef] [PubMed]
35. Hale, R.C.; Seeley, M.E.; La Guardia, M.J.; Mai, L.; Zeng, E.Y. A Global Perspective on Microplastics. *J. Geophys. Res. Ocean.* **2020**, *125*, e2018JC014719. [CrossRef]
36. Yuan, C.; Almuhtaram, H.; McKie, M.J.; Andrews, R.C. Assessment of microplastic sampling and extraction methods for drinking waters. *Chemosphere* **2022**, *286 Pt 3*, 131881. [CrossRef]
37. Harrold, Z.; Arienzo, M.M.; Collins, M.; Davidson, J.M.; Bai, X.; Sukumaran, S.; Umek, J. A Peristaltic Pump and Filter-Based Method for Aqueous Microplastic Sampling and Analysis. *ACS EST Water* **2022**, *2*, 268–277. [CrossRef]
38. Wright, S.L.; Kelly, F.J. Plastic and Human Health: A Micro Issue? *Environ. Sci. Technol.* **2017**, *51*, 6634–6647. [CrossRef]
39. Tirkey, A.; Upadhyay, L.S.B. Microplastics: An overview on separation, identification and characterization of microplastics. *Mar. Pollut. Bull.* **2021**, *170*, 112604. [CrossRef]

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Occurrence Characterization and Contamination Risk Evaluation of Microplastics in Hefei's Urban Wastewater Treatment Plant

Xiangwu Meng [1], Teng Bao [1,2,3,*], Lei Hong [1,2,3] and Ke Wu [1,2,3,*]

[1] School of Biology, Food and Environment, Hefei University, Hefei 230601, China
[2] Hefei Institute of Environmental Engineering, Hefei University, Hefei 230601, China
[3] Collaborative Innovation Center for Environmental Pollution Control and Ecological Restoration of Anhui Province, Hefei 230601, China
* Correspondence: tengbao1222@sina.com (T.B.); wuke@hfuu.edu.cn (K.W.)

Abstract: As one of the primary nodes in the flow of micro-plastics (MPs) in the environment, it is critical to examine and assess the Sewage Treatment, occurrence, and removal of MPs in waste treatment plant (WWTP). This research explored the shape, size, and composition of MPs at various stages of the WWTP process in the south of the city of Hefei, China, in dry and rainy weather conditions, as well as the removal effectiveness of MPs in a three-stage process. The collected MPs were quantitatively and qualitatively examined using an Osmosis electron microscope and micro-FTIR. The pollution risk of MPs in WWTP was assessed using the EU classification, labelling and packaging (CLP) standard and the pollution load index (PLI). The findings revealed that the average abundance of fibrous MPs was greatest in WWTP sewage and sludge, 49.3% and 39.7% in dry weather, and 50.1% and 43.2% in rainy weather, respectively. The average distribution of MPs in the 0–500 μm range was highest in WWTP wastewater and sludge, 64.9% and 60.4% in dry weather and 67.9% and 69.0% in rainy weather, respectively. Finally, the overall removal rate was 87.7% and 83.5%. At the same time, it has been demonstrated that MPs with varied compositions are strongly tied to human activities, and environmental conditions (such as rainy weather) also influence their source. In both dry and wet weather, the amount of polymers and the risk score were linked to the pollution risk of MPs in WWTP. In wet weather, the MPS pollution index was more variable. The pollution indices of MPs in row water and tail water were 2.40 and 2.46, respectively, which were heavily contaminated, and 1.0 and 1.2, which were moderately polluted. MPs in dewatered sludge had severely polluted indexes of 3.5 and 3.4, respectively. As a result, there is still MPs efflux or buildup in sludge during and after the WWTP process, which presents an ecological contamination concern.

Keywords: microplastics; wastewater treatment plant; dry and rainy weather; occurrence characteristic; pollution risk evaluation

Citation: Meng, X.; Bao, T.; Hong, L.; Wu, K. Occurrence Characterization and Contamination Risk Evaluation of Microplastics in Hefei's Urban Wastewater Treatment Plant. *Water* **2023**, *15*, 686. https://doi.org/10.3390/w15040686

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

Received: 4 January 2023
Revised: 2 February 2023
Accepted: 5 February 2023
Published: 9 February 2023

1. Introduction

Plastic is an organic polymer material that is extensively utilized in insulation, metal substitution, packaging, clothing, and other applications. By 2020, China's annual production of plastic products had reached 76.032 million tonnes, with 74.1 million tonnes of garbage, 30% of which was recycled, 32% disposed of in landfills, 31% burned, and roughly 7% lost. In Europe, the total quantity of recycled plastics is 4.6 million tonnes per year, with a 35% recycling rate [1,2]. Microplastics, MPs are a new category of pollutant described as plastic particles with a particle size of less than 5 mm [3]. Primary plastics and secondary plastics are the major sources: primary plastics are mostly formed by grinding particles and plastic beads included in industrial raw materials and cosmetics, as well as the loss of synthetic fibers in clothing caused by washing, etc. [4,5]. Secondary plastics are primarily created by photocatalysis, weathering, embrittlement, and cracking

of large abandoned plastics in the presence of light, water, and wind, which leads to the development of smaller microplastic particles [6,7].

Early MP research primarily focused on the distribution and movement of marine, lake, and river MPs, as well as the source, abundance, and biological toxicity of MPs. It has been documented that plastics distributed in the ocean are more readily broken down into microplastics by photocatalysis and water flow, and that tidal movement can reflow microplastics deposited in the ocean back into the freshwater environment [8]. MPs in the environment have been discovered to operate as a carrier for microorganisms such as pathogens [9], adsorbing organic substances (antibiotics, phthalate, Polycyclic aro-matic hydrocarbon, etc.), and heavy metals [10–13]. Microorganisms can colonise the surface of MPs and create biofilms, transferring a range of bacteria, including dangerous ones, to new environments [14]. Its potential ecotoxicity can be transmitted through the food chain [15–17], is easily acquired by zooplankton and higher animals such as humans, and accumulates in organisms, disrupting the flow of energy within organisms and posing a growth threat [18], even causing biological death [19], with the potential for irreversible risk. Furthermore, MPs has been found in human blood and faeces, as well as in the maternal placenta [20–23]. The primary sources of MPs in the body include daily consumption of table salt, bottled water, tap water, and seafood [24–26], whereas air-exposed microplastics may also be absorbed by inhaling airflow [27,28]. With the potential for ecotoxicity and far-reaching effects on the natural environment and human health, identifying pollution sources is critical for evaluating the pollution risks of MPs and establishing mitigation solutions. As a consequence, the Sewage Treatment monitoring of the whole MPs process was employed as a pilot study to determine the contamination risk.

The primary point at which MPs reach the natural environment from the urban water system is sewage treatment [29,30]. Sherri et al. examined 90 samples from 17 phases of the US sewage treatment process and discovered that up to 4 million MPs particles are still discharged into the natural environment per day following regular sewage treatment [31]. Xu et al. evaluated 11 sewage treatment plants in Changzhou, China, and discovered that the average concentration of MPs in the influent and effluent was 196.00 ± 11.89 n/L and 9.04 ± 1.12 n/L, respectively. The average removal rate of MPs was over 90%, with the highest percentage being 97.15% [32]. Zhang et al. assessed an MPs removal rate of 93.7% in the entering and departing water of the Turkish Sewage Treatment [33]. However, most of the MPs removed by the sewage treatment process are transferred and stored in sludge, and Esther et al., in their study of the WWTP in Vancouver, Canada, found that $1.76 \pm 0.31 \times 10^{12}$ MPs accumulate in the sewage treatment each year, of which, $(1.28 \pm 0.54) \times 10^{12}$ MPs settled into the primary sludge, $(0.36 \pm 0.22) \times 10^{12}$ MPs into the secondary sludge, and $(0.03 \pm 0.01) \times 10^{12}$ MPs were released into the natural environment [34]. Furthermore, Kay et al. reported that MPs abundance in river basins may increase with atmospheric deposition or agricultural soil infiltration in a study of MPs hosted upstream and downstream in six Sewage Treatment of distinct river basins [35]. Plastic film, microfibers, and inappropriately disposed of waste plastics used in agriculture and industry, on the other hand, degrade into fine plastic particles by a sequence of synergistic photocatalysis and physicochemical degradation processes [6,7,36]. These particles are extensively spread in the urban surface environment and in atmospheric flotsam, and may enter the sewage network when rainfall washes them away [37], which may then be transported to the Sewage Treatment, resulting in an increased treatment load [12,38,39]. The load of MPs, a frequent Persistent organic pollutant in WWTP such as Polycyclic aromatic hydrocarbon, would grow with increased rainfall [40], which would spread the degree of environmental contamination to some extent.

There is currently limited research on the evaluation of MPs pollution risk in urban WWTPs in Hefei City. This paper investigated the form, size, and composition features of MPs in each typical process step of a WWTP under two weather conditions, dry and rain, as well as the removal effectiveness of MPs in the three-stage treatment stage. The pollution risk of MPs in WWTP is analysed using the EU classification, labelling and packaging

(CLP) standard and the PLI Pollution load index model. This report serves as a reference for future MPs reductions in China's inland cities' WWTP.

2. Materials and Experimental Methods

2.1. Sample Sites

In this study, samples of sewage and sludge were collected from Hefei, China's Sewage Treatment in the city's southern region. The WWTP is equipped with an (Anaerobic-AnoxicOxic process, A^2O) with a daily capacity of 100,000 tonnes. The purified sewage is disposed of in the 15li River. Due to the fact that water quality and quantity indicators of the Sewage Treatment may vary depending on the weather, samples were obtained in July 2022 under dry (marked D) and rainy (marked R) conditions. Figure 1 and Table 1 showcases grid intakes (Row water, labeled D1 and R1), grid outlets (labeled D2 and R2), aeration grit chamber outlets (labeled D3 and R3), oxidation ditch outlets (labeled D4 and R4), in the secondary clarifier (D5 and R5), the outlet of the secondary clarifier (D6 and R6), the outlet of the denitrification deep bed filter (Tail water, D7 and R7). The sludge samples are dewatered sludge from the sludge pumping station (D8 and R8).

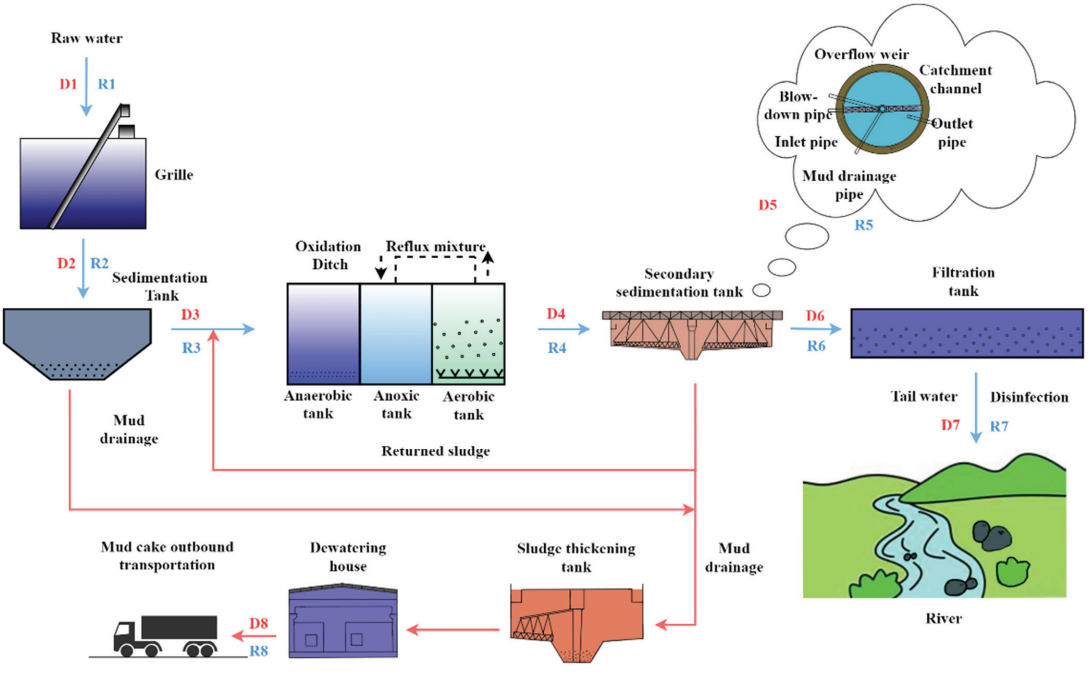

Figure 1. Wastewater treatment plant process flow design.

2.2. Sample Methods

The quantitative collection of MPs at the Sewage Treatment sample point is performed using a peristaltic pump with a custom stainless steel hopper connected to the pump's intake (20 mm inner diameter of the inlet) and a mesh (5 mm mesh aperture) covering the funnel mouth. Place the pump input pipe with a custom hopper 30 cm below the surface of the sewage for collecting samples, and store the sewage from the pump outlet pipe (10 L of sewage from each sample point) in 15 L stainless steel drums. For sludge samples, the dewatered sludge from the sludge pumping station (75–79% water content) was collected by wrapping 1 Kg samples of sludge in aluminium foil paper and placing them on a conveyor belt. Collect 1 Kg of sludge samples from three distinct points on the conveyor belt, combine the samples, and store them in a 5 L stainless steel drum. In addition, the Sewage Treatment

flow varies in real-time, and the temporal disparities between certain samples generate a data flow, resulting in variations in MPs' features(Table 2). Therefore, samples were collected at each sampling station at three separate times during the day (08:30–11:30, 13:30–16:30, 18:30–21:30), with Sewage Treatment flow sufficient for peak phases. After transferring the sample to the container and rinsing the collector with deionized water, the next collection activity may begin. The edges of the container's top lid are covered with aluminium foil before being returned to the laboratory. For testing and analysis, the container is placed in a refrigerated environment at 4 °C.

Table 1. WWTP process section layout design and description.

Sample Points D (Dry Weather), R (Rainy Weather)	Layout Design Description
Raw water or Grille Front (D1, R1)	Initial wastewater from WWTP. Sewage samples are collected at the grate.
After grille (D2, R2)	The first physical interception processing. Sewage samples are collected behind the grate.
After sedimentation Tank (D3, R3)	Gravity deposition of high-density contaminated impurities separates low density suspended matter to the next process. The sewage samples were collected after sand-settling.
After oxidation Ditch (D4, R4)	(Anaerobic-Anoxic-Aerobic) to maintain the flow of mixed sewage and activated sludge, initial removal of suspended substances. Sewage samples are collected behind the oxidation ditch.
Inside the secondary sedimentation tank (D5, R5)	The flow velocity and amount of water affect the cross-section of rainwater, and the suspended matter rises. Sewage samples are collected in the secondary sedimentation tank.
After secondary settling tank (D6, R6)	The mud and water are separated and the suspended impurities form flocculates and sink together. Sewage samples are collected after the secondary sedimentation tank is selected.
Tail water or After filtration tank (D7, R7)	WWTP outflow tail water. Sewage samples are collected after the denitrification filter.
Dehydrated sludge (D8, R8)	After being dehydrated by an enrichment centrifuge. Sludge samples are collected on the conveyor belt.

Table 2. Removal rate of MPs by tertiary treatment in WWTP.

Treatment Phase	Arrange	MPs Abundance (n/L)	Removal Efficiency (%)	Total Removal Rate of MPs (%)
Primary processing	D1 to D3, R1 to R3	101.9 ± 17.6 to 51.0 ± 7.3, 108.7 ± 20.1 to 81.2 ± 10.8	62.9%, 70.4%	87.7% (D), 83.5% (R)
Secondary treatment	D4 to D6, R4 to R6	71.9 ± 15.3 to 44.2 ± 5.5, 87.4 ± 21.3 to 53.6 ± 7.4	55.6%, 57.5%	
Tertiary or Advanced treatment	D6 to D7, R6 to R7	44.2 ± 5.5 to 18.2 ± 3.6, 53.6 ± 7.4 to 26.3 ± 5.1	44.9%, 34.6%	

2.3. Experimental Scheme

2.3.1. MPs Separation and Extraction

In the Pretreatment Experiment (Figure 2), 1 L of sewage and 10 g of dry sludge were collected to assess the properties of MPs in various WWTP process structures. Coarse filtration was primarily performed on sewage samples utilizing stacked layers of stainless steel screens with pore sizes of 4 mesh (5 mm), 18 mesh (1 mm), 600 mesh (25 μm), and 1000 mesh (15 μm) (m1). The deionized water is then transferred to a sand core filter (JOAN LAB, 0.8 μm, 1000 mL, Huzhou, China) for filtration (m2). The coarse filtering screen is rinsed three times in deionized water. Following filtering, the filter membrane (PTFE, LONGJIN, aperture 5 μm, diam 50 mm, Nantong, China) was placed in a beaker containing 75 mL of Fenton reagent, exposed to a digestion reaction (m3), and permitted to stand for 12 h [41]. The residue left on the screen is still impinged on by a pressured water bottle carrying deionized water, which is subsequently filtered (m2) and the filter membrane is also immersed in the digesting solution (m3). To guarantee that the material is transmitted, the digested mixture is filtered (m4) and the Beaker is rinsed frequently with deionized

water after digestion. To initiate density separation [42], the digestion mixture is filtered through a membrane and combined (m5) with 300 mL of a flotation solution (saturated NaCl solution, likewise filtered by a filter). Beakers containing plastic microparticulate membranes were then put on a Magnetic stirrer (HUXI, HMS-203D, Shanghai, China) to expedite material removal from the membrane and shaken at 65 °C and 500 rpm for 24 h (m6). After density flotation, the supernatant was filtered (m7) and the residue in the Beaker was put back into the floatation solution for a second floatation (m6 and m7). This process was done three times. After three cycles of density separation, the three filter films are maintained in glass petri dishes, with the petri dishes coated in aluminium foil to prevent plastic particles from dispersing. For the treatment of the sludge sample, the sludge was positioned flat in a glass Petri dish, and then the Petri dish was placed in an oven where the sludge was dried at 105 °C for 24 h. The ensuing digestion procedures are identical to those for wastewater treatment, except that the mass of the sludge sample and the ratio of the input of the digestion solution are 1 g:30–50 mL (the dose of the digestion solution is determined by the removal rate of the digested material).

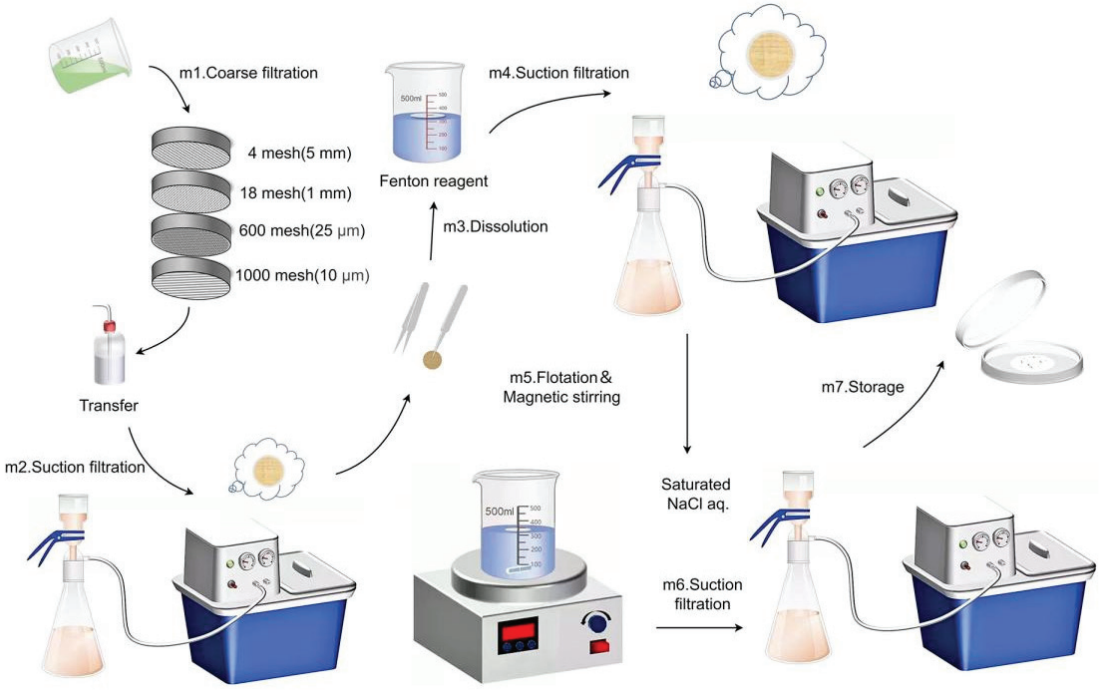

Figure 2. Experimental operation procedures.

2.3.2. Observation and Identification of Microplastics

Using an Osmosis electron microscope (AO-HK830-5870,Shenzhen, China), the morphological properties of putative MPs on a filter membrane were studied. Materials comprising plastic particles were dispersed over highly reflective glass in conjunction with micro-FTIR (Thermo Fisher Scientific Nicolet iN10, Waltham, MA, USA) in the region of 4000–400 cm^{-1} with a spectral resolution of 4 cm^{-1}; An average of 64 scans were recorded [43]. KnowItAl soft-ware (BIORAD Inc., Hercules, CA, USA) was employed. The acquired spectra were compared with those from the Knowitall FTIR library (Bio-Rad Inc.) and the national standard of the People's Republic of China (GB/T 40146-2021, China) to define the polymer type of MP [44,45] based on the distinctive functional groups and peak trend rate. To explore the surface properties of MPs that can adsorb organic pollutants

such as polycyclic aromatic hydrocarbon, a scanning electron microscope (SEM, Hitachi S4801-IM, Japan) was used.

2.3.3. Quantitative Methods for Microplastics

For the characterization and quantification of MPs, reference is made to Pivokonsky et al. technique's of quantitative analysis of MPs by sampling 25% of the circular sector of each filter [46]. The five-point sampling approach was employed for MPs average abundance statistics. First, the central sample point is positioned at the midway of the diagonal on the high reflection glass (24 mm × 50 mm), and four points on the diagonal are determined as sampling sites. The region of interest (ROI) is selected by modifying the facula based on the mode of reflection. The area of the high-reflection glass lens comprises five squares: upper left (U1), upper right (U2), middle (C3), bottom left (D4) and lower right (D5); each quadrat is the same distance from the lens's centre (Tables 3 and 4) [47]. Then, the material on the lenses was infrared scanned individually based on 5 squares selected by a micro-FTIR (Thermo Fisher Scientific Nicolet iN10) surface scan, and the morphological traits and chemical composition of MPs were identified. After that, utilizing Formula (1), the total number of N_m of MPs per litre of sewage or per gram of sludge collected throughout a single WWTP procedure is determined.

$$N_m = \frac{\sum_{i=0}^{5} * N_i * S_m}{5 S_f} \quad (1)$$

In Formula (1), N_i is the number of MPs on each quadrate (n/L), S_m is the contact area of impurities on a single high reflector, and $S_m \approx 9.26$ cm^2. S_f is the area of a single quadrate, and $S_f = 0.84$ cm^2. The length of a quadrate side is 12.5 mm, and its breadth is 6.72 mm.

2.3.4. Experimental Quality Control

All containers and devices for collecting and storing MPs are composed of stainless steel or quartz glass. Clean the container several times with deionized water before sample and keep it sealed. Furthermore, using a pure cotton lab coat and nitrile gloves while sampling and experimenting is necessary to eliminate the shedding of fibers from textiles and clothing, which increases the exceptional amount of MPs. All of the containers are sealed with aluminum foil because, during the oxidation and exothermic digesting processes, plastic particles with increasing water vapour may be adsorbed on the foil, requiring immediate attention to avoid loss. MPs samples were collected in a confined clean room for microscopic and infrared spectroscopy examination.

2.3.5. Data Analysis

For data preprocessing and analysis, Microsoft Excel 2019 was used, SPSS 26.0 (IBM Co., Ltd., Armonk, NY, USA) for data correlation analysis, and Origin 2018 (OriginLab, Farmington, ME, USA) and Microsoft Visio 2016 for data analysis and charting. The abundance of MPs particles in this sewage treatment is expressed as the mean standard error. The MPs abundances of the Sewage Treatment samples collected during the three time periods were measured at a single process stage, and the average of the MPs abundances of the samples collected during the three time periods was taken as the range of final considerations for MPs in a single process stage. Furthermore, the data examined the MPS removal effectiveness of WWTP stages 1, 2, and 3, as well as the whole process from raw water to tail water. The findings demonstrate that: Removal Efficiency (%) is the removal efficiency of three stages, Total removal rate of MPs (%) is the total removal rate for the whole process stage. Sections 2.4 and 3.4, Tables 5 and 6 analyse the ecological risk coefficient and the assessment of pollution load contained in the MPs to evaluate the pollution risk of the MPs in the WWTP. Moreover, all data were examined for normality

(Shapiro-Wilk test) and variance homogeneity (Levene test). The statistical significance threshold was established at $p < 0.05$.

2.4. Potential Ecological Risk Assessment of MPs

In order to restrict the spread of MPs pollution, it is necessary to quantify the potential ecological danger posed by MPs pollution. In this paper, the potential ecological risk of MPs in WWTP is evaluated using the MPS pollution load index (PLI) model, which was initially developed to evaluate the level of water pollution in estuaries; it is now expanded to calculate the value-at-risk of MPs [48]. The following is the formula:

$$CF_i = C_i / C_{oi} \tag{2}$$

In Formula (2), CF_i is defined as the ratio of MPs abundance (C_i) at each sampling point to MPs minimum abundance (C_{oi}) at each sampling point.

$$PLI_i = \sqrt{CF_i} \tag{3}$$

$$PLI_{zone} = \sqrt[n]{PLI_1 \times PLI_2 \times \ldots \times PLI_n} \tag{4}$$

In Formula (3) and (4), PLI_i represents the pollution load index of MPs for a single sample, whereas PLI_{zone} represents the pollution load index of MPs for WWTP.

$$H = \sum P_n \times S_n \tag{5}$$

In Formula (5), H is the MPs potential ecological risk index, the proportion of each MPs polymer type at each sampling site for the P_n, and S_n is the hazard score for the sample point MPs polymer [49] (Tables 5 and 6).

Table 3. MPs fixed-point quantification (WWTP in dry weather, Sewage (n/L), Sludge (n/10 g)).

Process Segment	D1	D2	D3	D4	D5	D6	D7	D8
Scanning points (U1, U2, C3, D4, D5)	125	93	62	137	104	78	34	260
				Shape (Formula (1))				
Fiber	5.9	1.7	1.9	4.3	1.7	2.0	0.9	5.6
Chip	2.8	1.3	1.2	1.1	1.3	0.9	0.3	3.9
Sheet	1.6	1.4	0.5	1.0	0.9	0.5	0.1	2.5
Particle	0.9	0.1	0.2	0.5	0.2	0	0	1.5
				Size (Formula (1))				
0–100 μm	2.8	1.8	1.4	3.0	1.4	1.1	0.7	4.9
100–500 μm	4.1	1.0	0.8	2.4	1.6	1.8	0.5	2.3
500–1000 μm	2.0	1.2	1.3	1.1	0.5	0.4	0.1	3.0
1000–2500 μm	1.7	0.6	0.3	0.4	0.7	0.1	0	1.7
2500–5000 μm	0.6	0	0	0.1	0.1	0	0	1.5
Actual MPs abundance, Formula (1)	101.9 ± 17.6	61.1 ± 9.3	51.0 ± 7.3	71.9 ± 15.3	68.1 ± 13.6	44.2 ± 5.5	18.2 ± 3.6	184.8 ± 28.6

Table 4. MPs fixed-point quantification (WWTP in rainy weather, Sewage (n/L), Sludge (n/ 10 g)).

Process Segment	R1	R2	R3	R4	R5	R6	R7	R8
Scanning points, (U1, U2, C3, D4, D5)	85	93	82	113	131	57	55	277
				Shape, (Formula (1))				
Fiber	5.0	2.3	1.9	2.3	3.8	2.8	1.7	5.5
Chip	2.8	1.6	1.2	0.1	1.0	0.9	0.1	3.0
Sheet	1.8	2.0	0.5	1.0	1.0	0.7	0.2	2.5
Particle	1.1	0.3	0.2	0	0.5	0	0	1.5
				Size, (Formula (1))				
0–100 μm	1.7	2.1	1.9	1.9	3.3	1.5	1.0	3.3
100–500 μm	4.0	1.8	1.3	1.4	3.7	1.8	0.8	5.2
500–1000 μm	1.3	1.4	0.6	2.4	1.4	0.5	0.1	2.1
1000–2500 μm	1.7	0.5	0.3	0.5	0.3	0.6	0	0.9
2500–5000 μm	1.1	0.4	0	0.1	0.1	0	0	1.1
Actual MPs abundance, Formula (1)	108.7 ± 20.1	77.9 ± 11	81.2 ± 10.8	87.4 ± 21.3	117.3 ± 22.4	53.6 ± 7.4	26.3 ± 5.1	178.4 ± 34.3

Table 5. Risk evaluation of MPs in WWTP (1).

Type of Polymer	PE		PP		PS		PET	
Hazard score (Highest level); S_n	11		1		4		30	
Process Segment	D	R	D	R	D	R	D	R
P_n (%)	11.30	15.89	6.88	12.33	9.83	8.60	10.81	8.60
H, (Formula (5))	1.24	1.75	0.07	0.12	0.39	0.34	3.24	2.58
Potential ecplogical risk level of MPs	I	I	I	I	I	I	I	I
PLI_i (Formula (2) and (3))	2.40	2.62	1.88	2.30	1.93	2.72	1.63	1.95

Table 6. Risk evaluation of MPs in WWTP (2).

Type of Polymer	PU		PA		PF		PVC	
Hazard score (Highest level); S_n	871		50		1450		30	
Process Segment	D	R	D	R	D	R	D	R
P_n (%)	5.65	6.54	24.32	21.68	17.94	14.95	13.27	11.40
H, (Formula (5))	49.22	56.98	352.70	314.39	1324.40	1104.15	663.52	570.20
Potential ecplogical risk level of MPs	II	II	III	III	III	III	III	III
PLI_i (Formula (2) and (3))	1.00	1.51	1.04	1.00	1.73	2.39	1.97	2.42
PLI_{zone} (Formula (4))	PLI_{zone} (Dry weather) value is 1.63 (moderately pollution), PLI_{zone} (Rainy weather) value is 2.03 (highly pollution)							

3. Results and Discussion

3.1. Distribution and Reduction of MPS in WWTP

In this investigation, 14 wastewater samples were collected from different typical WWTP stages (D1 to D8, R1 to R8) under dry (D) and rainy (R) weather conditions, together with two samples of dewatered sludge. These samples are used to illustrate the distribution and fluctuation of MPs following Sewage Treatment treatment at different phases of the procedure. Following are the abundance values of MPs at the WWTP under dry and rainy

circumstances. The abundance of MPs at D1 to D8 was 101.9 ± 17.6, 61.1 ± 9.3, 51.0 ± 7.3, 71.9 ± 15.3, 68.1 ± 13.6, 44.2 ± 5.5, 18.2 ± 3.6 n/L, 184.8 ± 28.6 n/10 g. The abundance of MPs was 108.7 ± 20.1, 77.9 ± 11.0, 81.2 ± 10.8, 87.4 ± 21.3, 117.3 ± 22.4, 53.6 ± 7.4, 26.3 ± 5.1 n/L, 178.4 ± 34.3 n/10 gat the R1 to R8 sampling sites, respectively (Tables 3 and 4).

Figure 3 depicts the overall distribution of MPs in the typical WWTP process phases, in which rainy conditions fluctuate significantly. The wastewater at R4 (4. After oxidation Ditch) and R5 (5. Inside the secondary sedimentation tank) was somewhat more turbid than that at D4 and D5, and the quantity of MPs at R5 increased by 19.8% as compared to D5. This is mostly attributable to the overflowing of sewage in the sewers, the rise in the treatment load on process equipment, and the increase in flow disruption when precipitation enters the Sewage Treatment. The impact of reflux in R4 was diminished, the settling time in R5 was shortened, and the performance of the activated sludge system was diminished [50]. These variables contribute to the difference between R5 and D5 MPs.

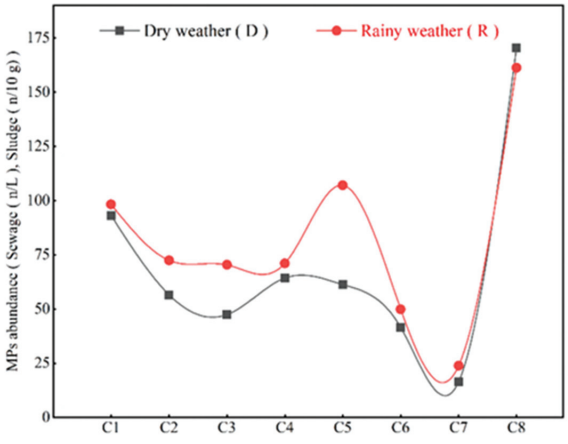

Figure 3. Comparison of the MPs variation trend for dry and rainy weather in typical process stage of WWTP. C: Craft (C1 to C8).

MPs abundance significantly decreased in the first stage of the WWTP, C1 to C3 (1. Row water, 2. After grille, 3. After sedimentation Tank), at rates of 33.3% and 25.0%, respectively (Figure 3). The interception of the grid and the role of flocculation and sedimentation in the grit chamber, which effectively precipitated suspended and colloidal material with a size of less than 100 μm in the grit chamber, were potential causes for the decline [51–53]. Table 2 demonstrates that the rates of primary removal were 62.9% and 70.4%, respectively. Nonetheless, the MP's D4 and R4 effluent abundances increased by 51.7% and 35.2%, respectively. Refluxing of the aeration tube in the aerobic portion modifies the structure of MPs, making it simpler for large plastics to be degraded by anaerobic processes, while breaking down into smaller plastic particles [54,55]. Compared to the D4 and R4 stages, the D5–D6 and R5–R6 (5. Inside the secondary sedimentation tank to 6. After the secondary settling tank) stages were partially settled by MPs. The removal rates of subsequent therapy were 55.6% and 57.5%, respectively. Tertiary treatment of filter sedimentation and disinfection only decreased contamination of treated water and chemical contamination indicators [56], with little influence on changes in MPs abundance. In three stages, the removal rates were 44.9% and 34.6%, respectively. The overall removal rates for raw water and discharge water were 87.7% and 83.5%, respectively. In addition, the MPs present in the sludge under both weather conditions were 184.8 ± 28.6 n/10 g, 178.4 ± 34.3 n/10 g. Compared to 79 sludge samples taken from 28 Chinese Sewage Treatment Facilities, the estimated average quantity of MPs in Sewage Treatment Sludge was 22.7 ± 12.1 n/g. MPs abundance significantly decreased in the first stage of the WWTP, C1 to C3 (1. Row water, 2. After grille, 3. After sedimentation Tank), at rates of 33.3% and 25.0%, respectively

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3.2. Source and Variation of MPs in Different Polymer Types in WWTP

The aggregate proportion of various MPs types in wastewater and sludge samples at typical WWTP stages in dry and rainy weather conditions was determined using micro-FTIR analysis (Tables 5 and 6). The plastics examined included PE (Polyethylene), PP (Polypropylene), PA (Polyamid), PVC (Polyvinylchloride), PS (Polystyrene), PET (PolyethyleneTerephthalate), PU (Polyurethane), PF (phenol-formaldehyde resin), which are the primary types of polymers that can be detected in the ordinary phases of a WWTP process in both rainy and dry weather, non-plastic is the impurity that interferes with the membrane and a non-plastic polymer. Figure 4 demonstrates that the average composition of the three plastic polymers PA, PF, and PE is greater than that of other polymers. In dry weather, the proportion of PA polymer was 24.32% and in rainy weather, it was 21.68%, followed by PF at 17.94% and PE at 11.30% and 15.89%. In the initial stage of physical treatment, the fine nylon fiber's PA interception effectiveness is limited. However, during the oxidation ditch and secondary sedimentation phases, enrichment is observed. This might be the secondary treatment (4. Oxidation ditch) stage because of the fluidity of the activated sludge, which results in the capture of the majority of polymers, including PA, at this point [51]. PE, PS and PA are frequent textile materials that are frequently derived from laundry. At the same time, the source of PE and PS can be used in personal care products as abrasive particles and in cosmetics as an absorbent. Most likely, PF and PU sources come from the wide use of electrical insulation and rubberized fabric. This is because an electrical processing factory is close to the WWTP. PVC and PET can be used in plastic greenhouses on agricultural land and come from various suppliers. The films are naturally aged and photocatalytic, resulting in debris and films that are washed away by rainfall, which will be dispersed in the plastic surface environment and transported to the municipal WWTP [57,58]. Based on how plastic polymer parts are spread out in the typical phases of the WWTP process, residential sewage is the main source of MPs. Rainfall is an outside factor that contributes to the spread of MPs.

3.3. Shape, Distribution and Size of MPs in Sewage Plants

Figures 5 and 6 illustrate the shape and size properties of MPs in sewage and sludge at various WWPT processes. The MPs was separated into four categories: fiber, chip, sheet, and particle. The dimensions are separated into five categories (Figure 7): 0–100, 100–500, 500–1000, 1000–2500, 2500–5000 μm. Figures 8 and 9 show that fibrous MPs in sewage and sludge are most numerous at each typical WWTP process stage, with 49.3% and 39.7% of sewage and sludge abundances in dry weather, respectively, and 50.1% and

43.2% of sewage and sludge abundances in rainy weather, respectively. Following that are particle, fragment, and film shapes. It can be established that the abundance of MPs in various forms is proportional to the source and composition of sewage, as well as the rate of change in sewage volume and pollutant content. Fibrous MPs have been discovered in sewage and sludge, primarily from washing-worn fabric fibres and synthetic fibres floating in the air [59–61]. Fragments with a particle size of smaller than 5 mm are more likely to have originated from plastic products as a result of environmental elements such as light, thermal oxidation, and physical friction during use or disposal [62]. The artificial generation of abrasives in industrial production and the consumption of personal care products and cosmetics containing significant quantities of plastic bead particles are the origins of the particle shape [4,63]. Figure 10 reveals that the source of the film is due in part to the usage of plastic bags by residents and in part to the extensive use of plastic greenhouses on agricultural land in the upper Sewage Treatment Basin. Direct sunlight and extreme precipitation, such as snow, and hail, expedited the deterioration of plastic green-houses, leaving shattered film residue on the soil and agricultural products [64].

Figures 8 and 9 demonstrate that MPs in the 0–500 μm size range had the greatest average distribution in WWTP, with sewage and sludge abundances of 64.9% and 60.4% in dry weather and 67.9% and 69.0% in rainy weather, respectively. The second is 500–1000 μm, or more than 1000 μm of plastic. The results revealed that the sludge was tightly concentrated with MPs fragments and fibers [65], with a microparticle-sized plastics content below 1000 μm ranging from 88.3–91.2%. During mud cake processing, MPs are fragmented into smaller plastic particles, thereby boosting MPs' abundance [66]. MPs with high density and large size may have been trapped by sedimentation in the region of 2500–5000 μm (1. Row water to 5. Inside the secondary sedimentation tank) and (6. After secondary settling tank). However, the fraction of MPs with a size of 0–500 μm expanded from 24.8% to 42.6%, most probably as a result of the aeration and activated sludge treatment process (4. After oxidation Ditch), which breaks down large plastics into smaller plastic particles [55,67]. The proportion of MPs with particle sizes ranging from 500–1000 μm remained steady, ranging between 11.2% and 18.8%. It demonstrates that larger particle-size MPs can be efficiently eliminated during the first and second treatment phases, but smaller MPs can be removed slower.

3.4. Contamination Risk Evaluation of MPs in WWTP

Sewage and sludge from WWTP are processed in various ways and still include MPs at each step. The lower MPs, along with the tail water and sludge from the dewatering pump house, will be dumped into the river's natural flow and could be used as fertilizer for farming and urban greening. These approaches, on the one hand, augment the pollutant load index of MPs at WWTP. On the other hand, MP migratory behaviour in the natural environment may provide an acute or chronic risk to the ecology, either directly or indirectly [68–70]. Firstly, when dividing the risk series of MPs in WWTP and its associated toxicity, the characteristics of MPs in each typical process stage are evaluated, and the European classification, labelling, and packaging (CLP) standard is referred to. The risk series is then divided into four grades, and the pollution load is divided into three types. The model was based on the risk index (H), and the danger levels varied from (I) (<10 slightly toxic) through (II) (10–100 moderately toxic), (III) (100–1000 highly toxic), and (IV) (>1000 very highly toxic). Each level is given an approximate risk rating, with each danger level (I–IV) rising tenfold. It was picked ten times because it differentiates between various degrees of toxicity risk. It is also a unique categorization criterion used in GHS to differentiate between acute and chronic risk categories in the aquatic environment [49]. Secondly, the three categories of pollution loads were determined using the pollution coefficients of the MPs pollution load index (PLIi) in a single sample and the MPs pollution load index (PLIzone) in the total research area: (<1 slightly pollution, 1–2 moderately pollution, >2 highly pollution). The pollution risk of MPs was eventually determined by combining it with the MPs risk index in WWTP [71].

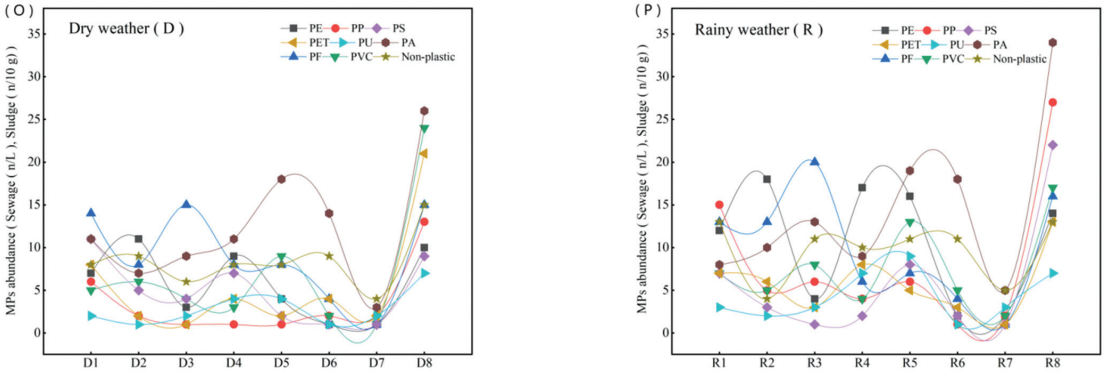

Figure 4. Variation of various MPs polymers in dry and rainy weather during a typical WWTP process stage. (**O**): Polymer in dry weather; (**P**): Polymer in rainy weather.

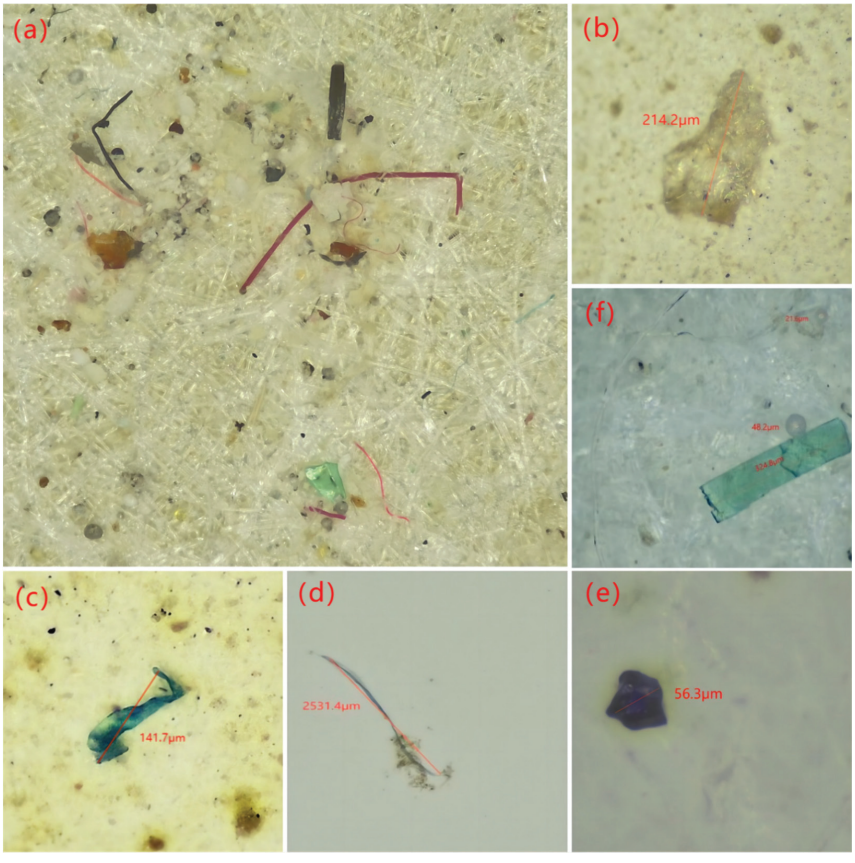

Figure 5. Multiple shapes of MPs identified in WWTP. (**a**): MPs in quadrat; (**b**,**c**): Chip; (**d**): Fiber; (**e**): Sheet; (**f**): Chip, Particle.

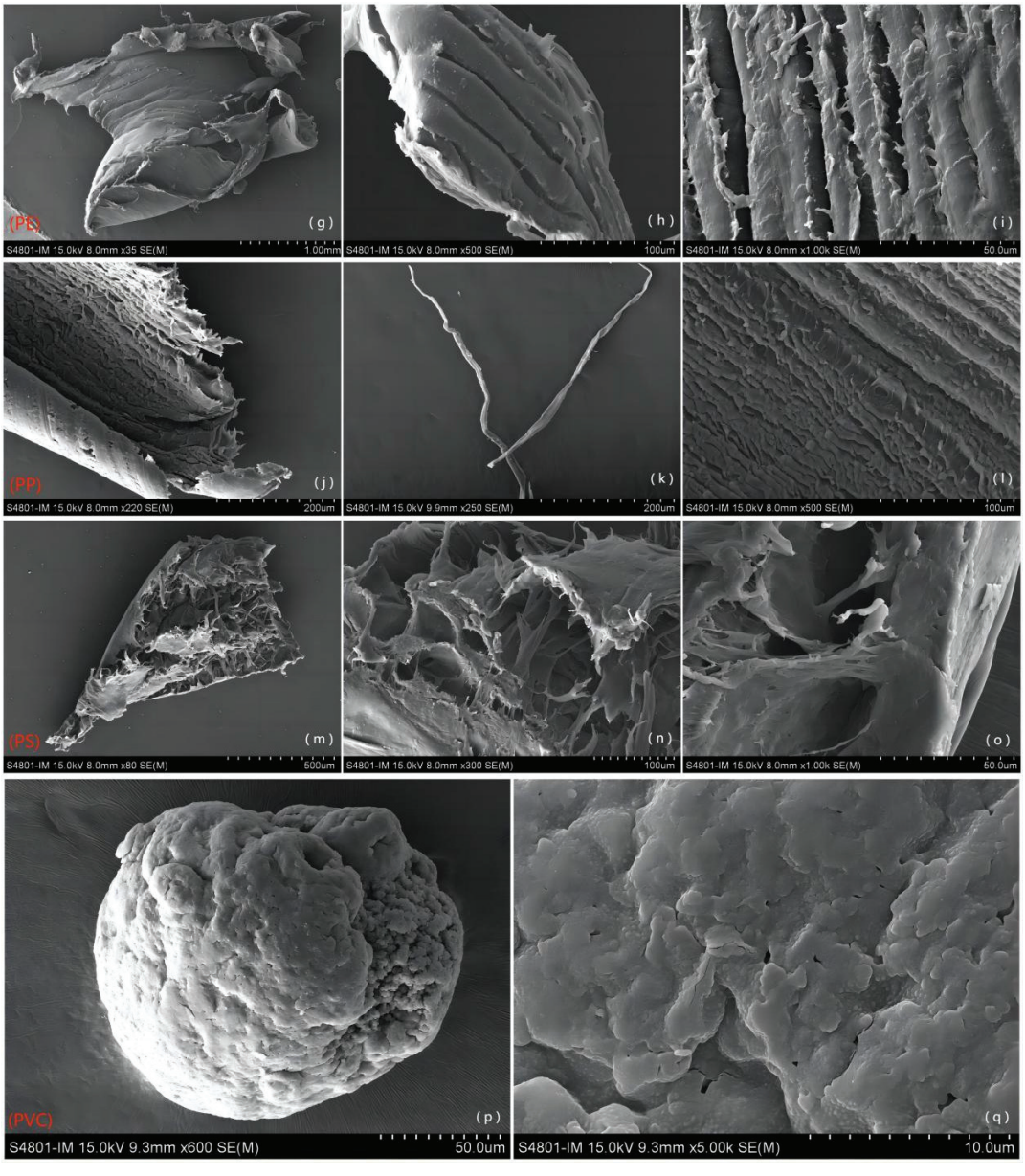

Figure 6. The surface-characteristic morphology of MPs. (**g–i**) are (PE); (**j–l**) are (PP); (**m–o**) are (PS); (**p**,**q**) is (PVC).

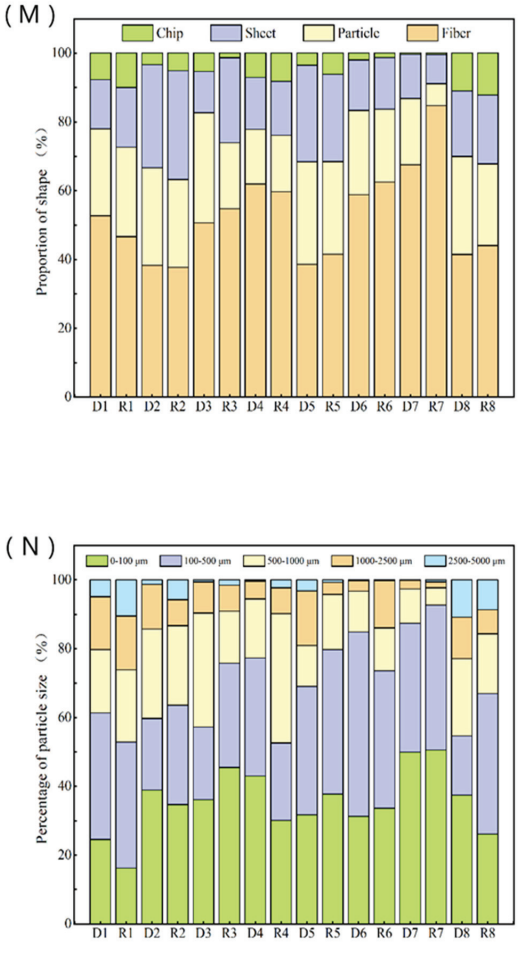

Figure 7. Characteristics of MPs in the sewage and sludge of the WWTP. (**M**): Shape of MPs, (**N**): Size of MPs.

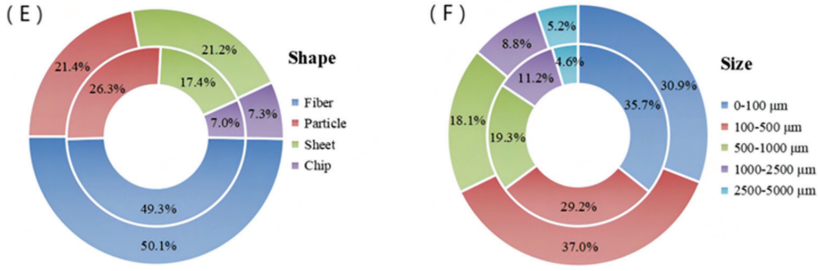

Figure 8. The aggregate relative proportions of MPs of varying shapes and sizes in sewage at the WWTP. (**E**): Shape, (**F**): Size; Dry and rainy weather conditions are represented by the inner and outer rings.

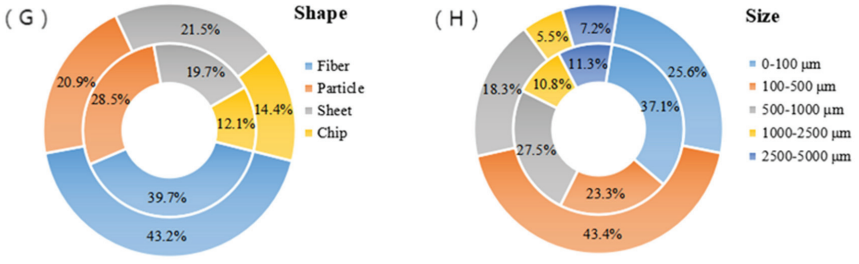

Figure 9. The overall relative proportions of MPs of different shapes and sizes in sludge at the WWTP. (**G**): Shape, (**H**): Size.

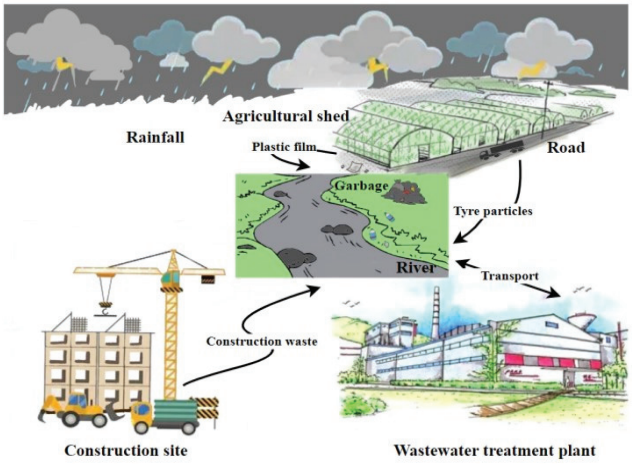

Figure 10. Migration of MPs in the freshwater environment.

Under both rainy and dry weather circumstances, the pollution index of MPs in Row water is 2.40 and 2.46, respectively, according to formula (5), indicating that the WWTP is significantly polluted. The tail water pollution index ranged from 1.0 to 1.2, suggesting considerable contamination. It demonstrates that the current procedure influences the expulsion of MPs. However, MPs in dehydrated sludge had a pollution index of 3.5 under dry weather circumstances and 3.4 under rainy weather conditions, indicating that they were highly polluting. The damage caused by sludge seems more substantial than that generated by sewage release. It is recommended that this substance be incinerated, since it is not suited for agricultural or urban greening fertilizer. Meanwhile, Tables 5 and 6 demonstrate that in dry and rainy conditions, the risk levels of PE, PP, PS PET polymer are (I), PU and PA are (II), and PVC and PF polymer are (III). The abundance of MPs 18.2 $\pm$ 3.6 n/L at sample point D7 was employed to calculate the C_{oi} for this investigation [47]. The PLI_i values of PP, PU, PVC, PS, PET, PA and PF in the typical process stages of WWTP are in the range of (1–2 moderately pollution) in dry weather (D1–D8), while PE polymers are in the (>2 highly pollution) range. The PLI_i values for PET, PA, and PF were in the (1–2 moderately pollution) range under rainy circumstances (R1–R8), whereas those for PE, PP, PU, PVC, and PS were in the (>2 highly pollution) range. Ultimately, the PLI_{zone} Index of the total MPs in the WWTP was determined using the formula (4). In dry weather (D1–D8), the PLI_{zone} value was 1.63, which was moderately polluted. In comparison, the PLI_{zone} value is 2.03, which is extremely polluted. Collectively, the percentage of different kinds of polymers and their hazard ratings were strongly connected with the pollution risk of MPs in the WWTP; the MPS Pollution Index was more volatile in rainy weather. The primary source of its effects is probably sewage sources, with non-point source migration

of pollution sources, including plastics, into the WWTP owing to rainfall, exacerbating the pollution risk of MPs in the WWTP [72].

4. Conclusions

In this paper, MPs abundances in sewage diminished most dramatically following primary and secondary treatment utilizing the WWTP, with average removal ratios of 59.3% and 64.0% during dry and rainy weather, respectively. However, sedimentation and disinfection from the three-stage treatment lessened the pollutant and Chemical Pollution Index further, and the MPs elimination effectiveness was only 44.9% and 34.6%, respectively. 87.7% and 83.5% of all MPs were terminated. Sewage and sludge from WWTPs had the most incredible average abundance of fibrous MPs, 49.3% and 39.7% in dry weather and 50.1% and 43.2% in rainy weather, respectively. With 64.9% and 60.4% in dry weather and 67.9% and 69.0% in rainy weather, respectively, WWTP wastewater and sludge had the greatest average distribution of MPs in the range of 0–500 μm. It demonstrates that the form and size of MPs are changing as a result of the WWTP process, and that sludge accumulation is considerable.

According to a micro-FTIR spectrometer, the predominant constituents of WWTP MPs include PP, PE, PS, PA, PET, PU, PF and PVC. It has been discovered that the types of polymers in question are strongly linked to human activities. The primary source of these polymers is sewage from homes, which can be affected by weather conditions like rain. Also, the pollution risk of MPs in the WWTP was related to the amount of polymers and their hazard scores both when it was dry and when it was raining The abundances of MPs in the WWTP's Row water were 101.9 $\pm$ 17.6 n/L and 108.7 $\pm$ 20.1 n/L, respectively. Extremely high pollution risk indices of 2.40 and 2.46 were calculated using the PLI pollution load index model. The concentrations of MPs in Tail water were 18.2 $\pm$ 3.6 n/L and 26.3 $\pm$ 5.1 n/L, with corresponding pollution risk indices of 1.0 and 1.2. The findings indicate that the current WWTP procedure influences the elimination of MPs. The abundances of MPs in dewatered sludge were 184.8 $\pm$ 28.6 n/10 g and 178.4 $\pm$ 34.3 n/10 g, and their pollution indices were 3.5 and 3.4, which were both extremely polluted. Consequently, there is a potential danger of ecological contamination since there is still an outflow of MPs from the WWTP process before and after treatment, with the tail water being discharged into the natural water body or deposited in the sludge, causing the movement of MPs in the environment. The interception and removal effectiveness of MPs need to be enhanced.

Author Contributions: Writing—original draft preparation, X.M.; conceptualization, writing—review and editing, T.B.; formal analysis, data curation, L.H.; supervision, project administration, K.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the National Key R&D Program of China (2020YFC1908601, 2020YFC1908602); National Special Item on Water Resource and Environment (2017ZX07603-003); Hefei University's major projects research grant (Grant No. 20RC42).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wright, S.L.; Kelly, F.J. Plastic and human health: A micro issue? *Environ. Sci. Technol.* **2017**, *51*, 6634–6647. [PubMed]
2. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, Use, and Fate of All Plastics Ever Made. *Sci. Adv.* **2017**, *3*, 1700782. [CrossRef] [PubMed]
3. Chakraborty, P.; Chandra, S.; Dimmen, M.V.; Hurley, R.; Mohanty, S.; Bharat, G.K.; Steindal, E.H.; Olsen, M.; Nizzetto, L. Interlinkage Between Persistent Organic Pollutants and Plastic in the Waste Management System of India: An Overview. *Bull. Environ. Contam. Toxicol.* **2022**, *109*, 927–936.
4. Lee, J.; Hong, S.; Song, Y.K.; Sang, H.H.; Yong, C.J.; Mi, J.; Heo, N.W.; Han, G.M.; Mi, J.L.; Kang, D. Relationships among the abundances of plastic debris in different size classes on beaches in South Korea. *Mar. Pollut. Bull.* **2013**, *77*, 349–354.
5. Liu, X.; Yuan, W.; Di, M.; Li, Z.; Wang, J. Transfer and fate of microplastics during the conventional activated sludge process in one wastewater treatment plant of China. *Chem. Eng. J.* **2019**, *362*, 176–182. [CrossRef]

6. Wagner, M.; Scherer, C.; Alvarez-Muoz, D.; Brennholt, N.; Bourrain, X.; Buchinger, S.; Fries, E.; Grosbois, C.; Klasmeier, J.; Marti, T. Mi-croplastics in freshwater ecosystems: What we know and we need to know. *Environ. Sci. Eur.* **2014**, *26*, 12. [CrossRef] [PubMed]
7. Silva, A.B.; Bastos, A.S.; Justino, C.I.L.; da Costa, J.P.; Duarte, A.C.; Rocha-Santos, T.A.P. Microplastics in the environment: Challenges in analytical chemistry—A review. *Anal. Chim. Acta* **2018**, *1017*, 1–19.
8. Lee, J.; Enfrin, M.; Dum, L.F.; Dumée, L.F.; Lee, J. Nano/microplastics in water and wastewater treatment processes—Origin, impact and potential solutions. *Water Res.* **2019**, *161*, 621–638.
9. Brennecke, D.; Duarte, B.; Paiva, F.; Caçador, I.; Canning-Clode, J. Microplastics as vector for heavy metal contamination from the marine environment. *Estuar. Coast. Shelf Sci.* **2016**, *178*, 189–195. [CrossRef]
10. Kida, M.; Koszelnik, P. Investigation of the Presence and Possible Migration from Microplastics of Phthalic Acid Esters and Polycyclic Aromatic Hydrocarbons. *J. Polym. Environ.* **2020**, *29*, 599–611.
11. Abbasi, S.; Keshavarzi, B.; Moore, F.; Shojaei, N.; Sorooshian, A.; Soltani, N.; Delshab, H. Geochemistry and environmental effects of potentially toxic elements, polycyclic aromatic hydrocarbons and microplastics in coastal sediments of the persian gulf. *Environ. Geol.* **2019**, *492*, 2–15.
12. Mclellan, S.L.; Huse, S.M.; Mueller-Spitz, S.R.; Andreishcheva, E.N.; Sogin, M.L. Diversity and population structure of sewage-derived microorganisms in wastewater treatment plant influent. *Environ. Microbiol.* **2010**, *12*, 378–392. [PubMed]
13. Li, J.; Shen, T.; Wang, H.; Li, S.; Wang, J.; Williams, R.G.; Zhao, Y.; Kong, X.; Zheng, L.; Song, Y. Insights into the Superstable Mineralization of Chromium(III) from Wastewater by CuO. *ACS Appl. Mater. Interfaces* **2022**, *14*, 37823–37832. [CrossRef] [PubMed]
14. He, S.; Jia, M.; Xiang, Y.; Song, B.; Xiong, W.; Cao, J.; Peng, H.; Yang, Y.; Wang, W.; Yang, Z.; et al. Biofilm on microplastics in aqueous environment: Physicochemical properties and environmental implications. *J. Hazard. Mater.* **2022**, *424*, 127286. [CrossRef] [PubMed]
15. Vcs, A.; Pgb, C.; Em, A.; Km, B. First study of its kind on the microplastic contamination ofsoft drinks, cold tea and energy drinks—Future research and envi-ronmental considerations. *Sci. Total Environ.* **2020**, *726*, 138580.
16. Luo, H.; Liu, C.; He, D.; Sun, J.; Li, J.; Pan, X. Effects of aging on environmental behavior of plastic additives: Migration, leaching, and ecotoxicity. *Sci. Total Environ.* **2022**, *849*, 157951. [CrossRef]
17. Botterell, Z.L.R.; Beaumont, N.; Dorrington, T.; Steinke, M.; Thompson, R.C.; Lindeque, P.K. Bioavailability and efects of microplastics on marine zooplankton: A review. *Environ. Pollut.* **2019**, *245*, 98–110. [CrossRef]
18. Wang, L.; Liu, Y.; Kaur, M.; Yao, Z.; Chen, T.; Xu, M. Phytotoxic Effects of Polyethylene Microplastics on the Growth of Food Crops Soybean (Glycine max) and Mung Bean (Vigna radiata). *Int. J. Environ. Res. Pub. Health* **2021**, *18*, 10629.
19. Toussaint, B.; Raffael, B.; Angers-Loustau, A.; Gilliland, D.; Kestens, V.; Petrillo, M.; Rio-Echevarria, I.M.; Van den Eede, G. Review of Micro- and Nanoplastic Contamination in the Food Chain. *Food Addit. Contam. Part A* **2019**, *36*, 639–673.
20. Schwabl, P.; Köppel, S.; Königshofer, P.; Bucsics, T.; Trauner, M.; Reiberger, T.; Liebmann, B. Detection of various microplastics in human stool: A prospective case series. *Ann. Intern. Med.* **2019**, *171*, 453–457.
21. Zhang, J.; Wang, L.; Trasande, L.; Kannan, K. Occurrence of Polyethylene Terephthalate and Polycarbonate Microplastics in Infant and Adult Feces. *Environ. Sci. Technol. Lett.* **2021**, *8*, 989–994.
22. Hal, A.; Mjmvv, A.; Shb, A.; Adva, B.; Gv, C.; Mhl, A. Discovery and quantification of plastic particle pollution in human blood. *Environ. Int.* **2022**, *163*, 107199.
23. Ragusa, A.; Svelato, A.; Santacroce, C.; Catalano, P.; Giorgini, E. Plasticenta: First evidence of microplastics in human placenta. *Environ. Int.* **2021**, *146*, 106274. [CrossRef] [PubMed]
24. Kim, S.K.; Song, N.S. Microplastics in edible Salt: A literature review focusing on Uncertainty related with Measured Minimum Cutoff Sizes. *Curr. Opin. Food Sci.* **2021**, *41*, 16–25. [CrossRef]
25. Jin, M.; Wang, X.; Ren, T.; Wang, J.; Shan, J. Microplastics contamination in food and beverages: Direct exposure to humans. *J. Food Sci.* **2021**, *86*, 2816–2837. [CrossRef]
26. Zhang, Q.; Xu, E.G.; Li, J.; Chen, Q.; Ma, L.; Zeng, E.Y.; Shi, H. A review of microplastics in table salt, drinking water, and air: Direct human exposure. *Environ. Sci. Technol.* **2020**, *54*, 3740–3751. [CrossRef]
27. Jenner, L.C.; Rotchell, J.M.; Bennett, R.T.; Cowen, M.; Tentzeris, V.; Sadofsky, L.R. Detection of microplastics in human lung tissue using μFTIR spectroscopy. *Sci. Total Environ.* **2022**, *831*, 154907.
28. Fournier, S.B.; D'Errico, J.N.; Adler, D.S.; Kollontzi, S.; Stapleton, P.A. Nanopolystyrene translocation and fetal deposition after acute lung exposure during late-stage pregnancy. *Part. Fibre Toxicol.* **2020**, *17*, 55.
29. Murphy, F.; Ewins, C.; Carbonnier, F.; Quinn, B. Wastewatertreatment works (WwTW) as a source of microplastics in theaquatic environment. *Environ. Sci. Technol.* **2016**, *50*, 5800–5808. [CrossRef]
30. Talvitie, J.; Mikola, A.; Koistinen, A.; Setälä, O. Solutions to microplastic pollution–Removal of microplastics from wastewater efuent with advanced wastewater treatment technologies. *Water Res.* **2017**, *123*, 401–407. [CrossRef]
31. Mason, S.A.; Garneau, D.; Sutton, R.; Chu, Y.; Ehmann, K.; Barnes, J.; Fink, P.; Papazissimos, D.; Rogers, D.L. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. *Environ. Pollut.* **2016**, *218*, 1045–1054. [CrossRef] [PubMed]
32. Xu, X.; Jian, Y.; Xue, Y.; Hou, Q.; Wang, L.P. Microplastics in the wastewater treatment plants (WWTPs): Occurrence and removal. *Chemosphere* **2019**, *235*, 1089–1096. [PubMed]

33. Zhang, K.; Shi, H.; Peng, J.; Wang, Y.; Xiong, X.; Wu, C.; Lam, P.K.S. Microplastic pollution in China's inland water systems: A review of findings, methods, characteristics, effects, and management. *Sci. Total Environ.* **2018**, *630*, 1641–1653. [CrossRef]
34. Gies, E.A.; LeNoble, J.L.; Noël, M.; Etemadifar, A.; Bishay, F.; Hall, E.R.; Ross, P.S. Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada Mar. *Pollut. Bull.* **2018**, *133*, 553–561.
35. Paul, K.; Robert, H.; Isobel, M.; Luke, B.; Niamh, M. Wastewater treatment plants as a source of microplastics in river catchments. *Environ. Sci. Pollut. Res.* **2018**, *25*, 20265–20266.
36. Thompson, R.C.; Moore, C.J.; Saal, F.V.; Swan, S.H. Plastics, the environment and human health: Current consensus andfuture trends. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2009**, *364*, 2153–2166. [PubMed]
37. Michelbach, S.; Wöhrle, C. Settleable Solids in a Combined Sewer System, Settling Characteristics, Heavy Metals, Efficiency of Storm Water Tanks. *Water Sci. Technol.* **1993**, *27*, 153–164. [CrossRef]
38. Gasperi, J.; Gromaire, M.C.; Kafi, M.; Moilleron, R.; Chebbo, G. Contributions of wastewater, runoff and sewer depositerosion to wet weather pollutant loads in combined sewersystems. *Water Res.* **2010**, *44*, 5875–5886. [CrossRef]
39. Hollender, J.; Zimmermann, S.G.; Koepke, S.; Keaoss, M.; Mcardell, C.S.; Ort, C.; Singer, H.; Gunten, U.V.; Siegrist, H. Elimination of organic micropollutants in a municipal wastewater treatment plant upgraded with a full-scale post-ozonation followed by sand filtration. *Environ. Sci. Technol.* **2009**, *43*, 7862–7869.
40. Noriatsu, O.; Takahiro, Y.; Tomonori, K.; Akiyoshi, O. Stormwater inflow loading of polycyclic aromatic hydrocarbons into urban domestic wastewater treatment plant for separate sewer system. *Water Sci. Technol.* **2019**, *79*, 1426–1436.
41. Hurley, R.; Lusher, A.; Olsen, M.; Nizzetto, L. Validation of a method for extracting microplastics from complex, organic-rich, environmental matrices. *Environ. Sci. Technol.* **2018**, *52*, 7409–7417. [PubMed]
42. Nuelle, M.T.; Dekiff, J.H.; Remy, D.; Fries, E. A new analytical approach for monitoring microplastics in marine sediments. *Environ. Pollut.* **2014**, *184*, 161–169. [PubMed]
43. Carr, S.A.; Liu, J.; Tesoro, A.G. Transport and fate of micro-plastic particles in wastewater treatment plants. *Water Res.* **2016**, *91*, 174–182. [PubMed]
44. Käppler, A.; Fischer, D.; Oberbeckmann, S.; Schernewski, G.; Labrenz, M.; Eichhorn, K.J.; Voit, B. Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? *Anal. Bioanal. Chem.* **2016**, *408*, 8377–8391.
45. Browne, M.A.; Galloway, T.S.; Thompson, R.C. Spatial patterns of plastic debris along estuarine shorelines. *Environ. Sci. Technol.* **2010**, *44*, 3404–3409.
46. Pivokonsky, M.; Cermakova, L.; Novotna, K.; Peer, P.; Cajthaml, T.; Janda, V. Occurrence of microplastics in raw and treated drinking water. *Sci. Total Environ.* **2018**, *643*, 1644–1651.
47. Liu, D.T.; Song, Y.; Li, F.F.; Chen, L.J. A detection method of small-sized microplastics based on micro-raman mapping. *CHN Environ. Sci.* **2020**, *40*, 4429–4438.
48. Tomlinson, D.L.; Wilson, J.G.; Harris, C.R.; Jeffrey, D.W. Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. *Helgolnder Meeresuntersuchungen* **1980**, *33*, 566–575.
49. Lithner, D.; Larsson, A.; Dave, G. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. *Sci. Total Environ.* **2011**, *409*, 3309–3324.
50. Wei, W.; Huang, Q.S.; Sun, J.; Wang, J.Y.; Wu, S.L.; Ni, B.J. Polyvinyl Chloride Microplastics Affect Methane Production from the Anaerobic Digestion of Waste Activated Sludge through Leaching Toxic Bisphenol-A. *Environ. Sci. Technol.* **2019**, *53*, 2509–2517.
51. Ghimici, L.; Nichifor, M. Flocculation by cationic amphiphilic polyelectrolyte: Relating efficiency with the association of polyelectrolyte in the initial solution Colloids Surf. A Physicochem. *Eng. Asp.* **2012**, *415*, 142–147.
52. Girish, C.R.; Singh, S.K.; Shenoy, S. Removal of suspended solids from dairy wastewater using flocculation. *Int. J. Mech. Eng. Technol.* **2017**, *8*, 99–105.
53. Zhu, S.; Khan, M.A.; Kameda, T.; Xu, H.; Wang, F.; Xia, M.; Yoshioka, T. New insights into the capture performance and mechanism of hazardous metals cr3+ and cd2+ onto an effective layered double hydroxide based material. *J. Hazard. Mater.* **2022**, *426*, 128062. [CrossRef] [PubMed]
54. He, Z.W.; Yang, W.J.; Ren, Y.X.; Jin, H.Y.; Wang, A.J. Occurrence, effect, and fate of residual microplastics in anaerobic digestion of waste activated sludge: A state-of-the-art review. *Bioresour. Technol.* **2021**, *331*, 125035. [CrossRef] [PubMed]
55. Conley, K.; Clum, A.; Deepe, J.; Lane, H.; Beckingham, B. Wastewater treatment plants as a source of microplastics to an urban estuary: Removal efficiencies and loading per capita over one year. *Water Res. X* **2019**, *3*, 100030. [PubMed]
56. Shima, Z.; Peta, A.N.; Llew, R.; Frederic, L. Wastewater treatment plants as a pathway for microplastics: Development of a new approach to sample wastewater-based microplastics. *Water Res.* **2017**, *112*, 93–99.
57. Koyuncuolu, P.; Gülbin Erden. Sampling, pre-treatment, and identifcation methods of microplastics in sewage sludge and their efects in agricultural soils: A review. *Environ. Monit. Assess.* **2021**, *193*, 175–177. [CrossRef]
58. Li, X.; Chen, L.; Mei, Q.; Dong, B.; Dai, X.; Ding, G.; Zeng, E.Y. Microplastics in sewage sludge from the wastewater treatment plants in China. *Water Res.* **2018**, *142*, 75–85. [CrossRef]
59. Ramasamy, R.; Aragaw, T.A.; Balasaraswathi Subramanian, R. Wastewater treatment plant effluent and microfiber pollution: Focus on industry-specific wastewater. *Environ. Sci. Pollut. Res.* **2022**, *29*, 51211–51233. [CrossRef]
60. Carney Almroth, B.M.; Åström, L.; Roslund, S.; Petersson, H.; Johansson, M.; Persson, N.K. Persson, Nils-Krister. Quantifying shedding of synthetic fibers from textiles, a source of microplastics released into the environment. *Environ. Sci. Pollut. Res.* **2017**, *25*, 1191–1199. [CrossRef]

61. Zhu, J.; Zhang, X.; Liao, K.; Wu, p.; Jin, H. Microplastics in dust from different indoor environments. *Sci. Total Environ.* **2022**, *833*, 155256. [CrossRef] [PubMed]
62. Galgani, F.; Hanke, G.; Werner, S.; De Vrees, L. Marine litter within the european marine strategy framework directive. *ICES J Mar. Sci.* **2013**, *70*, 1055–1064. [CrossRef]
63. Napper, I.E.; Bakir, A.; Rowland, S.J.; Thompson, R.C. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. *Mar. Pollut. Bull.* **2015**, *99*, 178–185. [CrossRef] [PubMed]
64. Ng, E.-L.; Lwanga, E.H.; Eldridge, S.M.; Johnston, P.; Hu, H.-W.; Geissen, V.; Chen, D. An overview of microplastic and nanoplastic pollution in agroecosystems. *Sci. Total Environ.* **2018**, *627*, 1377–1388. [CrossRef] [PubMed]
65. Hatinoğlu, M.D.; Sanin, F.D. Sewage sludge as a source of microplastics in the environment: A review of occurrence and fate during sludge treatment. *J. Environ. Manag.* **2021**, *295*, 113028. [CrossRef]
66. Harley-Nyang, D.; Memon, F.A.; Jones, N.; Galloway, T. Investigation and analysis of microplastics in sewage sludge and biosolids: A case study from one wastewater treatment works in the UK. *Sci. Total Environ.* **2022**, *823*, 153735. [CrossRef]
67. Hidayaturrahman, H.; Lee, T.G. A study on characteristics of microplastic in wastewater of South Korea: Identification, quantification, and fate of microplastics during treatment process. *Mar. Pollut. Bull.* **2019**, *146*, 696–702.
68. Senathirajah, K.; Attwood, S.; Bhagwat, G.; Carbery, M.; Wilson, S.; Palanisami, T. Estimation of the mass of microplastics ingested—A pivotal first step towards human health risk assessment. *J. Hazard. Mater.* **2021**, *404*, 124004.1–124004.15.
69. Xu, P.; Peng, G.; Lei, S.; Gao, L.; Li, D. Microplastic risk assessment in surface waters: A case study in the Changjiang Estuary, China. *Mar. Pollut. Bull.* **2018**, *133*, 647–654.
70. Kyriakopoulos, G.L.; Zamparas, M.; Kapsalis, V.C. Eutrophication control: The shif to invasive methods managing the internal nutrient loads. a bibliometric analysis. *Desalin. Water Treat.* **2022**, *267*, 177–185. [CrossRef]
71. Ranjani, M.; Veerasingam, S.; Venkatachalapathy, R.; Mugilarasan, M.; Bagaev, A.; Mukhanov, V.; Vethamony, P. Assessment of potential ecological risk of microplastics in the coastal sedime. *Mar. Pollut. Bull.* **2021**, *163*, 111969. [PubMed]
72. Kyriakopoulos, L.G.; Zamparas, G.M.; Kapsalis, C.V. Investigating the Human Impacts and the Environmental Consequences of Microplastics Disposal into Water Resources. *Sustainability* **2022**, *14*, 828.

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Social Cognitive Theory and Reciprocal Relationship: A Guide to Single-Use Plastic Education for Policymakers, Business Leaders and Consumers

Sarah Fischbach * and Brielle Yauney

Communication Division, Pepperdine University, Malibu, CA 90263, USA
* Correspondence: sarah.fischbach@pepperdine.edu

Abstract: Single-use plastic waste has become a growing concern in daily life. Community leaders are implementing programs to reduce the use of single-use plastic and change consumer behavior. This study, using the social cognitive theory framework for sustainable consumption, examines the reciprocal relationship among the following three factors: personal (green consumer values), environmental (bans and rebate/reward programs), and behavioral (consumer decision-making related to single-use plastic waste). The study surveyed consumers (N = 330) across the United States who watched a video on the effects of single-use plastic waste on health and well-being. The results indicate that states with bans or rebate/reward programs tend to have higher green consumer values and consumers in those states report less use of single-use plastic waste. Education level also has a significant impact on green consumer values and plastic waste usage. The study provides a resource guide for decision makers to implement programs in five areas: (1) Business Resources, (2) Public Policy Resources, (3) Non-Profit Resources, (4) Education Resources, and (5) Personal Resources. The study also suggests potential areas for future research.

Keywords: single-use plastic; bans and rebate programs; sustainability; green consumer values; solution guide

Citation: Fischbach, S.; Yauney, B. Social Cognitive Theory and Reciprocal Relationship: A Guide to Single-Use Plastic Education for Policymakers, Business Leaders and Consumers. *Sustainability* **2023**, *15*, 3946. https://doi.org/10.3390/su15053946

Academic Editors: Grigorios L. Kyriakopoulos, Antonis A. Zorpas, María Rocío Rodríguez Barroso and Vassilis J. Inglezakis

Received: 15 December 2022
Revised: 16 February 2023
Accepted: 16 February 2023
Published: 21 February 2023

1. Introduction

Increasing concerns by policymakers toward sustainability is directed toward single-use plastic waste [1]. Acceptance of the "disposable society," commonly referred to as the "single-use society," has detrimental impacts on the planet and consumers. The Environmental Protection Agency [2] considers source reduction to be the highest priority method for addressing plastic marine litter as it decreases the amount of trash there is to control, clean up, and dispose. County supervisors and city officials including Government agencies have pieced together regulations for their city, counties, and states as the global production of plastic has increased and the volume of plastic entering our ocean continues to increase [3]. The intent of this research is to provide guidance for business leaders, non-profits, and policymakers to actively engage and contribute to the United Nation's agenda to build more responsible consumption and production practices by 2030 [4]. Applying the social cognitive framework for sustainable consumption [5], the study provides solutions to the reciprocal relationship of the following three factors: (1) environment, (2) consumers, and (3) behavior. Our study not only introduces theory to assist policymakers and community leaders, but also provides solutions for more responsible consumption awareness toward single-use plastic waste.

To make significant progress mitigating marine plastic pollution, a wide range of solutions must be implemented simultaneously at multiple systemic and governmental levels. According to a new report commissioned by the Pew Charitable Trust in 2020, reforming the entire plastics economy—including source reduction, strategic single-use plastic substitutions, new product design, and reimagining recycling and disposal systems—will be critical

to addressing the ocean plastic problem as no single solution will meaningfully reduce global plastic pollution [6]. Therefore, our research attempts to extend this conversation by providing structured solutions for companies to meet organizations where they are in their goal development/execution.

Many business leaders, non-profit organizations, and government officials attempt to curb single-use plastic waste and increase awareness of the need to reduce single-use waste. Nonprofit organizations such as Blue Ocean Project [7] and Plastic Pollution Coalition [8] have developed teams of businesses and consumers to increase awareness of the benefits of reducing single-use product waste. Plastic Pollution Coalition continues to grow their global alliance of over 1200 organizations and business and thought leaders committed to building a world free of plastic pollution by highlighting the impact of plastic pollution on humans, animals, and the environment [8]. For-profit organizations such as TerraCycle [9] and Loop Store [10] have found ways to encourage sustainable practices through their business models. Terracycle's business model begins to eliminate the idea of waste by finding ways to recycle and collect typically non-recycled items, diverting millions of pounds of valuable resources from landfills all over the world [10]. In addition, companies such as Ohoo Water [11] and Dropps [12] have developed products that use plastic alternative packaging and rethink the engineering of existing products as we receive them now. Our study extends research on social cognitive theory [13] to provide businesses and policymakers a place to start by delivering data in support of rebates and bans and therefore offering a solutions guide.

The paper proceeds as follows. First, the relevant literature is discussed. This discussion is followed by an explanation of the research design. Results of the study are then presented. To conclude, a discussion of the findings, as well as managerial implications and future avenues of research related to this study, are presented.

2. Literature Review

2.1. Sustainability and Plastic Pollution

Despite all the work conducted by academics, governments, non-governmental organizations, and business communities, there is still significant need for additional work [14]. Research has shown there is a gap between positive attitudes toward sustainability and people's actual consumption behavior [14,15]. Sustainability can refer to the endurance of both ecological and biological processes and systems [16]. Plastic pollution is a subset of the sustainability conversation that looks at how plastic enters waterways and into drinking water, animals, food, and the human body. Research in marketing and public policy research mostly focuses on packaged goods, but there is a need to move to consider automobiles, appliances, and housing [14]. This may explain why researchers have found a mismatch among consumers. In addition, when sustainable consumption behavior is considered a private consumer decision rather than a civic duty, marketing strategies and company perception override personal obligations [17]. For example, if Coca Cola campaigns that they will use only 20% recycled plastic across the United States, this moves the responsibility away from the consumer and back to the organization [18].

Plastic pollution is often measured by what is termed "marine debris" or the measurement of plastic pollution found in the ocean. Data inconsistencies led to the first comprehensive assessment of the magnitude and extent of trash in streams and the nearshore Southern California Bight which was facilitated in 2013 by the Southern California Coastal Water Research Project (SCCWRP) [19]. Compared to 1994 seafloor surveys, the amount of seafloor trash had nearly doubled, and the extent of plastic material found had increased threefold [20]. In addition, coastal cleanups have provided some of the best region-wide data on marine debris specifically addressed in Southern California. Data collected between 1989–2014 found that 36.5% of ocean litter found in California was a form of food and beverage packaging (i.e., food wrappers, bottle caps, utensils, straws, and bottles), matching the prevalence of cigarette butts, California's top litter item [21].

2.2. Plastic Pollution and Social Cognitive Theory

To explore more closely the values of consumer behavior, we made use of a social cognitive framework for sustainable consumption [13]. The core concepts of the model explore (1) personal, (2) environmental, and (3) behavioral factors in consumer decision-making. All three elements work together to influence the desired outcome, which is in this case the reduced use of single-use plastic waste. The feedback loop referred to in the model is the reciprocal determinism. Reciprocal determination is derived from the self-determination theory mainly used in studying customer relationships [22]. Research on green consumer behaviors and self-determination has been investigated by marketing scholars to explore different motivations in consumption [23,24].

Research on sustainability-related consumption behaviors has often observed effects such as spillover effects on pro-environmental behaviors [25,26] and licensing and rebound effects [27,28]. Reciprocal determinism does not predict the positive behavior, rather, relationships may be presented with existing factors representing the feedback loop of behaviors [13]. Research on sustainability has explored areas such as the role of religiosity [29] and standardized labeling disclosures [30] to enhance sustainability information. In this study, we highlight the social cognitive framework by testing (1) personal (green consumer values), (2) environmental (consumer location, rebates, bans), and (3) behavioral (consumer decision-making) factors in the reciprocal determinism feedback loop. The study aims to better understand how these elements work together to reduce use of single-use plastic waste [13,31] (see Figure 1).

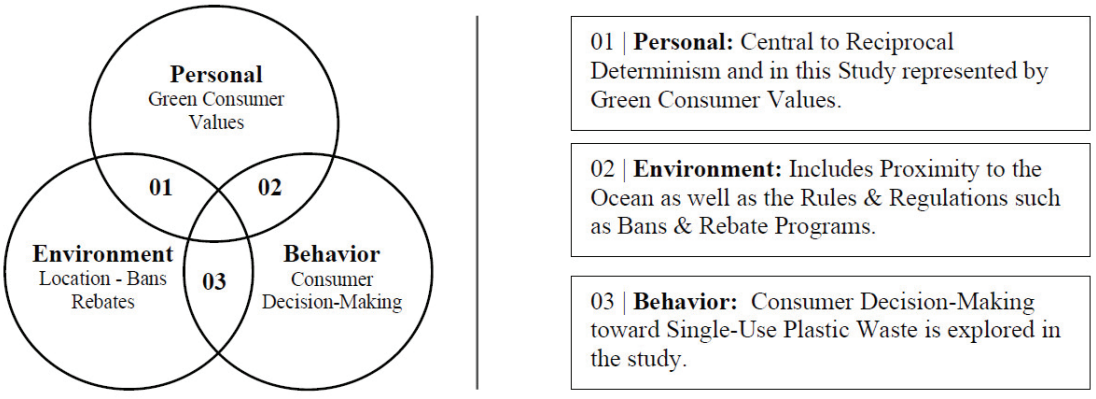

Figure 1. Social cognitive theory and self-determinism [13,31].

Green consumer values are defined as those that tend to consider the environmental impact of purchases and consumption behavior [32]. Recently the study of green consumption has explored the inconsistent results of green consumption and have rejected previous findings that young people are more inclined to consume green products [33]. Current research on social structures, particularly age or generation, has looked at the reshaping of green perceptions and overall purchasing behavior [33]. Straughan and Roberts's [34] research, conducted with a sample of 235 college students, concluded that young consumers were more concerned about the environment and therefore were more likely to be potential green product consumers. Ham et al.'s [33] findings argue that each generation exhibits different personal and company beliefs. Overall, regardless of the generation, the consumer's belief of green product intent to purchase is the strongest predictor in company benefit [33]. Our study takes these findings into consideration and explores whether consumers' level of education influences their green consumer values in terms of single-use plastic waste. Our study takes these findings into consideration and explores whether consumers within close proximity to the ocean may be more likely to hold higher green consumer values in terms of single-use plastic waste.

2.3. Policy and Green Consumer Values

Data collection by the California Coastal Commission [21] led to the conclusion that plastic is making its way into the environment at unprecedented rates, and that solutions to manage marine debris must be implemented to protect oceans and coastlines. In executing its response to such dire findings, California has become a world leader in the management of plastic pollution in the marine and coastal environment. California was the first state to ban plastic bags and one of the first to ban microbeads in personal care products. In addition to these measures, 296 state and local laws preventing the sale or use of plastic bags, plastic straws, and expanded polystyrene food packaging have been passed in the state since 2014, [35], inspiring similar legislation around the globe. California, with its densely populated coastline where 77% of residents live within 20 miles of the ocean [36], struggles to manage the influx of plastic debris into the surrounding environment, notably into nearby sensitive coastal habitats, many of which are classified as Marine Protected Areas. The direct and indirect costs of such waste are high. For example, in California, towns and taxpayers spend approximately $500 million each year in marine protected areas on beach trash cleanup [37].

Research has explored the voluntary simplicity, collaborative consumptions, and boycotts that anti-consumption types are embedded in concepts of sustainability [38]. Taking this model further, we utilized single-use plastic legislation on bans and rebates. The Footprint Foundation [39] gathers and analyzes single-use plastic legislation across the United States. As of July 2019, only eight states have a ban on single-use plastic bags: California, Hawaii, New York, Connecticut, Delaware, Maine, Oregon, and Vermont. A map of polystyrene bans and bans under consideration (see Figure 2) clearly shows that single-use plastic bans and legislation are heavily focused on coastal regions. As of 2021, Maine became the first state to ban plastic foam containers and Seattle, WA became the first city to ban straws in 2018. In states such as Iowa, we see a different story being told by the government and citizens. Iowa barred locales from banning single-use plastic bags, however, several restaurants in Des Moines refuse to offer plastic straws, and grocery stores such as HyVee offer discounts for bringing back the single-use plastic bags [40]. Our study takes these findings into consideration and explores whether consumers within states that have bans on single-use plastic are more likely to hold higher green consumer values.

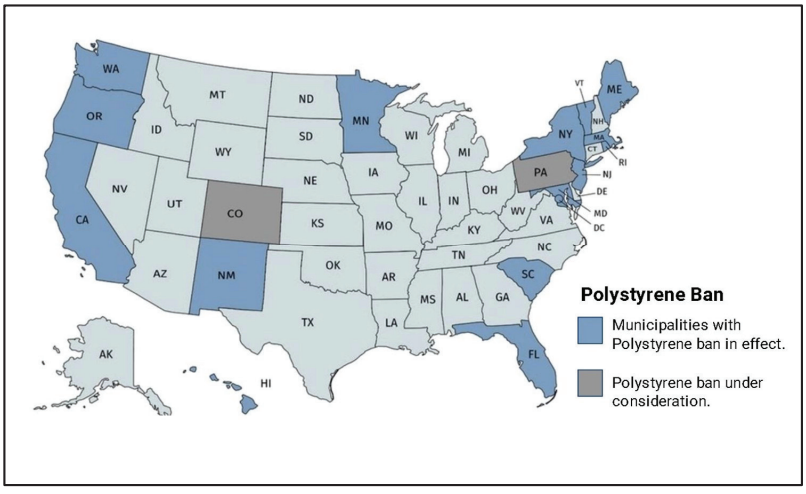

Figure 2. Footprint Foundation's single-use plastic map, May 2022.

2.4. Recycling and Single-Use Plastic Waste

Beverage container deposit laws, or bottle bills, are designed to reduce litter and capture bottles, cans, and other containers for recycling. Ten states and Guam have a deposit-refund system for beverage containers. Deposit amounts vary from two cents to 15 cents, depending on the type of beverage and volume of the container. To explore the effects of such bans on green consumer values, we take the use of bans and explore deposits and rebates reward systems put into place by state policymakers. Therefore, we divided the states by those that provide rewards or rebates and those that do not [41]. The positive connotations over reusable products in the form of a rebate or deposit may be a way to hold each other accountable for the recycling of single-use plastic products. As our study focuses on consumers' perceptions of single-use plastic waste, we analyzed if consumers in states with rebates on plastic are more likely to report using less single-use plastic products. These states include California, Connecticut, Hawaii, Iowa, Maine, Massachusetts, Michigan, New York, Oregon, and Vermont.

Consumers have the capacity to change irrespective of past circumstances, yet research suggests a continued reluctance amongst consumers to transform their behavior toward making eco-friendly choices [42]. That is, consumers are choosing not to use reusable items and many consumers are uncomfortable with making this seemingly substantial change to their purchasing and consuming behaviors [42]. Many consumers seem to overlook the impact that the individual has on the overall condition of the environment. It may be that consumers do not realize the impact of a single consumer when it comes to single-use plastic items and the increase of use-and-throw away items. In order to make the changes to preserve the environment, moving away from disposable products needs to be a community-wide effort. There may be a positive result when reusable products are enforced and consumers can hold each other accountable in making substantial action. Bans may lead to consumers reporting less use of single-use plastic products. These expectations lead to the following hypotheses:

Hypothesis 1 (H1). *Consumers who live near the ocean (environment) are more likely to have a greater concern for (1) green consumer values and (2) consumer behavior reducing single-use plastic waste (personal).*

Hypothesis 2 (H2). *States with bans (environment) on single-use plastic are more likely to have higher levels of green consumer values (personal) than states without such legislation.*

Hypothesis 3 (H3). *States with deposit/rebate programs (environment) for single-use plastic are more likely to have higher levels of green consumer values (personal) than states without such legislation.*

Hypothesis 4 (H4). *States with bans (environment) on single-use plastic are more likely to have greater concern for (1) green consumer values and (2) consumer behavior reducing single-use plastic waste (personal).*

3. Methodology and Analysis

3.1. Method

Participants we recruited through a Qualtrics Q-panel with selection of participants evenly distributed across the U.S. Participants were asked to complete a survey through the Qualtrics Q-panel that would take approximately 10 min. Each participant was asked to review the Plastic Pollution Coalition [43] video produced in collaboration with Jeff Bridges to educate consumers on the impacts of single-use plastic waste on humans and their environment. The video explores the effects of single-use plastic waste on humans, the environment, and animals, and made a call to action to make change. After each video, participants were asked to answer a series of questions on (1) Green Consumer Value Questions [32] and (2) Consumer Behavior and Sustainability, specifically single-use plastic

waste as developed by the research team. Along with these questions, participants were also asked to answer demographic profiles including gender, age, marital status, education, and employment status. Participants watched the video embedded into the Qualtrics survey on the YouTube platform.

3.2. Measures

The Green Consumer Value Questions were adapted from questions previously used to assess an individual consumer's tendency to consider the environmental impact of their purchases and consumption behaviors [32]. These questions were rated on a 5-point Likert scale from 5 = agree a lot, 4 = agree, 3 = neither agree nor disagree, 2 = disagree, and 1 = disagree a lot. Cronbach's alpha ($\alpha = 0.93$) showed strong reliability in the green value scale questions. Finally, participants were presented with two questions related specifically to consumer behavior and single-use plastic waste. These questions include "Are you likely to consider single-use plastic waste alternatives when making purchase choices?" and "Do you make an effort toward sustainability (e.g., reusable bags, paper straws). These questions were rated on a 5-point Likert scale from 1 = definitely no, 2 = probably no, 3 = neither agree nor disagree, 4 = probably yes, and 5 = definitely yes.

4. Results

A total of 358 participants completed the study with a usable number of participants at N = 342 with the overall completion rate of 96%. The participants in the Q-panel were 55% female with a median age between 18–45 years and were distributed across the United States with 39 states represented in the sample. We chose a sample of states from the middle of the country, including Iowa, Illinois, Kansas, Minnesota, Missouri, Tennessee, and Kentucky, to represent regions that are not near the ocean (N = 184 participants). We also selected a sample of coastal states that border major bodies of salt water, such as the Pacific Ocean and Gulf of Mexico, these states were Florida, California, Oregon, and Washington (N = 158 participants).

The results of the study found that proximity to the ocean did not have a significant impact on consumers' green values or decisions related to sustainability and reducing single-use plastic usage. In a multivariate analysis that divided states based on their proximity to the ocean, the findings showed that location did not have a significant effect on green consumer values ($F = 2.36$, $p = 0.126$), nor did it affect consumer decisions related to reducing single-use plastic usage ($F = 2.81$, $p = 0.10$). Table 1 presents the means, standard deviations, F-statistic, and *p*-value for coastal and non-coastal sample regions and (1) the green consumer value and (2) consumer behavior toward single-use plastic. The hypothesis that proximity to the ocean would impact individuals' green values was not supported.

Table 1. Proximity to the Ocean Study Results.

		Non-Coastal (N = 158)	**Coastal (N = 158)**	
		Mean (SD)	**Mean (SD)**	**F (*p*)**
H1 (1)	Green Consumer Values	3.87 (1.02)	4.04 (0.88)	2.36 (0.126) *
H1 (2)	Reducing Single-Use Plastic Waste	4.36 (0.79)	4.23 (0.65)	2.81 (0.10) *

Note: * Results not significant based on proximity to the ocean.

The study's findings suggest that statewide bans on single-use plastic may have a positive impact on consumers' green values. By May 2022, states that had implemented plastic bans included California, Hawaii, New York, Connecticut, Delaware, Maine, Oregon, and Vermont. The researchers conducted a multivariate analysis (MANOVA) by dividing the data into two categories - states with bans and states without bans - and using Green

Consumer Values as the fixed variable. The results indicated that states with bans had more significant green consumer values than those without bans (F = 8.39, $p < 0.00$). Refer to Table 2 for means, standard deviations, and MANOVA results. This supports the hypothesis (H2) that states with bans would have higher green consumer values.

Table 2. Bans Results.

		No-Ban (N = 213)	Ban (N = 129)	
		Mean (SD)	**Mean (SD)**	**F (*p*)**
H2	Green Consumer Values	3.83 (1.02)	4.14 (0.84)	8.39 (0.00)
H3	Reducing Single-Use Plastic Waste	4.39 (0.76)	4.15 (0.65)	8.97 (0.00)

Next, the consumer behavior usage of single-use plastic was reported with two consumer decision-making measures. We ran a MANOVA on the same divided population sample (those states with bans and those without bans) and the fixed variable of Consumer Decision. The mean score was combined for the following questions on "reporting on the consumers usage of alternatives to single-use plastic" and "reporting on the consumer's effort towards making sustainable decisions." The consumers that lived within states with bans on plastic waste reported more consumer decision-making towards sustainable practices (F = 8.97, ($p < 0.00$). Refer to Table 2 for means, standard deviation, and MANOVA results. H3 is supported.

To solidify the positive results, the data was then divided into two groups by states with deposits on single-use plastic and those without. States with a Statewide Rebate Program (N = 144) and those with No Statewide Rebate Program (N = 198). These states are slightly different than those with bans on single-use plastic, however the results were still favorable in that those states with statewide rebate programs reported higher green consumer values (F = 7.33, $p < 0.00$) and use of alternative solutions by reducing use of single-use plastic, Consumer Decision toward reducing single-use plastic waste (F = 6.32, $p < 0.01$). Table 3 presents the means, standard deviations, f-statistics, and p-value for the comparison between the states with rebate programs and those without. H4 is supported.

Table 3. Rebate Results.

		No-Rebate (198)	Rebate (N = 144)	
		Mean (SD)	**Mean (SD)**	**F (*p*)**
H4 (1)	Green Consumer Values	3.84 (1.01)	4.11 (0.87)	7.33 (0.00)
H4 (2)	Reducing Single-Use Plastic Waste	4.39 (0.76)	4.19 (0.68)	6.32 (0.01)

5. Discussion

The findings support the importance of effective policy making around issues of single-use plastic waste. Applying a social cognitive framework to better assess the multiple factors to include (1) personal factors: green consumer values; (2) environmental factors: location, bans, rebates; and (3) behavioral factors: consumer decision-making, supports that reciprocal determinism provides ways to challenge reduction of single-use plastic waste in consumer decision-making. In the data analysis, H1 was not supported, which explored the environmental factor of a consumer's proximity to the ocean. However, the data supported H2–H4, which included environmental factors such as bans and rebate/reward

programs. The results found that consumers who lived within states that had bans and/or rebate programs were more likely to have stronger green consumer values and indicated a reduction in the use of single-use plastic waste. Therefore, one of the most valuable results of the study is the idea that states that support single-use plastic waste reduction might take it one step further to implement strategies to enforce these polices across the state rather than leaving the practices up to the individual consumer.

In the study, the educational video developed by the Plastic Pollution Coalition addresses environmental concerns of single-use plastic waste rather than the human health effects of single-use plastic waste. Oftentimes, the marketing campaigns on single-use plastic waste mitigation are focused on the ocean and ocean animals. However, as the research has found, there may need to be more of a focus on the effects on human health and the environment, not just the effects on animals in the ocean. Upon examination of the results, we found some of the demographic findings to be noteworthy. Our findings indicated that there was a significant correlation between individuals' level of education and their values towards green consumption and decision-making regarding single-use plastic waste ($F = 5.02$, $p < 0.00$, and $F = 4.96$, $p < 0.00$, respectively). Table 4 presents the mean, standard deviation, f-statistic, and p-value on the level of education and green consumer values and consumer decision-making toward single-use plastic waste. This suggests that individuals with more education on the topic of single-use plastic waste may be more likely to change their behavior. While implementing bans and rebates can enforce change, providing education at all levels may have a even greater impact on individuals' behavior. Further research is needed to understand the impact of education on individual consumer shopping habits. In future studies, it would be beneficial to examine the extent to which individuals are familiar with education and campaigns related to single-use plastic waste in order to gain a better understanding of their overall effects.

Table 4. Education Level Comparison Results.

		Green Consumer Values	Consumer Behavior
Level of Education	**Total per Group**	**Mean (SD)**	**Mean (SD)**
High School Degree	N = 54	3.85 (1.24)	4.32 (0.78)
Some College	N = 110	3.79 (0.96)	4.51 (0.74)
4-year Degree	N = 96	3.75 (0.95)	4.33 (0.74)
Professional/Doctorate Degree	N = 103	4.15 (0.89)	3.94 (0.50)
F statistic (p-value)		$F = 5.02$ ($p < 0.00$)	$F = 4.96$ ($p < 0.00$)

In the demographic section of the study, the study included three questions related to political views on climate change and pollution. These questions aimed to understand the respondents' views on how politicians should address pollution and environmental issues, and how those factors may affect voter behavior. The questions were as follows: Q1: "I feel politicians should be more concerned about climate change and pollution"; Q2: "Environmental factors influence the way I vote for political leaders"; and Q3: "I am single issue voter, concerned only with environmental policy." Our findings indicated that there is a relationship between individuals' political views and their attitudes towards green consumer value and consumer decision-making toward single-use plastic waste. Table 5 presents the means, standard deviations, f-statistic, and p-value for the three political view questions and shows the relationship between respondents' political views and their values towards green consumption and decision-making regarding single-use plastic waste. This suggests that individuals' political views may influence their values in green consumption and therefore their behavior toward single-use plastic waste. However, the study did not examine the direct impact of political affiliation (i.e., Democrat and Republican) on

consumer behavior change. Further research is needed to fully understand the relationship between political affiliation and policy toward single-use plastic waste.

Table 5. Political View Results.

		Political View Q1	Political View Q2	Political View Q3
	Mean (SD)	**F (*p*)**	**F (*p*)**	**F (*p*)**
Green Consumer Values	3.95 (0.96)	37.82 (0.00)	47.12 (0.00)	9.03 (0.00)
Consumer Behavior	4.30 (0.73)	88.71 (0.00)	68.51 (0.00)	15.71 (0.00)

As programs are introduced to states based on legislation enforcement of bans, rebates, and rewards, consumers' mere exposure to the campaigns can influence their consumer decision-making. In addition, states with bans, rebates, and rewards provide single-use plastic waste marketing material such as trash cans and imagery of plastic waste, such as PSA video education about the effects of single-use plastic waste. These efforts may make a difference in the consumer's decision-making toward single-use plastic waste.

Personal locus in the model addresses green consumer values. Green consumer values continue to provide a guide for sustainable consumption habits in consumer behavior research [33]. In this study, we did not find support in the proximity to the ocean (location) of the consumers for increased green consumer values. However, we did find support for those states that had bans and/or deposit/rebate programs as reporting higher levels of green consumer values (personal). This supports the need for more state government legislation around single-use plastic waste programs to encourage consumers to reduce their use of single-use plastic waste. Our study results support the effectiveness of government bans and rebate or reward programs in changing consumer behaviors toward higher green consumer values.

6. Conclusions, Limitations and Future Research Orientations

6.1. Conclusions

Using a social cognitive framework, these findings support the developing of regulation around single-use plastic waste reduction. Therefore, we have done an exploratory analysis of resources available to guide decision makers. There are many solutions that policymakers, businesses, non-profits, educators, and consumers could use to become more aware of the challenges faced by single-use plastic waste. Governmental organizations working to investigate and prevent the adverse impacts of marine debris include NOAA Marine Debris Program [44], for example, which has already funded many initiatives to address these important organizational issues. These educational programs need the continued support of policymakers and the promotion of tools that include consumer-citizens responsibilities [45,46]. For example, consumer-citizens' responsibilities may include recycling and reducing one's use of single-use plastic waste. Prothero et al. [14] calls for an increase in public environmental and social awareness through environmental education in the school system, educational programming on television, and campaigns using social media. To assist in the solutions initiative, this study categorizes existing solutions into a database for decision makers to use in their efforts to prevent single-use plastic waste. To date we have compiled over 100 resources. The guidebook of solutions to single-use plastic waste mitigation can be found by following the QR Code (see Policy Solution Resource, Figure 3) or following the link to the database https://tinyurl.com/SUPtogether (accessed on 10 December 2022).

Figure 3. Policy Solution Resource.

A closer analysis of the resources gathered through a six-month analysis period shows 26 resources that are specifically tailored towards education on single-use plastic waste. Of those resources, 17 came from the United States based on headquartered locations including Washington D.C., CA, CO, HI, NY, and VT, with international locations represented by resources from Australia, UK, Netherlands, Switzerland, New Zealand, and Kenya. This collection of over 100 policy solutions has been categorized into five major resource groups: (1) Business Resources (N = 25), (2) Public Policy Resources, (N = 5) (3) Nonprofit Resources, (N = 15) (4) Education Resources, (N = 25); and (5) Personal Resources, (N = 33). A structured search was conducted to find other means of communicating the single-use plastic message beyond the video produced by the Plastic Pollution Coalition. The results of a six-month search utilizing Google Trends to narrow the results applying keywords such as "what are single-use plastic", "single-use plastic", "single-use plastic ban", and "plastic bag bans" developed categories for (1) name of resource, (2) content type, (3) policy solution, and (4) link to information.

The solution guide provides those in practice with the necessary information to continue to build their own tailored single-use plastic waste solutions. The tools and links to more information include books, videos, websites, educational kits, documentaries, movies, packaging alternatives, and many more. The current research adds to the existing knowledge by expanding the discussion on sustainability with a focus on single-use plastic. In addition, the paper synthesizes many resources as a form of a meta-analysis, enabling stakeholders to meet their needs as well as provide scalable solutions for future endeavors. In addition, the guide provides tools to guide policymakers and governments on how to implement a ban or rebate program. For example, the Product Stewardship Institute (PSI) provides an extended producers responsibility and the Break Free from Plastic Pollution Act guides businesses on the steps to take to implement a program that works best for industries. There are many free resources available for adaption to fit diverse needs. For example, the state of Maine provides a 'guide on single-use plastic carry-out bag ban.' These resources are listed as 'business solutions' and 'public policy solutions' on https://tinyurl.com/SUPtogether (accessed on 10 December 2022).

6.2. Limitations and Future Research

This study is not without its limitations. Green consumer values were used to assess sustainability perspectives of the survey results. As the use of the term sustainability becomes more politically polarized, we may need to re-evaluate or relabel the use of the word sustainability in ways similar to the rebranding of global warming as climate change. In the current study, survey respondents were asked demographic questions by state, making it hard to assess the effects of city and community bans. For example, Iowa was considered a Republican state without statewide plastic waste bans, however communities within Iowa are choosing to enforce city bans on plastic waste counter to the statewide programs [40]. A future study exploring city-by-city green consumer values would add to the existing research.

Further, a future study incorporating the additional resources found in Figure 3 would be valuable. For example, participants' familiarity with the Plastic Pollution Coalition, Jeff Bridges, or single-use plastic causes needs to be addressed in the future. Finally, analyzing results in a time series would be valuable to address participants' experience with single-use plastic waste. This may engage the discussion for others to seek out more information that we would not fully understand within a complete time series analysis.

Another study that is worth undertaking would be to address an individual's actual consuming habits. For example, do respondents use reusable shopping bags in the supermarket in the plastic ban state and the no ban state? We know that bans and rebates are making a difference in consumer behavior change. A next step would be to analyze factors of motivation at the individual level. Many non-profit organizations such as the Plastic Pollution Coalition call on individuals to 'take action'. Compiling a list of single-use

plastic decision-making habits and comparing this regulation would add value to the existing study.

The English proverb *"birds of a feather flock together"* refers to how individuals with similar thinking and lifestyles gravitate toward each other and live within similar communities. Our findings suggest that individuals living in states with bans or rebate/reward programs for green consumer behavior may already be more inclined towards green consumption, and therefore more likely to report their use or lack of use of single-use plastic alternatives. Additionally, these individuals may have already been exposed to or participated in campaigns related to reducing single-use plastic waste. As we continue this study, we will also investigate participants' experiences with educational programs related to single-use plastic waste. If our findings are supported by further research, it could help bring together like-minded businesses, consumers, and decision makers in communities that share not only similar lifestyles but also a commitment to sustainability issues, such as reducing single-use plastic waste.

Author Contributions: Conceptualization, S.F.; Methodology, S.F.; Resources, B.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Pepperdine University protocol code 18-12-930 on 8 January 2019.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Choi, A. Why Plastic Waste Is a C-Suite Issue. 2020. Available online: https://hbr.org/2020/04/why-plastic-waste-is-a-c-suite-issue (accessed on 10 December 2022).
2. Environmental Protection Agency. Sustainable Materials Management: Non-Hazardous Materials and Waste Management Hierarchy. 2017. Available online: https://www.epa.gov/smm/sustainable-materials-management-non-hazardous-materials-and-waste-management-hierarchy (accessed on 10 December 2022).
3. UrthPact. Plastics Bans in the US: Where They Are and How to Comply with Them. 2021. Available online: https://www.urthpact.com/plastics-bans-in-the-us-where-they-are-and-how-to-comply-with-them/ (accessed on 10 December 2022).
4. United Nations. Sustainable Development Goals Report. 2019. Available online: https://unstats.un.org/sdgs/report/2019/ (accessed on 10 December 2022).
5. Mende, M.; Scott, M. May the Force Be with You: Expanding the Scope for Marketing Research as a Force for Good in a Sustainable World. *J. Public Policy Mark.* **2021**, *40*, 116–125. [CrossRef]
6. Pew Charitable Trusts. Breaking the Plastic Wave: A Comprehensive Assessment of Pathways towards Stopping Ocean Plastic Pollution. 2020. Available online: https://www.pewtrusts.org/-/media/assets/2020/07/breakingtheplasticwave_report.pdf (accessed on 10 December 2022).
7. Blue Ocean Project. Positive Effects on the Environment. 2021. Available online: https://oceanblueproject.org/ (accessed on 10 December 2022).
8. Plastic Pollution Coalition. 2021. Available online: https://www.plasticpollutioncoalition.org/ (accessed on 10 December 2022).
9. Terracycle. What Is Terracycle? 2022. Available online: https://www.terracycle.com/en-US/ (accessed on 10 December 2022).
10. Loop Store. See You in Stores Soon. 2022. Available online: https://loopstore.com/ (accessed on 10 December 2022).
11. Ohoo Water. Ohoo Water, the Edible Waterbottle. 2022. Available online: http://www.oohowater.com/ (accessed on 10 December 2022).
12. Dropps. Want Green Cleaning Products that Work? 2022. Available online: https://www.dropps.com/ (accessed on 10 December 2022).
13. Phipps, M.; Ozanne, L.K.; Luchs, M.G.; Subrahmanyan, S.; Kapitan, S.; Catlin, J.R.; Gau, R.; Naylor, R.W.; Rose, R.L.; Simpson, B.; et al. Understanding the inherent complexity of sustainable consumption: A social cognitive framework. *J. Bus. Res.* **2012**, *66*, 1227–1234. [CrossRef]
14. Prothero, A.; Dobscha, S.; Freund, J.; Kilbourne, W.E.; Luchs, M.G.; Ozanne, L.K.; Thøgersen, J. Sustainable Consumption: Opportunities for Consumer Research and Public Policy. *J. Public Policy Mark.* **2011**, *30*, 31–38. [CrossRef]

15. United Nations Environment Programme. *Talk the Walk: Advancing Sustainable Lifestyles through Marketing and Communications*; United Nations Global Compact and Utopias: New York, NY, USA, 2005.
16. Farmer, A.; Breazeale, M.; Stevens, J.L.; Waites, S.F. Eat Green, Get Lean: Promoting Sustainability Reduces Consumption. *J. Public Policy Mark.* **2017**, *36*, 299–312. [CrossRef]
17. Frey, B.S.; Oberholzer-Gee, F. The Cost of Price Incentives: An Empirical Analysis of Motivation Crowding-Out. *Am. Econ. Rev.* **1997**, *87*, 746–755.
18. Elkin, E. Coca-Cola Moves from Plant-Based Bottles to Recycled Ones, Bloomberg Business Week. 2021. Available online: https://www.bloomberg.com/news/articles/2021-02-09/coca-cola-wants-to-reduce-plastic-pollution-with-recycled-bottles#xj4y7vzkg (accessed on 10 December 2022).
19. Moore, S.; Sutula, M.; Von Bitner, T.; Lattin, G.; Schiff, K. Southern California Bight 2016 Regional Monitoring Program: Trash and Marine Debris. 2013. Available online: https://www.sccwrp.org/search-return/?q=moore (accessed on 10 December 2022).
20. Moore, C.J. How much plastic is in the ocean? You tell me! *Mar. Pollut. Bull.* **2015**, *92*, 1–3. [CrossRef]
21. California Coastal Commission. California Coastal Cleanup Day History. 2019. Available online: https://www.coastal.ca.gov/publiced/ccd/history.html#top10 (accessed on 10 December 2022).
22. Sun, X.; Foscht, T.; Eisingerich, A.B. Does educating customers create positive word of mouth? *J. Retail. Consum. Serv.* **2021**, *62*, 102638. [CrossRef]
23. Gilal, F.G.; Zhang, J.; Gilal, R.G. Integrating intrinsic motivation into the relationship between product design and brand attachment: A cross-cultural investigation based on self-determination theory. *Eur. J. Int. Manag.* **2020**, *14*, 1. [CrossRef]
24. Lee, S.; Pounders, K.R. Intrinsic versus extrinsic goals: The role of self-construal in understanding consumer response to goal framing in social marketing. *J. Bus. Res.* **2019**, *94*, 99–112. [CrossRef]
25. Thorgersen, J.; Gronhoj, A. Electricity savings in Households—A Social Cognitive Approach. *Energy Policy* **2009**, *38*, 7732–7743. [CrossRef]
26. Verplanken, B.; Aarts, H.; Van Knippenberg, A. Habit, information acquisition, and the process of making travel mode choices. *Eur. J. Soc. Psychol.* **1997**, *27*, 539–560. [CrossRef]
27. Small, K.A.; Van Dender, K. Fuel Efficiency and Motor Vehicle Travel: The Declining Rebound Effect. *Energy J.* **2007**, *28*, 25–51. [CrossRef]
28. Sorrell, S.; Dimitropoulos, J.; Sommerville, M. Empirical estimates of the direct rebound effect: A review. *Energy Policy* **2009**, *37*, 1356–1371. [CrossRef]
29. Kaplan, B.; Easwar, I. Motivating Sustainable Behaviors: The Role of Religiosity in a Cross-Cultural Context. *J. Consum. Aff.* **2021**, *55*, 792–820. [CrossRef]
30. Cho, Y.-N.; Soster, R.L.; Burton, S. Enhancing Environmentally Conscious Consumption through Standardized Sustainability Information. *J. Consum. Aff.* **2018**, *52*, 393–414. [CrossRef]
31. Bandura, A. Human Agency in Social Cognitive Theory. *Am. Psychol.* **1989**, *44*, 1175–1184. [CrossRef]
32. Haws, K.L.; Winterich, K.P.; Naylor, R.W. Seeing the world through GREEN-tinted glasses: Green consumption values and responses to environmentally friendly products. *J. Consum. Psychol.* **2013**, *24*, 336–354. [CrossRef]
33. Ham, C.-D.; Chung, U.C.; Kim, W.J.; Lee, S.Y.; Oh, S.-H. Greener than Others? Exploring Generational Differences in Green Purchase Intent. *Int. J. Mark. Res.* **2021**, *64*, 376–396. [CrossRef]
34. Straughan, R.D.; Roberts, J.A. Environmental segmentation alternatives: A look at green consumer behavior in the new millennium. *J. Consum. Mark.* **1999**, *16*, 558–575. [CrossRef]
35. Romer, J. The Latest Plastic Bag Laws and Map, Surfrider Foundation. 2019. Available online: https://www.surfrider.org/coastal-blog/entry/the-latest-plastic-bag-laws-and-maps (accessed on 10 December 2022).
36. Kildow, J.; Colgan, C.S. California's Ocean Economy. National Ocean Economics Program. 2005. Available online: https://cbe.miis.edu/noep_publications/8/ (accessed on 10 December 2022).
37. Monroe, L. Waste in Our Waterways: Unveiling the Hidden Costs to Californians of Litter Clean-Up. Available online: https://www.nrdc.org/sites/default/files/ca-pollution-in-waterways-IB.pdf (accessed on 10 December 2022).
38. Seegebarth, B.; Peyer, M.; Balderjahn, I.; Wiedmann, K.P. The Sustainability Roots of Anti-Consumption Lifestyles and Initial Insights Regarding Their Effects on Consumers' Well Being. *J. Consum. Aff.* **2016**, *50*, 68–99. [CrossRef]
39. Footprint Foundation. Single Use Plastic Legislation U.S. Bans at a Glance. 2022. Available online: https://footprintusfoundation.org/single-use-plastic-legislation/ (accessed on 10 December 2022).
40. Kilen, M. Plastic Problem: What One Tiny Iowa Town Did to Combat It, and How Yours Ranks, Des Moines Register. 2018. Available online: https://www.desmoinesregister.com/story/news/2018/05/02/earth-month-plastic-bags-pollution-grimes-alleman-iowa-mwa/573983002/ (accessed on 20 May 2022).
41. NCSL National Conference of State Legislatures. State Beverage Containers Deposit Laws. 2020. Available online: https://www.ncsl.org/research/environment-and-natural-resources/state-beverage-container-laws.aspx (accessed on 10 December 2022).
42. Price, L.L.; A Coulter, R.; Strizhakova, Y.; E Schultz, A. The Fresh Start Mindset: Transforming Consumers' Lives. *J. Consum. Res.* **2018**, *45*, 21–48. [CrossRef]
43. Plastic Pollution Coalition. Open Your Eyes. 2016. Available online: https://www.youtube.com/watch?time_continue=2&v=9znvqIkIM-A (accessed on 10 December 2022).

44. NOAA Marine Debris Program. Who We Are. 2021. Available online: https://marinedebris.noaa.gov/who-we-are (accessed on 10 December 2022).
45. Hansen, U.; Schrader, U. A Modern Model of Consumption for a Sustainable Society. *J. Consum. Policy* **1997**, *20*, 443–468. [CrossRef]
46. McGregor, S. Towards a rationale for integrating consumer and citizenship education. *J. Consum. Stud. Home Econ.* **1999**, *23*, 207–211. [CrossRef]

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Review

A Review of the Current State of Microplastic Pollution in South Asian Countries

Lee Tin Sin [1,*], Vineshaa Balakrishnan [1], Soo-Tueen Bee [2,*] and Soo-Ling Bee [3]

[1] Department of Chemical Engineering, Lee Kong Chian Faculty of Engineering and Science, Universiti Tunku Abdul Rahman, Jalan Sungai Long, Bandar Sungai Long, Cheras, Kajang 43000, Selangor, Malaysia
[2] Department of Mechanical and Material Engineering, Lee Kong Chian Faculty of Engineering and Science, Universiti Tunku Abdul Rahman, Jalan Sungai Long, Bandar Sungai Long, Cheras, Kajang 43000, Selangor, Malaysia
[3] School of Materials and Mineral Resources Engineering, Engineering Campus, Universiti Sains Malaysia, Nibong Tebal 14300, Pulau Pinang, Malaysia
* Correspondence: leets@utar.edu.my (L.T.S.); beest@utar.edu.my (S.-T.B.)

Abstract: Microplastic contamination has become a concerning topic of study in recent decades. This review discusses the development of microplastic pollution based on a selection of South Asian countries consisting of Bangladesh, Iran, Philippines, Thailand, India, Indonesia, and Vietnam. The condition of microplastic pollution related to the abundance of microplastic found in various environments as well as the presence of microplastics in food and the air, is covered in this review. Several reports found that drinking water sourced from taps was found to have about 83% of microplastic particles in the year 2017 based on results from 14 nations, and in the year 2018, 260 bodies of water for human consumption in 11 countries were found to have about 93% of microplastic particles. Micro debris pollution in seas and oceans worldwide is predicted to be at an amount of 236,000 metric tons based on a statistical report. A mean value of 30 micro debris per liter of glacier water was recovered from the top of Mount Everest, whereas about 2200 small particles per liter were discovered in the deep waters of the Mariana Trench. The main environments that are severely microplastic-contaminated are water-based places such as rivers, estuaries, and beaches. The presence of microplastics in food items, such as tea bags, sugar, shrimp paste, and salt packets, has been reported. In terms of impacts on the environment, microplastic contamination includes the ingestion of microplastics by aquatic creatures in water environments. The impacts on terrestrial environments relate to microplastics sinking into the soil, leading to the alteration of the physicochemical parameters of soil. Meanwhile, the impacts on the atmospheric environment include the settling of microplastics on the external bodies of animals and humans.

Keywords: microplastics; pollution; contamination; environmental impacts; South Asian

Citation: Sin, L.T.; Balakrishnan, V.; Bee, S.-T.; Bee, S.-L. A Review of the Current State of Microplastic Pollution in South Asian Countries. *Sustainability* **2023**, *15*, 6813. https://doi.org/10.3390/su15086813

Academic Editors: Grigorios L. Kyriakopoulos, Antonis A. Zorpas, Vassilis J. Inglezakis and María Rocío Rodríguez Barroso

Received: 12 November 2022
Revised: 14 February 2023
Accepted: 15 February 2023
Published: 18 April 2023

1. Introduction

Plastics are polymeric materials mainly made up of hydrocarbons that are obtained from charcoal, petroleum, and fossil gas, which can be molded and shaped by utilizing pressure or heat methods. For instance, aerospace industries have been utilizing plastics more than metal alloys for the construction of the interiors of aircraft, structural elements, and navigation components due to the lighter weight of a plastic component than its metal counterparts. For safety aspects, some translucent, lightweight plastic materials have been found to exhibit high impact resistance, and these have additionally helped the aerospace industry in saving fuel costs over the years. Plastics also have other outstanding properties, such as low electrical conductivity, less density, high toughness, and transparency [1]. As such, the utilization of plastic is widely preferred by many industries around the globe, such as automotive, packaging, manufacturing, logistics and freight forwarding, medical

and healthcare services, and many more industries. Although plastics have many good properties, plastics decompose in landfills at a rate slower than any other waste, thus leading to a high accumulation of plastic waste, which results in the degradation of plastic materials into macro and microplastics [2]. In 2017, the usage of plastic drastically increased to 350 million metric tons compared with only 1.5 million metric tons in 1950 [3].

Plastics dumped in landfill normally undergo interment in terrestrial areas, and some of these waste plastics have a high probability of reaching aquatic environments. Most plastic waste moves downstream through rivers into marine environments. Research has shown that an approximation of 8 million metric tons to 11.5 million metric tons of plastic were detected in marine environments [2]. The marine debris most commonly found in oceans is known as microplastics, and the plastics vary in shape and size, with a size usually smaller than five millimeters. Plastic products, such as bottles, synthetic clothes, plastic bags, and cosmetics, have been identified as the main contributing sources to the formation of microplastics [4]. Microplastics that degenerate from larger parts of plastic waste into tiny plastic pieces are known as secondary microplastics. On the other hand, microbeads are one type of microplastic consisting of small pieces of polyethylene plastic products commonly used in health and beauty products as exfoliants, such as in toothpaste and facial cleansers, as well as plastic pellets utilized by industrial manufacturers and synthetic textile industries to produce plastic fibers such as nylon [4]. These microbead particles are capable of flowing through water filtration membranes effortlessly from households and subsequently being discharged into marine environments [4–8].

On the other hand, air pollution caused by microplastics occurs in the form of airborne particles and may cause health issues when inhaled. These microplastics are non-biodegradable and thus highly increase the accumulation of these microplastics on land and in marine environments. Marine deposits in seas and oceans that contain microplastics may settle straight into liquid columns or implicitly via tides as well as sediments that are washed into the water from cliffs. According to *The Guardian*, drinking water sourced from taps was found to contain about 83% of small microplastic particles in the year 2017 based on 14 nations, and in the year 2018, out of 260 water bodies used for human consumption tested in 11 countries, about 93% of microplastic particles was reported [9]. At the same time, Sebille et al. [10] reported that worldwide micro debris pollution throughout the seas and oceans is predicted to be 236,000 metric tons based on a statistical report. A mean value of 30 micro debris per liter of glacier water was recovered from the top of Mount Everest, whereas about 2200 small particles per liter were discovered in the deep waters of the Mariana Trench.

Microplastics have been identified in high quantities in about 114 species on land and in water [4]. About 80% of microplastic pollution in the environment has been determined to be from various sources. It has been shown that numerous invertebrate ocean and sea animals, such as crabs and crustaceans, have microplastics in their digestive tracts and tissues. Fish and air species, such as birds, ingest floating microplastics in seas and oceans and from water surfaces at a high rate. These animals misinterpret plastic pieces as food sources. Much of the data have been reported through global analysis, yet statistics related to microplastic pollution in Asian countries microplastic currently remain unknown. With this arising issue, a characterization study on microplastic pollution conditions in South Asian countries is worthy of investigation. In this review, the characterization of the microplastics present in South Asian countries was studied. The conditions of microplastic pollution were analyzed, and the impacts on the environment were further discussed in this review [4].

2. Countries of Study

2.1. Pakistan

According to Dowarah and Devipriya (2019) [11], the Arabian Sea contains approximately 0.2 million tons of polymer waste sent via the Mighty Indus. Pakistan generates roughly 0.6 million tons of polymer. Irfan et al. (2020) [12] reported that polymers are pro-

jected to make up almost 6% of the country's overall solid waste, which is above 40 million tons, with most debris discarded in exposed areas. Most of the megacities of the country face significant challenges in fixing the issues related to urban waste. Moreover, the precise volume of polymers in freshwater environments as well as microplastic contamination on surface water, are often overlooked [12]. The Ravi River is one of the rivers that flows by Lahore and is also known as the city's most prominent housing, industrial, and commercial center. The Ravi River accumulates various sorts of polymer waste, and this polymer waste eventually degenerates into microplastics [12]. In addition to the Ravi River, the Arabian Sea has a coastal area in Pakistan that is about 1000 km, where a high amount of microplastics is located [13]. The literature was studied based on the water surface and sediment of the Ravi River and Arabian Sea of Pakistan, where samples collected around the Ravi River originated from a combination of drainage, canals, and wastewater from households, known as sullage [12,13]. The microplastics concentrated on the surface water of the Arabian Sea was an average of 582.12 particles per liter, with a standard deviation of 246.14, whereas at the Ravi River, a mean value of 2074 pieces/m^3, with a standard deviation of 3651 [12,13]. With these results, both the Ravi River in Lahore and the Arabian Seacoast have been deemed to be polluted based on the surface water values. According to Business Recorder (2016) [14], Lahore is a megacity that ranks second as the city with the highest population in Pakistan. The Ravi River has become a severely contaminated waterway as a result of by-products from various businesses and tons of metropolitan wastewater [14].

The shapes of the microplastic identified in both areas of study were fragments, sheets, beads, foams, and fibers, where fragments were found to be the highest, 56.1%, in the Ravi River; subsequently, fibers accounted for 38.6%, and foams, sheets, and beads were found to be below 3% [12]. According to Ahmed et al. [13], fibers were abundantly found in surface water samples from the Arabian Sea, with a percentage of 99.77% and a balanced percentage accounted for by beads, sheets, and fragments [13]. The micro debris, with a size range between 300 μm and 500 μm, was primarily found in the surface water samples of the Ravi River, whereas particles of 55 μm were primarily located in the Arabian Sea specimens; subsequently, 500 μm-sized particles accounted for 35.53%, and there was a balanced percentage for 250 μm-sized particles [12,13]. A significant fraction of the large-dimensioned micro debris floating on the water might relate to the source's vicinity and a decreased time frame for fragmentation to occur [15,16].

The microplastics concentrated in the dregs of the Arabian Sea were, an average, 987.40 particles per liter, with a standard deviation of 617.06, whereas in the Ravi River, there was a mean value concentration of 3726 pieces/m^2, with a standard deviation of 9030 [12,13]. These values also show that the microplastic pollution in the sediment specimens is similar to the pollution levels of the water surface specimens. In the collected sediments, the specimens from the Ravi River, a higher segment of fragments was distinguished, resulting in an amount of 83.1% out of the total microplastics extracted from the dregs; subsequently, fibers amounted to 11.8% and were balanced by other polymers, such as beads and foams [12]. The Arabian Seashore samples exhibited higher fractions of fiber, similar to that of surface water specimens, with a value of 99.24% out of the total 4900 microplastics from the dregs, subsequently below that of the 0.8% of the other shapes, such as fragment forms, beads, and sheets [13]. Microplastics, ranging in size between 150 and 300 μm, were significantly identified in the dregs collected from the Ravi River, whereas the Arabian shore specimens have a greater proportion of micro debris in the size range of 55 μm, with a percentage of 43.98% out of the total microplastics categorized in the sediment samples, and the least was found to be 22.82% for the size of 250 μm [12,13].

From both pieces of research conducted in Pakistan, it is evident that fibers were found in both locations of the Ravi River and Arabian Seashore, with a high amount of fibers found in the dregs and water surface specimens collected from the Arabian Seacoast and Ravi River. The primary driver of fiber microplastics into sewers is the discharge resulting from household chores, such as laundry, which substantially contributes to the amount of

these microplastic particles found throughout the river. According to Irfan et al. (2020), the occurrence of a large number of fragmented microplastics in water specimens of sullage may be due to the decomposition of openly disposed plastic wastes dumped in the nearby surroundings [12]. In addition, plastic waste may be flushed into rivers and lakes during monsoon periods when there are massive amounts of waste discarded near drains and canals [12].

2.2. Saudi Arabia

In the year 2019, in the Middle East, Saudi Arabia was known as having one of the largest industries for the production of goods packaging, with an estimated value of USD 8.07 billion and residents using about 40 kg of polymer bags annually. The European Union reported that such usage is approximately twenty times more than the worldwide mean [17,18]. According to Al-Lihaibi et al. [18], Saudi Arabia has an approximate headcount of 34 million citizens. In earlier decades, major increases in the population of countries and industrialization have resulted in massive quantities of solid waste disposal [19]. The improper disposal of non-biodegradable items, such as reusable cans and totes, produces large quantities of hard waste, specifically during the holy periods of Ramadan and Haj. According to the research, only 10 to 20% of the total recyclables were successfully recovered and repurposed [20,21].

According to Pico et al. [22], Riyadh is a prominent economic, commercial, and production industry hub, covering a population of about 7.6 million residents, with many manufacturing companies generating polymer products that are capable of utilizing a great number of plastics. On the other hand, Al-Jubail is also one of the biggest petrochemical production regions that produce polymer resins and synthetic materials [22]. A study based on these two cities was conducted by obtaining surface water specimens. The mean concentrations of microplastics in Riyadh and Al-Jubail were 3.2 ± 0.2 items per liter and 0.2 ± 0.1 items per liter, respectively [22]. These mean results indicate that these towns have been contaminated with microplastics. Based on Figure 1, fibers are highly distinguished, accounting for 60% of the total microplastics extracted from the specimens (with only 1% lesser fiber found in Riyadh), subsequently fragment-shaped and spherules microplastics account for 15% each, and the remaining percentage consists of other kinds of minor shapes [22]. Most of the micro debris extracted was between 50 μm and 1000 μm, with the highest percentage of 35% in the group ranging from 50 μm to 100 μm, followed by 25% in the group ranging from 100 μm to 250 μm [22].

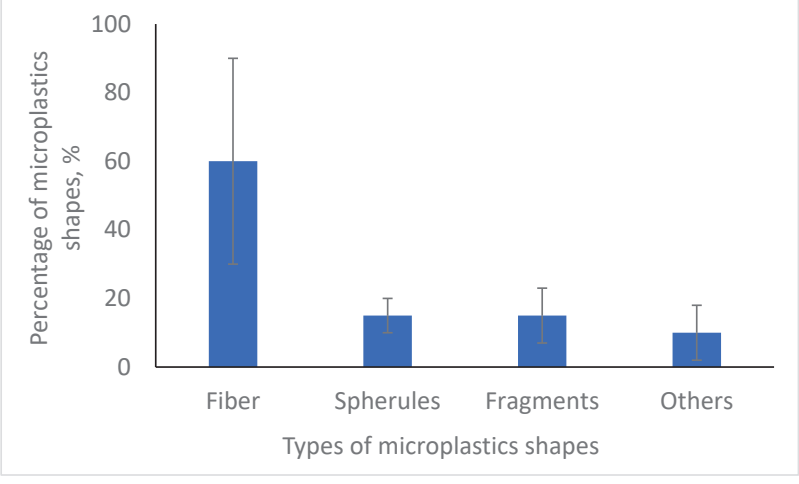

Figure 1. Percentage of microplastic shapes from surface water specimens (re-plot from data of Riyadh and Al-Jubail [22].

Based on Figure 2, polypropylene (PP) was the most abundant polymer from the surface water specimens sourced from Riyadh and the second highest in Al-Jubail, with a percentage difference of 6%. While polyethylene was found to be the most significant identified in Al-Jubail, with 24%, and a percentage of 19% of polyethylene was found in Riyadh.

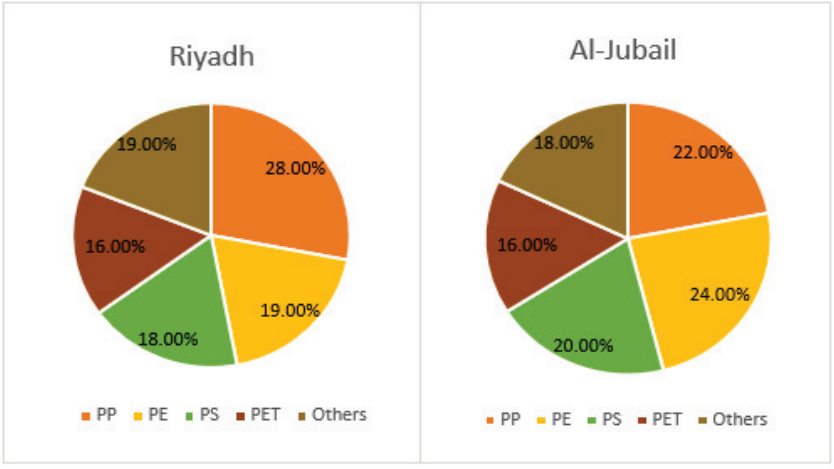

Figure 2. Average percentage of polymers from surface water specimens in Riyadh and Al-Jubail towns (Replot from data in Pico et al. [22].

Jeddah, with a populace of 3.4 million people, is known to be one of Saudi Arabia's biggest towns, and it is situated on the Red Sea's eastern shore. According to Anjum et al. [21], the town generates roughly two million tons of trash per annum. A study on microplastic contamination has been conducted on four different areas linked to the Jeddah shoreline. Sediments were collected from open sandy beaches and wastewater-releasing areas near the surroundings of Jeddah. Overall, the sediment specimens were found to contain approximately 119 particles per kg [23]. The open beaches were found to have a significant number of unevenly shaped fragments and foams, whereas sewage discharge sites have higher numbers of granular and fiber shapes [23]. The presence of granular shapes may be due to the discharge of microplastics in sewage. The polymers that were present in the dregs of both sampling locations are polyethylene terephthalate, polystyrene, polyester, and other minor polymers with percentages of 37%, 12%, 4%, and the remaining 53%, respectively [23]. The remaining proportions of microplastics could not be classified due to the use of low-quality equipment.

In summary, Saudi Arabia is a country facing increasing microplastic pollution; however, limited studies have been conducted to classify the presence and abundance of microplastics in the country. According to Pico et al. [22], the presence and quantity of microplastics can be impacted by the type of industry present in the metropolis and the growth of the town. Therefore, towns may have similar populations yet distinct characteristics, with varying numbers and distributions of microplastics, which is well evident by the greater abundance of micro debris in Riyadh compared to Al-Jubail [22]. In addition, public beaches may be polluted due to tourists and tourism-related events [23]. The high amount of fibrous and granular microplastics at sewage discharge ports may be due to the deposition of sewage at those ports and residues from clothing generated as fibers from laundry emanating via untreated wastewater [23].

2.3. Bangladesh

The characterization of the microplastics present in certain well-known areas in Bangladesh was established and studied. There are two main types of samples collected in

this study, sediment and water, based on six locations across the country. Water sampling was undertaken in two different research areas, which were urban lakes and rivers. Lakes are well noted for providing essential ecological amenities, such as providing a place for wildlife habitation, farming, flood defenses, water storage areas, weather regulation, and tourist activities [24,25]. In contrast to natural waterways, several urban lakes are specifically designed to handle urban overflow and collect rainwater, causing it to be vulnerable to anthropogenic contamination, ecological strain, and microplastics, becoming highly prevalent in wetlands due to greater anthropogenic occurrence [26–29].

Water specimens were collected from lakes in Dhaka, a highly populated and fast-expanding megacity area. In this area, microplastic contamination is mainly due to abundant plastic degradation caused by anthropogenic activities. While in another study, samples were taken from Karnafully river, where there are about eight million people residing close to the river situated in Chattogram, Bangladesh's second largest town and a corporate hub. The dominant shape of microplastic in the lake were films, micro pellets, and fragments, with a percentage of 40.91%, 36.36%, and 22.73%, respectively, whereas in the river, with a percentage of 83%, fibers were the most common type of micro debris, followed by fragments [30,31]. More than 50% of microplastics of a size smaller than 1 mm were highly located in the Karnafully river water samples, as shown in Table 1. According to Hossain et al. [30], strong tides, semidiurnal tide motion, and sea transportation could generate sufficient turbulence in the river and shred the plastic materials into tiny particles [30]. In terms of the plastic polymers found in both of the areas, high-density polyethylene (HDPE) was the most abundant found, with 60% in urban lakes, and polyvinyl chloride (PVC) and polycarbonate (PC) were present with equal percentages of 20% [31]. However, there was no information on the polymer types from the river water sample analysis.

Table 1. Characterization of microplastic present in environments of Bangladesh.

Location	Environment	Type of Sample	Dominant Shape	Size	Dominant Polymers	Reference
Cox's Bazar	Natural Beach	Beach sediment	Fibers (53%) Films (20%) Fragments (12%) Microbead (9%) Others (6%)	1–5 mm (59%) 0.5–1 mm (27%) <0.5 mm (14%)	-	[32]
Kuakata Beach	Beach	Beach sediment	Fibers (55%) Fragment (15%) Films (14%) Microbead (10%)	1–5 mm (55%) 0.5–1 mm (31%) <0.5 mm (14%)	PET, PE and PP (45.5%)	[33]
St. Martin's Island	Coastal beach	Coastal sediment	Fibers (50%) Films (26%) Foam (14%) Fragment (10%)	1–5 mm (25%) 0.5–1 mm (57%) <0.5 mm (18%)	Rayon (32%) Nylon (17%) PE (16%) PUR (11%) Others (24%)	[34]
Cox's Bazar	Beach	Beach Sediment	Fragment (64%) Foam (15%) Fibers (9%) Bead (6%) Film (6)	-	PP (47%) PE (23%) PS (9%) UD (9%) Others (12%)	[35]

Table 1. *Cont.*

Location	Environment	Type of Sample	Dominant Shape	Size	Dominant Polymers	Reference
Karnafully river	River	Surface water	Fibers (83%) Fragment (17%)	1–5 mm (24%) <1 mm (59%)	-	[30]
		Sediment	Fibers (56%) Fragments (34%) Film (6%) Pellets (3%)	<1 mm (39%)	-	
Dhaka	Urban Lakes	Water	Film (40.91%) Micro pellets (36.36%) Fragments (22.73%) Fiber (13.63%)	-	HDPE (60%) PVC (20%) PC (20%)	[31]
		Sediment	Film (33.33%) Fiber (30.55%) Fragment (27.77%)	-	HDPE (42.85%) PC (28.57%) CA (14.28%) PP (14.28%)	

Based on other research conducted on sediment samples, the sediment samples were categorized into various types: coastal sediments, beach sediments, and sediments from lakes and rivers [30,32–34,36]. St. Martin Island is currently the sole coral area in the country, and the number of travelers visiting the island has risen in current times, and tourist industry development plans have also grown, leading the number of ecological contaminants to surge on the island [36]. According to Tajwar et al. [34], many travelers visit St. Martin for its picturesque coral coast. Coastal sediment analysis from this island showed more than 50% fiber-shaped microplastics, similar to the studies from different researchers based on sediment extracted from Cox's Bazar, Kuakata Beach, and Karnafully river, in which fibers were dominant [30,32,33]. Cox's Bazar is also known to be a favored tourist spot due to the mountains and dunes surrounding the beach. An implication that fiber accumulation on the island and Cox's Bazar beach could be due to the utilization of swimwear and UV-protective garments produced from synthetic fibers as well as the traveler's activities in the area [32,34]. However, in another study by Rahman et al. [35], fragments were the highest found, with 64%, and 9% of the beach sediment samples from Cox's Bazar were fibers. This may be due to the sampling location, which was different from that of [32], and the sampling collection time was from august to yearend, including the traveler visiting season.

According to Banik et al. [33], increased fiber might be due to the generation of waste related to the textiles industry, such as apparel manufacturing, household launder released into the rivers, and fishing-related waste in the Bay of Bengal [33]. In the sediment sample from the urban lake, films, fibers, and fragments were almost equally dominant, where films are generated from the breakage of plastic bags and fragments potentially originate from remnants of polymer trash that arise from the direct disposal of solid waste [31,37]. Dhaka city deals with large amounts of pollutants discharged from drains, municipal waste disposal, and the discharge of unprocessed household and corporate effluents [31]. The sizes of microplastic from the sediment samples were mostly 1–5 mm with high percentages, as shown in Table 1; however, Karnafully river sediment specimen microplastics were mostly smaller than 1 mm, which may have decomposed as a result of aging and wear. The

decomposition of plastic waste into smaller microplastics was affected by environmental factors and hydrodynamic fluctuations [38].

In addition, Kuakata beach samples were found to consist of polyethylene terephthalate (PET), polyethylene (PE), and polypropylene (PP). The different types of plastic waste were highly extracted from the collected beach samples [33]. According to Banik et al. [33], PET and PP may originate from garments and fabric items as these are vastly utilized. PE is a type of polymer that is frequently utilized in meal packaging sheets and bottles for the products of gels and cleansers [39,40]. This type of polymer was evidently located in Cox's Bazar sediment samples, while the PP-type waste was found to be the highest, 47% [35]. In contrast, regarding St. Martin sampling, rayon followed by nylon polymers were highly found in the island specimens. According to Lebreton et al. [41], the presence of these polymers along the seaside was mainly due to the utilization of hard polymers in meshes and cords, which tend to be produced using these polymers [34,41]. Conversely, the waste from high-density polyethylene (HDPE) was extracted the most, 43%, in the sediment sample analysis from the lakes of Dhaka city, and this observation is mainly attributed to the increased utilization of plastic products made of PE. Subsequently, polycarbonate waste was extracted with 28% from the sediment sample [31].

Microplastic pollution is prevalent in the twain terrestrial and maritime ecosystems of Bangladesh based on the research carried out by professional organizations [42]. The Bay of Bengal is significantly polluted with microparticles from both internal and external sources. The sediments or water stream may have contributed to the emergence of microparticles in the area, according to Browne et al. [43] and Thompson [44]. They mentioned that microplastics can flow easily through filtration units of wastewater and sewerage purification plants [32]. Furthermore, winds may carry microparticles from one section to another section of the Bay of Bengal toward the shoreline and dunes. These three key streams are known as Padma, Meghna, and Jamuna in Bangladesh, and these could perhaps facilitate the transfer phenomenon [32].

The presence of microplastics in sugar, teabags, salt, and environments, such as rivers and islands, has raised alarm concerning the severe pollution conditions of these microplastics in the country. The survival of coral reefs in Bangladesh is a matter of study by researchers; St. Martin is currently the only coral island in Bangladesh and has more than 3000 travelers per year visiting the island. According to Gazi et al. [45], various studies have shown that coral reefs have considerably decreased recently [34]. Coral reefs are the ocean's biggest diverse biome and serve as coastal screens to preserve the shore from the sea's damaging effects, and these corals consume aquatic microorganisms for survival [46]. However, the presence of microplastic pollutants in the sea has affected coral viability as aquatic microorganisms could ingest the micro debris. Eventually, this condition has resulted in reducing the number of coral reefs in the ocean.

In addition, a recent study has shown the existence of microplastics in sugar and teabags based on two different analyses conducted based on five retail sugars and five flavored teabags, including branded packets purchased from various retailers in Dhaka City. These analyses showed a mean of 343.7 ± 32.08 microplastics per kg of sample sugar, with a greater prevalence of small fibers and spherules where the particles were mostly 300 μm in size. On the other hand, the teabag analysis was found to have high amounts of microplastics in unopened tea bags compared with the opened tea bag, with a greater frequency of fibers and fragments [47,48]. Bangladesh's sugar sector contributes significantly to the development of remote area facilities, labor opportunities for villagers, farmer income, forex savings, and quality enhancement to jaggery businesses and waste sectors [49]. Moreover, the country is a huge tea manufacturing nation, producing about 82.13 million kg of tea, based on figures from 2018. They also estimated an increased rate of 1.89% annually for the following years, leading to the country being ranked as the 10th biggest tea manufacturer worldwide [50,51].

Recent statistics on sugar utilization per individual in the country showed a peak in the year 2019, with a mass of 6.18 kg, which was 6% higher than in the year 2018 [52]. With

these sugar intake statistics, the continued consumption of sugar containing microplastic contaminants has the possibility to result in a yearly intake of tons of microparticles, on average about 10.2 tons comprising a variety of forms, sizes, and polymer constituents [47]. According to Afrin et al. [48], the release of microplastic particles through tea packets in Dhaka town can result in an estimated microplastic release rate of 10.9 million grams yearly. This high release rate can spread higher amounts of microplastic to various habitats [48]. Furthermore, the presence of microplastics in salts has also become a matter of study in Bangladesh. According to Parvin et al. [53], a mean amount of approximately 2676 microplastics per kg was discovered in retail sea salt purchased from various regional markets and grocery stores. Equally, both the purified and improperly refined salts consist of a significant number of microplastic contaminants [53]. The increased amounts of polymer pollution or microplastic contamination throughout coasts and salt-farming areas may be the reason for the discovery of microplastics in sea salt.

Based on a study conducted by Rakib et al. [54], microplastics have been detected in sea salt specimens collected from salt pans at Bangladesh's biggest salt-farming region located in the Maheshkhali Channel. They also concluded that the amount of microplastics detected reached an average value between 74.7 and 136.7 particles per kg [54]. In addition to that, microplastics have been discovered in sediment samples from St. Martin Island that amounted to 250 micro debris per kg [34]. Based on the beach sediment analysis conducted at Cox's Bazar, microplastics amounted to an average of 368.68 ± 10.65 particles per kg [32] and 8.1 ± 2.9 microparticles per kg [35]. Both studies conducted by Hossain et al. [32] and Rahman et al. [35] on Cox's Bazar indicate that the differences in values may be inferred from the different sampling points of the sediments and other natural environmental factors, such as the wave-induced transfer of microplastics. The wave-induced transfer could transfer microplastics across a larger distribution area. In addition, based on a recent analysis of one of the river streams in Bangladesh known as the Karnafully stream, this stream was found to consist of micro debris that amounted to a mean of 2.11 ± 1.15 microplastics per liter of top water specimen. Furthermore, the daily load of microparticles flushed into the Bay of Bengal through the river during summer was computed to be 61.3×10^9 microplastics per day [30].

Stream water is utilized for commercial, agricultural, and domestic purposes. However, the river is currently heavily contaminated with hazardous organic and inorganic contaminants [30,55]. The stream absorbs water inflow from numerous channels and tributaries along the way toward the Bay of Bengal and contains significant amounts of particulate and liquid waste originating from corporate, domestic, and rural areas in Chattogram [56]. Furthermore, farmers employ various insecticides and manures to improve yield, and the agricultural residues contaminate the stream water via canal outflows as agricultural works are widespread close to the river [56]. Approximately 750 sectors, such as garments, packaging, recycling, laundry, coating, automotive, heavy machinery, etc., explicitly or implicitly release unprocessed waste or effluent into the Karnafully water stream in which the closest municipal areas to Chattogram discard waste into the stream, significantly contributing to microplastic prevalence [30]. Statistics have shown that as of 2018, the town's municipal waste from different sources reached 2289 tons a day, including plastic items, which amounted to 8.3% [57].

Several potential factors that lead to elevated levels of micro debris include the disposal of defective fish meshes, cords, buoys, and fishing boxes at the Karnafully stream bank. The flow sections of this river along the country's business centers are likewise contaminated with micro debris, which could be a result of inadequate facilities to manage wastewater in urban areas nearby the Karnafully River [30]. The abundance of the microplastics discovered at the shore, beach, and river areas denotes the development of microparticles in the water throughout the Bay of Bengal that eventually build up in sea salts. Moreover, according to Parvin et al. [53], exposure to small plastic debris through salt has been predicted to constitute up to an average of 13088 microparticles annually. These data were computed based on the average quantity of microplastics, about 2676 microplastics per

kg, discovered from the sea salt analysis as well as the average per day salt intake level of 13.4 g by the people living in Bangladesh [53,58].

2.4. India

The characterization of microplastic in India was analyzed based on a few environments in India, such as estuaries, city streets, beaches, and coastal areas. Karthikapally and Panmana are situated on a hollow saline liquid lagoon known as the Kayamkulam estuary. The estuary region holds a significant quantity of sediments due to the low slope around the junction, with either backwaters or ponds [59]. A high amount of fiber-shaped micro debris, which amounted to 61.08%, was discovered in the estuarine sample [59], which is in line with analysis conducted at the Vellar estuary. They reported that fiber was predominant in the estuarine sample, 58.46% in the sediment and 79.29% in the estuarine water [60]. The minor form of small particles in the Vellar estuarine sediment were fragments and glitter, with 12.33% and 21.83%, respectively, which is in contrast to the estuarine sediment at Kayamkulam, with filaments and fragments as the minority of shaped microplastics [59,60]. Clay, gault in black, and silt, including alluvial deposits, make up the estuarine cliffs where the deposits affect the estuarine area. The ground of the Vellar estuarine is known to be Tamil Nadu's finest fertile firth [59,60]. The majority of the micro debris range in sizes of less than 1000 μm, as shown in Table 2.

Table 2. Characterization of microplastic present in environments of India.

Location	Environment	Type of Sample	Dominant Shape	Size	Dominant Polymers	Reference
Kayamkulam Estuary	Estuary	Estuarine sediment	Fiber (61.08%) Filament (33.94%) Fragment (4.52%)	1000–3000 μm (29.86%) <1000 μm (62.90%) >3000 μm (7.24%)	PE (22.62%) Polyester (42.98%) PP (34.38%)	[59]
Chennai	City Streets	Street dust	Fragment (92.46%) Fiber (7.54%)	-	PVC (24%) Superflex (14%) Microcrystalline (19%) PTFE (14%) HDPE (9%)	[54]
Marina Beach	Beach	Beach sediment	Fragments (33%) Fiber (15%) Foam (12%) Film (10%) Pellet (6%)	0.1–3 mm <2 mm (50%)	PE (29.9%) PP (16.9%) PVC (10.9%) Polyester (9.9%) Nylon (7.9%)	[61]
Karnataka	Beach	Beach sediment	Fragments (46%) Fiber (34%) Films (12%) Pellet (8%)	0.1–1 mm (49%) 1–2 mm (27%) 2–5 mm (24%)	PE (37%) PP (26%) PS (17%) Nylon (9%) Others (11%)	[62]
Puducherry	Beach	Beach sediment	Fragments (56.32%) Fiber (15.85%) Film (13.54%) Foam (8.27%) Others (6.02%)	1000–3000 μm (31.40%) 300–500 μm (34.88%) 500–800 μm (20.93%)	-	[11]

Table 2. *Cont.*

Location	Environment	Type of Sample	Dominant Shape	Size	Dominant Polymers	Reference
Vellar Estuary	Estuary	Estuarine water	Fibers (79.29%) Fragments (13.61%)	-	LDPE (58%) PP (36%) Others (6%)	[60]
		Estuarine sediment	Fiber (58.46%) Glitter (21.83%) Fragment (12.33%) Others (7.39%)	-	LDPE (45%) PP (35%) PVC (5%) PVA (4%) Others (11%)	
Odisha Coast	Coast	Coast sediment	Fragments (70.65%) Fibers (27.05%) Spherules (2.30%)	500–1000 μm (27.84%) 250–500 μm (21.11%) 100–250 μm (15.67%) >1 mm (23.90%)	12 Polymers detected (E.g HDPE, PVC, BR, PP, PTFE, SMMA)	[63]
South Andaman Coast	Coast	Coast sediment	Fragments (75.76%) Fibers (21.76%) Spherules (2.46%)	500–1000 μm (39.71%) 250–500 μm (22.67%) <100 μm (28.65%) Others (8.94%)	13 polymers detected (e.g., PP, BR PVC, ABS, Nylon 6)	[63]

PE is the third highest microplastic type found in the Kayamkulam estuarine [60], which contrasts with the sampling conducted in the Vellar estuary, where LDPE was most prominent in both water and sediment estuarine specimens [60]. Polyester-type waste was the majority discovered in the Kayamkulam estuary, amounting to 42.98% [59]. Moreover, based on a study conducted by Venkatramanan et al. [61], a lower percentage of polyester-type microplastics was found in the sediments collected from Marina beach. The beach samples have a large number of PE microplastics, 29.9%, followed by PP microplastics, with 16.9% [61]. This is a popular site in the state that attracts roughly 50,000 people a day for vacation holidays, eventually causing a severe accumulation of plastic litter on the beach. There is a lack of awareness and knowledge of both people and the government related to the detrimental impact of plastic waste contamination on the environment and human health. This may be the main reason that the pollution caused by plastic contamination is not regarded as a severe issue at Marina beach [61]. The predominant presence of PE and PP discovered are similar to the study conducted in Karnataka, where 37% and 26%, respectively, were extracted [62]. Moreover, the presence of nylon in Karnataka and Marina was a result of the utilization of fishing nets in that area.

The main human activities near Karnataka beach, the visitation of travelers, businesses, fishing ports, and touchdown areas, could have contributed to the presence of micro debris in that location [62]. In addition, common forms of microparticles extracted from Karnataka sediment samplings are fragments and fiber-shaped small debris [62], in line with the studies carried out using Puducherry beach sediments [11] and sediment samplings from Marina beach [61], as shown in Table 2. The abundant dimensions of microplastics discovered on all three beaches were between 1 and 3 mm. Furthermore, the significant presence of fibers and fragments was similar to the samplings of sediment from the coasts of Odisha and South Andaman, with more than 90% on both coasts, respectively [63,64]. Both Odisha and South Andaman coasts had 27.84% and 39.71%, respectively, of microplastics sized between 500 and 1000 μm [63,64]. In terms of the polymer types, both coasts had a few similar polymers discovered, such as PP, PVC, and BR, with no percentages to indicate dominance in both analyses. In another analysis based on Chennai Road dust, various

types of polymer waste were observed, such as PVC, which is the major micro polymer with 24%. Subsequently, microcrystalline waste was found with 19% to be the second highest, followed by PTFE and Superflex 200, both 14%, respectively [65].

PVC waste may have originated from the extensive utilization of item packaging, powerlines along the streets, and automobile wear-offs that end up on the street [65]. According to Thomas [66], ductwork and bottles used to store volatile chemicals and caustic substances frequently include PTFE. PTFE is mainly utilized in the production of cookware and kitchen utensils, serving as a non-stick layer [65]. Microcrystalline products serve as important elements in various industries, such as food, where they are utilized as stabilizing agents, especially in the soft drink industry, to serve as emulsifiers. Microcrystalline products are also used in beauty products as thickeners [65]. In the road sample, fragments and fiber were the major micro debris detected, which is similar to the beach sediment and coast sample sediment analysis conducted in certain places in India, as shown in Table 2. The urban cluster in Chennai has a population of around 10.9 million people living in the town based on figures from 2020. As a result, compared to nearby coastal towns along the country's East Coast, Chennai residents utilize plastic in significantly greater quantities [61]. The small plastic debris discovered on the streets is one of the concerning factors as these small particles could potentially enter land and marine habitats through several routes facilitated by automobile movement, snow, rainfall, and wind.

In an evaluation, roughly 85% of the polymer waste in the country is improperly handled and has the propensity to enter the ecosystem, particularly surface water networks [67]. According to CPCB (2018) statistics, 62 million tons of municipal waste were produced in the country in 2015. They also found that 82% of the waste was collected, and the remaining percentage was discarded as waste [68]. In addition, based on Joshi and Ahmed [69], a continued reliance on landfill as a disposal pathway for municipal solid waste treatment by urban government authorities may lead to an upsurge in municipal solid waste accumulation of nearly 300 million tons annually by the year 2051. This is because of the significant population growth that contributes to the generation of plastic waste [69,70]. Additionally, according to Jambeck et al. [71], approximately 0.24 million tons of polymer waste are dumped into the sea each year; India is fourth in the world in terms of the improper management of polymer waste [72]. In a recent study conducted by Yadav, Sethulekshmi and Shriwastav [73], the probability of susceptibility to micro debris through some major sources, such as consumer water, food, air, etc., was conducted by collecting samples from respective sources [73]. The predicted daily vulnerability in consuming water, breathing air, and consuming food were estimated to be around 382 ± 205, 594 ± 269, and 1036 ± 493 microplastics, respectively, per individual [73]. These results are based on conjecture and presumption. However, the actual findings could be considerably higher than the predicted approximations.

In addition, Yadav, Sethulekshmi and Shriwastav [73] reported that the existence of friction while handling, packaging edge-cutting, storage processes, and shipping could lead to the breakdown of the wrapping materials into smaller pieces. This source of microplastic contamination is considered secondary micro debris present in prepared meals. In addition, small particles have been reported to be present in salt based on a few salt specimen studies. The majority of coastal areas in India produce salt, such as Gujarat and Tamil Nadu, which are known as the largest salt producers in India. Generally, salt pans utilize sea or estuary water as their basis for the generation of salt, although there are certain salt pans that still employ groundwater [60]. In research conducted by Nithin et al. [60], approximately 3.67 to 21.33 nos/10 g microparticles were detected in the salt. In this area of study, brine liquids via the Vellar estuarine were employed for the salt pans as a basis for the generation of salts [60]. Nylon and LDPE microplastic were the predominant polymer types discovered in the salt [60], which is relevant to the Vellar estuarine. LDPE-type microplastic was the major polymer found in the water and sediment specimens. It can be deduced that LDPE microplastics may have settled in the salt as Vellar estuarine fluids are utilized as a source for the generation of salt. In addition, another salt pan analysis at Tuticorin, where the salt

is generated through the utilization of marine and borewell liquids, showed the presence of approximately 54 ± 13.4 and 12 ± 9.5 particles per kg, respectively [74].

With the obtained data, the researchers inferred that an individual might consume 216 and 48 particles annually from marine salt and bore well salt, respectively. On average, it is predicted that individuals consume 5 g of salt per day [74,75]. Seth and Shriwastav [76] deduced that the presence of micro debris might be due to the notion that marine salt is the direct output of shore liquids. Furthermore, based on CPCB yearly statistics, Gujarat is one of the top three areas for generating plastic waste, as reflected in its ecosystem, corresponding with the findings concerning an analysis of Gujarat fine salt, where about pieces of 1075 small debris were discovered [77]. Moreover, a mean amount of fewer than 700 microplastics per kg was extracted from retail salt packets [78]. The utilization of marine liquid, bore well liquid, and brine liquid originating from the estuarine showed that the presence of microplastics in water or sediment samples has a direct influence on the presence of microplastics in salt. Additionally, river streams that contain microplastics have a high possibility of transferring the micro debris to lakes that mainly receive water via river streams. One such lake in India that receives water via two different rivers, Vadavaru and Sengal, is known as Veeranam lake.

Veeranam lake is among the oldest and biggest basins in South India [79]. In the analysis, sediment specimens were collected from the lake's bottom, and it was revealed that there was an average amount of 309 microplastics per kg of sediment [79]. In addition to the transfer of micro debris via river streams, fishing activities have also contributed to microparticle abundance, as a significant quantity of worn-off plastic polymers from fishery equipment might seep into the lake. Additionally, to further explain the situation related to microplastics in India, in mid-August 2021, a news article was released stating that residential faucets exhibited the presence of microplastics in faucet water samples [80]. A test conducted by obtaining faucet water specimens and water purification facilities in Goa showed about 288 microparticles consisting of 26 kinds of plastic polymers [81]. Moreover, *The Hindustan Times* [82] stated that water purification facilities might moderately lower the number of small particles in water; however, they are not completely removed. This statement was justified with a report based on an analysis conducted by NIO in which purified water from the treatment facilitates showed the presence of microplastics in the range of 1 to 3.8 small particles per liter of water [82]. These microplastics in tap water can be hazardous to humans if ingested; however, there is no research or proof to show ingestion by humans or the related risk factors in India to date.

Furthermore, India is known to be the second highest flood-prone nation, with many areas of the country susceptible to flooding [83]. The microplastic issue in certain areas of India is severely impacted by the heightened occurrence of floods, together with the large volume of improperly handled plastic waste [84]. In fact, such a situation has caused top waters, sediments from deep waters, and beach areas to accumulate almost double the amount of an overall average of 246 to 506 microplastics after floods, as landed in Kerala in the year 2018, which was one of the five most severe flood occurrences in the country, as stated by World Meteorological Organization [84]. The after-flood sample study showed that the mean amount of microplastic in the top water was 174,016 ± 103,470 particles per kilometer and 28 ± 7 and 68 ± 19 particles per kg in deep water and shore sediment specimens, respectively [84], which were considerably higher after the floods. Commonly, after flood occurrences, strong waves overrun shores, inducing buried small particles to be swept toward sea waters, generating an overabundance of microplastics throughout seawaters.

2.5. Indonesia

Based on water and sediment sample analysis, foams were dominantly discovered from a few places in Indonesia, such as at Surabaya, which involved a few sampling spots: bays, beaches, and shore water specimens, with a percentage of 58.44% [85], sediment from wildlife reserve mangrove with an abundance of 13.33 ± 8.54 particles per kg [86]

and Bantan bay sediments with a percentage of 30.4% [87]. The emergence of foams is caused by pieces or shards of Styrofoam [87]. Surabaya serves as a hub for maritime transit, fishing, as an attraction site, and as a home for fisherman [87]. The water specimen analysis from a few spots of Surabaya showed fragments. They are the second highest type of small particles extracted, and large amounts of particles, ranging between 300 and 1000 µm from the overall microplastics, accounted for 94.02% [87]. The river water of Surabaya at different depth zones showed the highest number of fragments located at the top and mid-level of the water. Whereas films were the highest at the low level of the river, with the greatest abundance of large microparticles in all three depth zones, as shown in Table 3 [88].

Table 3. Characterization of microplastics present in Indonesia.

Location	Environment	Type of Sample	Dominant Shape	Size	Dominant Polymers	Reference
Northern Surabaya	Bay, Beach and Coastal	Water	Fibers (3.32%) Foams (58.44%) Fragments (34.51%) Granule (3.73%)	500–1000 µm (48.54%) 300–500 µm (45.48%) >1000 µm (5.86%)	PS (58.44%) PE (18.42%) PP (18.80%) Polyester (2.40%)	[87]
Makassar City	Tamangapa landfill	Dug well water	Fiber (72%) Fragments (28%)	<2 mm (86%)	-	[89]
Muara Angke Wildlife Reserve	Mangrove	Sediment	Foams Fragments	<1000 µm	PS (44.62%) PP (29.23%) PE (15.38%)	[90]
Surabaya	River	Surface water, Middle water and Bottom water	Film, Fragments Foam Pellet Fiber	LMP SMP	LDPE PP PS PE PET	[88]
Banten Bay	Bay	Sediment	Foam (30.4%) Fragments (26.5%) Granule (24.4%) Fiber (18.7%)	500–1000 µm (53%) 100–500 µm (17%) 1000–5000 µm (23%)	CP PET PP PE	[86]
Majalaya	Ciwalengke River	Sediment	Fiber (91%) Fragments (9%)	LMP > SMP	-	[20]
		Water	Fiber (65%) Fragments (35%)	SMP > LMP	-	
Surabaya	Jagir estuary and nearby Wonorejo Coast	Sediment	Fiber (57%) Film (36%) Fragment (7%)	LMP (68%) SMP (32%)	Polyester (56.7%) LDPE (24.6%) PP (18.8%)	[91]
Jakarta	Bay	Sediment	Fragments Fiber Film	-	PE PP PS PA	[92]
Bali	Benoa Bay	Surface water	Fragments (73.19%) Foam (17.02%) Fiber (6.38%) Granule (3.40%)	500–1000 µm (37.9%) >1000 µm (35.7%) 300–500 µm (22.1%)	PP (17.6%) PE (17.6%) PS (17.6%) Others (38.3%)	[93]

In another analysis, Surabaya town, related to an estuarine named the Jagir estuary and the Wonorejo coast, sediment sampling showed that fibers are the majority form of microplastic pollution, followed by films (Firdaus et al., 2020). According to Firdaus et al. [91], it was deduced that the presence of fibers may be due to expanding laundry industries, in which above 50% of industries lacked proper water effluent purification facilities. This predominance of fiber is in line with another study of the Ciwalengke River [20], where both water, as well as sediment, contain significant amounts of fiber micro debris, followed by fragments. In addition, large microplastics were majority extracted in the sediment specimens compared to smaller-sized micro debris and vice versa, as discovered in the water test. The Ciwalengke water stream is bordered by areas where people work in the fabric industry and impoverished families that reside in the slums with improper waste disposal facilities, which may have led to microplastic accumulation [20]. The presence of similarly shaped microplastics was also discovered in dug well liquids at the Tamangapa landfill, with a significant predominance of particles dimensions below 2 mm, which was more than 80% [89]. Makassar is a highly populated town where the waste from the town is discarded at the Tamangapa landfill [89], and it is possible that the liquids from landfills have seeped into the groundwater, eventually into dug wells, leading to microplastic contamination. While public activities close to the dug well could have contributed to the contamination as well.

The dominance of foam-shaped microparticles in the sediment at Bantan Bay [86] was in contrast to research conducted at Jakarta Bay, where fragments were extracted mostly from the sediments [92], as well as in the Benoa Bay water analysis, with a percentage of 73.19% of fragmented microplastics [92]. Bantan Bay is known to obtain water via waterways and rivers that might have possibly accepted a significant inflow of household waste, particularly Styrofoam, causing an abundance of foam in the area [86]. Benoa Bay is utilized by people that reside close to this area for aquaculture activities and crab and fish production, leading to the existence of PP, PE, and PS as the major plastic polymers [93]. Indeed, similar kinds of polymers were discovered in Jakarta Bay sediment specimens [92], mangrove sludge specimens that accounted for a cumulative percentage of more than 80% [90] as well as water specimens from three different sampling spots at northern Surabaya that accounted to a cumulative percentage of 96% [87]. In addition, LDPE was discovered at the Jagir estuarine as the second-highest pollutant, followed by polyester, the leading plastic polymer [91]. LDPE was discovered in different depth zones of Surabaya River stream analysis, in which the author deduced that the presence of LDPE in the lower depths of water may be due to the formation of biofilms over time through biofouling that may vertically disseminate microplastics to lower depths of the river [91].

In Indonesia, there are roughly 925 polymer factories that have the capacity to manufacture 4.68 million t of plastic items per year. As of the past 5 years, there has been an increment of 5% in overall yearly manufacturing [91]. According to Jambeck et al. [71], statistical data were obtained that showed, solely in the sea environment, 4.8 to 12.7 MMT of polymer waste dumped into the sea each year, leading to Indonesia becoming the second-largest contributor of plastic waste in oceans, raising concern among the federal and provincial government of the country. Mangrove forests could become a hub for the buildup of micro debris, which was verified with the large abundance of small plastic particles in non-moist sediment specimens taken from the Muara Angke Wildlife spot, which totalled 28.09 ± 10.28 microplastics per kg. The downstream of a river stream in Muara Angke regularly collects enormous amounts of municipal waste, notably plastic waste [94], whereas the upper section of the river stream is a large city known as Jakarta with remote areas, causing solid waste disposal to become a huge burden [90].

According to Syakti et al. [95], the country is a rising nation with an increasing populace; however, solid waste handling is inadequate, causing it to become a major issue. Based on statistical data from 2020, the predicted quantity of the yearly utilization of plastic drinking containers totaled 971,000 metric t [96], wherein residential households produce the majority of solid waste disposal, 39.8%. Surabaya city improperly manages solid waste

disposal, with roughly 37.1% of the waste being mismanaged out of an overall amount of 1562.2 t [91]. Moreover, a statement suggested that solid waste production may grow to 76% by the year 2025, with more than 13% of the waste comprising polymers, which may result in worsening microplastic contamination in the coastal areas of the country via rivers [91]. This statement has become partially true, as in one study based on the Surabaya water stream, microplastic contamination was high, with an average of 22.29 particles/m^3 [88]. The channels to the river stream that drain waters may serve as a conduit for microparticle contamination in the Surabaya River via households and commercial inputs [97].

In addition to that, other environments, such as northern coastal areas as well as the Jagir estuarine polluted with microplastics, accounted to a mean of 0.49 items per liter of water and a range of 92 to 590 microplastics per kg of sludge, respectively [87,91]. The abundance at the estuarine is related to improper solid waste management due to inadequate systems and facilities. Furthermore, public participation in reducing solid waste is minimal, causing concern. The residents living in the Tenggilis Mejoyo region stated that 63% of people do not minimize or isolate household solid waste at the source [98]. As a result, significant amounts of solid waste are discarded into river streams, leading to the estuarine at Jagir that runs through heavily populated urban areas becoming a dumping site, according to World Bank Group [99], and solid waste facilities are only partially available [91]. Further, one of the spots, Banten Bay, was also contaminated with micro debris, which accounted for a maximum amount of 431 pieces per kg of dry sediment. Bays are other prominent sites where a significant quantity of household waste might enter the area via river streams and nearby ports [88]. Importantly, slum zones are defined as dwellings without fixed rooftops, limited personal restrooms, and inadequate drains.

Another river known as Ciwalengke has numerous sorts of small-sized plastic particles that amount to a concentration of 5.85 $\pm$ 3.28 items/liter and 3.03 $\pm$ 1.59 items per 100 g of both river liquid and sediment specimens, respectively. From this, it can be deduced that the close proximity of the slums, characterized by inadequate sanitary infrastructure, waste disposal, and open washing, to the nearby river stream, could have caused the pollution to occur [20]. Moreover, the raw water supply and purified water were contaminated with microplastics that amounted to 26.8 to 35 and 8.5 to 12.3 items per liter of liquid, respectively, in Surabaya [100]. Surabaya is known to provide water for consumption by purifying the raw water obtained from the town's river, supplying roughly 92.5% of the people living in the town. Films were retrieved from the purified water, although films were absent in the raw water specimens [100]. In addition, the microplastic contamination in the country currently seems to have higher links to the COVID-19 pandemic due to the large production and utilization of PPE. Based on a recent study conducted in Jakarta Bay, water specimens from the upstream of rivers that enter Jakarta contain microplastics, with a mean value of 9.02 $\pm$ 4.68 item/m^3, comprising a significant number of fiber-dimensioned particles [85]. This was deduced after a clear indication of where the secondary microplastics originated from by a separate source that showed a rise in percentage from 3.33% to 30% between the months of March and December 2020 [85]. The assumption that PPE, specifically face covers, could have contributed to the fiber microplastics was in line with research that showed waste from face covers made up 9.83% of small particles in most of the rivers of Jakarta in the month of March 2020 [85].

In order to further explain the conditions of microplastic contamination in Indonesia, a study revealed that micro debris was detected in specimens of human excrement as well as everyday goods retrieved from an agricultural population of an upland village. Micro debris amounted to between 6.94 and 16.55 μg/g and was discovered in human excrement, with a predominance of polypropylene (PP)-shaped microparticles that accounted to 10.19 μg/g [101]. Microparticle pollution was absent in both daily water consumption and the main meals of the individuals chosen for the study [101]. The water used for daily consumption was known to be obtained via natural springs that did not contain micro debris pollution [101]. However, a traditional food known as tempeh was filled with micro debris, containing 11.08 μg/g, presumably caused by the wrappers [101]. Moreover, high

levels of microparticles were discovered in toothpaste as well as the salt utilized for cooking by the populace, with mean concentrations of 2.06 μg/g of PP, which was predominantly detected in the human excrement samples [101].

2.6. Vietnam

In Vietnam, the characterization of microplastics was carried out based on environments such as beaches, ponds, roads, and rivers. One of Vietnam's largest and most important coastal towns is Da Nang. Based on a beach sediment study at this location, about 745 microplastics in the beach sediment were found, with fiber-shaped particles being detected as dominant, with a percentage of 99.2%, with the remainder being fragment-type pollutants. Both shapes were primarily found to have sizes between 300 and 2100 μm [102]. Such microplastic pollution may have originated from industrial effluent discharge comprised mainly of that from the clothing and fabric sectors [42]. The presence of fiber and fragment shapes was similar to that of another study conducted in the Tien Giang region, a beach where these two shapes totaled a cumulative percentage of 88.6%, dominantly composed of PE and PP, which amounted to percentages of 44% and 47%, respectively [103].

However, contrasting findings were obtained from the beaches of Vung Tau and Can Gio, where granules and pellets, respectively, were abundant [103,104], however, these were totally absent on Da Nang beach. The granule-shaped microplastic, primarily composed of PS polymer [103], were caused by Styrofoam and divers, as commonly, pool accessories, including pull buoys and kickboard containing Styrofoam release pieces during utilization. Polymer PE and PS accounted for a cumulative percentage of 78% in Vung Tau [103], whereas in Can Gio, sediment specimen. Meanwhile, an additional polymer of PP can be observed at a percentage of 32% [104], with the remaining 68% comprising PE and PS. Several rivers tend to transport internal waste and debris from mangrove swamps that converge near Can Gio, wherein the area is affected by ocean tides [104]. In terms of size, Can Gio beach specimens have larger microplastics of 2.8 to 5 mm, which amounted to 71.46% [104] compared to the beaches in Vung Tau and the Tien Giang region, where 42% were much smaller particles of 0.5 to 1 mm [103]. They are relatively reduced in size compared to the Da Nang sediment specimens, as shown in Table 4. This may be caused by the collection of samples during different seasons for the three research studies, as commonly, microplastics further degrade into smaller pieces during summer seasons and when exposed to high tides.

Table 4. Characterization of microplastic at certain environment in Vietnam.

Location	Environment	Type of Sample	Dominant Shape	Size	Dominant Polymers	Reference
Da Nang	Beach	Sediment	Fiber (99.2%) Fragment (0.8%)	300 to 2100 μm (76.1%)	-	[102]
Northern Vietnam	Red River Delta and Tien Yen Bay	Mangrove sediment	Fiber	0.3–5 mm	PE, PP, PS, PET, PA	[105]
Tien Giang (TG) Region and Vung Tau (VT) Town	Beach	Sediment	TG: Fragments and Fibers (88.6%) VT: Granules (72%)	0.5–1 mm (42.1%) 1–2.8 mm (30.0%) 2.8–5 mm (27.9%)	TG: PE (44%) PP (47%) VT: PS (40%) PE (38%)	[103]
Ho Chi Minh town	Saigon river	Surface water	Fiber Fragment	50–250 μm	Polyester PE PP PS	[106]

Table 4. *Cont.*

Location	Environment	Type of Sample	Dominant Shape	Size	Dominant Polymers	Reference
Hanoi City	Aquaculture pond 1 and pond 2	Sediment	Pond 1: Fiber (62%), Fragment (38%) Pond 2: Fiber (81%) Fragment (19%)	-	PE (40%) PP (50%)	[107]
Da Nang	Road	dust	-	100 μm–5 mm (4.1 ± 3.5 pieces/g dry weight)	PE, PP, PS, PET	[108]
Da Nang	Phu Loc drainage canal	Surface water	Fiber (77.2%) Fragment (21.1%)	Fiber (1335.6 μm in average) Fragment (163,285 μm^2 in average)	PE (24.4%) PET (22.0%) PP (22.0%)	[109]
		Sediment	Fiber (86.1%) Fragments (12.4%)	Fiber (840.1 μm in average) Fragment (74,225 μm^2 in average)		
Ho Chi Minh town	Can Gio Beach	Beach sediment	Pellets (41.31%) Fragment (38.5%)	0.5–1 mm 1–2.8 mm 2.8–5 mm	PP (32%) PE (25.69%) PS (42.31%)	[104]

Based on the latest analysis, a drainage canal in Da Nang was polluted with microplastics, primarily with fibers and fragments [109], which amounted to a cumulative percentage of 98.3% in a top water sample of the canal and 98.5% in a sediment specimen. Phu Loc canal is known to be crucial in the transport of sewage out of congested housing zones, passing into Da Nang Bay [109]. A cumulative percentage of 68.4% was ascribed to PE, PET, and PP polymers from both types of specimen sampling. This presence implied that smaller streams, including Phu Loc, tend to accumulate a greater quantity of microplastics than bigger streams, particularly in situations where the river serves as a drainage conduit in heavily populated urban districts [109]. A similar presence of the polymer types in the Phu Loc canal was observed from street dust obtained from the Da Nang metropolis, with sizes that varied between 100 μm and 5 mm [108]. This implied that the reduced size might be due to the disintegration of microplastics on the street over a lengthy period as the streets are incompletely paved [108]. Moreover, based on the river stream, approximately 10% of the urban sewage generated is managed using activated sludge; hence the majority of the town's effluents are dumped straight into streams of the Saigon River as well as its waterways [106].

Generally, microplastics were detected throughout the surface water of the Saigon River, primarily fibers, followed by fragments, where the fibers mostly comprised synthetic forms of fiber, with a percentage of 92% [106]. This is in line with a study conducted on the Red River Delta and Tien Yen Bay, where fibers were dominant in mangrove sediment, accounting for a maximum cumulative of 4253 particles per kg [105]. Microplastics of a size range between 0.3 and 5 mm were spotted, and this may be due to plastic degradation as well as the minimal dynamic conditions within the mangrove swamps [3,105]. Additionally, an aquaculture pond located in Hanoi showed a significant abundance of microplastics, comprised of recurrent fiber-shaped particles that accounted for a cumulative percentage of 143% based on two different sampling ponds and fragments accounted for 57%. According to Le et al. [107], the production and grade of fishery products may be impacted by the

liquid and sediment conditions of an aquaculture lake. The sediment analysis of this lake showed the predominance of PE and PP, accounting for 90% of the total microplastics extracted. Both lakes may be contaminated due to the polluted Nhue streams that enter the lake as well as the human activities surrounding the lake [107].

In Vietnam, roughly over 3000 islets of different sizes have been discovered around the country, providing many opportunities for growth related to marine industries, including aquaculture, tourism, and maritime transportation [110]. Approximately 8 million tons of polymer waste are dumped in the marine area annually, causing the country to be ranked as having the highest global polymer waste dumping rate [111]. A substantial volume of plastic waste is generated per day, mainly consisting of plastic wrappers that are difficult to decay. The plastic waste is mainly produced by over 2000 polymer factories, of which 450 produce wrapper materials [112]. The country has experienced substantial growth in plastic production; as in 1990, the utilization of plastic was 3.8 kg per individual, drastically surging to 41.3 kg per individual in 2018. That information is congruent with the fact that residential solid waste comprising plastic escalated to 13.9% in 2017 compared to 2019, with a percentage of 5.5% [113,114]. According to the government, based on the year 2019, statistics showed more than 2 million tons of polymer waste produced in Vietnam, of which a significant percentage was found to be circulating in streams, ponds, estuaries, and wetlands.

Moreover, based on a study, it was revealed that each home in the country utilizes roughly a mean value of 10 polymer bags per day and a monthly base consumption of 1 kg [114]. According to Lahens et al. [106], the frequency of micro debris differed between 10 and 223 microplastic/m^3, as sampled from various sites of Saigon streams [106]. This means that approximately over 200,000 tons of plastic waste are released yearly via Ho Chi Minch City, where roughly 40,000 tons are interred, and the remaining amount is recovered or released into the ecosystem [111]. The author also claimed that the plastic recovery equipment utilized in the country's largest towns is both inefficient and unsafe for recycling [111]. In addition to the statistical reports, the conditions caused by microplastic contamination are further explained with the detection in salts and wastewater purification facilities. Vietnam's salt production is based on the protracted drying of moisture and salt precipitation retrieved via saltwater due to the unavailability of salt mines [115]. Foods containing salt consumed by Vietnam individuals are increasing, reaching approximately 10 g per day, as salt is a primary ingredient in a majority of the country's spices [116].

The utilization of processed salt for everyday dishes has become increasingly prevalent, whereas unbranded finer salts are manually made at home or by local industries [115]. Unbranded salt packets from Can Gio have the lowest number of microparticles compared to the suburban regions, which were much larger, amounting to 56.98 ± 23.81 and 402.52 ± 168.43 microplastics per kg, respectively, in which both locations have an abundance of PET-type polymers [115]. The author deduced that the presence of this type of polymer might be due to the settling and staying of PET on salt throughout crystallization, as the polymer has a greater density [115]. Moreover, processed salts contain roughly 140 microplastics, which may be due to crushing as well as refining [115]. In another study, market salt packets showed an average amount of 340 ± 26 particles per kg, whereas pure salt contained an average of 878 ± 101 particles per kg [117]. According to the WHO, the individuals in the country ingest two times more salt than suggested, 10 g per day, meaning that this double salt intake rate can cause individuals in Vietnam to consume 637 to 1270 particles annually [117,118].

Furthermore, micro debris pollution from atmospheric fallout is another situation occurring at the Phuoc Hiep depot. In this analysis, micro debris was observed that amounted to 1791 particles comprised of fibrous and fragmented shapes [119]. Based on the concentration value, the amount was higher by a factor of two during the summer season, accounting for 1801.2 particles/m^2 per day, compared to the wet season, accounting for 912.5 particles/m^2 per day [119]. It was deduced that the reduced amount in the wet seasons could possibly be caused by rain, which provides a limiting effect on the number of

air contaminants [119]. In addition, in terms of the extent of microplastic contamination, the beach spots of Da Nang showed roughly 745 particles in the sediment [102]. According to the Nguyen et al., [102], more than 30570 dwellings and visitor lodgings located around Da Nang's 16 shore districts are partly linked to the town's sewage network, where domestic effluents of purified and non-purified liquids are released straight to seashores [102]. The overflowing of effluents at coastlines frequently occurs, particularly during the monsoon period, and the peak number of travelers could possibly further lead to the accumulation of microplastics around the beach. The above-mentioned observation unfolding on Da Nang beach was in line with another study related to shore sediment in two different areas, the Tien Giang region and Vung Tau, which showed mean concentration values that varied between 2.5 and a maximum of 44.6 particles per kg of non-moisture beach sand [103]. Moreover, the Da Nang study related to road dust and a drain canal of Phu Loc also revealed serious contamination of microplastics in a range of 1.78 to a maximum of 9.65 g dry road dust/m^2 [108] and to a mean of 1482.0 $\pm$ 1060.4 particles/m^3 on the top of the water as well as a greater abundance of 6120 $\pm$ 2145.7 particles per kg in the canal [109].

2.7. Iran

The socioeconomic shifts have caused an increased utilization of plastics and polluted important coastal and marine environments with microplastics. One such place is Chabahar bay, a well-known tourist attraction of great commercial significance [39]. A microplastic characterization study was conducted based on surface water and sediment samples collected from this bay. In the sediment specimen analysis, microplastic fragments, pellets, and fibers were identified that accounted for a cumulative percentage of 85.85% in the top water sample. In fact, fibers were predominant, followed by fragments and films, which amounted to a cumulative percentage of 89.44%. The fibers may have been prevalent due to the presence of grill nettings in wastewater that comes from households and recreational areas [120]. In Qatar, about 1.5% of fragments out of overall microplastics were discovered on the surface water; however, in Chabahar bay, the percentage of fragments was higher, about 42.54%, implying that microplastics in Chabahar have eroded over time [39].

Microplastics with a size of less than 1 mm were predominant and primarily contained PE and PET plastic polymers, comprising 67% of the total microplastics extracted from both sediment and water specimens. This was suggested to be due to the fact that the sample collection spots were near populated, industrial, and tourism areas [39]. The abundance of microplastics less than 1 mm in size was in line with another study conducted in the Caspian Sea, where 66% of the coastal sediment specimens were microplastics smaller than 1 mm [121], as shown in Table 5. This sea is known to be a significant maritime ecosystem that is susceptible to a variety of commercial, shipping, and oil spill contamination events [121]. The analysis showed high amounts of fibers, followed by fragments [121], which accounted for 98% of the total number of microplastics. The shapes discovered in the Caspian Sea coast are in line with studies conducted on the Aras River, where fibers were dominant in both water and sediment specimens, amounting to 52.6% and 54.4%, respectively, followed by fragments [122]. Estuarine sediment from the Qarasu estuary amounted to 98% fibers, followed by fragments [123], and in the Hashilan wetland, primarily fibers, in both the top water and sediment, were found in the specimen analysis [124], as shown in Table 5.

Table 5. Characteristics of microplastic in Iran at various environments.

Location	Environment	Type of Sample	Dominant Shape	Size	Dominant Polymers	Reference
Oman Sea	Chabahar Bay	Sediment	Fragments (32.22%) Pellet (27.37%) Fiber (26.26%)	<1 mm (61.32%)	PE (38%) PET (29%)	[39]
		Surface water	Fiber (42.54%) Fragment (28.66%) Film (18.24%)	<1 mm (42.35%)		
Mazandaran Province	Caspian Sea coast	Coast Sediment	Fragment (43.30%) Fiber (54.75%)	250–500 µm (43%) 500–1000 µm (23%)	PS PE	[121]
Hormozgan Province	Bandar Abbas beach	Sediment	Foam (64.01%) Fragment (30.96%)	LMP (67%) SMP (33%)	EPS, PE, PET, PP	[125]
Northwest Iran	Aras River and Reservoir	Sediment	Fiber (54.4%) Fragment Film Foam	0.1–5 mm 3–4 mm 1–2 mm	PE (37%) PS (30%) Cellophane (24.4%)	[122]
		Surface water	Fiber (52.6%) Fragment Film Foam			
Gorgan Bay	Qarasu estuary	Estuarine sediment	Fiber (72%) Fragment (26%)	1–2 mm (33%) 0.5–1mm (41%)	PP (33%) PE (24%) PA (21%)	[123]
Kermanshah Province	Hashilan Wetland	Sediment	Fiber (95.77%)	<100 µm (38.03%) 100–250 µm (29.58%)	PP, PS, PE	[124]
		Water		<100 µm (33.33%) 100–250 µm (25%)		

It has been suggested that the main causes of the significant concentration of fiber-shaped microplastic in the examined Caspian Sea coast specimens were probably residential sewage intake through waterways and intensive fishing practices [121]. In the Aras River and reservoir, the larger plastics tend to erode more, showing higher amounts of disintegrated microplastics, leading to fiber abundance [122]. Moreover, the Aras River is also one of the primary contributors of fiber and fragments to the ocean environments of the Caspian Sea due to the Aras River flowing into this sea. For the Qarasu estuary, the abundance of fibers in the Qarasu stream estuarine and, consequently, the Gorgan Bay, may be the result of residential sewage entering the streams, contributing to the microparticles present in the Caspian Sea because the Qarasu River is connected via a similar estuarine and flows into the sea [123]. Wetland ecotones are known to be important components of ecosystems that support the rapid transfer of gases, sediments, groundwater, and adjoining soils [124]. In the Hashilan Wetland, the prevalence of microfiber pieces is presumably transported into the research area by the air due to the low density of the environment [126].

In terms of sizes, most of the studies, as shown in Table 5, revealed microplastic sizes of less than 1 mm; however, in the beach sediment of Bandar Abbas, the number of large microparticles was much higher than the number of small microparticles [125]. This study demonstrated a contrasting microplastic shape, predominantly foams, with a percentage of 64.01%, followed by fragments [125]. The province of Hormozgan is a focal point of key industries in the town. The metropolis is established both regionally and globally due to

its huge docks, including Rajaee as well as Bahonar, along with important sectors, notably shipyards, petrochemical factories, aluminum production, and electricity plants. The type of polymer found in this Bandar Abbas beach was primarily expandable polystyrene (EPS), leading to presence of foam-shaped microplastics. They originate from fragile shipment items, protective food packages, hot beverage drinking cups, and feeders for costly materials and machines [125]. However, this is in contrast to the Caspian Sea, which has an abundance of PS and PE [122], the Aras River with PE and PS, which accounted for 67% [122]. While in the Hashilan Wetland, PS, PE, and PP [126], and the Qarasu estuary, has a cumulative of 78% of PE, PP and PA [123].

Sewage originating from ships, lighthouses, and water-balancing equipment is a substantial cause of maritime ecosystem contamination, leading to microplastics in Chabahar Bay [39]. The Caspian Sea exhibited about 1830 microparticles, with a mean of 107.6 pieces per kg of sediment from the coast due to moist weather patterns and the shoreline attributes of the Caspian beach attracting intense increased tourist visitation [121]. According to UNEP [127], areas near towns with larger populations and tourists tend to have increased marine debris surrounding Iranian shores [127]. Moreover, in another study on the coast of Bandar Abbas, roughly 195,000 microparticles provided a mean of 3252 ± 2766 particles/m^2 [125]. In addition, the Qarasu estuary and Aras River, connected to the Caspian Sea, had an average of 217.8 ± 132.6 microplastics/kg in the sediment and 12.8 ± 10.5 microplastics/m^3 in the top waters collected from the Aras River, whereas the Qarasu estuarine had 182 ± 111 microplastics/kg in the sediment [122,123].

The Aras stream is known as the primary supply of irrigation for many industrial facilities as well as farming regions, passing through various metropolises before reaching the Caspian Sea [122]. The microplastics in this stream could have been contributed from urban tributaries that access the stream, carrying a significant quantity of microplastics toward the Aras stream [122]. According to Li et al. [128], micro debris contamination tends to adopt a comparable trend similar to clay deposits, which are lightweight and transferred throughout aquatic waters from greater energy habitats with larger current rates to lesser energy conditions. Hence, this could have led to a greater number of microparticles in Qarasu estuarine sediment with its increased clay content [123]. In another study, a mangrove jungle close to the Bidkhoun urban region showed significant microparticle pollution, amounting to a mean of 416 particles/m^2, which was considered to be due to inadequately processed sewage water as well as nearby urban developments [129].

To further explain the current conditions of microplastics in Iran, in a recent study, microplastics were discovered in the atmosphere at Ahvaz town, with levels varying between 0.002 and 0.017 microplastics/m^3 and primarily fiber-shaped [124]. A slightly higher concentration of micro debris was located in Asaluyeh County, varying between 0.3 and 1.1 microplastics/m^3, which was predominantly composed of fibers [130]. The Ahvaz site is close to housing and commercial sectors, which indicates that the local origins of the fiber particles are significant due to fewer signs of degradation [131]. Fiber-shaped microscopic atmospheric particles have the ability to travel large distances and directly contaminate crop soils as well as water sources when the particles are rinsed off by rain or other factors. According to the US EPA estimation, with a mean of 100 milligrams per day of atmospheric particle absorption for adults and 200 milligrams a day for adolescents, roughly a maximum of 50 and 100 microplastics per day may be taken in from atmospheric dust by adults and adolescents, respectively [130].

Furthermore, 3459 pieces of micro debris were discovered in wastewater runoff in Iran with a mean value of 70.66 ± 14.12 microplastics per liter of liquid, which revealed fibers as the dominant particle shape [132]. The intended and unintended release of household polymer waste that enters the Gulf waterways are projected to rise due to the growing volume of humans brought about by industrialization and huge constructions. Bandar Abbas sewage management facility's effluent discharges have an estimated volume of 2040 particles/m^3, in which presumably about 120 million microplastics are released throughout the Persian Gulf on daily basis [132]. A dense solid substance called wastewater sludge is generated

during the purification of sewage [133], wherein the sludge contains large amounts of nutrients that are utilized in woodlands as well as for agricultural purposes as soil manure or enhancer [134]. Micro debris was located in the sludge of the Sari purification plant that varied between 129 ± 17 and a maximum of 238 ± 31 particles per gram of non-moisture sludge [134].

Most of micro debris is removed in water treatment facilities and captured in sludges for application on crop soils and woodlands, which may result in the potential accessibility of microplastics to rivers, lakes, and marine ecosystems via rain wash-off, wind, or irrigation. The purposeful intake of organic geosolids, such as soil, slit, ground stones, and ant pile earth, is known as geophagy [135]. Several modern indigenous people are still engaged in geophagy at times for nutritional or cleansing reasons. Human geophagy may have developed covertly as a result of the utilization of soils in cooking, such as red ochre sediment, which has been applied for ages to enhance meals, acting as a spice in Iran [135]. Soil is combined with salt that is either used in the preparation of seafood or in suspension in freshwater to produce loaves or food dressings [135]. This has become a concerning matter, as based on a current study, a mean value of 0.05 microplastics per gram has been detected in the soil obtained from Hormoz island, with an abundance of fiber-shaped particles [135].

The abundance was suspected to be due to the accumulation of locals or visitors that wore garments made from fabrics or linens, air precipitation during the mid of collecting and wrapping [135]. The yearly intake of micro debris is estimated to be roughly 200 with a daily highest presumptive use of salt by one standard individual of about 5 g or equivalent to per day absorption of spices obtained from the soil at Hormoz islet [135]. The presence of microplastics in marine environment can be related to the occurrence of dust storms in Iran that happened in 2018. Dust storms entail a huge variety of effects on ecosystems, meteorology, and the climate; for instance, excessive dust volumes may influence atmospheric warmth, cloud creation, and convectional movements and have been suggested to transport iron and various minerals toward marine environments [135,136]. With a presumed content of microparticles inside soils that serving as the origin, dusts that are approximately 0.02 particles per gram may contain roughly 2 trillion microplastics suspended by storms, dropping about 1.5 trillion microparticles locally as well as 150 billion pieces of micro debris across the marine environment [135,137].

Around 40 trillion microplastics may be transferred and mobilized into the atmosphere each year as a result of dust cyclones [137], in which dust cyclones may act as substantial intermediates for micro debris dispersion throughout marine and terrestrial ecosystems. Flakes of snow are big, low in density, and move at slower terminal speeds compared to rain, causing the flakes to absorb small atmospheric particles and settle on the ground [138]. In Northern Iran, where snowfall occurs, microplastics were obtained and studied that showed the presence of micro debris, which amounted to a total of 348 microplastics, which were primarily in the form of fiber, with a percentage of more than 90% [138]. An implication is that this could have occurred due to airborne microparticles that fell to the ground during the rainfall season, settling deep into the snow; additionally, travelers, as well as mountaineers, might directly contribute substantially to the amount of small plastic particle pollution.

Another major issue when the snow melts is related to the fact that micro debris contaminants are conveyed toward water bodies through runoff from impermeable areas, soil, aquifers, as well as various vegetated surfaces [139]. Moreover, dust in the classrooms of schools and indoor buildings was recently found to be contaminated with microplastics. Epidermal skin, pet fur, plant residue, food remnants, microbes, fabrics, ashes, building debris, acrylic flakes, soil particles, and sprays are among the diverse as well as heterogeneous components that make up indoor dust [140,141]. A few samples taken from Shiraz secondary school classrooms showed the presence of 188566 microplastics in the dust of a classroom floor, with a high proportion of fiber-form particles [142]. It has been suggested that the presence of these microplastics may be due to access via windows, open classroom

doors, and being transported under footwear or on garments from the outside environment [142]. The other relevance of this pathway is probably influenced by local geology, the closeness of the school to roadway congestion and factories, and the level of interior airflow inside the classrooms.

A teenager may be exposed to an estimated daily amount of 113 pieces of micro debris, assuming that a teenager occupies 40% of their time in lesson rooms and ingests 40 milligrams of debris [142]. In annual terms, teenagers are vulnerable to roughly 40 thousand microparticles in lesson rooms alone [142]. In various internal buildings, dust specimens taken from the Bushehr area and Shiraz showed micro debris contamination that amounted to 1212 and 1362 microparticles, respectively [143]. Some of the sample collection locations were a children's playschool and a mosque, from which it can be reasoned that it could be due to being worn off from children's toys as well as mosque ground rugs that may accumulate dirt and fiber-shaped microplastics. Based on the authors, the utilization of air conditioners and chiller devices throughout the year could result in elevated amounts of micro debris in the dust of internal buildings, especially in summer towns, such as Bushehr and the Shiraz metropolis [143].

2.8. Thailand

Based on the beach sediment analysis conducted in Rayong province in Thailand, microplastics were detected, predominantly fragments, accounting for 50% of those present in the sand [144], which was in contrast to the beach areas surrounding the Phuket coast, where fiber was significantly the most accounted for, with a percentage of 85.6% [145]. However, the Rayong province water specimen study showed more fibers than fragments, amounting to 80% of the total, and it was suggested that this was due to the fact that fishing operations produce fiber particles through the use of nets that contain nylon fibers utilized for catching fish [144]. Phuket Province, with one of the country's biggest shorelines, is tremendously popular for travelers, with an estimated 13 million people traveling there each year [145]. The increased levels of visitors to the beaches of Phuket could be regarded as a possible source of fiber abundance, as the fibers may have worn off from the garments worn by travelers.

The prevalence of microparticles was noticeably larger at beach spots that serve as docks or departing locations where intense shipping activities could result in greater concentrations of microplastic waste, which amounted to 188.3 ± 34.48 particles per kg of sediment specimen [145]. The polymer types found in Phuket were PET, PS, and PP, which were larger in number. The presence of PET was due to the close vicinity of the site to a boat yard and traveler visitation [145]. However, PET was not discovered at the Rayong Province sampling spots, but PP and PS were similarly found with more abundance than PE, accounting for more than 70%. This was suggested to be due to the fact that PE may have arisen from the recent growth of the fishing and leisure industries [144]. Additionally, fragment-shaped microplastics are highly found in river and estuary combined areas, such as the Chao Phraya River and estuarine area, having an abundance of fragments in water and sediment specimens [146]. The sampling points of the river and estuarine combination of the internal Gulf of Thailand yielded the same shape of microparticles in liquid specimens [147]. The decreased number of fibers at the CPRE was because of fewer sewage purification facilities surrounding the area [147].

The internal bay of Thailand was one of a location that received an influx of Chao Phraya River drainage, which was concluded due to the similarity of dominant microplastic shapes in the water specimens of both locations. Both river estuary sediment as well as water yielded the same dominant types of polymers, PE and PP, as shown in Table 6. The larger amounts of these two polymers at CPRE was assumed to be due to the large discharge into Thailand [148]. For the internal bay of Thailand, the abundance of microplastics was different during the rain period and summer period. During the monsoon season, the waterways carry micro debris into the marine environment from the ground due to the rising freshwater flow, causing microplastic pollution [147]. Fragments amounted to being

the most dominant form in the Bandon Bay water specimens, accounting for a maximum of 77.1%, with more PP polymer [149]. However, in the previous year, water and sediment specimens obtained from Tapi-Phumduang, which flows into Bandon Bay, predominantly contained fibers with greater amounts of Rayon [150].

Some of the sampling points in Bandon Bay are considered fishing sites and places for rearing aquatic animals, particularly clams and prawn farming spots [151], and these practices have led to a significant amount of fishing gear being abandoned at the bay [149]. The prevalence of fragments from studies conducted by Ruangpanupan et al. [149] suggested that weaved poly bags are frequently utilized, particularly at sampling points where aquaculture was prominent for bundling manure, prawn feed, and shellfish field border poles to prevent tidal detrition. In order to conceal the border poles, which are about 100 in numbers, more than one sack is utilized per pole in which these weaved poly bags disintegrate into micro debris when in contact with environmental elements, such as breeze attrition and UV rays from the sun, that will eventually cause the accumulation of microplastics in the vicinity [149]. Moreover, fibers were found in beach sediments surrounding the shore of Phuket, the Bay of Thailand, and Bang Yai estuarine sediment with a prevalence of films [3,145,152]. The majority of the microplastics found in various environments in Table 6 were below 1 mm in size.

Table 6. Characteristics of microplastics at various environment in Thailand.

Location	Environment	Type of Sample	Dominant Shape	Major Size	Dominant Polymers	Reference
Rayong Province	Beach and river estuary	Beach sand	Fragment (50%) Fiber (41%)	100–500 μm (58%)	PE (75%) PP (12.0%) PS (13%)	[144]
		Surface water	Fiber (80%) Fragment (19%)	100–500 μm (46%)	PE (73%) PP (17%) PS (10%)	
Coast of southern Thailand	Tapi-Phumduang River and Bandon Bay	Surface water and sediment	Fiber Fragment	<1 mm	Rayon, PP, PE, PET	[150]
Gulf of Thailand	Bandon Bay	Surface water	Fragment (66–77.1%) Foam	0.5–1 mm (22–23.4%) <2 mm (61.13%)	PP (57%) PE (30%)	[149]
Pak Nam, Samut Prakan	Chao Phraya River Estuary (CPRE)	Surface water	Fragments Fiber Films	-	PP PE	[146]
		Sediment	Fragments	0.05–0.3 mm	PE (50%) PP (21%) PS (7%)	
Phuket Province	Bang Yai canal	Estuarine sediment	Fiber (80%) Films	0.1–5 mm	Polyester PP PE PET	[152]
Gulf of Thailand	Coast	Sediment	Fiber	0.1–0.5 mm 0.5–1 mm Both 71%	Rayon Polyester	[3]
Inner Gulf of Thailand	River estuaries and coastal sea	Water	Fragment Film Fiber	1000–5000 μm	PP PE	[147]
Phuket Coastline	Beaches surrounding Phuket Coastline	Sediment	Fiber (85.6%) Film (3.3%)	20–300 μm (max 56.7%) >300 μm (max 50%)	PET PS PP PVC	[145]

The high volume of travelers touring Phuket province has induced an upsurge in textile washing, possibly causing the Bang Yai estuarine specimens to be contaminated with fibrous microparticles. Rayon microplastics were visibly present at two locations in

the country: the Bay of Thailand and the Tapi-Phumduang stream ([3,150]. However, contrasting findings were seen in different environments, which showed a higher abundance of PE, PP, and PS polymer types, those which are commonly derived from food wrappers, as shown in Table 6.

Thailand holds a significant position in the worldwide plastic sector, ranking as the 11th largest plastic manufacturer globally and the second largest based in the Asian region [153]. Based on studies and statistics from 2020, the issue of Thailand's maritime has been exacerbated, causing the country to be classified as the 6th largest worldwide emitter of plastics into the sea [154]. Rayong province is a popular vacation spot, with a record of drawing up to 7.7 million people in 2018. However, based on the current analysis, the location contaminated with microplastics amounted to a mean of 338.89 $\pm$ 264.94 particles per kg in sand specimens, and a much higher mean of 1781.48 $\pm$ 1598.36 microplastics/m^3 in the top water of the sea [144]. Another study based on water and sediment obtained from the Tapi-Phumduang river yielded micro debris with a maximum value of 2.81 particles per liter of water [150]. The authors concluded that this river obtains water from Ratchaprapa reservior and the reservoirs' downstream watershed is surrounded by extensive palm and rubber plantations [150]. The river water enters Bandon Bay, passing through towns that could be a source of microplastics and tributaries, which may serve as microparticle-holding networks [155]. Evidently, cockle picking entails bottom dredging, which may revitalize sediments, leading them to move and enter the surrounding water and possibly shifting formerly deposited microplastics inside the bay [150]. Further investigations shed light on microplastic movement from the river channel into the bay, particularly during the monsoon period, which was thought to account for the majority of the influx of particles from land entering Bandon Bay. As studies have shown, the number of micro debris is three times more during large tidal cycles [151]. The movement of microplastics from rivers such as the Tapi-Phumduang stream into Bandon Bay has definitely caused an abundance of microplastics in the bay; where in a recent study, the bay contains 7899 detected based on water specimen analysis [149].

Currently, Bandon Bay is at risk of degradation due to the growth of commerce and population, leading to excessive resource utilization as well as a significant volume of waste being generated [156]. According to Ruangpanupan et al. [156], the surface tide moving counterclockwise could transport microparticles from the north of the bay to the midsection and move to the southern point. With the utilization of typical concentrations of micro debris in liquid over a tide cycle, the load of small-sized plastics in the bay was computed to be 22.4 billion particles on a daily basis during the monsoon period [149]. Moreover, estuarine areas were also contaminated, such as the Chao Phraya River Estuarine (CPRE), which is a primary saline aquaculture spot, generating roughly 7105 tons of fish per year. It showed an overall total of 2704 microplastics in the estuary's water specimens collected [3] and the estuarine sediment obtained at the Bang Yai channel, which flows starting from Kathu falls, traveling through huge segments of Phuket metropolis and ending at Thailand's west side coast, yielding 463 microplastics [152].

At CPRE, some of the collection points were close to large freight ships, which frequently navigate near the estuary's midsection and where bow waves are produced, dispersing water across two banks [146]. The Bang Yai channel receives huge amounts of water coming from both household and factory effluents, causing the canal to be a contributor to the numerous microplastic contaminants present in the sea [157], and the canal inlet is in close proximity to a sewage purification facility as well as landfill [152]. In addition to that, the Bang Yai canal was found to have considerably greater amounts of microplastics than the specimens obtained from the shore of the Gulf of Thailand that amounted to a maximum of 362.5 particles per kg of sediment [3] In a recent study based on the inner gulf of the country, the numbers amounted to only 21.29 $\pm$36.21 items per liter of top water specimens [147]. This gulf of the country's environment is susceptible to human activities close to this environment [158], from which a hypothesis can be made that runoff transports

a significant volume of sediment containing a large amount of plastic, primarily originating from aquaculture practices.

In addition, a significant number of micro debris tends to be left behind as the sea widens, reducing hydrodynamic pressure; hence, sediment within the confines of the sea is more likely to collect and accumulate microplastics [3]. For instance, in the inner gulf, top water movement could have caused the dispersion and lodging of particles [147]. The quantity of secondary micro debris is predicted to have a maximum value of 79140.8 tons annually, permeating into various ecosystems in Thailand [159]. To further explain the condition of microplastics in Thailand, microplastic contamination has been discovered in shrimp paste, amounting to between 6 and a maximum of 11.3 items per 10 g of paste [160]. One of the goods that the country's fishermen pursue is shrimp paste, as various Thai dishes depend heavily on this paste, particularly chili dips [160]. An implication is that the micro debris could have arisen during the making of the paste with other ingredients, such as salt, source of water, and krill that was already contaminated with particles [160]. Furthermore, wastewater purification facilities have accumulated microplastics between 77 ± 7.21 and 10.67 ± 3.51 items per liter of wastewater [161].

In the year 2020 alone, roughly 6300 tons of polymer waste were manufactured per day in the country. The sizes of the micro debris in the sewage purification facility were highly prevalent in the 0.05 to 0.5 mm range [161]. According to the authors, the percentage of microparticles rose significantly in an aeration unit, up to 40.7%, in which the debris becomes trapped in sludge and not entirely removed from the purification plant [161]. The micro debris is then redispersed after the sludge has been recirculated and placed back into the aeration unit [162]. The estimated leak of microplastics from purification plant effluent day into nearby freshwater habitats is 1280 ± 421 million pieces per [161]. In the sludge, roughly 26.3 ± 12.6 thousand microplastics per kg may be retained in the non-moisture sludge [161], which is then applied to farming and could eventually adversely affect flora as well as wildlife due to the presence of microparticles in the soil.

2.9. Philippines

Based on coastal environments, sediments obtained from the shores of Negros Oriental yielded major amounts of fiber-shaped microplastics [162]. This was in line with specimens obtained from the Cayagan de Oro shore, with a predominance of fibers followed by fragments [163]. However, in terms of polymer types, rayon, PET, and PVC were discovered at Negros Oriental, which is in extreme contrast to the Cagayan de Oro area, which showed the predominance of LDPE, HDPE, and PP [162,163]. The fiber presence at the Cagayan de Oro area shore situated at Macajalar Bay was explained to be due to the fact that household sewage drainage released into streams probably transported the particles to the shore [163]. However, certain specimen collection spots in the seaport zone showed lower counts of fibers and a greater number of fragment-shaped microplastics. It was concluded that the transfer of fiber or any form of microplastic from the land to the ocean might have been hampered by the constructed environment of the harbor and the constrained water movements [43,163].

Based on river habitats, as shown in Table 7, fragments, films, and pellets were commonly located in the top water and sediment specimens obtained from five different rivers [164] and the Metro Manila stream [165] where both are situated in Manila Bay. High counts of fragments consisted of PP-type polymer, whereas films were more prevalent at stream entrances, which are encircled by large housing communities and where open disposal and pervasive waste discarding are noticed [164]. Pellets were collected from the Meycauayan stream top-level liquid where the potential cause is spillage from polymer production sectors that are in close proximity to this stream [164,165]. A line that was located in the sediments of Manila Bay [164] may be attributed to the number of angling practices, aquaculture, and docks for kayaks, which are present near the sample collection sites. Lines could also be present from straws, cords, and catchment netting from kayaks that are stationed close to seafood shops around the stream's entrance [164]. The types of

polymers found in both research studies were PP and PE [164,165], and this was in line with an analysis conducted at the Molawin watershed [166] and Cayagan de Oro shore sediment analysis [43], as shown in Table 7.

Table 7. Characteristics of microplastic in various environments at Philippines.

Location	Environment	Type of Sample	Dominant Shape	Major Size	Dominant Polymers	Reference
Negros Oriental	Coastal of Negros Oriental	Coastal sediment	Fiber	<2000 μm (majority 1000 μm)	Rayon PET PVC	[162]
Manila Bay	Canas River, Meycauayan River, Pasig River and Tullahan River	Surface water and Sediment	SW: Films Fragment Pellets S: Line	SW: 1–2.36 mm S: 2.36–5 mm	PP PE	[164]
Manila Bay	Baseco Port	Bay Sediment	Fragment (84.1%) Fiber (4.1%) Pellet (4.1%) Film (3.1%)	1.6 ± 1.4 mm (average)	-	[167]
Makiling Forest Reserve, Los Banos, Laguna	Molawin Watershed	Sediment	Fragment Fiber	100–200 μm	PP PE	[166]
Macajalar Bay	Cagayan de Oro Coast	Coastal sediment	Fiber Fragment	-	LDPE HDPE PP PE	[163]
Manila Bay	Metro Manila River	Surface water and sediment	Fragment Film Pellets	-	PP PE PS	[165]

Molawin creek yielded a predominance of fragments, followed by fiber, amounting to 71.33 pieces and 20 pieces per 100 g of non-moisture sand specimens, respectively, where both were majorly discovered on the lower bank of the watershed [166]. Microplastics that are larger than 2 mm were majorly found on the upper section of the bank, and a decreased number of particles with sizes greater than 2 mm was discovered in the middle section of the stream, followed by the lower section of the bank [166], which could be due to temperature differences between the upper and lower sections of the bank, as higher temperatures may lead to the faster disintegration of polymers. In addition to that, similarly shaped microplastics were discovered at Baseco port, where the sediments yielded fragments and fibers [167] that amounted to a cumulative percentage of 88.1%. Smaller counts of pellets and films were also found in the environment of the study. Baseco port is a harbor that is well-noted as a place where unofficial immigrants reside [168]. Overcrowding and the shortage of urban spaces have ultimately resulted in an unclean and disorganized colony [169], becoming a major driver of the improper handling and dumping of waste. Approximately 550 waste bags were gathered from the Baseco region during a communal cleaning [170]. The port of Baseco showed more microparticle pollution in rising water sediment specimens [167], which can be explained by the area's proximity to an overcrowded populated area.

3. Impacts to Environment

The impacts on the environment due to microplastic pollution in South Asian countries are related to mainly three environments: the marine environment, terrestrial environment, and atmospheric environment. In the marine environment, the main impact is the ingestion

of microplastics by aquatic organisms due to the high number of microplastics in the sea as well as the aquatic organisms misinterpreting the microplastics as food for consumption. In the terrestrial environment, the impacts mainly relate to the sinking of microplastics into soils that are mainly used for growing crops or agriculture purposes, causing changes to the physicochemical properties of the soil. In terms of the atmospheric environment, the direct impact of microplastics in the atmosphere is the settling of these particles onto the human body, such as skin, hair follicles, and a few other receptors.

3.1. Bangladesh

The impacts on the environment due to microplastic pollution in Bangladesh are related to mainly two environments: the marine environment and the terrestrial environment. Based on the marine environment, studies from researchers have shown microparticle intake by sea fish as well as microparticle presence in dried fish in Bangladesh. Based on a study conducted by Parvin, Jannat and Tareq [171], microparticles were detected in the gastrointestinal (GI) tracts of commercial fish acquired from two fish selling spots known as Ashulia and Savar situated in Dhaka town [171]. The occurrence in the fish samples is related to the presence of high amounts of microplastics that have been discovered in Dhaka lakes [31], and these fish selling spots sell fish obtained from streams, waterways, and lakes around Dhaka, which are then delivered straight to regional shops [171]. Based on a few studies, an analysis of fish samples purchased from the Kuakata fish selling market [172], caught from Jamuna stream [70] as well as Hilsa Shad of an anadromous migrating breed that are prevalent and numerous throughout the Bay of Bengal [173], showed the presence of microparticles in the GI tracts of the species.

The fish species' feeding spots, known as demersal, benthopelagic, and pelagic, have an influence in terms of the number of microplastics taken in by the fish in the specified zones. According to Pauly [174], fish species that reside and eat close to the bottom of the sea's column are called demersal fish species, whereas benthopelagic fish species are found slightly beyond the bottom of the sea's column, and pelagic fish eat near the wide surface area of the sea's column [171]. In most of the studies conducted based on commercial fish samples in Bangladesh, the demersal eating area of fish tend to have a higher number of microplastics present in the digestive tracts compared to the other two feeding spot fish samples. In the research conducted by Parvin, Jannat and Tareq [171], compared to fish species from benthopelagic and pelagic feeding areas, one of the demersal fish species known as *Mystus vittatus* has a substantially greater frequency of small particles, amounting to 9 ± 1 micro-particles per fish [171]. This statement is in line with analysis carried out on demersal-type fish from Jamuna stream, *P. hammur* [70] and Wallago attu obtained from Kuakata [172], with an abundance of 3.8 and 3.5 ± 1.93 microplastics per species, respectively.

According to Siddique et al. [173], about 19.13 ± 10.77 microplastics per individual Hilsa Shadfish were discovered due to misunderstanding microparticles as feed; furthermore, the particles may gather during the filtration of marine food, such as plankton [173]. Moreover, small particles have also been extracted from dried fish, with about 41.33 particles per gram in a Bombay duck species and 46 micro debris per gram in a ribbon fish species [175]. Bangladesh's financial economy has relied heavily on its dried fish industry for many years. In Bangladesh, marine fish curing has indeed traditionally been performed for a lengthy period of time, and it is predicted that approximately close to 50% of the overall sea fish catchments are dried [175]. Micro debris could impair food flow or obstruct swallowing organs, eventually covering the GI tract; as further evidenced by Parvin, Jannat and Tareq. [171] upon the intake of micro debris by fish, the species tend to experience tiredness and loss of appetite. However, these health effects stated are based on other countries and research based on effects on fish health and physical changes to the body after the intake of microplastics in Bangladesh is still a matter of study.

One of the impacts on the terrestrial environment in Bangladesh is the microplastic in soils, wherein microplastics tend to reduce the effectivity of the soil used for agriculture. According to Jung et al. [176] and Schroter et al. [177], soils serve as a medium for several

functions, including carbon isolation and the biogeochemical cycle, as well as boosting biodiversity [178]. Small particles may alter the physio-chemical characteristics of the soil, which could exert a negative effect on biodiversity as well as a range of soil functions, including the rate of organic waste decomposition in the soil. In a study conducted by Afrin, Uddin and Rahman [178], microplastics were detected from soil specimens taken at an urban landfill spot in Dhaka [178]. The moisture content of the soil is indeed a crucial factor. The permeability properties of the soil may be affected in situations of excessive moisture content in the soil, which may impact the soil's shear strength [178]. In this research, the soil specimens have percentages between 15.48% and 56.54%, much greater than the ideal limit, as shown in Table 8, with an inference that it may cause changes to metabolic functions as well as erosion [178].

Table 8. Standards for physicochemical compositions of soil.

Compositions	Standard
Moisture content	10–45%
pH	7 ± 1
Electrical conductivity	200–1200 µS/cm
Alkalinity	6.5–7.5
Total organic carbon (TOC)	<0.5%
Data obtained from Afrin, Uddin and Rahman [178]	

In addition, the electrical conductance of the soil and soil alkalinity acts as key parameters of the environmental health of the soil. As per the research, the electric conductance and alkalinity in the soil was 0.1 μS/cm to 2.43 μS/cm and 6.7 µS/cm to 14.33 µS/cm, respectively [178]. Both values obtained were far from the optimum limits, where an electrical conductance below 200 μS/cm and high alkalinity values imply low fertility in the soil and fewer nutrients in the soil for intake by crops [178]. The total organic carbon in the soil may affect several physical, as well as chemical, properties of the soil, including the ability to store nutrients and the sturdiness of soil, prospective soil color, and nutrient cycling. From the research soil sample, the total organic carbon was between 0.18% and 1.09%, a little more than the norm, as shown in Table 8 [178]. Huerta Lwanga et al. [179] inferred that the presence of micro debris in soils might affect structural features, including the raising of porosity, altering the solid form, and also equalizing with soil aggregates, wherein these modifications may influence the microbial behavior of the soil [179].

3.2. India

In India, the impacts of microplastics on the environment are significantly related to the marine environment in which the ingestion of microplastics by aquatic biotas, such as shrimp, mussels, clams, and fish is largest and these are commonly consumed by the residents of India. In a study conducted by Daniel, Ashraf and Thomas [84], "*Fenneropenaeus indicus*", with the local name of white shrimp, were obtained from Cochin, a heavily industrialized and crowded town where fishing is a significant industry and the presence of small particles was found in the supple tissue of shrimp [84]. The shrimp analysis showed a mean amount of about 0.39 ± 0.6 small particles per white shrimp, roughly a percentage of 30.9% [84], which is currently still closely consistent with a study conducted in 2018 at a different location in India; for instance, on the Northwest Coast, where about 31.7% of small particles were extracted from the intestines of crustaceans [72]. White shrimp are known to reside and forage in muddy depths, where microplastics commonly build up, thus causing the crustacean to be vulnerable to small plastic debris [72].

These presences are highly concerning as crustaceans have a strong market demand both locally and internationally due to being a desirable and nutritious seafood that considerably supports the country's seafood industry [84]. Oysters and fish on the coast of

Tuticorin are also vulnerable to microplastics, wherein small plastic debris was detected in an average amount ranging from 0.1 to 1.73 microplastics per gram of oyster tissue specimens from the Tuticorin coast with significant amounts of fiber-shaped microplastics [8]. While based on another study, the micro debris detected in the fish samples amounted to a mean of 1.49 microplastics in the GI tracts per fish, predominantly fiber and fragment small particles [75]. These findings are relatable, as based on a sediment specimen analysis conducted in Tuticorin, microplastics with an average concentration of 83 ± 49 microdebris/m^2 with a predominance of fiber micro debris [180] were discovered. These sediments were collected from various points near the coast of Tuticorin, in which it can be inferred that the microparticles from the sediment might have transferred to the water of the coast induced by the action of the waves.

Tropical and subtropical areas are home to large populations of oysters that are known to be benthic organisms that live in close proximity to the coast, deeps seas, estuaries, as well as lagoons; additionally, oysters are filter-feeding species that exclude debris in water in order to feed [8]. The consumption of microplastics by oysters is highly probable, as according to Patterson et al. [8], food intake from the feeding method of oysters is through a suction force produced by its protractile jaw to increase effectiveness when hunting prey. In terms of the fish sample analysis, epipelagic fish that reside in deep waters have greater particle numbers in their GI tracts compared to mesopelagic fish that reside closer to the surface of coastal water [74]. Moreover, based on another alien fish sample study, wherein the species was known to have entered the Vembanad pond in the course of huge floods that hit Kerala in 2018, microplastics were extracted from the species with an overall count of 69 pieces [72], which was in line with a sediment specimen inspection conducted on Venbanad lake that was contaminated with microplastics accounting to 252.8 debris pieces/m^2 in mean [72].

Based on these findings, it can be deduced that the prevalence of small plastic debris in the intestines of an alien species, which provides a clear indication of the level of pollution in the pond water, might have ingested the plastic debris via land inputs as well as river streams that drain liquids into Venbanad ponds. In another piece of research, seafood, such as shrimp, squid, and crabs that are marketed as a source of food for humans, was analyzed in Kerala to evaluate the abundance of microplastics ingested by the crustaceans and squid. The crustaceans and squid showed a microplastic count with a mean value of 0.07 ± 0.3 small particles per shellfish, whereas the consumable tissue specimens of the shellfish had a mean of 0.0027 ± 0.012 microplastics per gram of tissue sample [181]. According to Daniel et al. [181], in crabs, the route for small plastic waste to enter their body is via ventilation, whereas, for squids, cephalopods migrate vertically throughout the daylight and nightfall, congregating toward deep sea depths during the day and rise to the surface of the sea after nightfall, leading the cephalopods to ingest a greater abundance of small particles as a result of exposure due to persistent movement through various sea zones.

Daniel et al. [181] author inferred that the prevalence and nonexistence of microplastics inside the consumable tissue of shrimp depend on the gut holding period and stomach elimination mechanism, which act as major drivers, as a longer holding period in the stomach may disintegrate plastic waste into microplastics that can move into the consumable tissue of the shrimp. Fragments were the dominant shapes located in the consumable tissue parts of the crustaceans and cephalopods [181], which is similar to a few studies conducted on the beaches and coasts of India, as shown in Table 2. Again, fragments were highly accounted for in the sediment specimens in which there are possibilities for the transfer of fragments from sediment to the waters of the coast and beaches due to wind and sea waves. Furthermore, in a recent analysis of yellow clams, mollusks that feed through filtering pieces of organic materials as well as planktons floating in liquid and that dwell continuously on a substrate by submerging to rough and thin sand bottoms, it was found that they were contaminated with microplastics, with 19,826 extracted pieces and an average of 138.61 ± 94.74 items per yellow clam in Maharashtra [182]. This em-

phasizes the fact that when clams expand and open their mouths wider, these clams sieve a larger body of water, causing the clams to ingest a greater amount of the micro debris present in the water [182]. According to FAO, a predicted amount of 4.9 kg of shellfish is consumed yearly per individual worldwide; taking this into consideration with a mean of 2.7 ± 12 microparticles per kg in the tissue of these specimens, an individual may roughly ingest 13 ± 58 microplastics yearly through the consumption of shellfish alone [181].

According to a survey of bivalve intake conducted across local households, a mean value of 120.59 g bivalve tendons is consumed each meal per individual; by taking this into consideration, approximately a mean of 3917.79 ± 144.71 microplastics are ingested per person annually [181]. Moreover, annually, refrigerated squid alone could transfer 776.5 million small particles outside of India through international trade (Daniel et al., 2021). Attributed to their ability to function as a carrier of substances that might threaten the security of seafood, microplastics are thought to be a gateway for hazards carried by seafood [181]. Raw and cured kinds of white shrimp are commonly served in meals. For raw shrimp, the top layer exoskeleton is eliminated to lower the chances of the human ingestion of micro debris. On the contrary, humans are likely subjected to some level of micro debris from the consumption of shrimp hindguts that have not been extracted, particularly in the case of mini shrimp [84]. According to statistics from the Government of India (2018), an approximated amount of 4584 tons of cured shrimp is produced yearly in the country [81]. As a preliminary estimation, around 733.44 million small plastic particles are assumed to be transferred via the intake of cured shrimp cycle alone based on an estimated mean amount of small plastic particles in shrimps of 16 per 100 g [84].

In a recent study based on the terrestrial environment, soils were particularly impacted by microplastic contamination in urban areas because of increased anthropogenic activities and the fact that the soils of urban areas could potentially serve as sinking sites for micro debris [183]. The collected soil specimens in this research were soils mainly used for agricultural activities. The soil exhibited physicochemical changes from standard crop soils due to microplastic pollution. Based on the analysis, the soil has a lower bulk density and followed by a moderately lesser pH in soil specimens, which was an average of 5.84 [183] compared to the standard 7 ± 1 [178]. In addition, the soil specimens showed greater soil organic and nitrogen contents, closer to the threshold level, and the author inferred that a greater level of nitrogen in soils might result in leaching [183]. Various forms of plastic particles have the tendency to accumulate in urban soils that may potentially disintegrate into tiny particles, leading to detrimental consequences for soil flora as well as fauna.

3.3. Indonesia

In Indonesia, the ingestion of microplastic by fish is prominent in the country's marine environment. One of the fish, *Gambusia affinis*, was polluted with small-sized particles. According to Rautenberg et al. [184], this species is an ecological stabilization species due to its broad range and biological significance in the human food cycle. The digestive area and gills of the species were filled with micro debris. Both of the studied organs are relevant to the sampling location, where the sampling points with the highest microparticle concentration of about 5192.59 items/m^3 contained fish with greater amounts of micro debris in their gills and digestive tracts, which amounted to 2861.11 items per gram and 4125 items per gram, respectively [185]. According to Buwono, Risjani and Soegianto [186], micro debris in the gills tends to build up during the filtration operation at the point of respiration and feeding, whereas the deposition of small particles in their digestive tracts is assumed to be due to the species' jaw opening that allows the entrance of both large and minute pieces [186,187]. Large amounts of residential waste that are generated by densely populated areas reach the marine habitat, increasing the composition of micro debris in the water.

In addition, according to McCauley [188], fish are a widespread species that contribute significant ecological and economic value that can act as stress monitors in marine environments [186]. *Gambusia affinis* are regarded as foreign, aggressive fish in the country as

they can rapidly breed with a high level of adaptability to different environments [189]. In a study, an oxidative stress test in relation to the microplastics showed that the species had positive feedback to the test, which was due to the consumption of microparticles. According to Jabeen et al. [190] and Movahedinia et al. [191], microparticles may be caught in the gills, leading to hypoxia strand cracks, reduced respiratory capacity, and the destruction of the gills, which may eventually raise the risk of illness in the species [192]. Furthermore, microplastic contamination has affected marine urchins and seagrass, with small particles detected that amounted to 23.7 ± 2.99 particles per sea urchin and a cumulative of 0.34 ± 0.07 particles/cm^2 of seagrass at Barranglompo islet [193]. This island is polluted due to extensive human activity and poor waste handling, particularly plastic waste, which is dispersed over various portions of the area, leading to the entrance of degraded microplastics into deep marine seagrass habitats. These particles may further travel to sea creatures, mainly those that feed on seagrass, as particles may become attached to the fronds of seagrasses.

Marine urchins are known to be significantly consumed by the local residents of the islet due to their considerable economic and rich mineral content [194]. This organism is known to be a generalist feeder that can intake large amounts of microplastics from seawater, particularly from the unintentional consumption of sediments from the bottom of the sea or sea grass fronds [193]. Similar to sea urchins, another substantial food in the country of economic value is Skipjack tuna due to the prevalence of this fish throughout the whole maritime territorial environment, and the country holds the rank as one of the top breeders and suppliers globally [195]. However, this species was found to be filled with micro debris, amounting to a mean of 76.72 g in the GI tract alone [195]. Moreover, the accumulation of particles in sandfish was revealed to amount to 2.01 ± 1.59 pieces per species. This species ingests a huge number of sediments that could be filled with microorganisms, phytoplankton, and shellfish [196], and due to their inability to filter-feed the particles, the species might ingest microplastics. This species is captured for regional human consumption but also farmed in industrial fish cultures [196]. The country is known to be a significant place for sandfish species, and there has been a surge in the sandfish market [197].

Additionally, a mean particle concentration of 1.97 items per species of blue panchax was discovered at Ciliwung estuarine, as debris has accumulated at the estuarine due to tourism-related activities as well as dumping from resorts and eateries in close proximity [198]. In addition, microplastic contamination in water can cause physicochemical changes to water standards. Based on a study related to Brantas river water, the physicochemical changes of the water were analyzed. The analysis showed that the temperature of the stream was 21 °C in the upper section of the stream and 31 °C in the lower section [186], lower than the standards shown in Table 9. The growth of flora that safeguards the upper section of the stream could have caused the temperature of the upper section to be lower than the standards. Next, the overall suspended solids (TSS) measurements varied between 39.0 and 750.1 mg/L, whereas the overall dissolved solids (TDS) showed readings between 195.3 and 375.0 mg/L [186], and the TDS is within the standards shown in Table 9. Increased amounts of TSS downstream may be a result of larger quantities of soluble salt, high pesticides and urban waste, sewage waste, decaying vegetation, and riverbank degradation. According to Risjani et al. [199], the streams genotoxicity could have been mediated by the elevated TSS levels, which are produced through sediment inflow into the Brantas river [186].

Table 9. Standards for physicochemical parameters of water.

Parameters	Standard	Reference
Temperature	25–32 °C	[200]
Total suspended solids (TSS)	<400 mg/L	[186]
Total dissolved solid (TDS)	<1000 mg/L	[186]
pH	6–9	[186]
Dissolved oxygen (DO)	>4 mg/L	[186]
BOD	1–8 mg/L	[200]
Nitrate	<10 mg/L	[186]
Phosphate	<1 mg/L	[200]

In terms of pH, the scale read between 6.8 and 7.6 [186], and these readings are within the standards shown in Table 9 and are ideal for the survival of the species in the river. The DO varied between 1.4 and 8.1 mg/L [186], in which certain sampling points had values of more than 4 mg/L, above the standard established by Supenah et al. (2015) as deduced by the organic contaminants predominant in pollution, especially domestic waste from household chores [201]. Lastly, the BOD values ranged between 5.9 and 20.8 mg/L [186], and the lower section of the river showed a maximum BOD of 20.8 mg/L, relatively higher than the standards. According to Widodo et al. [202], the BOD of water is important in measuring the amount of available oxygen needed by organisms to break down organic matter under aerobic conditions. The greatest BOD content was located in the lower section of the stream, which could be due to pollution from organic waste associated with the close-to-high level of nitrate, which varied between 1.7 and 9.2 mg/L, as well as the moderate level of phosphate, which varied between 0.4 and 1.2 mg/L [186].

3.4. Vietnam

In Vietnam, the studies that had been conducted to investigate the impact of microplastics pollution on marine environments were limited. One of the impacts is ingestion by clams, wherein clams collected from the Ban Sen community showed the presence of microplastics that amounted to a range between 0.25 ± 0.16 and a maximum of 0.67 ± 2.98 items per clam [203]. Ban Sen contains roughly 150 families and about 2.6 million clam cages, where, in 2020, roughly 2.250 tons of aquatic resources, including clams and oysters, were produced [203]. Based on two different sampling sites, Quang Ninh and Nam Dinh, the authors stated that juvenile clams from Quang Ninh contain a greater number of microparticles in their tissue compared to mature clams and vice versa at Nam Dinh [203]. The species has an abundance of pellet-shaped microplastics, contrasting with many studies conducted based on different environments where fibers and fragments are more prevalent.

This country is an attractive site for fishing and seafood sector growth [204]. Mollusks are significant trade and nutritional resource for the people in the country, contributing greatly to economic growth, and according to VASEP, mollusk cultivation in 2020 alone yielded an overall total of 375,000 tons. Oysters obtained from Da Nang growing bay accounted for 445 microplastics overall and an average amount of 18.54 ± 10.08 pieces per oyster sample [204]. The authors deduced that this abundance might be attributed to the generation of domestic waste, leading to shellfish capturing areas being highly polluted [204]. Da Nang is very much contaminated with microplastics in various environments, such as in drainage canals [109], street dust [108], and beaches [102]. Microplastics with fragmented shapes were located in the oysters [204] significantly more than fibers, which were predominantly seen in several studies in Da Nang, and it has been suggested that the limited specific surface area of the fragment-shaped particles could have caused

them to settle and concentrate in deep waters [205], leading to a higher number of microplastics in oysters.

Polymer types, such as nylon and MUF, were identified in the oysters, and it was deduced that the greater density of these polymers could have led to them sinking into deep waters [204]. MUF is a prevalent thermoset resin for producing wood compounds as well as varnishes to enhance waterproofing [204]. Oysters are cultivated specifically around seaside regions in structures dug into the seabed or on strings dangling on rafts, and these species are more susceptible to consuming small-sized particles that are deposited in freshwater in situations when there is insufficient feed for the oysters [204]. Additionally, in another bivalve analysis, the species showed roughly 2.60 pieces per bivalve, with a predominance of PP and polyester [206].

3.5. Iran

The presence of microplastic contamination in Iran has impacted waterbodies and terrestrial and atmospheric environments. Based on the water environment, one of the impacts was micro debris ingestion by fish species at a wetland called Anzali, which is located close to the Caspian Sea, which has a significant role in sustaining the welfare of the community through leisure and angling activities. The fish species captured from this wetland showed about 358 microplastics in the bodies of the species [207]. This area is contaminated with many different sources, including factory waste, crop waste, hospital wastewater, and dirty waterways, placing a severe strain on the wetland and eventually leading to microplastic contamination in the water. There are possibilities for the species to have unintentionally consumed substances that are present on plant surfaces, retained in sediment, or confined inside biofilm structures [207]. The fish species analyzed were omnivores, and such species might absorb microplastics through sediment or flora's exterior during consumption of benthic creatures, as the amount of debris is greater in sediments and showed a maximum value of 3690 items per kg of sediment in Anzali swamp [208].

Omnivores tend to retain particles in the stomach for lengthier periods of time, which may p result in a larger volume of micro debris in the digestive tracts of these species [207]. Furthermore, the stomachs of fish from the Karkheh basin, which is close to the Qarasu stream, a significant stream in the country of the Kermanshah metropolis that supplies water to farming areas, were contaminated with microparticles that varied between 1.5 and 15 pieces per fish with a predominance of fibers [209]. This is relatable as the Qarasu estuarine that connects to the waterways of the area is polluted with microplastics. Studies based on ingestion by crustaceans, shellfish, and other aquatic organisms are limited in Iran. Based on the terrestrial ecosystem, a soil specimen analysis conducted recently in Ahvaz town on urban and manufacturing soils showed microplastics that amounted to an overall count of 10,940 pieces [210]. Micro debris in topsoil could have impacted flora interactions with the topsoil by changing the morphology, liquid dynamics, and the biological growth of plants.

According to Nematollahi et al. [210], microparticles may influence the manner in which earthworms build their tunnels, leading to higher amounts of micro debris seeping into ground liquids. Ahvaz's atmospheric air contains microplastics that have been analyzed and shown in a recent study [130]; thus, air precipitation could have contributed considerably to transfer into soils from the town's urban as well as commercial sectors, leading to high amounts in deep soils [210]. In terms of the atmospheric environment, the direct impact of microplastic contamination in such an environment is human exposure, wherein the micro debris is located on the external parts of a human body. The impact of the atmospheric ecosystem was proven with the presence of micro debris found on the human skull, epidermis, hair strands, as well as hands and spittle, in a study conducted at a few locations in Iran [138]. A cumulative of 16,000 microparticles was found in which the male population had a greater count compared to the female population chosen for the research [138].

The prevalence of atmospheric particles, particularly fibers, was abundantly noticed on hair follicles, which was deduced to be a consequence of meteorological conditions that could influence the regional variations of microplastics on skulls and hair follicles [138]. It is assumed that Bushehr's humid climate could have encouraged the adherence of microplastics to the participants selected for the study [138]. Additionally, the utilization of helmets by men and head veils by Iranian women serve as primary settling sites for micro debris onto skull and hair follicles. The presence of microplastics in Iran's land dust, as well as airborne micro debris, is an emerging matter due to deposition onto the human body; thus, more studies have to be conducted in Iran to further explain the situation and its consequences.

3.6. Thailand

In Thailand, the environmental impact primarily relates to the marine environment, where ingestion by sea animals has been observed, yet limited research has been carried out in the country. One of the conducted studies was based on the blood of cockles as well as green mussels acquired from a few carefully chosen markets and rearing spots. In this study, the blood of the cockles retrieved from the rearing spot yielded fewer microplastics, 6 ± 1 pieces, compared to the market-purchased cockles, which yielded 11 ± 5 pieces per cockle, whereas green mussels also yielded similar results of 96 ± 19 for market-purchased and lower values of 11 ± 7 pieces per mussel from the rearing spots [211]. Approximately 193,000 tons of mollusk and an overall 21% brine aquaculture yield are delivered each year by green mollusks [212]. According to Tuan-Ta et al. [211], the increased quantity of microparticles in commercialized bivalves could be caused by pollution during wrapping and shipping from rearing areas to marketplaces [211].

Additionally, bivalves for sale may be polluted by precipitated atmospheric microplastics as the bivalves are presented for sale in an exposed area [213]; hence leading to more particles being observed at the marketplace than the rearing spots. Mussels are farmed using a variety of plastic equipment, including cords made of polypropylene and buckets comprising polyethylene, to assist in suspending the bivalve over the water's surface, whereas cockles are raised by employing a sowing cultivation approach, as cockles are prone to residing about 2 m inside of a mudflat via burying [211]. There was a predominance of fragments in the bivalves exposed to nylon located in the marketplace, which might be due to shipping and wrapping [211]. In addition, sessile invertebrates surfaced as a species that ingested microplastics, amounting to a maximum of 0.6 pieces per gram [214]. According to this study, the key elements that influenced the concentration of the micro debris consumed by sea creatures were the degree of contamination in the intertidal ecosystem and duration away from habitat spots as well as anthropogenically impacted places near beachfronts [214].

One of the spots where invertebrate samples were collected was Angsila, which appeared to have improper management measures enforced to handle plastic contamination, and as a result, economic shellfish and fish cultivation techniques have been negatively impacted in the region, eventually leading to an increased buildup of microplastics [214]. Mussels and clams that have ingested microplastics were collected from Bandon Bay with water flowing from the Tapi-Phumduang stream with a prevalence of fibers and fragments inside the tissue specimens of both the species. This is in line with the predominance of microplastic shapes discovered in the top water and deep sediment acquired from the Bandon environment [150]. This bay is a significant mariculture area for its abundance of commercially valuable shellfish [150], and it has been suggested that stream release combined with ocean practices, such as angling and mariculture, could be a crucial source and channel for microparticles to access Bandon Bay. Furthermore, at Songkhla, prawn and catfish, which are popular foods in the area, were acquired, and the abdomen of these species contained a total of roughly 172 microparticles, extracted with a predominance of fiber-shaped forms [215].

Catfish are known to be a benthic species that dwells inside Songkhla pond and are widely distributed during the monsoon period until the end of the period, whereas yellow shrimp are distributed in the outer part of the pond nearly year-long [215]. Prawns have commercial importance, notably at this pond, where there is a high demand from marketplaces and surrounding nations, such as Malaysia and Singapore, due to their excellent flavor, delicate shells, and suitability for a variety of cuisines [215]. The authors predicted that amid the COVID-19 pandemic, the majority of workers remained at home, which could have led to more frequent laundry, removing extra fibers, which then infiltrate drains, canals, and ponds, polluting ground and ocean animals [215]. The polyester polymer type could have arisen from the cleaning of face covers made of fabric, whereas rayon may be derived via laundry-generated garment lint. Moreover, it is possible that pieces of shattered angling nets would float and gather on the sea surface at the outflow of the pond, eventually flowing into the Gulf of Thailand [215].

Due to high waves, liquid from the ocean in the Gulf of Thailand would access Songkhla Lake via the pond's opening, and vice versa, the situation occurs in the mid of lesser tides [215], leading to microplastic contamination in the Gulf of Thailand. Sating Phra Division, situated close to the lower bay of Thailand, is among the fastest-growing regions in terms of commercial expansion, and microplastic ingestion in demersal as well as pelagic types of fish was demonstrated [216]. The abdomens of demersal species showed a little larger mean count of microplastic, amounting to 5.41 particles per gram compared to pelagic fish, which exhibited a mean count of 4.61 particles per gram, and the author deduced that this occurrence could be the result of abundant polymer waste present on the surface water of the sea [216]. Owing to the firmness and nature of plastic, the majority of polymer waste tends to float on water top, wherein pelagic species could misinterpret plastic as feed for consumption. After reaching the sea, polymers tend to remain at the top layer of the seawater for a specific amount of time before disintegrating into smaller shards and moving to different trophic zones in the sea, including the center zone and, eventually, to deeper zones of water. Hence, the above has become a leading cause for the abundance of microplastics in pelagic fish abdomens.

3.7. Philippines

The impact on the environment in the Philippines mainly relates to marine environments. Based on the marine environment, ingestion by aquatic species is prominent in the Philippines. Market fish species obtained from Cebu islet consumed microparticles that came to an overall count of 635 particles from 81 species, with over a 90% predominance of fiber pieces [217]. According to the authors, the area of study is crowded with a high number of people, approximately 1.68 million people compared to other towns within the country [217], which may have led to micro debris pollution in the area. In another study based on the same island, whale shark excrement contained a count of 393 microparticles, with larger amounts of fragments shape and the PP plastic polymer group [218]. The imperiled whale shark could devote about 7.5 h daily eating at the top level of the water, which permits the species to filter roughly 326,000 L of water every hour [219]. In addition, whale sharks can effectively consume small particulates, notably fish larvae via cross-flow screening (Motta et al., 2010 [219]) and hence potentially also consume microplastics.

Whale sharks that feed at the top level of waters in Cebu could have potentially consumed roughly 14,000 microplastics daily [218], presuming seawater filter efficiency is 326,000 L per hour and an average of 7.5 h daily devoted to eating at the top level of the water [219]. In addition, microparticles were observed in cultivated green mussels that varied between 0.27 and 0.41 pieces per gram at Bacoor Cove. This bay has a thriving mussel market and is regarded as being among the country's leading mussel growers [220]. Fibers were most counted supposedly due to the widespread usage of PP cords used by numerous mussel growers at the bay, whereas the subsequent prevalence of fragments can possibly be related to the utilization of readily compostable polymer bags made from oxo-biodegradable PE [220]. Additionally, based on oyster analysis in the country from

three different locations, 11.8 pieces in an oyster were extracted recently [221], which is in line with another study conducted at the Bombong estuarine, where about 40 small particles were extracted from oysters grown in the aquaculture spot.

The microplastic shapes observed in green mussels were also found in the oyster specimens. In addition, many different fish species have been shown to have ingested microparticles from the aquatic environment; the occurrence of microplastics are also found in rabbitfish and mullets, which contained 85 and 33 pieces in their bodies, as obtained from different areas of East Visayas [222]; an abundance of 97 microplastics in the gastroenteric of all rabbitfish species taken from the Tanon Strait [223] and Negros. This observation of similar fish species showed an ingestion of 0.6 pieces per species [162]. Rabbitfish have been analyzed in many studies, as this species is among the main fish that are highly consumed by the people in the country due to the flavor and rich protein content of the species. Mullets consume sand as well as dirt along with tiny algae or natural substances [222], causing the species to have high chances of ingesting microplastics that are under intertidal strata as mullets are known as benthic creatures [224]. Angling is a leading sector, with a mean intake level of roughly 40 kg of fish or derivatives from the species by an individual yearly, leading the Philippines to be the largest worldwide in these terms [225].

As rabbit fish species are deep water consumers, the species have the potential to persistently intake significant amounts of microparticles in situations of over-exploitation of coastal habitats, notably excessive clearing of algae as well as the constant dumping of polymer into waters [223]. Additionally, habitat splitting occurs frequently among rabbit fish that shares the same resources [226]; therefore, they could be susceptible to different microplastics, as rabbit fish have such an ecological approach. In addition, "sardinella lemuru", commonly called sardine, are a significant aquatic food resource in the country, and those primarily caught offshore are filled with microplastics in the abdomen of the species, amounting to 2238 pieces from 600 species collected [227]. This important fishing area yields roughly 50 to 60 percent of the overall yearly output, which is then converted into products, such as canned or bottled sardines [227]. The high presence of microparticles in sardines from different areas in Northern Mindanao was explained by the magnitude of the human population, which could be the reason, as in this study, the overall number of microplastic consumed by the species substantially correlated with the number of people living close to the collection areas [227].

4. Summary of Study

Microplastic contamination is prevalent in all South Asian countries. In summary, based on the coastal environment, fiber and films were more commonly observed in coastal studies in Bangladesh and the Philippines and, in contrast, fibers and smaller amounts of fragments were observed in India, Indonesia, Iran, and Thailand. The common polymers detected in coastal areas are rayon, nylon, PVC, HDPE, LDPE, polyester, and PP based on the seven countries. For beach areas, fibers, fragments, foams, and pellets were located in Bangladesh, India, Vietnam, Iran, and Thailand, all studies where PP, PE, PS, EPS, and PET polymer forms were discovered. In terms of river environments, Bangladesh, Indonesia, and Iran showed primarily fiber polymers, whereas Thailand and Vietnam have a subsequent abundance of fragments, and the Philippines is the sole country that has additional shapes of microplastics, such as films, pellets, and lines in the streams. The polymer types predominantly located in river streams were PP, PE, polyester, LDPE, and PS in all six South Asian countries. Based on lake and wetland specimen analysis involving water and sediments, the prominent shapes of fibers, films, and pellets were observed with HDPE, PVC, and PP polymer types in Bangladesh, Vietnam, and Iran, whereas Indonesian bay environments were characterized by foam, followed by fragments, which contrasts with the detection of microplastics in Iran, Thailand, and the Philippines that exhibited fragment dominance, followed by fiber and pellet shapes. In all four of these countries, the polymer types in the bay are PE, PET, PP, and PS. Mangrove area specimens in Vietnam showed fiber dominance with larger sizes, contrasting to the findings in Indonesia that

yielded foam and fragments smaller than 1000 μm; however, the polymer types were identical in both countries, with PE, PP, and PS. Watershed analysis in Vietnam and the Philippines showed common polymers of PP, PE, and PET in the shapes of fibers and fragments. Based on the estuarine environment, fiber is the most dominant shape and is mostly below 1 mm in size of polyester, PP, LDPE, and PE in India, Indonesia, Iran, and Thailand. In all the studies conducted based on the seven South Asian countries, the microplastics range in size between 100 μm and 5000 μm, with the majority having a size below 1000 μm. Fibers are commonly observed in every country and are mainly related to household launder waste as well as wearing off of garments, leading to the accumulation of fiber-shaped microplastics, and another contributing factor is the higher population in the area, which could create more launder effluents.

In addition, in every country, polyethylene was observed, where PE and PP are known to be in plastic products, such as plastic bags, bottles, wrappers, and more items. The high utilization and improper disposal of plastics made up of PE or PP polymer leads to the accumulation of microplastics, as plastics are not naturally biodegradable products, and these plastics tend to degrade due to high UV or climate-induced reasons. Additionally, aquaculture-related activities, mainly fishing, lead to the generation of microplastics of different forms in most of the South Asian countries reviewed in this study. Moreover, microplastics tend to further disintegrate into smaller sizes, as low as 300 μm, which is a result of aging and wear brought about by environmental factors as well as hydrodynamic fluctuations. One of the reasons for the accumulation of microplastics in huge water environments such as seas, bays or coastal areas is due to the influx of water from rivers, lakes, waterways, or canals, which are already contaminated with microplastics. In terms of the current conditions of microplastics in South Asian countries, the main environments that are highly contaminated are water-based places, such as rivers, estuarine, beaches, and seas, as the transfer of microplastic to water environments is easy due to air movement and rainfall. Moreover, a few food items that are commonly utilized, such as sugar, teabags, shrimp paste, and salts, have been contaminated with microplastics based on recent research conducted in some of the countries.

In brief, the condition of microplastics in each South Asian country relates to the abundance of microplastics found in various environments as well as the presence of microplastics in the food and air. The main environments that are highly contaminated are water-based places, such as rivers, estuarine, beaches, and seas, as the transfer of microplastic to water-based places is easy due to air movement and rainfall. The majority of the countries reviewed were characterized by the presence of microplastics in food items, such as tea bags, sugar, and, notably, salt packets. Microplastics have also been present in the atmosphere in a few countries, such as Indonesia, Iran, and Vietnam. The presence of microplastics in the air leads to the settling of these particles in various areas, such as classrooms and indoor buildings as well as being carried by snow particles and dust. In terms of the impacts on the environment, every South Asian country has been affected in the marine environment, where ingestion by aquatic life has been widely observed, such as in shrimp, oysters, clams, mussels, and fish, wherein this ingestion by seafood species could be a vulnerable pathway for human exposure to microplastics.

5. Future Works

The Sustainable Development Goals (SDGs), global goals that are a set of separate but interrelated objectives precisely crafted to provide everyone on Earth a brighter future, comprising numerous milestones and evaluation markers focused toward 2030. The purpose of Sustainable Development is to promote progress that meets current requirements without affecting the resource supply of subsequent generations [228]. Two Sustainable Development Goals that can be related to this study are SDG 6, clean water and sanitation, and SDG 14, for water-based life. As the microplastics issue is highly concerning in terms of water-based environments, achieving both SDGs is highly important. Accessibility to clean water and sanitation, as well as the sustainable maintenance of freshwater supply,

are crucial, and they relate to Sustainable Development Goal 6 in that they boost economic output and act as powerful multipliers for newly made expenditures in healthcare and for educational purposes [229]. In terms of SDG 14, this goal focuses on the protection and appropriate utilization of marine resources. According to GSMA [230], by 2100, a roughly 100 to 150 percent increase in acidity could occur, causing damage to over 50% of aquatic species and, in 2050, it is predicted that plastics could be higher in number than fish species present in oceans.

One of the targets of SDG 14 is to minimize and eliminate marine contamination by inhibiting and drastically decreasing all forms of marine contamination, especially that caused by land-based practices, such as fertilizer pollution and ocean waste disposal by 2025 [230]. Moreover, one of the targets of SDG 6 is to enhance water purity by 2030 by decreasing contamination, stopping dumping, limiting the discharge of dangerous chemicals and substances, lowering the amount of unprocessed wastewater as well as vastly expanding recovery and ethical reusing worldwide [229]. Hence, to achieve the aim of SDG 6 and SDG 14, rapid measures should be taken through the implementation of new and advanced rules related to plastic utilization, and more efficient water processing facilities as microplastic contamination from plastics is becoming more prevalent. Persistent improper disposals may cause more microplastic to be generated, and thus, more efficient disposal systems and facilities must be developed, and greater attention has to be given to developing and developed countries to prevent more accumulation and dispersion of these microplastic to food and other environments. Higher enforcement of plastic usage policies with stricter laws is needed, as the increased usage of plastic will cause many folds of microplastic generation. In order to minimize fiber abundance, the innovation of good microplastic-filtrating systems in laundry machines could possibly be a promising way to reduce fiber microplastics from residential areas, as fibers were prominently noticed in every South Asian country viewed in this study, and the addition of extra processing or filtration systems in purification facilities to remove other forms of microplastics. The characterization of microplastics in South Asian countries and the current condition of microplastic pollution, and the impacts on the environment, were researched in this study. Regarding the current conditions, the presence of microplastics in the food chain was mainly observed in salt, sugar, tea bags, and many more food items in certain South Asian Countries. Wide research has to be conducted regarding the microplastics found in food and beverage items in South Asian Countries to locate the possible routes of the microplastics to food and beverage goods as well as the potential consequences to humans due to the ingestion of food and beverages contaminated with microplastics. Microplastic contamination in the human body must be studied to better understand the level of dispersion of microplastics.

Author Contributions: Conceptualization, L.T.S. and S.-T.B.; writing—original draft preparation, V.B.; writing—review and editing, L.T.S., S.-T.B. and S.-L.B.; supervision, L.T.S.; project administration, S.-T.B. and S.-L.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Universiti Tunku Abdul Rahman Internal Funding 6251-B04.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

μm	micrometer
mm	millimeter
cm	centimeter
kg	kilogram
MMT	Million Metric Tons
AR	Awano River
AsR	Asa River
AyR	Ayaragi River
MR	Majime River
EPS	Expanded Polystyrene
PAN	Polyacrylonitrile
PDAP	Polydiallyl phthalate
PE	Polyethylene
PET	Polyethylene terephthalate
PES	Polyester
PMMA	Polymethyl methacrylate
PS	Polystyrene
PTFE	Polytetrafluoroethylene
PP	Polypropylene
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
SIS	Seto Inland Sea
SJ	Sea of Japan
HDPE	High Density Polyethylene
CA	Cellulose Acetate
LMP	Large Microplastics
SMP	Small Microplastics
ABS	Acrylonitrile Butadiene Styrene
PPE	Personal Protective Equipment

References

1. Abbasi, S.; Jaafarzadeh, N.; Zahedi, A.; Ravanbakhsh, M.; Abbaszadeh, S.; Turner, A. Microplastics in the atmosphere of Ahvaz City, Iran. *J. Environ. Sci.* **2023**, *126*, 95–102. [CrossRef] [PubMed]
2. Weldon, N.A. Chapter 8-The Environmental Impacts of Plastic Pollution. 2020. Available online: https://www.sciencedirect.com/science/article/pii/B9780128178805000086 (accessed on 31 January 2022).
3. Wang, Y.; Zou, X.; Peng, C.; Qiao, S.; Wang, T.; Yu, W.; Khokiattiwong, S.; Kornkanitnan, N. Occurrence and distribution of microplastics in surface sediments from the Gulf of Thailand. *Mar. Pollut. Bull.* **2020**, *152*, 110916. [CrossRef] [PubMed]
4. Rogers, K. "Microplastics". Encyclopedia Britannica. 2020. Available online: https://www.britannica.com/technology/microplastic (accessed on 31 January 2022).
5. Chatziparaskeva, G.; Papamichael, I.; Zorpas, A.A. Microplastics in the coastal environment of Mediterranean and the impact on sustainability level. *Sustain. Chem. Pharm.* **2022**, *29*, 100768. [CrossRef]
6. Gkatzioura, A.; Zafirakou, A.; Zorpas, A.A. Wastewater treatment plants' contribution in microplastics' contamination of surface water. Effluent concentration and detection techniques review. *Desalination Water Treat.* **2021**, *227*, 26–33. [CrossRef]
7. Loizia, P.; Voukkali, I.; Chatziparaskeva, G.; Navarro-Pedreño, J.; Zorpas, A. Measuring the Level of Environmental Performance on Coastal Environment before and during the COVID-19 Pandemic: A Case Study from Cyprus. *Sustainability* **2021**, *13*, 2485. [CrossRef]
8. Patterson, J.; Jeyasanta, K.I.; Sathish, N.; Booth, A.M.; Edward, J.P. Profiling microplastics in the Indian edible oyster, *Magallana bilineata* collected from the Tuticorin coast, Gulf of Mannar, Southeastern India. *Sci. Total. Environ.* **2019**, *691*, 727–735. [CrossRef]
9. Kim, H.; Lee, J.-Y. Emerging Concerns about Microplastic Pollution on Groundwater in South Korea. Microplastics-Macro Challenge for Environmental Sustainability. 2020. Available online: https://www.mdpi.com/2071-1050/ (accessed on 1 February 2022).
10. Sebille, E.V.; Wilcox, C.; Lebreton, L.; Maximenko, N.; Hardesty, B.D.; Franeker JA, V.; Eriksen, M.; Siegel, D.; Galgani, F.; Law, K.L. A global inventory of small floating plastic debris. *Environ. Res. Lett.* **2015**, *10*, 124006. Available online: https://www.researchgate.net/publication/286243165_A_global_inventory_of_small_floating_plastic_debris (accessed on 31 January 2022). [CrossRef]
11. Dowarah, K.; Devipriya, S.P. Microplastic prevalence in the beaches of Puducherry, India and its correlation with fishing and tourism/recreational activities. *Mar. Pollut. Bull.* **2019**, *148*, 123–133. [CrossRef]

12. Irfan, M.; Qadir, A.; Mumtaz, M.; Ahmad, S.R. An unintended challenge of microplastic pollution in the urban surface water system of Lahore, Pakistan. *Env. Sci. Pollut. Res.* **2020**, *27*, 16718–16730. Available online: https://www.researchgate.net/publication/339704099_An_unintended_challenge_of_microplastic_pollution_in_the_urban_surface_water_system_of_Lahore_Pakistan (accessed on 25 March 2022). [CrossRef] [PubMed]
13. Ahmed, Q.; Ali, Q.M.; Bat, L.; Oztekin, A.; Memon, S.; Baloch, A. Preliminary Study on Abundance of Microplastic in Sediments and Water Samples along the Coast of Pakistan (Sindh and Balochistan)-Northern Arabian Sea. *Turk. J. Fish. Aquat. Sci.* **2021**, *22*, TRJFAS19998. Available online: https://www.trjfas.org/uploads/pdf_14866.pdf (accessed on 26 March 2022). [CrossRef]
14. Business Recorder. World Economy and Business, Aug 2016, pg 11. Available online: epaper.brecorder.com/2016/08/18/11---page/784169-news.html (accessed on 25 March 2022).
15. Isobe, A. Percentage of mcirobeads in pelagic microplastics within Japanese coastal waters. *Mar. Pollut. Bull.* **2016**, *110*, 432–437. [CrossRef] [PubMed]
16. Pan, Z.; Guo, H.; Chen, H.; Wang, S.; Sun, X.; Zou, Q.; Zhang, Y.; Lin, H.; Cai, S.; Huang, J. Microplastics in the Northwestern Pacific: Abundance, distribution, and characteristics. *Sci. Total. Environ.* **2019**, *650*, 1913–1922. [CrossRef]
17. Oteef, M.D.Y.; Elhassan, M.S. Plastic toys and child care articles as a source of children exposure to phthalates and other plasticisers in Saudi Arabia. *Int. J. Environ. Anal. Chem.* **2020**, *102*, 4316–4330. Available online: https://www.tandfonline.com/doi/abs/10.1080/03067319.2020.1784407 (accessed on 25 March 2022). [CrossRef]
18. Alharbi, N.S.; Qattan, M.Y.; Alhaji, J.H. Towards Sustainable Food Services in Hospitals Exapnding the Concept of 'Plate Waste' to 'Tray Waste'. *Sustainability* **2020**, *12*, 6872. Available online: https://www.mdpi.com/2071-1050/12/17/6872 (accessed on 15 February 2022). [CrossRef]
19. Ouda, O.K.M.; Raza, S.A.; Nizami, A.S.; Rehan, M.; Al-Waked, R.; Korres, N.E. Waste to energy potential: A case study of Saudi Arabia. *Renew. Sustain. Energy Rev.* **2016**, *61*, 328–340. [CrossRef]
20. Alam, F.C.; Sembiring, E.; Muntalif, B.S.; Suendo, V. Microplastic distribution in surface water and sediment river around slum and industrial area (case study: Ciwalengke River, Majalaya district, Indonesia). *Chemosphere* **2019**, *224*, 637–645. [CrossRef]
21. Anjum, M.; Miandad, R.; Waqas, M.; Ahmad, I.; Alafif, Z.O.A.; Aburizaiza, A.; Barakat, M.; Akhtar, T. Solid waste management in Saudi Arabia: A review. *J. Sci. Food Agric.* 2016. Available online: https://www.researchgate.net/publication/303348044_Solid_waste_management_in_Saudi_Arabia_A_review (accessed on 20 March 2022).
22. Pico, Y.; Soursou, V.; Alfarhan, A.H.; El-Sheikh, M.A.; Barcelo, D. First evidence of microplatics occurance in mixed surface and treated wastewater from two major Saudi Arabian cities and assessment of their acological risk. *J. Haz-Ardous Mater.* **2021**, *416*, 125747. [CrossRef] [PubMed]
23. Al-Lihaibi, S.; Al-Mehmadi, A.; Alarif, W.M.; Bawakid, N.; Kallenborn, R.; Ali, A.M. Microplastics in Sediments and Fish from the Red Sea Coast at Jeddah (Saudi Arabia). Environemental Chemistry. 2019. Available online: https://www.researchgate.net/publication/336576075_Microplastics_in_sediments_and_fish_from_the_Red_Sea_coast_at_Jeddah_Saudi_Arabia (accessed on 29 March 2022).
24. Woodward, R.T.; Wui, Y.S. The economic value of wetland services: A meta-analysis. *Ecol. Econ.* **2001**, *37*, 257–270. [CrossRef]
25. Eriksen, M.; Mason, S.; Wilson, S.; Box, C.; Zellers, A.; Edwards, W.; Farley, H.; Amato, S. Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Mar. Pollut. Bull.* **2013**, *77*, 177–182. [CrossRef]
26. Driedger, A.G.; Dürr, H.H.; Mitchell, K.; Van Cappellen, P. Plastic debris in the Laurentian Great Lakes: A review. *J. Great Lakes Res.* **2015**, *41*, 9–19. [CrossRef]
27. Floehr, T.; Xiao, H.; Starke, S.B.; Wu, L.; Hou, J.; Yin, D.; Zhang, X.; Ji, R.; Yuan, X.; Ottermanns, R. Solution by dilution?—A review on the pollution status of the Yangtze River. *Environ. Sci. Pollut. Res.* **2013**, *20*, 6934–6971. Available online: https://pubmed.ncbi.nlm.nih.gov/23608976/ (accessed on 16 June 2022).
28. Ismail, N.S.; Müller, C.E.; Morgan, R.R.; Luthy, R.G. Uptake of Contaminants of Emerging Concern by the Bivalves *Anodonta californiensis* and *Corbicula fluminea*. *Environ. Sci. Technol.* **2014**, *48*, 9211–9219. Available online: https://pubmed.ncbi.nlm.nih.gov/25017714/ (accessed on 16 June 2022). [CrossRef]
29. Wong, T.; Geiger, W. Adaptation of wastewater surface flow wetland formulae for application in constructed stormwater wetlands. *Ecol. Eng.* **1997**, *9*, 187–202. [CrossRef]
30. Hossain, J.; AftabUddin, S.; Akhter, F.; Nusrat, N.; Rahaman, A.; Sikder, M.N.A.; Monwar, M.; Chowdhury, M.S.N.; Jiang, S.; Shi, H.; et al. Surface water, sediment, and biota: The first multi-compartment analysis of microplastics in the Karnafully river, Bangladesh. *Mar. Pollut. Bull.* **2022**, *180*, 113820. [CrossRef] [PubMed]
31. Mercy, F.T.; Alam, A.K.M.R.; Akbor, M.A. Abundance and Characteristics of Microplastics in Major Urban Wetlands of Dhaka, Bangladesh. 2022. Available online: https://assets.researchsquare.com/files/rs-1455552/v1/eff (accessed on 16 June 2022).
32. Hossain, M.B.; Banik, P.; Nur, A.-A.U.; Rahman, T. Abundance and characteristics of microplastics in sediments from the world's longest natural beach, Cox's Bazar, Bangladesh. *Mar. Pollut. Bull.* **2020**, *163*, 111956. [CrossRef]
33. Banik, P.; Hossain, M.B.; Sun, J.; Choudhury, T.R.; Liba, S.I.; Yu, J.; Noman, A.; Nur, A.U. Microplastics in Sediment of Kuakata Beach, Bangladesh: Occurrence, Spatial Distribution, and Risk Assessment. Marine Science. 2022. Available online: https://www.researchgate.net/publication/359929908_Microplastics_in_Sediment_of_Kuakata_Beach_Bangladesh_Occurrence_Spatial_Distribution_and_Risk_Assessment (accessed on 16 June 2022).
34. Tajwar, M.; Shreya, S.S.; Gazi, Y.; Hasan, M.; Saha, S.K. Microplastic contamination in the sediments of the Saint Martin's Island, Bangladesh. *Reg. Stud. Mar. Sci.* **2022**, *53*, 102401. [CrossRef]

35. Rahman, S.M.A.; Robin, G.S.; Momotaj, M.; Uddin, J.; Siddique, M.A.M. Occurrence and spatial distribution of microplastics in beach sediments of Cox's Bazar, Bangladesh. *Mar. Pollut. Bull.* **2020**, *160*, 111587. [CrossRef] [PubMed]
36. Bhuyan, M.S.; Sharif, A.S.M.; Islam, M.S. Beach Pollution and Sustainable Tourism in the St. Martin's Island. *Glob. J. Environ. Res.* **2019**, *3*, 1–6. Available online: https://www.researchgate.net/publication/333998732_Beach_Pollution_and_Sustainable_Tourism_in_the_St_Martin\T1\textquoterights_Island (accessed on 16 June 2022).
37. Nor, N.H.M.; Obbard, J.P. Microplastics in Singapore's coastal mangrove ecosystems. *Mar. Pollut. Bull.* **2014**, *79*, 278–283. [CrossRef] [PubMed]
38. Yang, L.; Luo, W.; Zhao, P.; Zhang, Y.; Kang, S.; Giesy, J.P.; Zhang, F. Microplastics in the Koshi River, a remote alpine river crossing the Himalayas from China to Nepal. *Environ. Pollut.* **2021**, *290*, 118121. [CrossRef]
39. Hosseini, R.; Sayadi, M.H.; Aazami, J.; Savabieasfehani, M. Accumulation and distribution of microplastics in the sediment and coastal water samples of Chabahar Bay in the Oman Sea, Iran. *Mar. Pollut. Bull.* **2020**, *160*, 111682. [CrossRef]
40. Wu, F.; Wang, J.; Jiang, S.; Zeng, H.; Wu, Q.; Chen, Q.; Chen, J. Effect of cascade damming on microplastics transport in rivers: A large-scale investigation in Wujiang River, Southwest China. *Chemosphere* **2022**, *299*, 134455. [CrossRef] [PubMed]
41. Lebreton, L.-M.; Greer, S.; Borrero, J. Numerical modelling of floating debris in the world's oceans. *Mar. Pollut. Bull.* **2012**, *64*, 653–661. [CrossRef]
42. Environment and Social Development Association (ESDO). Microbeads! Unfold Health Risk and Environmental Pollutant. 2016. Available online: https://esdo.org/wp-content/uploads/Microbeads-final-report-10.11.16-1.pdf (accessed on 17 June 2022).
43. Browne, M.A.; Galloway, T.S.; Thompson, R.C. Spatial Patterns of Plastic Debris along Estuarine Shorelines. *Environ. Sci. Technol.* **2010**, *44*, 3404–3409. [CrossRef]
44. Thompson, R.C. Microplastics in the Marine Environment: Sources, Consequences and Solutions. In *Marine Anthropogenic Litter*; Springer: Berlin/Heidelberg, Germany, 2006; pp. 185–200.
45. Gazi, M.Y.; Mawsumi, T.J.; Ahmed, M.K. Detection of Coral Reefs Degradation using Geospatial Techniques around Saint Martin's Island, Bay of Bengal. *Ocean. Sci. J.* **2020**, *55*, 419–431. [CrossRef]
46. Meyers, R.A. Encyclopedia of Sustainability Science and Technology. 2020. Available online: https://link.springer.com/book/9781493927746 (accessed on 18 June 2022).
47. Afrin, S.; Rahman, M.; Hossain, N.; Uddin, K.; Malafaia, G. Are there plastic particles in my sugar? A pioneering study on the characterization of microplastics in commercial sugars and risk assessment. *Sci. Total. Environ.* **2022**, *837*, 155849. [CrossRef]
48. Afrin, S.; Rahman, M.; Akbor, A.; Siddique, A.B.; Uddin, K.; Malafaia, G. Is there tea complemented with the appealing flavor of microplastics? A pioneering study on plastic pollution in commercially available tea bags in Bangladesh. *Sci. Total. Environ.* **2022**, *837*, 155833. [CrossRef] [PubMed]
49. Rahman, M.S.; Khatun, S. Sugarcane and Sugar Industry in Bangladesh: An Overview. *Sugar Tech* **2016**, *18*, 627–635. Available online: https://link.springer.com/article/10.1007/s12355-016-0489-z (accessed on 19 June 2022). [CrossRef]
50. Saha, J.; Adnan, K.M.; Sarker, S.A.; Bunerjee, S. Analysis of growth trends in area, production and yield of tea in Bangladesh. *J. Agric. Food Res.* **2021**, *4*, 100136. [CrossRef]
51. Atlas Big, World Tea Production by Country. 2021. Available online: https://www.atlasbig.com/en-ie/countries-by-tea-production (accessed on 19 June 2022).
52. Helgi-Library. Sugar Consumption per Capita in Bangladesh. 2022. Available online: https://www.helgilibrary.com/indicators/sugar-consumption-per-capita/bangladesh/ (accessed on 19 June 2022).
53. Parvin, F.; Nath, J.; Hannan, T.; Tareq, S.M. Proliferation of microplastics in commercial sea salts from the world longest sea beach of Bangladesh. *Environ. Adv.* **2022**, *7*, 100173. [CrossRef]
54. Rakib, R.J.; Al Nahian, S.; Alfonso, M.B.; Khandaker, M.U.; Enyoh, C.E.; Hamid, F.S.; Alsubaie, A.; Almalki, A.S.A.; Bradley, D.A.; Mohafez, H.; et al. Microplastics pollution in salt pans from the Maheshkhali Channel, Bangladesh. *Sci. Rep.* **2021**, *11*, 23187. [CrossRef]
55. Uddin, R.; Hossain, M.; Akter, S.; Ali, M.E.; Ahsan, A. Assessment of some physicochemical parameters and determining the corrosive characteristics of the Karnaphuli estuarine water, Chittagong, Bangladesh. *Water Sci.* **2020**, *34*, 164–180. [CrossRef]
56. Hossain, M.S.; Islam, M.S.; Chowdhury, M.A.T. Shore Based Pollution Sources of the Karnafully River and the Effects of Oil-Grease on the Riverine Environment. *J. Geo-Environ.* **2005**, *5*, 55–66. Available online: https://www.researchgate.net/publication/235004579_Shore_Based_Pollution_Sources_of_the_Karnafully_River_and_the_Effects_of_Oil-Grease_on_the_Riverine_Environment (accessed on 19 June 2022).
57. Masum, M.M.H.; Riyad, S.I.; Riaz, R.R. Solid Waste Management in Chattogram City Corporation Area. *Solid Waste Manag.* 2018. Available online: https://www.researchgate.net/publication/329980487_SOLID_WASTE_MANAGEMENT_IN_CHATTOGRAM_CITY_CORPORATION_AREA (accessed on 19 June 2022).
58. Zaman, M.M.; Choudhury, S.R.; Ahmed, J.; Khandaker, R.K.; Rouf, M.A.; Malik, A. Salt Intake in an Adult Population of Bangladesh. *Glob. Heart* **2017**, *12*, 265–266. Available online: https://fp.brecorder.com/2016/10/2016101994395/ (accessed on 14 March 2022). [CrossRef]
59. Radhakrishnan, K.; Sivapriya, V.; Rajkumar, A.; Akramkhan, N.; Prakasheswar, P.; Krishnakumar, S.; Hussain, S. Characterization and distribution of microplastics in estuarine surface sediments, Kayamkulam estuary, southwest coast of India. *Mar. Pollut. Bull.* **2021**, *168*, 112389. [CrossRef]

60. Nithin, A.; Sundaramanickam, A.; Sathish, M. Seasonal distribution of microplastics in the surface water and sediments of the Vellar estuary, Parangipettai, southeast coast of India. *Mar. Pollut. Bull.* **2021**, *174*, 113248. [CrossRef] [PubMed]
61. Venkatramanan, S.; Chung, S.; Selvam, S.; Sivakumar, K.; Soundhariya, G.; Elzain, H.E.; Bhuyan, S. Characteristics of microplastics in the beach sediments of Marina tourist beach, Chennai, India. *Mar. Pollut. Bull.* **2022**, *176*, 113409. [CrossRef]
62. Yaranal, N.A.; Subbiah, S.; Mohanty, K. Distribution and characterization of microplastics in beach sediments from Karnataka (India) coastal environments. *Mar. Pollut. Bull.* **2021**, *169*, 112550. [CrossRef]
63. Patchaiyappan, A.; Ahmed, S.Z.; Dowarah, K.; Jayakumar, S.; Devipriya, S.P. Occurrence, distribution and composition of microplastics in the sediments of South Andaman beaches. *Mar. Pollut. Bull.* **2020**, *156*, 111227. [CrossRef]
64. Patchaiyappan, A.; ZakiAhmed, S.; Dowarah, K.; Khadanga, S.S.; Singh, T.; Jayakumar, S.; Thirunavukkarasu, C.; Devipriya, S.P. Prevalence of microplastics in the sediments of Odisha beaches, southeastern coast of India. *Mar. Pollut. Bull.* **2021**, *167*, 112265. [CrossRef]
65. Patchaiyappan, A.; Dowarah, K.; Ahmed, S.Z.; Prabakaran, M.; Jayakumar, S.; Thirunavukkarasu, C.; Devipriya, S.P. Prevalence and characteristics of microplastics present in the street dust collected from Chennai metropolitan city, India. *Chemosphere* **2020**, *269*, 128757. [CrossRef]
66. Thomas, E.M. *HVAC Principles and Applications Manual: Technology & Engineering*; McGraw-Hill Education: Berkshire, UK, 1998; p. 288.
67. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci. Adv.* **2017**, *3*, e1700782. [CrossRef] [PubMed]
68. Ranjani, M.; Veerasingam, S.; Venkatachalapathy, R.; Mugilarasan, M.; Bagaev, A.; Mukhanov, V.; Vethamony, P. Assessment of potential ecological risk of microplastics in the coastal sediments of India: A meta-analysis. *Mar. Pollut. Bull.* **2021**, *163*, 111969. [CrossRef] [PubMed]
69. Joshi, R.; Ahmed, S. Status and challenges of municipal solid waste management in India: A review. *Cogent Environ. Sci.* **2016**, *2*, 1139434. [CrossRef]
70. Khan, H.S.; Setu, S. Microplastic Ingestion by Fishes from Jamuna River, Bangladesh. *Environ. Nat. Resour. J.* **2022**, *20*, 1–11. [CrossRef]
71. Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K.L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347*, 768–771. [CrossRef]
72. Kumar, V.E.; Ravikumar, G.; Jeyasanta, K.I. Occurrence of microplastics in fishes from two landing sites in Tuticorin, South east coast of India. *Mar. Pollut. Bull.* **2018**, *135*, 889–894. [CrossRef]
73. Yadav, H.; Sethulekshmi, S.; Shriwastav, A. Estimation of microplastic exposure via the composite sampling of drinking water, respirable air, and cooked food from Mumbai, India. *Environ. Res.* **2022**, *214*, 113735. [CrossRef]
74. Sathish, M.N.; Jeyasanta, I.; Patterson, J. Microplastics in Salt of Tuticorin, Southeast Coast of India. *Arch. Environ. Contam. Toxicol.* **2020**, *79*, 111–121. [CrossRef]
75. Sathish, M.N.; Jeyasanta, I.; Patterson, J. Occurrence of microplastics in epipelagic and mesopelagic fishes from Tuticorin, Southeast coast of India. *Sci. Total. Environ.* **2020**, *720*, 137614. [CrossRef]
76. Seth, C.K.; Shriwastav, A. Contamination of Indian sea salts with microplastics and a potential prevention strategy. *Environ. Sci. Pollut. Res.* **2018**, *25*, 30122–30131. [CrossRef]
77. Vidyasakar, A.; Krishnakumar, S.; Kumar, K.S.; Neelavannan, K.; Anbalagan, S.; Kasilingam, K.; Srinivasalu, S.; Saravanan, P.; Kamaraj, S.; Magesh, N. Microplastic contamination in edible sea salt from the largest salt-producing states of India. *Mar. Pollut. Bull.* **2021**, *171*, 112728. [CrossRef] [PubMed]
78. Sivagami, M.; Selvambigai, M.; Devan, U.; Velangani, A.A.J.; Karmegam, N.; Biruntha, M.; Arun, A.; Kim, W.; Govarthanan, M.; Kumar, P. Extraction of microplastics from commonly used sea salts in India and their toxicological evaluation. *Chemosphere* **2020**, *263*, 128181. [CrossRef] [PubMed]
79. Bharath, M.; Natesan, U.; Ayyamperumal, R.; Kalam, N.; Muir, D.; Manikandabharath, K.R.; Srinivasalu, S. Microplastics as an emerging threat to the freshwater ecosystems of *Veeranam lake* in south India: A multidimensional approach. *Chemosphere* **2021**, *264*, 128502. [CrossRef] [PubMed]
80. The Indian Exspress. 'Microplastics in Tap Water': Goa Says Drinking Water Safe. 13 August 2021. Available online: https://indianexpress.com/article/india/microplastics-in-tap-water-goa-says-drinking-water-safe-7451311/ (accessed on 23 June 2022).
81. The Times of India. Microplastics Found in Goa's Tap Water, Reveals Study. 11 August 2021. Available online: https://timesofindia.indiatimes.com/city/goa/microplastics-found-in-goas-tap-water-reveals-study/articleshow/85249794.cms (accessed on 23 June 2022).
82. The Hindustan Times. Tap Water in Goa Contains Potentially Hazardous Microplastics: Study. 12 August 2021. Available online: https://www.hindustantimes.com/india-news/tap-water-in-goa-contains-potentially-hazardous-microplastics-study-101628743834050.html (accessed on 23 June 2022).
83. Joy, J.; Kanga, S.; Singh, S.K. Kerala Flood 2018: Flood Mapping by Participatory GIS Approach. *Meloor Panchayat* **2019**, *10*, 197–205. Available online: https://www.researchgate.net/publication/334508520_Kerala_Flood_2018_Flood_Mapping_by_Participatory_GIS_approach_Meloor_Panchayat (accessed on 23 June 2022).
84. Daniel, D.B.; Ashraf, P.M.; Thomas, S.N. Impact of 2018 Kerala flood on the abundance and distribution of microplastics in marine environment off Cochin, Southeastern Arabian Sea, India. *Reg. Stud. Mar. Sci.* **2022**, *53*, 102367. [CrossRef]

85. Cordova, M.R.; Ulumuddin, Y.I.; Purbonegoro, T.; Puspitasari, R.; Afianti, N.F.; Rositasari, R.; Yogaswara, D.; Hafizt, M.; Iswari, M.Y.; Fitriya, N.; et al. Seasonal heterogeneity and a link to precipitation in the release of microplastic during COVID-19 outbreak from the Greater Jakarta area to Jakarta Bay, Indonesia. *Mar. Pollut. Bull.* **2022**, *181*, 113926. [CrossRef]
86. Falahudin, D.; Cordova, M.R.; Sun, X.; Yogaswara, D.; Wulandari, I.; Hindarti, D.; Arifin, Z. The first occurrence, spatial distribution and characteristics of microplastic particles in sediments from Banten Bay, Indonesia. *Sci. Total. Environ.* **2019**, *705*, 135304. [CrossRef]
87. Cordova, M.R.; Purwiyanto, A.I.S.; Suteja, Y. Abundance and characteristics of microplastics in the northern coastal waters of Surabaya, Indonesia. *Mar. Pollut. Bull.* **2019**, *142*, 183–188. [CrossRef]
88. Lestari, P.; Trihadiningrum, Y.; Wijaya, B.A.; Yunus, K.A.; Firdaus, M. Distribution of microplastics in Surabaya River, Indonesia. *Sci. Total. Environ.* **2020**, *726*, 138560. [CrossRef]
89. Natsir, M.F.; Selomo, M.; Ibrahim, E.; Arsin, A.A.; Alni, N.C. Analysis on microplastics in dug wells around Tamangapa Landfills, Makassar City, Indonesia. *Gac. Sanit.* **2021**, *35*, S87–S89. [CrossRef]
90. Cordova, M.R.; Ulumuddin, Y.I.; Purbonegoro, T.; Shiomoto, A. Characterization of microplastics in mangrove sediment of Muara Angke Wildlife Reserve, Indonesia. *Mar. Pollut. Bull.* **2021**, *163*, 112012. [CrossRef] [PubMed]
91. Firdaus, M.; Trihadiningrum, Y.; Lestari, P. Microplastic pollution in the sediment of Jagir Estuary, Surabaya City, Indonesia. *Mar. Pollut. Bull.* **2020**, *150*, 110790. [CrossRef] [PubMed]
92. Azizi, A.; Setyowati, W.N.; Fairus, S.; A Puspito, D.; Irawan, D.S. Microplastic pollution in the sediment of Jakarta Bay, Indonesia. *IOP Conf. Series Earth Environ. Sci.* **2021**, *930*, 012010. [CrossRef]
93. Suteja, Y.; Atmadipoera, A.S.; Riani, E.; Nurjaya, I.W.; Nugroho, D.; Cordova, M.R. Spatial and temporal distribution of microplastic in surface water of tropical estuary: Case study in Benoa Bay, Bali, Indonesia. *Mar. Pollut. Bull.* **2021**, *163*, 111979. [CrossRef]
94. Cordova, M.R.; Nurhati, I.S. Major sources and monthly variations in the release of land-derived marine debris from the Greater Jakarta area, Indonesia. *Sci. Rep.* **2019**, *9*, 18730. [CrossRef]
95. Syakti, A.D.; Bouhroum, R.; Hidayati, N.V.; Koenawan, C.J.; Boulkamh, A.; Sulistyo, I.; Lebarillier, S.; Akhlus, S.; Doumenq, P.; Wong-Wah-Chung, P. Beach macro-litter monitoring and floating microplastic in a coastal area of Indonesia. *Mar. Pollut. Bull.* **2017**, *122*, 217–225. [CrossRef]
96. Statista Research Department. *Global Waste Generation: Statistics and Fact*; Statista Research Department: New York, NY, USA, 2021; Volume 2013–2019.
97. Mani, T.; Hauk, A.; Walter, U.; Burkhardt-Holm, P. Microplastics profile along the Rhine River. *Sci. Rep.* **2016**, *5*, 17988. Available online: https://www.nature.com/articles/srep17988 (accessed on 22 June 2022). [CrossRef]
98. Trihadiningrum, Y.; Laksono, I.J.; Dhokhikah, Y.; Moesriati, A.; Radita, D.R.; Sunaryo, S. Community activities in residential solid waste reduction in Tenggilis Mejoyo District, Surabaya City, Indonesia. *J. Mater. Cycles Waste Manag.* **2015**, *19*, 526–535. [CrossRef]
99. World Bank. *The World Bank Annual Repoart 2018 (English)*; World Bank Group: Washington, DC, USA, 2018; Available online: http://document.worldbank.org/curated/en/630671538158537 24/The-World-Bank-Annual-Report-2018 (accessed on 22 June 2022).
100. Radityaningrum, A.D.; Trihadiningrum, Y.; Mar'Atusholihah; Soedjono, E.S.; Herumurti, W. Microplastic contamination in water supply and the removal efficiencies of the treatment plants: A case of Surabaya City, Indonesia. *J. Water Process. Eng.* **2021**, *43*, 102195. [CrossRef]
101. Wibowo, A.T.; Nugrahapraja, H.; Wahyuono, R.A.; Islami, I.; Haekal, M.H.; Fardiansyah, Y.; Sugiyo, P.W.W.; Putro, Y.K.; Fauzia, F.N.; Santoso, H.; et al. Microplastic Contamination in the Human Gastrointestinal Tract and Daily Consumables Associated with an Indonesian Farming Community. *Sustainability* **2021**, *13*, 12840. [CrossRef]
102. Nguyen, Q.A.T.; Nguyen, H.N.Y.; Strady, E.; Nguyen, Q.T.; Trinh-Dang, M.; Vo, V.M. Characteristics of microplastics in shoreline sediments from a tropical and urbanized beach (Da Nang, Vietnam). *Mar. Pollut. Bull.* **2020**, *161*, 111768. [CrossRef] [PubMed]
103. Hien, T.T.; Nhon, N.T.T.; Thu, V.T.M.; Quyen, D.T.T.; Nguyen, N.T. The Distribution of Microplastics in Beach Sand in Tien Giang Province and Vung Tau City, Vietnam. *J. Eng. Technol. Sci.* **2020**, *52*, 208–221. [CrossRef]
104. Nhon, N.T.T.; Nguyen, N.T.; Hai, H.T.N.; Hien, T.T. Physical and Chemical Characteristics of Microplastic in Beach Sand in Can Gio, Ho Chi Minh City, Vietnam. *Res. Square* **2021**, *in press*. [CrossRef]
105. Dung, L.V.; Duc, T.H.; Linh, L.T.K.; Ly, T.T.D.; Duong, H.A.; Hao, N.T.M. Depth Profiles of Microplastics in Sediment Cores from Two Mangrove Forests in Northern Vietnam. *J. Mar. Sci. Eng.* **2021**, *9*, 1381. [CrossRef]
106. Lahens, L.; Strady, E.; Kieu-Le, T.-C.; Dris, R.; Boukerma, K.; Rinnert, E.; Gasperi, J.; Tassin, B. Macroplastic and microplastic contamination assessment of a tropical river (Saigon River, Vietnam) transversed by a developing megacity. *Environ. Pollut.* **2018**, *236*, 661–671. [CrossRef] [PubMed]
107. Le, D.N.; Hoang, T.T.H.; Duong, T.T.; Lu, X.; Pham, T.M.H.; Phung, T.X.B.; Le, T.M.H.; Nguyen, T.D. First observation of microplastics in surface sediment of some aquaculture ponds in Hanoi city, Vietnam. *J. Hazard. Mater. Adv.* **2022**, *6*, 100061. [CrossRef]
108. Yukioka, S.; Tanaka, S.; Nabetani, Y.; Suzuki, Y.; Ushijima, T.; Fujii, S.; Takada, H.; Van Tran, Q.; Singh, S. Occurrence and characteristics of microplastics in surface road dust in Kusatsu (Japan), Da Nang (Vietnam), and Kathmandu (Nepal). *Environ. Pollut.* **2019**, *256*, 113447. [CrossRef]
109. Nguyen, Q.A.T.; Vu, T.B.H.; Nguyen, Q.T.; Nguyen, H.N.Y.; Le, T.M.; Vo, V.M.; Trinh-Dang, M. Urban drainage channels as microplastics pollution hotspots in developing areas: A case study in Da Nang, Vietnam. *Mar. Pollut. Bull.* **2022**, *175*, 113323. [CrossRef]

110. Hoang, A.T.; Pham, X.D. An investigation of remediation and recovery of oil spill and toxic heavy metal from maritime pollution by new absorbent material. *J. Mar. Eng. Technol.* **2018**, *20*, 159–169. [CrossRef]
111. Hai, D.M. Current situation and solutions for the environmental pollution in vietnam. *J. Mech. Eng. Res. Dev.* **2019**, *42*, 06–10. [CrossRef]
112. Ministry of Natural Resources and Environement (MONRE). Plastic Waste Management in Vietnam. 2019. Available online: https://www.env.go.jp/en/recycle/asian_net/Annual_Workshops/2019_PDF/Session1/S1_12_Vietnam_ANWS2019.pdf (accessed on 11 November 2022).
113. Ministry of Natural Resources and Environement (MONRE). National Environment Report 2019 pdf. 2019. Available online: https://www.vd-office.org/en/national-state-of-environment-report-2019-on-solid-waste-released/ (accessed on 11 November 2022).
114. Ministry of Natural Resources and Environement (MONRE). Ocean Plastic Waste Records pdf. 2020. Available online: https://www.oecd.org/ocean/topics/ocean-pollution/marine-plastics-pollution-Malaysia.pdf (accessed on 11 November 2022).
115. Khuyen, V.T.K.; Le, D.V.; Anh, L.H.; Fischer, A.R.; Dornack, C. Investigation of Microplastic Contamination in Vietnamese Sea Salts Based on Raman and Fourier-Transform Infrared Spectroscopies. *Environ. Asia* **2021**, *14*, 1. [CrossRef]
116. Jensen, P.N.; Bao, T.Q.; Huong, T.T.T.; Heckbert, S.R.; Fitzpatrick, A.L.; LoGerfo, J.P.; Le Van Ngoc, T.; Mokdad, A.H. The association of estimated salt intake with blood pressure in a Viet Nam national survey. *PLoS ONE* **2018**, *13*, e0191437. [CrossRef] [PubMed]
117. Ha, D.T. Microplastic contamination in commercial sea salt of Vietnam. *Vietnam. J. Sci. Technol.* **2021**, *59*, 333–344. [CrossRef]
118. World Health Organization. 2022. Available online: https://www.who.int/ (accessed on 15 August 2021).
119. Thinh, T.Q.; Sang, T.T.N.; Viet, T.Q.; Tam, L.T.M.; Dan, N.P.; Strady, E.; Chung, K.L.T.; Alpes, C.U.G. Preliminary assessment on the microplastic contamination in the atmospheric fallout in the Phuoc Hiep landfill, Cu Chi, Ho Chi Minh city. *Vietnam. J. Sci. Technol. Eng.* **2020**, *62*, 83–89. [CrossRef]
120. Gallagher, A.; Rees, A.; Rowe, R.; Stevens, J.; Wright, P. Microplastics in the Solent estuarine complex, UK: An initial assessment. *Mar. Pollut. Bull.* **2015**, *102*, 243–249. [CrossRef]
121. Mehdinia, A.; Dehbandi, R.; Hamzehpour, A.; Rahnama, R. Identification of microplastics in the sediments of southern coasts of the Caspian Sea, north of Iran. *Environ. Pollut.* **2020**, *258*, 113738. [CrossRef]
122. Vayghan, A.H.; Rasta, M.; Zakeri, M.; Kelly, F.J. Spatial distribution of microplastics pollution in sediments and surface waters of the Aras River and reservoir: An international river in Northwestern Iran. *Sci. Total. Environ.* **2022**, *843*, 156894. [CrossRef] [PubMed]
123. Gholizadeh, M.; Cera, A. Microplastic contamination in the sediments of Qarasu estuary in Gorgan Bay, south-east of Caspian Sea, Iran. *Sci. Total. Environ.* **2022**, *838*, 155913. [CrossRef]
124. Abbasi, S. Prevalence and physicochemical characteristics of microplastics in the sediment and water of Hashilan Wetland, a national heritage in NW Iran. *Environ. Technol. Innov.* **2021**, *23*, 101782. [CrossRef]
125. Nabizadeh, R.; Sajadi, M.; Rastkari, N.; Yaghmaeian, K. Microplastic pollution on the Persian Gulf shoreline: A case study of Bandar Abbas city, Hormozgan Province, Iran. *Mar. Pollut. Bull.* **2019**, *145*, 536–546. [CrossRef]
126. Abbasi, S.; Turner, A.; Sharifi, R.; Nematollahi, M.; Keshavarzifard, M.; Moghtaderi, T. Microplastics in the school classrooms of Shiraz, Iran. *Build. Environ.* **2021**, *207*, 108562. [CrossRef]
127. United Nations Environment Program (UNEP). Marine Litter: A Global Challenge. 2009. Available online: https://www.unep.org/resources/report/marine-litter-global-challenge-0 (accessed on 25 July 2022).
128. Li, M.; Zhang, X.; Yi, K.; He, L.; Han, P.; Tong, M. Transport and deposition of microplastic particles in saturated porous media: Co-effects of clay particles and natural organic matter. *Environ. Pollut.* **2021**, *287*, 117585. [CrossRef]
129. Maghsodian, Z.; Sanati, A.M.; Ramavandi, B.; Ghasemi, A.; Sorial, G.A. Microplastics accumulation in sediments and Periophthalmus waltoni fish, mangrove forests in southern Iran. *Chemosphere* **2020**, *264*, 128543. [CrossRef] [PubMed]
130. Abbasi, S.; Rezaei, M.; Ahmadi, F.; Turner, A. Atmospheric transport of microplastics during a dust storm. *Chemosphere* **2022**, *292*, 133456. [CrossRef] [PubMed]
131. Abbasi, S.; Keshavarzi, B.; Moore, F.; Turner, A.; Kelly, F.J.; Dominguez, A.O.; Jaafarzadeh, N. Distribution and potential health impacts of microplastics and microrubbers in air and street dusts from Asaluyeh County, Iran. *Environ. Pollut.* **2018**, *244*, 153–164. [CrossRef]
132. Naji, A.; Azadkhah, S.; Farahani, H.; Uddin, S.; Khan, F.R. Microplastics in wastewater outlets of Bandar Abbas city (Iran): A potential point source of microplastics into the Persian Gulf. *Chemosphere* **2020**, *262*, 128039. [CrossRef]
133. Demirbas, A.; Edris, G.; Alalayah, W.M. Sludge production from municipal wastewater treatment in sewage treatment plant. *Energy Sources Part A Recovery Util. Environ. Eff.* **2017**, *39*, 999–1006. [CrossRef]
134. Petroody, S.S.A.; Hashemi, S.H.; van Gestel, C.A. Transport and accumulation of microplastics through wastewater treatment sludge processes. *Chemosphere* **2021**, *278*, 130471. [CrossRef]
135. Amiri, H.; Hoseini, M.; Abbasi, S.; Malakootian, M.; Hashemi, M.; Jaafarzadeh, N.; Turner, A. Geophagy and microplastic ingestion. *J. Food Compos. Anal.* **2021**, *106*, 104290. [CrossRef]
136. Goudie, A.S. Dust storms: Recent developments. *J. Environ. Manag.* **2009**, *90*, 89–94. [CrossRef] [PubMed]
137. Abbasi, S.; Alirezazadeh, M.; Razeghi, N.; Rezaei, M.; Pourmahmood, H.; Dehbandi, R.; Mehr, M.R.; Ashayeri, S.Y.; Oleszczuk, P.; Turner, A. Microplastics captured by snowfall: A study in Northern Iran. *Sci. Total. Environ.* **2022**, *822*, 153451. [CrossRef] [PubMed]

138. Abbasi, S.; Turner, A. Human exposure to microplastics: A study in Iran. *J. Hazard. Mater.* **2020**, *403*, 123799. [CrossRef]
139. Chen, L.; Zhi, X.; Shen, Z.; Dai, Y.; Aini, G. Comparison between snowmelt-runoff and rainfall-runoff nonpoint source pollution in a typical urban catchment in Beijing, China. *Environ. Sci. Pollut. Res.* **2017**, *25*, 2377–2388. [CrossRef]
140. Butte, W.; Heinzow, B. Pollutants in house dust as indicators of indoor contamination. *Rev. Environ. Contam. Toxicol.* **2002**, *175*, 1–46. Available online: https://pubmed.ncbi.nlm.nih.gov/12206053/ (accessed on 11 November 2022). [PubMed]
141. Turner, A.; Simmonds, L. Elemental concentrations and metal bioaccessibility in UK household dust. *Sci. Total. Environ.* **2006**, *371*, 74–81. [CrossRef]
142. Abbasi, S.; Turner, A. Dry and wet deposition of microplastics in a semi-arid region (Shiraz, Iran). *Sci. Total. Environ.* **2021**, *786*, 147358. [CrossRef]
143. Kashfi, F.S.; Ramavandi, B.; Arfaeinia, H.; Mohammadi, A.; Saeedi, R.; De-La-Torre, G.E.; Dobaradaran, S. Occurrence and exposure assessment of microplastics in indoor dusts of buildings with different applications in Bushehr and Shiraz cities, Iran. *Sci. Total. Environ.* **2022**, *829*, 154651. [CrossRef] [PubMed]
144. Prarat, P.; Hongsawat, P. Microplastic pollution in surface seawater and beach sand from the shore of Rayong province, Thailand: Distribution, characterization, and ecological risk assessment. *Mar. Pollut. Bull.* **2021**, *174*, 113200. [CrossRef]
145. Akkajit, P.; Tipmanee, D.; Cherdsukjai, P.; Suteerasak, T.; Thongnonghin, S. Occurrence and distribution of microplastics in beach sediments along Phuket coastline. *Mar. Pollut. Bull.* **2021**, *169*, 112496. [CrossRef] [PubMed]
146. Ta, A.T.; Babel, S. Microplastics pollution with heavy metals in the aquaculture zone of the Chao Phraya River Estuary, Thailand. *Mar. Pollut. Bull.* **2020**, *161*, 111747. [CrossRef]
147. Vibhatabandhu, P.; Srithongouthai, S. Influence of seasonal variations on the distribution characteristics of microplastics in the surface water of the Inner Gulf of Thailand. *Mar. Pollut. Bull.* **2022**, *180*, 113747. [CrossRef] [PubMed]
148. Wichaiutcha, N.; Chavalparit, O. 3Rs Policy and Plastic Waste Management in Thailand. *J. Mater. Cycles Waste Manag.* **2019**, *21*, 10–22. Available online: https://www.researchgate.net/publication/327229173_3Rs_Policy_and_plastic_waste_management_in_Thailand (accessed on 25 July 2022). [CrossRef]
149. Ruangpanupan, N.; Ussawarujikulchai, A.; Prapagdee, B.; Chavanich, S. Microplastics in the surface seawater of Bandon Bay, Gulf of Thailand. *Mar. Pollut. Bull.* **2022**, *179*, 113664. [CrossRef]
150. Chinfak, N.; Sompongchaiyakul, P.; Charoenpong, C.; Shi, H.; Yeemin, T.; Zhang, J. Abundance, composition, and fate of microplastics in water, sediment, and shellfish in the Tapi-Phumduang River system and Bandon Bay, Thailand. *Sci. Total. Environ.* **2021**, *781*, 146700. [CrossRef]
151. Ratchatapattanakul, N.; Kazuya, W.; Yuki, O.; Yasuyuki, K. Living under the State and Storms: The History of Blood Cockle Aquaculture in Bandon Bay, Thailand. *South Asian Stud.* **2017**, *6*, 3–30. [CrossRef]
152. Jiwarungrueangkul, T.; Phaksopa, J.; Sompongchaiyakul, P.; Tipmanee, D. Seasonal microplastic variations in estuarine sediments from urban canal on the west coast of Thailand: A case study in Phuket province. *Mar. Pollut. Bull.* **2021**, *168*, 112452. [CrossRef]
153. Khanunthong, A. Industry Outlook 2021–2023: Plastics. 2021. Available online: https://www.krungsri.com/en/research/industry/industry-outlook/Petrochemicals/Plastics/IO/io-plastics-21 (accessed on 28 July 2022).
154. Marks, D.; Miller, M.A.; Vassanadumrongdee, S. The geopolitical economy of Thailand's marine plastic pollution crisis. *Asia Pac. Viewp.* **2020**, *61*, 266–282. [CrossRef]
155. Yan, M.; Nie, H.; Xu, K.; He, Y.; Hu, Y.; Huang, Y.; Wang, J. Microplastic abundance, distribution and composition in the Pearl River along Guangzhou city and Pearl River estuary, China. *Chemosphere* **2018**, *217*, 879–886. [CrossRef]
156. Department of Marine and Coastal Resources, Thailand (DMCR). The Information of Marine and Coastal Resources of Suratthanal Province, Ministry of Natural Resources and Environment Bangkok. 2018. Available online: https://www.iyor2018.org/organization/department-marine-coastal-resources-thailand/ (accessed on 30 July 2022).
157. Regional Environment Office 15 (REO 15). Report of the Results of the Monitoring and Evaluation of the Environmental Quality Management Plan Region 15 during 2007–2011. 2012. Available online: https://enviro2.doe.gov.my/ekmc/wp-content/uploads/2017/02/FA-EIA-GUIDELINE-IN-MALAYSIA-1.pdf (accessed on 11 November 2022).
158. Liebmann, B.; Koppel, S.; Konigshofer, P.; Bucsics, T.; Reiberger, T.; Schwabl, P. Assessment of Microplastic Concentrations in Human Stool-Final Results of a Prospective Study. 2018. Available online: https://www.researchgate.net/publication/328702413_ASSESSMENT_OF_MICROPLASTIC_CONCENTRATIONS_IN_HUMAN_STOOL_-_FINAL_RESULTS_OF_A_PROSPECTIVE_STUDY (accessed on 30 July 2022).
159. Sukrita, P.P.; Prasongsom, P.P. Microplastics: Origin, Environmental impact, Food and Beverage contamination and Management methods. *Sci. Technol.* **2020**, *28*, 72–80. [CrossRef]
160. Sutthacheep, M.; Phoaduang, S.; Chamchoy, C.; Klinthong, W.; Salakphet, C.; Silpparasarn, A.; Sangiamdee, D.; Yeemin, T. The particles of microplastics in shrimp paste from the Gulf of Thailand and the Andaman Sea. *Sci. Technol.* **2021**, *4*, 27–34.
161. Tadsuwan, K.; Babel, S. Microplastic abundance and removal via an ultrafiltration system coupled to a conventional municipal wastewater treatment plant in Thailand. *J. Environ. Chem. Eng.* **2022**, *10*, 107142. [CrossRef]
162. Bucol, L.A.; Romano, E.F.; Cabcaban, S.M.; Siplon, L.M.D.; Madrid, G.C.; Bucol, A.A.; Polidoro, B. Microplastics in marine sediments and rabbitfish (*Siganus fuscescens*) from selected coastal areas of Negros Oriental, Philippines. *Mar. Pollut. Bull.* **2019**, *150*, 110685. [CrossRef] [PubMed]

163. Esquinas, G.G.M.S.; Mantala, A.P.; Atilano, M.G.; Apugan, R.P.; Galarpe, V.R.K.R. Physical characterization of litter and microplastic along the urban coast of Cagayan de Oro in Macajalar Bay, Philippines. *Mar. Pollut. Bull.* **2020**, *154*, 111083. [CrossRef]
164. Osorio, E.D.; Tanchuling, M.A.N.; Diola, M.B.L.D. Microplastics Occurrence in Surface Waters and Sediments in Five River Mouths of Manila Bay. *Front. Environ. Sci.* **2021**, *9*, 719274. [CrossRef]
165. Tanchuling, M.A.N.; Osorio, E.D. The Microplastics in Metro Manila Rivers: Characteristics, Sources, and Abatement. In *Plastics in the Aquatic Environment-Part I: Current Status and Challenges*; Springer International Publishing: Cham, Switzerland, 2020; Volume 111, pp. 405–426. [CrossRef]
166. Limbago, J.S.; Bacabac, M.M.A.; Fajardo, D.R.M.; Mueda, C.R.T.; Bitara, A.U.; Ceguerra, K.L.P.; Lopez, M.R.C.; Posa, G.A.V.; Nacorda, H.M.E. Occurrence and Polymer Types of Microplastics from Surface Sediments of Molawin Watershed of the Makiling Forest Reserve, Los Baños, Laguna, Philippines. *Environ. Nat. Resour. J.* **2020**, *19*, 57–67. [CrossRef]
167. Castro, A.L.J.; Monsada, A.M.; Cruz, K.D. Occurrence of microplastics in the sediments of Baseco Port area at Manila Bay, Philippines. *IOP Conf. Series Earth Environ. Sci.* **2021**, *958*, 012009. [CrossRef]
168. Mercado, R.M. People's Risk Perceptions and Responses to Climate Change and Natural Disasters in BASECO Compound, Manila, Philippines. *Procedia Environ. Sci.* **2016**, *34*, 490–505. [CrossRef]
169. Cutini, V.; Di Pinto, V.; Rinaldi, A.M.; Rossini, F. Proximal Cities: Does Walkability Drive Informal Settlements? *Sustainability* **2020**, *12*, 756. [CrossRef]
170. Department of Environment and Natural Resources, (DENR) Baseco Cleanup Yileds 550 Sacks of Garbage. 2019. Available online: https://denr.gov.ph/index.php/news-events/press-releases/857-basecocleanup-yields (accessed on 30 July 2022).
171. Parvin, F.; Jannat, S.; Tareq, S.M. Abundance, characteristics and variation of microplastics in different freshwater fish species from Bangladesh. *Sci. Total. Environ.* **2021**, *784*, 147137. [CrossRef]
172. Ghosh, G.C.; Akter, S.M.; Islam, R.M.; Habib, A.; Chakraborty, T.K.; Zaman, S.; Kabir, A.E.; Shipin, O.V.; Wahid, M.A. Microplastics contamination in commercial marine fish from the Bay of Bengal. *Reg. Stud. Mar. Sci.* **2021**, *44*, 101728. [CrossRef]
173. Siddique, M.A.M.; Uddin, A.; Rahman, S.M.A.; Rahman, M.; Islam, S.; Kibria, G. Microplastics in an anadromous national fish, Hilsa shad Tenualosa ilisha from the Bay of Bengal, Bangladesh. *Mar. Pollut. Bull.* **2021**, *174*, 113236. [CrossRef]
174. Pauly, D. Tropical fishes: Patterns and propensities. *J. Fish Biol.* **1998**, *53*, 1–17.
175. Hasan, J.; Islam, S.M.; Alam, S.; Johnson, D.; Belton, B.; Hossain, M.A.R. Shahjahan Presence of microplastics in two common dried marine fish species from Bangladesh. *Mar. Pollut. Bull.* **2022**, *176*, 113430. [CrossRef] [PubMed]
176. Jung, M.; Reichstein, M.; Ciais, P.; Seneviratne, S.I.; Sheffield, J.; Goulden, M.L.; Bonan, G.; Cescatti, A.; Chen, J.Q.; de Jeu, R.; et al. Recent decline in the global land evapotranspiration trend due to limited moisture supply. *Nature* **2010**, *467*, 951–954. [CrossRef]
177. Schroter, D.; Cramer, W.; Leemans, R.; Prentice, I.C.; Araujo, M.B.; Arnell, N.W.; Bondeau, A.; Bugmann, H.; Carter, T.R.; Gracia, C.A.; et al. Ecosystem service supply and vulnerability to global change in Europe. *Science* **2005**, *310*, 1333–1337. [CrossRef]
178. Afrin, S.; Uddin, K.; Rahman, M. Microplastics contamination in the soil from Urban Landfill site, Dhaka, Bangladesh. *Heliyon* **2020**, *6*, e05572. [CrossRef] [PubMed]
179. Lwanga, E.H.; Vega, J.M.; Quej, V.K.; Chi, J.D.L.A.; del Cid, L.S.; Chi, C.; Segura, G.E.; Gertsen, H.; Salánki, T.; van der Ploeg, M.; et al. Field evidence for transfer of plastic debris along a terrestrial food chain. *Sci. Rep.* **2017**, *7*, 1–7. [CrossRef]
180. Jeyasanta, K.I.; Sathish, N.; Patterson, J.; Edward, J.P. Macro-, meso- and microplastic debris in the beaches of Tuticorin district, Southeast coast of India. *Mar. Pollut. Bull.* **2020**, *154*, 111055. [CrossRef]
181. Daniel, D.B.; Ashraf, P.M.; Thomas, S.N.; Thomson, K. Microplastics in the edible tissues of shellfishes sold for human consumption. *Chemosphere* **2020**, *264*, 128554. [CrossRef]
182. Naidu, B.C.; Xavier, K.; Shukla, S.P.; Jaiswar, A.K.; Nayak, B.B. Comparative study on the microplastics abundance, characteristics, and possible sources in yellow clams of different demographic regions of the northwest coast of India. *J. Hazard. Mater. Lett.* **2022**, *3*, 100051. [CrossRef]
183. Sarkar, A.; Deb, S.; Ghosh, S.; Mandal, S.; Quazi, S.A.; Kushwaha, A.; Hoque, A.; Choudhury, A. Impact of anthropogenic pollution on soil properties in and around a town in Eastern India. *Geoderma Reg.* **2021**, *28*, e00462. [CrossRef]
184. Rautenberg, G.E.; Ame, M.V.; Monferran, M.V.; Bonansea, R.I.; Hued, A.C. A Multi-Level Approach Using Gambusia Affinis as a Bioindicator of Environmental Pollution in the Middle-Lower Basin of SuquÃ—A River. *Ecol. Indic.* **2015**, *48*, 706–720. [CrossRef]
185. Buwono, N.R.; Risjani, Y.; Soegianto, A. Contamination of microplastics in Brantas River, East Java, Indonesia and its distribution in gills and digestive tracts of fish Gambusia affinis. *Emerg. Contam.* **2021**, *7*, 172–178. [CrossRef]
186. Buwono, N.R.; Risjani, Y.; Soegianto, A. Distribution of microplastic in relation to water quality parameters in the Brantas River, East Java, Indonesia. *Environ. Technol. Innov.* **2021**, *24*, 101915. [CrossRef]
187. Lehtiniemi, M.; Hartikainen, S.; Näkki, P.; Engström-Öst, J.; Koistinen, A.; Setälä, O. Size matters more than shape: Ingestion of primary and secondary microplastics by small predators. *Food Webs* **2018**, *17*, e00097. [CrossRef]
188. McCauley, R.W. Determining the Health of Fish Communities—Parallels with Human Medicine. *J. Great Lakes Res.* **1990**, *16*, 635–636. [CrossRef]
189. Adam, M.A.; Kilawati, Y.; Risjani, Y.; Maftuch, M. Clinical Symptoms of Gambusia Fish (Gambusia Affinis) after Exsposure to Cadmium Absorbed in the Gills. Pollution Research. 2019. Available online: https://www.researchgate.net/publication/352800241_CLINICAL_SYMPTOMS_OF_GAMBUSIA_FISH_GAMBUSIA_AFFINIS_AFTER_EXPOSURE_TO_CADMIUM_ABSORBED_IN_THE_GILLS (accessed on 20 July 2022).

190. Jabeen, K.; Li, B.; Chen, Q.; Su, L.; Wu, C.; Hollert, H.; Shi, H. Effects of virgin microplastics on goldfish (Carassius auratus). *Chemosphere* **2018**, *213*, 323–332. [CrossRef]
191. Movahedinia, A.; Abtahi, B.; Bahmani, M. Gill histopathological lesions of the sturgeons. *Asian J. Anim. Vet. Adv.* **2012**, *7*, 710–717. [CrossRef]
192. Buwono, N.R.; Risjani, Y.; Soegianto, A. Oxidative stress responses of microplastic-contaminated *Gambusia affinis* obtained from the Brantas River in East Java, Indonesia. *Chemosphere* **2022**, *293*, 133543. [CrossRef]
193. Sawalman, R.; Werorilangi, S.; Ukkas, M.; Mashoreng, S.; Yasir, I.; Tahir, A. Microplastic abundance in sea urchins (*Diadema setosum*) from seagrass beds of Barranglompo Island, Makassar, Indonesia. *IOP Conf. Series Earth Environ. Sci.* **2021**, *763*, 012057. [CrossRef]
194. Archana, A.; Babu, K.R. Nutrient composition and antioxidant activity of gonads of sea urchin *Stomopneustes variolaris*. *Food Chem.* **2016**, *197*, 597–602. [CrossRef] [PubMed]
195. Andreas; Hadibarata, T.; Sathishkumar, P.; Prasetia, H.; Hikmat; Pusfitasari, E.D.; Tasfiyati, A.N.; Muzdalifah, D.; Waluyo, J.; Randy, A.; et al. Microplastic contamination in the Skipjack Tuna (*Euthynnus affinis*) collected from Southern Coast of Java, Indonesia. *Chemosphere* **2021**, *276*, 130185. [CrossRef] [PubMed]
196. Bulleri, F.; Ravaglioli, C.; Anselmi, S.; Ranzi, M. The sea cucumber Holothuria tubulosa does not reduce the size of microplastics but enhances their resuspension in the water column. *Sci. Total Environ.* **2021**, *781*, 146650. [CrossRef]
197. Riani, E.; Cordova, M.R. Microplastic ingestion by the sandfish Holothuria scabra in Lampung and Sumbawa, Indonesia. *Mar. Pollut. Bull.* **2021**, *175*, 113134. [CrossRef] [PubMed]
198. Cordova, M.R.; Riani, E.; Shiomoto, A. Microplastics ingestion by blue panchax fish (*Aplocheilus* sp.) from Ciliwung Estuary, Jakarta, Indonesia. *Mar. Pollut. Bull.* **2020**, *161*, 111763. [CrossRef] [PubMed]
199. Risjani, Y.; Yunianta; Couteau, J.; Minier, C. Cellular immune responses and phagocytic activity of fishes exposed to pollution of volcano mud. *Mar. Environ. Res.* **2014**, *96*, 73–80. [CrossRef] [PubMed]
200. Delzer, G.C.; Mckenzie, S.W. Five Day Biochemical Oxygen Demand. 2003. Available online: https://pubs.usgs.gov/twri/twri9a7/twri9a7_nfmchap7_2_bod.pdf (accessed on 6 August 2022).
201. Supenah, P.; Widiastuti, E.; Priyono, R.E. Study of the quality of condong river water affected by the trusmi cirebon batik industry wastewater. *Biosfera* **2015**, *32*, 110. [CrossRef]
202. Widodo, T.; Budiastuti, M.T.S.; Komariah, K. Water Quality and Pollution Index in Grenjeng River, Boyolali Regency, Indonesia. *Caraka Tani J. Sustain. Agric.* **2019**, *34*, 150. [CrossRef]
203. Hue, H.; Dong, L.; Hien, T.; Nguyen, T.; Pradit, S. Assessment of microplastics contamination in commericial clams in the coastal zone of vietnam. *Appl. Ecol. Environ. Res.* **2021**, *19*, 4977–4991. [CrossRef]
204. Do, V.M.; Dang, T.T.; Le, X.T.T.; Nguyen, D.T.; Phung, T.V.; Vu, D.N.; Pham, H.V. Abundance of microplastics in cultured oysters (*Crassostrea gigas*) from Danang Bay of Vietnam. *Mar. Pollut. Bull.* **2022**, *180*, 113800. [CrossRef]
205. Li, Y.; Chen, G.; Xu, K.; Huang, K.; Wang, J. Microplastics Environmental Effect and Risk Assessment on the Aquaculture Systems from South China. *Int. J. Environ. Res. Public Health* **2021**, *18*, 1869. [CrossRef] [PubMed]
206. Nam, P.N.; Tuan, P.Q.; Thuy, D.T.; Quynh, L.T.P.; Amiard, F. Contamination of microplastic in bivalve: First evaluation in Vietnam. *J. Sci. Earth* **2019**, *41*, 252–258. [CrossRef]
207. Rasta, M.; Sattari, M.; Taleshi, M.S.; Namin, J.I. Microplastics in different tissues of some commercially important fish species from Anzali Wetland in the Southwest Caspian Sea, Northern Iran. *Mar. Pollut. Bull.* **2021**, *169*, 112479. [CrossRef] [PubMed]
208. Rasta, M.; Sattari, M.; Taleshi, M.S.; Namin, J.I. Identification and distribution of microplastics in the sediments and surface waters of Anzali Wetland in the Southwest Caspian Sea, Northern Iran. *Mar. Pollut. Bull.* **2020**, *160*, 111541. [CrossRef] [PubMed]
209. Makhdoumi, P.; Hossini, H.; Nasmara, Z.; Mansouri, K.; Pirsaheb, M. Occurrence and exposure analysis of micro-plastic in the gut and muscle tissue of riverine fish in Kermanshah province of Iran. *Mar. Pollut. Bull.* **2021**, *173*, 112915. [CrossRef] [PubMed]
210. Nematollahi, M.J.; Keshavarzi, B.; Mohit, F.; Moore, F.; Busquets, R. Microplastic occurrence in urban and industrial soils of Ahvaz metropolis: A city with a sustained record of air pollution. *Sci. Total. Environ.* **2022**, *819*, 152051. [CrossRef]
211. Ta, A.T.; Pupuang, P.; Babel, S.; Wang, L.P. Investigation of microplastic contamination in blood cockles and green mussels from selected aquaculture farms and markets in Thailand. *Chemosphere* **2022**, *303*, 134918. [CrossRef]
212. Koedprang, W. Culture of Green Mussel, Perna Viridis in Thailand. 2013. Available online: https://fishconsult.org/?p=9397 (accessed on 7 August 2022).
213. Danopoulos, E.; Jenner, L.C.; Twiddy, M.; Rotchell, J.M. Microplastic Contamination of Seafood Intended for Human Consumption: A Systematic Review and Meta-Analysis. *Environ. Health Perspect.* **2020**, *128*, 126002. [CrossRef]
214. Thushari, G.G.N.; Senevirathna, J.D.M.; Yakupitiyage, A.; Chavanich, S. Effects of microplastics on sessile invertebrates in the eastern coast of Thailand: An approach to coastal zone conservation. *Mar. Pollut. Bull.* **2017**, *124*, 349–355. [CrossRef]
215. Pradit, S.; Noppradit, P.; Goh, B.; Sornplang, K.; Ong, M.; Towatana, P. Occurrence of microplastics and trace metals in fish and shrimp from songkhla lake, thailand during the covid-19 pandemic. *Appl. Ecol. Environ. Res.* **2021**, *19*, 1085–1106. [CrossRef]
216. Azad, S.M.O.; Towatana, P.; Pradit, S.; Patricia, B.G.; Hue, H.T. Ingestion of microplastics by some commercial fishes in the lower Gulf of Thailand: A preliminary approach to ocean conservation. *Agric. Technol.* **2018**, *14*, 1017–1032.
217. Abinon, B.S.; Camporedondo, B.S.; Mercadal, E.M.B.; Olegario, K.M.R.; Palapar, E.M.H.; Ypil, C.W.R.; Tambuli, A.E.; Lomboy, C.A.L.M.; Garces, J.J.C. Abundance and characteristics of microplastics in commercially sold fishes from Cebu Island, Philippines. *Aquat. Biol.* **2020**, *8*, 424. [CrossRef]

218. Yong, M.M.H.; Leistenschneider, C.; Miranda, J.A.; Paler, M.K.; Legaspi, C.; Germanov, E.; Araujo, G.; Burkhardt-Holm, P.; Erni-Cassola, G. Microplastics in fecal samples of whale sharks (Rhincodon typus) and from surface water in the Philippines. *Microplast. Nanoplast.* **2021**, *1*, 17. [CrossRef] [PubMed]
219. Motta, P.J.; Maslanka, M.; Hueter, R.E.; Davis, R.L.; de la Parra, R.; Mulvany, S.L.; Habegger, M.L.; Strother, J.A.; Mara, K.R.; Gardiner, J.M.; et al. Feeding anatomy, filter-feeding rate, and diet of whale sharks Rhincodon typus during surface ram filter feeding off the Yucatan Peninsula, Mexico. *Zoology* **2010**, *113*, 199–212. [CrossRef]
220. Bilugan, Q.M.; Limbago, J.S.; Gutierrez, R.L. Detection and quantification of microplastics from cultured green mussel Perna viridis in Bacoor Bay, Cavite, Philippines. *Sustinere J. Environ. Sustain.* **2021**, *5*, 90–102. [CrossRef]
221. Jambre, K.G.E. Density of Microplastics in Philippine Cupped Oyster (*Crassostrea iredalei*). *Int. J. Adv. Multidiscip. Stud.* **2022**, *1*. Available online: https://www.researchgate.net/publication/358234028_Density_of_Microplastics_in_Philippine_Cupped_Oyster_Crassostrea_iredalei?enrichId=rgreq-d84c6b3e779661a6231927d7293915f3 (accessed on 12 August 2022).
222. Cabansag, J.B.P.; Olimberio, R.B.; Villanobos, Z.M.T. Microplastics in some fish species and their environs in Eastern Visayas, Philippines. *Mar. Pollut. Bull.* **2021**, *167*, 112312. [CrossRef]
223. Paler, M.K.O.; Leistenschneider, C.; Migo, V.; Burkhardt-Holm, P. Low microplastic abundance in *Siganus* spp. from the Tañon Strait, Central Philippines. *Environ. Pollut.* **2021**, *284*, 117166. [CrossRef]
224. Naidoo, T.; Smit, A.J.; Glassom, D. Plastic ingestion by estuarine mullet *Mugil cephalus* (Mugilidae) in an urban harbour. KwaZulu-Natal, South Africa. *Afr. J. Mar. Sci.* **2016**, *38*, 145–149. [CrossRef]
225. Lamarca, N. Fisheries Country Profile: Philippines. 2017. Available online: http://www.seafdec.org/fisheries-country-profile-philippines (accessed on 12 August 2022).
226. Ebrahim, A.; Martin, T.S.H.; Mumby, P.J.; Olds, A.D.; Tibbettss, I.R. Differences in diet and foraging behaviour of commercially important rabbitfish species on coral reefs in the Indian Ocean. *Coral Reefs* **2020**, *39*, 977–988. [CrossRef]
227. Palermo, J.; Labrador, K.; Follante, J.; Agmata, A.; Pante, M.; Rollon, R.; David, L. Susceptibility of Sardinella lemuru to emerging marine microplastic pollution. *Glob. J. Environ. Sci. Manag.* **2020**, *6*, 373–384. [CrossRef]
228. Wings. Essay on Sustainable Dvelopment: Format & Examples. 2020. Available online: https://leverageedu.com/blog/essay-on-sustainable-development/ (accessed on 24 August 2022).
229. UNEP. Sustainable Development Goal 6. 2022. Available online: https://www.unep.org/explore-topics/sustainable-development-goals/why-do-sustainable-development-goals-matter/goal-6 (accessed on 24 August 2022).
230. UNEP. Sustainable Development Goal 14. 2022. Available online: https://www.unep.org/explore-topics/sustainable-development-goals/why-do-sustainable-development-goals-matter/goal-14 (accessed on 24 August 2022).

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Assessment of Microplastics in Green Mussel (*Perna viridis*) and Surrounding Environments around Sri Racha Bay, Thailand

Jitraporn Phaksopa *, Roochira Sukhsangchan *, Rangsiwut Keawsang, Kittipod Tanapivattanakul, Bojara Asvakittimakul, Thon Thamrongnawasawat and Suchai Worachananant

Department of Marine Science, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand
* Correspondence: jitraporn.p@ku.th (J.P.); roochira682@gmail.com (R.S.)

Abstract: Microplastic pollution is a common problem in the coastal and marine environment, especially in the transferential process through trophic levels. This study analyzed the characteristics of microplastics in the seawater, sediments, and green mussels (*Perna viridis*) around Sri Racha Bay, Thailand, during the dry and wet season in 2020. This area is a semi-enclosed bay highly affected by extensive green mussel farms and anthropogenic activities. Qualitative and quantitative analysis of microplastics was undertaken using a stereomicroscope and micro-Fourier transform infrared spectrometer (ATR-μ-FT-IR). During the wet season (July), the average abundance of microplastics was found to be relatively higher in water (2.06 ± 1.78 particles/m^3) and sediment (69.35 ± 22.29 items/Kg D.W.) than those found in the dry season (April) (0.85 ± 0.25 items/m^3 in water and 48.30 ± 28.17 items/Kg D.W.). Overall, the most abundant microplastic polymers were PE, PP, and poly in water, but PE, nylon, and PP in sediments. In green mussels, microplastic counts were 0.15 ± 0.41 and 0.22 ± 0.57 items/individual in the dry and wet season, respectively, and PET, PP, and nylon were the three most abundant. Some inter-specific differences were found, but no evidence for a sampling sites or seasons was highlighted, although the tendency was higher during the wet season. The excessive riverine freshwater discharge transported terrestrial plastic debris into the estuarine system; hence, higher microplastic contamination in surface seawater and sediment was evidenced. The presence of colorants in organisms revealed an anthropogenic origin through the use of a wide array of applications. This study provides thoughtful insights for coastal area management and food-safety planning.

Keywords: FTIR; green mussel; microplastic pollution; sea floating raft culture; Gulf of Thailand

Citation: Phaksopa, J.; Sukhsangchan, R.; Keawsang, R.; Tanapivattanakul, K.; Asvakittimakul, B.; Thamrongnawasawat, T.; Worachananant, S. Assessment of Microplastics in Green Mussel (*Perna viridis*) and Surrounding Environments around Sri Racha Bay, Thailand. *Sustainability* **2023**, *15*, 9. https://doi.org/10.3390/su15010009

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

Received: 4 November 2022
Revised: 16 December 2022
Accepted: 16 December 2022
Published: 20 December 2022

1. Introduction

Plastic debris in aquatic ecosystems has become a serious concern due to its high consumption and the high potential of accumulation by its long degradation time. Eventually, plastic debris can be broken down into smaller particles via photo-oxidative, chemical, and biological mechanisms [1–4]. These fragments are defined by sizes into nano-plastics (less than a few micrometers), microplastics (approximately less than 5 mm), and mesoplastics. Plastics and microplastics enter the marine and coastal environments by human activities such as fishing, tourism, marine transport, shipping, and industries [5,6] or from land to the sea through river discharge [7,8]. Of these, microplastics are the ones receiving close attention from various researchers due to their potential as a threat to marine lives. Some toxic pollutants can be adsorbed on the microplastic surface once in the marine environment [9–11]. Microplastics are likely to be taken up by marine organisms. When microplastics are ingested, additives and adsorbed chemicals can be released and pose potential risks to their health [12–16].

The distribution of microplastics has been reported in marine environments in many regimes, such as in water [17–22] and sediments [23–25]. Recent studies have shown

that microplastics have been detected in a large number from marine species of various geographical areas and trophic levels [26–32]. Additionally, microplastics may be transferred along marine food webs [33,34]. Consequently, microplastic pollution has potentially threatened marine ecosystems and human health. Thus, it is significant to expand our knowledge of the occurrence, abundance, and effect of microplastics in marine ecosystems.

In Thailand, microplastic pollution has been reported in many areas, such as estuarine water at the Chaopraya River mouth [35], beaches [36,37], sediments in the urban estuary in Phuket Province [38], and marine organisms [31,39,40]. Unfortunately, the degree and evaluation of microplastics in coastal farming areas are rarely established and unclear. This issue needs to be addressed, and the possible transferential pathway of microplastics from surrounding to marine organisms must be determined.

Bivalves have been extensively used as a bio-indicator species for environmental monitoring for pollutants such as heavy metals, microplastics, and persistent organic pollutants due to their immobile nature, immense distribution, high tolerance towards contaminants, and the ability to filter a large volume of seawater. Moreover, bivalves, especially mussels, are directly connected with other marine predators on the higher trophic level, as well as human health. Green mussels (*Perna viridis*) are selected in this study because of their value economically as highly sought-after seafood in Thailand. Low prices and convenience can lead to a direct transfer of microplastics if green mussels are contaminated. The mussel is a filter feeder; therefore, its activities depend on the environment (water temperature, salinity, and currents) and the concentration of food particles in water. The ability to accumulate various pollutants makes green mussels reliable bio-indicators for marine pollution [41–43]. The cultivation of green mussels is highly extensive in Sri Racha Bay or the Sri Racha Coast, Chonburi Province, which is strategically located in the special economic zone of Thailand, namely the Eastern Economic Corridor (EEC). This area has been well-known for green mussel farming in Thailand for decades. The cultivation of green mussels is mainly undertaken by installing sea-floating rafts. While green mussel farming is crowded in marine areas, Sri Racha Bay is also terrestrially densely packed with surrounding land use, including residential areas, industrial zones, recreational spaces, and an entertainment estate. Hence, some previous studies demonstrated the corrosion of coastal water quality and heavy metal contamination around this area [44,45].

This research proposed to investigate the spatial and seasonal accumulation of microplastics in green mussels, seawater, and sediments around Sri Racha Bay. Understanding the microplastic contamination across seawater, coastal sediments, and the uptake in green mussels is the key to evaluating the ecological risk that microplastic pollution poses to ecological functions and human consumers. This study can contribute useful information and knowledge in understanding microplastic distribution in aquatic environments for further developing a coastal management plan.

2. Materials and Methods

2.1. Study Area and Sample Collection

The presence and distribution of microplastics in marine environments around Sri Racha Bay surface waters and sediment were evaluated during two seasons in 2020: April (dry season) and July (rainy or wet season). Samples were collected from 5 sampling stations located about 5 km further offshore and spanned 10 km along the coastline of Sri Racha Bay, Chonburi Province (Figure 1A). The five locations were selected based on different influential factors: location 1 (S1) is impacted by waste from the food factory, location 2 (S2) is the outburst of Sukhrep canal, which runs through coastal community area and starch factory, and location 3 (S3), location 4 (S4), and location 5 (S5) are influenced by community activities in coastal areas.

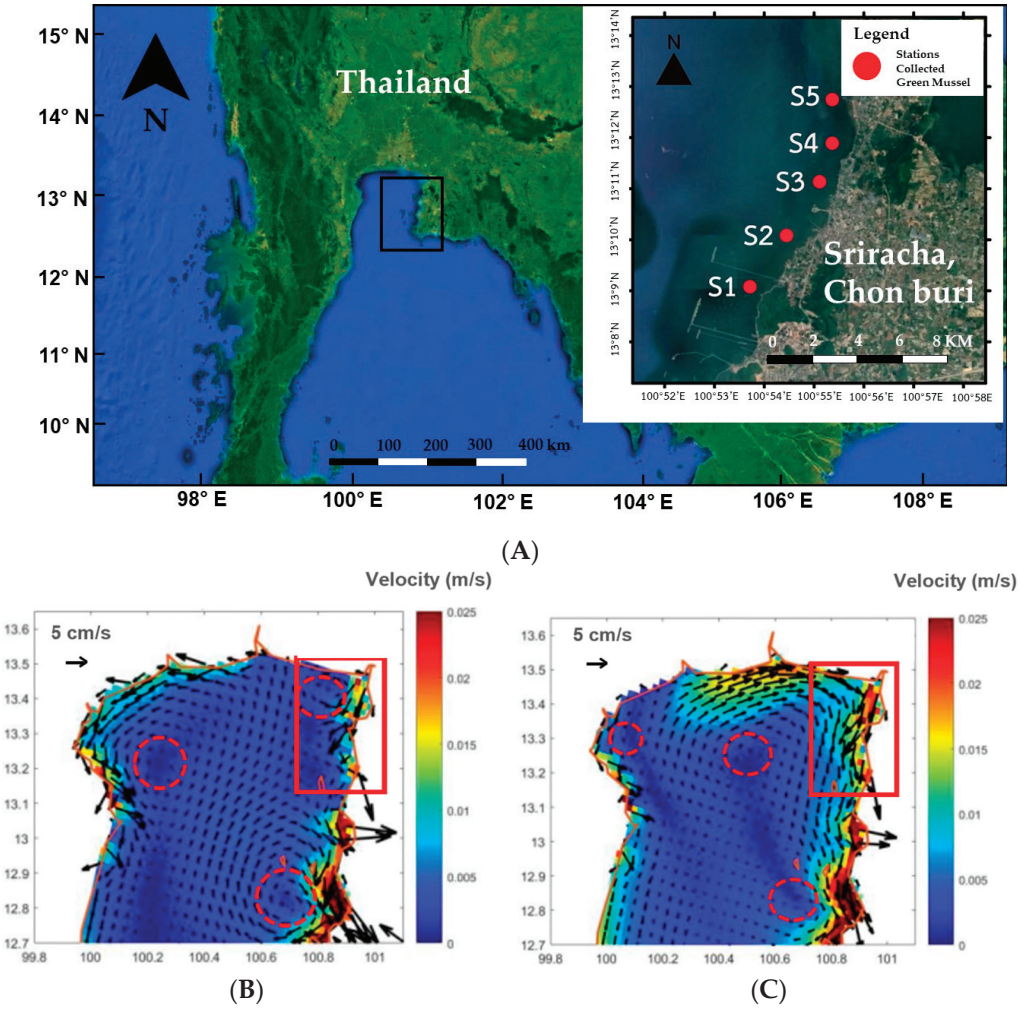


Figure 1. (**A**) Sampling sites location in Sri Racha Bay, Thailand (**B**) Currents around the upper Gulf of Thailand in April (**C**) Currents around the upper Gulf of Thailand in July (Reprinted with permission from Lamsawat et al., 2020 [46]. 2022, Pakorn Lamsawat). Circles and frames show gyre and study area, respectively.

The hydrodynamics of Sri Racha Bay are complex and influenced by wind and tidal currents. The general trend of the current runs from the north to the south in the dry season and the opposite direction in the rainy season [46] (Figure 1B,C).

The level of microplastics from seawater and sediments also becomes a consideration in choosing the sampling location. A neuston net (75 cm in diameter, 300 cm in length, and 0.35 mm in mesh size), originally designed for sampling of zooplankton, fish larvae, and fish eggs near the sea surface, was used to collect mesoplastics and microplastics. A flowmeter was installed at the mouth of the neuston net to measure the water volume passing through during sampling. The neuston was deployed off the port side. The samplers were towed at a ship's speed of 2–4 knots for 15 min in the upstream flow direction. Water characteristics, i.e., salinity and temperature, were documented and are listed in Table 1.

Table 1. Microplastic abundance in seawater and sediments in Sri Racha Bay.

	Locations	MP Piece/m^3 in Water		MP Piece/Kg D.W. in Sediments		MP Number in Mussels		Salinity (PSU)		Water Temp (°C)	
		Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
S1	13° 8′40.65″ N 100°53′46.83″ E	0.64	1.59	18.58	43.34	13	11	28.86	30.10	31.20	30.00
S2	13° 9′46.59″ N 100°54′36.21″ E	1.22	0.92	30.96	80.50	8	20	28.25	30.20	31.00	30.40
S3	13°10′54.36″ N 100°55′15.82″ E	0.70	0.57	37.15	49.54	5	9	32.38	31.25	30.95	29.80
S4	13°11′53.24″ N 100°55′39.86″ E	0.69	5.04	86.69	74.30	13	35	31.10	30.80	30.80	30.70
S5	13°12′46.13″ N 100°55′40.74″ E	0.99	2.20	68.11	99.07	31	37	31.80	31.15	31.50	31.00

Three replications of sediment samples were taken by a stainless-steel Ekman grab sampler (6″ × 6″ × 6″). About 1–2 kg of wet sediments were collected and immediately kept in a clean container. Sediment samples were wrapped with an aluminum foil and stored in the freezer until further analysis in the laboratory.

A hundred green mussels were collected from each location. All mussels acquired were adult mussels (shell sizes of 2–9 cm) attached to cultivated rope at depths of 2 and 5 m. All green mussels were then stored in iceboxes to be transferred to the laboratory and kept in the freezer for analysis preparation.

2.2. Microplastic Extraction

2.2.1. Sea Water

Sea water samples were treated solely with wet peroxide oxidation to remove organic matter according to [35], with minor modifications. First, each sample was filtered through metal sieves (5 mm and 1 mm) to remove all natural or artificial litter objects with the larger size, and the residual particles on the sieve were stored and rinsed into a 50 mL glass bottle using the distilled water. All residual particles were dried in an oven, sorted using visual identification, and then identified. The filtered water sample was transferred into a 500 mL clean glass beaker. At first, 20 mL of 30% H_2O_2 was added, and the mixture was stirred by a magnetic stir bar on a hot plate at 60 °C for 30 min to digest the organic matter from the water sample. This step was repeated until the organic matter disappeared in the beaker. For the separation method, a NaCl solution was chosen, and prepared by dissolving 337 g of NaCl in 1000 mL of distilled water. We also performed the floatation method, where separation was achieved based on differences in density to produce samples to be visually analyzed using a stereomicroscope [47]. The NaCl solution was added to increase the density of the aqueous solution. After settling for 4 h, the supernatant was filtered under vacuum through a fiberglass Whatman filter (1.2 um pore size; 47 mm diameter) and nylon (330 µm mesh size). This process was repeated three times to increase the recovery rate of microplastics. The supernatant was filtered under vacuum through a fiberglass Whatman filter (1.2 um pore size; 47 mm diameter) and nylon (330 µm mesh size). This process was repeated three times. Following this, microplastics with filters were kept in an aluminum foil and dried in the oven at 60 °C. Finally, all samples were kept in glass petri dishes and covered with a glass lid for further analysis.

2.2.2. Sediments

Each sediment sample was placed in a glass beaker with deionized water and agitated with a metal spatula to isolate large clumps of sediment. Samples were dried at 60 °C in an oven for 24 h. The dried sample was placed in a glass beaker and 20 mL of 30% H_2O_2 and 20 mL of 0.05M iron (II) solution were added. The sediment solution was left at room temperature for 5 min, then was placed on a heating magnetic stirrer and heated to 60 °C for 30 min to increase the organic digesting rate. Following this, samples were settled about 4 h or until all visible organic materials disappeared. The separation and filtration processes were performed in the same manner as for the water samples.

2.2.3. Green Mussels

All samples were frozen at −20 °C until further laboratory analysis. Subsequently, after defrosting for 1 h, samples were washed with distilled water to remove large matter. Basic measurements (body length and wet weight) were recorded of each sample without byssus. Following this, each individual sample was dissected in a metal tray using a scalpel, forceps, and scissors. Samples were carefully removed from their shells to prevent contamination, and then immediately placed into clean beakers and covered with aluminum foil to minimize the risk of contamination.

In order to degrade organic matter and enable the detection of microplastic particles, samples were subjected to hydrogen peroxide digestion according to [42], with minor modifications. Initially, each sample was transferred into a 500 mL clean glass beaker. About 20 mL 10% KOH solution was added, and the mixture was stirred for 1 h to increase the organic matter digestion rate. Following this, 20 mL of 30% H_2O_2 was added, and the mixture was heated on a hot plate at 60 °C with a magnetic stirrer until H_2O_2 was evaporated. After 24 h, around 100 mL of the filtered NaCl solution was added, and the mixture was stirred for 5 min for density separation. After settling for 4 h, the sample was filtered under vacuum through filters (20 μm pore size; 47 mm diameter). This process was repeated three times to increase the recovery rate of microplastics. The filter was placed in Petri dishes and dried in an oven at 60 °C while being covered in aluminum foil. Finally, all samples were kept in the desiccator for further analysis. During the digestion procedures, three procedural blanks were also run without samples in parallel with samples containing the digestion solutions, and any particles detected in these blanks were characterized as contamination.

2.3. Identification of Microplastics

All potential microplastic particles were subjected to visual examination by the stereomicroscopy and identified by their colors and shapes. All plastic-like items from the samples were sorted and quantified by color (blue, black, white, yellow, red, and transparent), shape (fragments—irregular pieces; pellets—spherical and ovoid debris; fibers—thin and elongated pieces) with a stereomicroscope (Olympus SZ51). Polymer types were identified using a Fourier transform infrared microscopy system (μFT-IR; Spotlight 200i FT-IR microscopy system, PerkinElmer, Waltham, MA, USA) in reflection mode with 30 × 30 μm aperture size, using 24 scans and spectral resolution of 4 cm^{-1}. Each spectrum was recorded after 4 accumulations ranging from 400 to 4000 cm^{-1}. Following this, each obtained spectrum was compared to the polymer database (PerkinElmer Polymer database) and the type of plastic was determined when the research score was higher than 80%. The number of microplastics were expressed as particles/m^3 in seawater, particles/Kg D.W. in sediments, and items/individual in mussel.

2.4. Contamination Control

Lab coats and gloves were always worn throughout the microplastic analysis to prevent contamination. Before working, the workplace was cleaned with 70% alcohol; all glassware was rinsed three times with distilled water and covered with aluminum foil before usage. The distilled water and saturated NaCl solution were filtered by a vacuum pump with a WHATMAN® GF/C filter (1.2 μm pore size; 47 mm diameter). For the blank test, 500 mL of filtered NaCl solution without any samples was used, following the extraction and separation procedure detailed above. Following this, the dried filters were observed under the stereomicroscope. There were no microplastics found in the blank-test filters, indicating that there was no microplastic contamination during the laboratory work.

2.5. Statistical Analysis

All results are presented as mean ± standard deviation of the mean (SD). The microplastic abundances in surface seawaters, sediments, and green mussels with the significant differences between the groups (locations and seasons) were determined by ANOVA and *t*-test (significance level at 0.05). Linear regressions were used to determine the relationship between microplastic in mussels, seawaters, and sediments. Additionally, Pearson correlation was used to determine the relationship between microplastic abundance from seawater and sediment samples, and mussel sizes and microplastic abundances. Statistical significance was accepted at p-value < 0.05. Statistical analyses were performed with SPSS Statistics 27.0 (IBM Corp., New York, NY, USA).

3. Results

3.1. Water

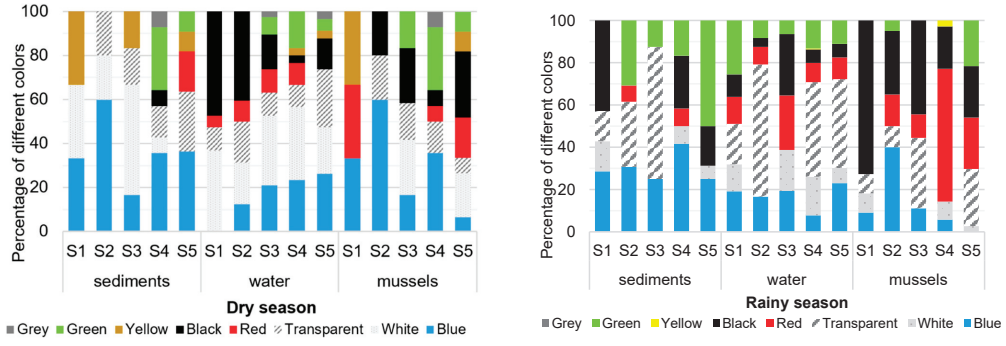
The present study evidenced higher salinity (31.08 ± 1.05 PSU) and water temperature (30.70 ± 0.53 °C) during the dry season and lower salinity (31.09 ± 0.47 PSU) and water temperature (30.38 ± 0.49 °C) during the wet season (Table 1). Microplastics were detected in all locations, but numbers ranged considerably from 0.69 to 1.22 particles/m^3 in the dry season and from 0.57 to 5.04 particles/m^3 seawater in the wet season. Results showed that average abundance of microplastics in the dry season (0.85 ± 0.25 particles/m^3) was lower than that in the wet season (2.06 ± 1.78 particles/m^3). The concentration of microplastics was highest at S2 in the dry season and S4 in the wet seasons. The density of microplastics in each station followed a descending order: S2 > S5 > S3 > S4 > S1 in the dry season and S4 > S5 > S1 > S2 > S3 in the wet season. There were eight colors found in the samples. Colors of particles varied widely, but transparent, white, and blue were the most common in water in all locations; meanwhile, red, green, yellow, and grey were observed in fewer contents (Figure 2A).

The majority of identified particles in seawater were dominated by fibers (10.47 to 40.70%), sheets (0 to 55.88%), and granules (2.44 to 44%) (Figure 2B). That no pellets were found in this study suggests there are no primary microplastics in this area. A total of seven polymers were identified from all samples (Figure 2C). Polypropylene (6.45 to 32.85%) and polyethylene (0 to 66.39%) were commonly observed due to their extensive usage and ease of degradation. Others (PET, nylon, polystyrene, and PVC) were less prevalent. Primarily polystyrene was found at S2 and S3, and PVC was found at S1 only in the dry season. Interestingly, the statistic test showed no significant difference between the number of microplastics related to sample location and season.

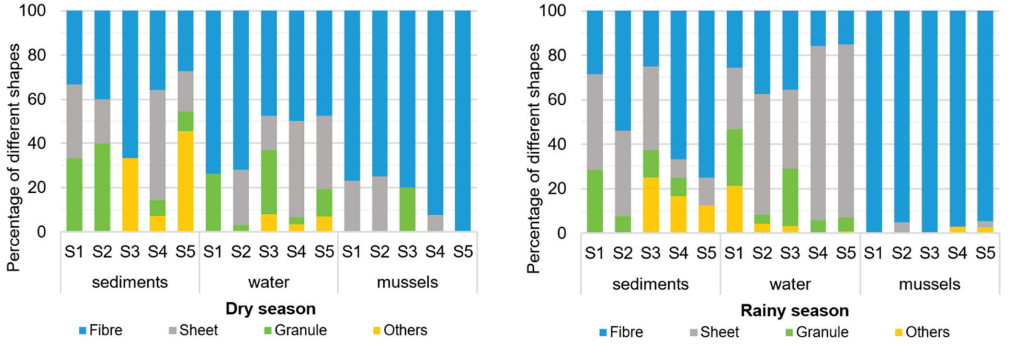
3.2. Sediments

The number of microplastic particles observed at the different sampling stations in the Sri Racha Bay was presented in Table 1. The items ranged from 18.58 to 86.69 particles/Kg D.W. weight with average of 48.30 ± 28.17 particles/Kg D.W. in the dry season and from 43.34 to 99.07 particles/Kg D.W. with average of 69.35 ± 22.29 particles/Kg D.W. in the wet season. Polyethylene was the most common plastic type. Generally, the highest concentrations of microplastics were found at S4 in the dry season and S5 in the wet season, with concentrations of up to 86.69 and 99.07 items/Kg D.W., respectively.

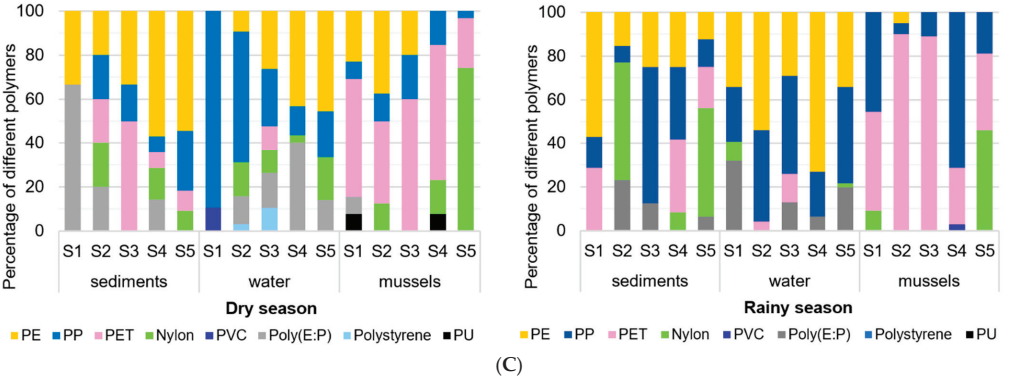
The colors of the observed microplastics collected were white, transparent, black, grey, red, blue, yellow, and green. Figure 2A shows the proportion of microplastics of different colors varying spatially and seasonally. Blue (35.90% and 30.40%, respectively), transparent (17.95% and 17.90%, respectively), and green (12.82% and 26.80%) were the dominant colors in the dry and rainy (or wet) seasons, while white and yellow were found in low quantities, most of them only in the dry season. The microplastic shapes were mainly fibers, sheets, granules, and infrequent occurrences of other shapes (such as fragment and rod). Figure 2B shows that fibers were the dominant microplastic shape in both seasons (38.46% and 55.36%, respectively). The proportion of sheets in these two seasons was also relatively high (28.21% and 25.0%, respectively).



(**A**)



(**B**)



(**C**)

Figure 2. Seasonal variation in relative composition of microplastics in sediments, water, and green mussels (*Perna viridis*) in five stations during the dry and rainy season around Sri Racha Bay, Thailand, according to color, shape, and polymer type. (**A**) Colors (**B**) Shapes (**C**) Polymer types.

As shown in Figure 2C, all items were identified using micro-Fourier transform infrared spectrum and classified into five categories: PP, PET, nylon, PE, and Poly (ethylene-co-propylene). The polymer types of the microplastics were diverse in all locations and both seasons, indicating the extents of the microplastic sources. The polymer types of the microplastics detected in the dry season were categorized based on occurrence as PE (46.15%) with the highest proportions, followed by PP (15.38%), PET (15.38%), and poly (12.82%). In the rainy season, nylon (28.6%) was the most frequent, followed by PE (23.2%), and PP (23.2%), and PET (16.1%). The study investigated the different degrees of microplastic pollution because the polymer types and composition were various at the same

sampling sites and seasons. This finding might suggest either difference or homogeneity of microplastic sources among sites each season.

In general, microplastics were present across all locations, and no significant differences could be detected between the five sampling sites and between seasons. This result might indicate the spatial homogeneity of microplastic occurrence in the study area.

3.3. Green Mussels

A total of 1000 green mussels (from five stations in two seasons) were analyzed for microplastics. The range (mean ± SD) of length, width, and soft tissue weight of the green mussels were 2.05–9.00 cm (5.55 ± 0.88 cm), 1.43–5.10 cm (2.66 ± 0.37 cm), and 1.01–9.54 g (3.21 ± 1.36 g), respectively. The whole abundance of microplastics found in green mussels was around 0.07 ± 0.19 items/gram of wet weight soft tissues and around 0.19 ± 0.50 items/individual (N = 1000). The average microplastic concentration in the dry season (0.15 ± 0.41 items/individual) was lower than those in the rainy season (0.22 ± 0.57 items/individual). In both seasons, the highest microplastics frequency was observed at S5, while S3 was the slightest (Figure 3a). Microplastic abundances significantly differed statistically among locations (ANOVA: F = 9.816; df = 4, 995; $p < 0.01$).

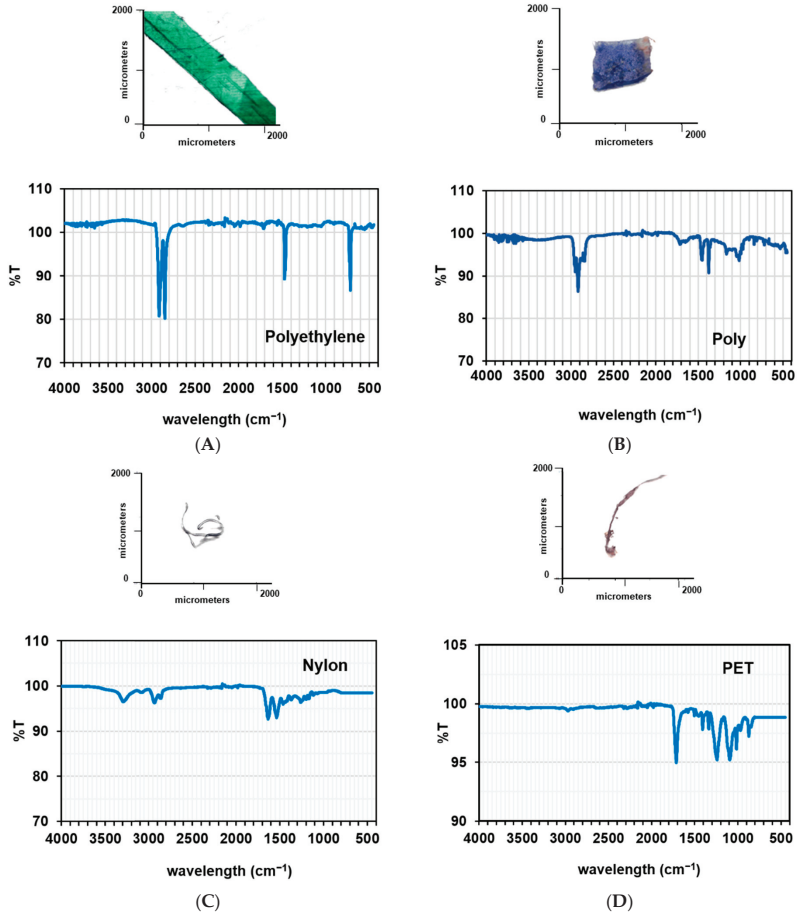


Figure 3. FT-IR analysis and image of representative microplastics found in Sri Racha Bay. (**A**) Green sheet in sea water (PE); (**B**) Blue sheet in sea water (Poly); (**C**) Green fiber in mussel (Nylon); (**D**) White fiber in sediments (PET).

All 182 identified microplastics in green mussels were grouped according to color, shape, and polymer type (Figure 2). Spatially and seasonally, the plastics categorized as fiber were the most observed, reaching 94%. Surprisingly, neither collection sample presented contamination by primary source microplastics—pellets. Three most frequently found colors were black (27%), white (25%), and red (23%). Five different types of microplastics were identified. PET, PP, and nylon were the dominant plastics with respective proportions of 45%, 40%, and 24%.

Overall, types of microplastic seemed to be influenced both spatially and seasonally (Figure 2). At S4 and S5, PE particles were absent while other particulars were still present. Additionally, the component of nylon at S5 differed significantly compared to any other sites. This finding hinted the difference of microplastic types in green mussel varied among sampling sites and might provide some explanation as to the site-selecting rationale.

4. Discussion

4.1. Abundance and Characteristics of Microplastics in Surface Seawater

The sampling was conducted at five locations, as shown in Figure 1. The microplastics spread sporadically across area both in sediment and seawater. The abundance of microplastics is relatively low compared with other coastal water areas from the Tolo Harbor, Hongkong [48], Hainan, China [49] and higher than those in the East China Sea [18]; Goiana Estuary, Brazil [50]; Tamar Estuary, Southwest England [51]; or the Japan Sea [52] (Table 2). Therefore, Sri Racha Bay could be classified as moderately microplastic polluted areas. Comparing internally with other crucial mariculture areas, such as Ban Don Bay in the southern part of Thailand [39], microplastic abundance here is significantly lower. These results suggest that the topography and coastal circulation are the essential factors in distributing the microplastics in each area.

All surface seawater samples contained microplastic particles, with concentrations ranging from 0.57 to 5.04 particles m^{-3}; however, there is no significant differences across study sites and between seasons. It should be noted that while there is no difference statistically, the amount of microplastics observed in the rainy season was distinctively high. The hydrodynamic conditions (comparatively high water velocity) and input source from the river discharge (high rainfall) may lead to the distribution in this area during the rainy season. The higher concentration in the rainy season is not unique to here; studies in some estuaries and marginal seas in China [22] also revealed high concentrations. The river runoffs might be the culprit that ties microplastics to coastal water [35,53,54]. This finding provides the rationale for designing sampling that includes seasonal variation for further monitoring.

While there was no statistical difference amongst sites, it should be noted that the abundance of microplastics in the northern part of the bay (station S4 and S5) was higher than in the southern part (station S1, S2, and S3) in the rainy season. In particularly, location S5 is adjacent to the famous tourism beach and seafood factories, and station S4 has a canal that run though a coastal community. This might be the main source of microplastics around these two stations.

Overall, recent studies showed that there are many factors influencing the distribution and abundance of microplastics in water samples, including the human population, wind direction and resulting water currents and waves, entrance of waste waters and sewage, size and shapes of microplastics, shipping, and anthropogenic activities (such as fisheries and industry) in the neighborhood [1,55,56]. Our results found that the reason for microplastic pollution may be caused by the circulation currents produced by tides and wind. Additionally, the water circulation pattern in the upper Gulf of Thailand (GOT) was dominated by river discharge and was distinguished from the circulation in the other parts of the GOT, where it is influenced by the South China Sea waters [57]. Recent studies [58,59] suggested that wind and currents may influence the transportation of the materials in surface seawater as well as the abundance and distribution of microplastics. A previous study on winds and hydrodynamics of the Upper Gulf of Thailand [46] demonstrated

that winds mostly blow from the south-west. In Sri Racha Bay, winds may significantly modify surface currents and affect vertical flow changes. The prevailing winds create surface and near-bottom currents, carrying suspended materials from the northern parts (or further northward) towards the south of the bay. Hence, the current directions should be considered as one of the reasons for the lower microplastic abundance in southern stations and the high microplastic abundance in northern stations.

According to polymer types (Figure 2), most microplastics in seawater were PP and PE (67% and 85% in dry and rainy seasons). These polymers are the most common for packing, plastic bags, and material in maricultures. These confirmed that the microplastics in seawater are very closely related to coastal activities like any other anthropogenic stressed areas, such as Jiaozhou Bay [60] and Xincun Lagoon, China [61]. However, the main types of microplastics in Sri Racha Bay differed from Ban Don Bay, where the majority was rayon [39]. It inferred that source of microplastics might be diverse, while having a similar type of mariculture activity.

4.2. Abundance and Characteristics of Microplastics in Sediments

Similar to seawater, microplastics were found sporadically across five stations. The observed microplastics in sediment showed no significant difference seasonally, though those observed microplastic numbers in the rainy season are higher than those in the dry season. In contrast, microplastic abundance were varied significantly amongst location ($p < 0.05$). As expected, total microplastic concentrations were highest around S4 and S5, but we still cannot be confirm the relation of these observed concentrations to local anthropogenic activities. However, microplastics in sediments are transported more slowly than in water because of the stability of sediments [62]. Additionally, many factors affect the spatial distribution in sediments: source, hydrodynamics, and sediment characteristics [26,63,64]. Therefore the distance from the source might be the main factor affecting the spatial distribution in this area. The low flushing rate may explain this observation. Microplastics floating into such compartments could get trapped around this area because of the rafting patches, eventually settling on the bottom instead of flushing out of this area. This result could explain the higher microplastic concentrations in these stations. Our findings suggest that seasonal variations should elicit caution when monitoring the microplastic abundance in sediments.

Different sampling and analysis processes could make direct comparison difficult and uncertain. Therefore, some studies that have similar methods were selected. Compared with the abundance of microplastics in different regions reported in previous studies as shown in Table 2, the microplastic quantity in the coastal sediment in Sri Racha Bay is lower than those in the Gulf of Thailand [65], an estuary in Phuket [38], and the Tunisian coast [66], whilst distinctively high compared to Tokyo Bay [67]. The abundance was more heightened than in Ban Don Bay, Thailand [39].

Fiber was the dominant shape found in the sediment samples here, followed by sheets and granules. Hence, a high abundance of microfibers in sediment might impact the local benthic fauna in various ways [26,68,69]. Additionally, some microfibers might originate from the rope material used in the fishery activities in the study area [2,18]. FT-IR was performed to identify polymer types of all samples. The majority component of microplastics in sediment is polyethylene, nylon, and PET, possibly originating from food packaging, clothes, and fishing gear, with a variation in proportion during both seasons. Additionally, nylon is used in fishing gear and equipment, such as discarded or abandoned nets, and could also be considered an important microplastic source. Due to its density being higher than seawater, nylon is likely to deposit in sediment. Moreover, the sediment beneath mussel rafts had a more refined texture of mud and silt, with a higher degree of accumulation [70]. Nylon was also detected as forming the majority in sediments in some recent studies [5]. There was no rayon in our study but rayon was the majority in sediments in other areas, such as Phuket [38] and Ban Don Bay [39]. These confirm that the microplastics in sediments are very closely related to coastal activities

that agree with other adjacent human activities. Nylon is used in maricultures and since station S3, S4 and S5 are the location for green mussel farms (raft and bamboo-pole culture), the farms could also be considered an important microplastic source together with discarded or abandoned ropes.

Consequently, our results support that microplastic pollutants primarily came from urban and farming activities. Due to the spatial pattern of microplastics in the sediments, the microplastics found here were homogenous. This pattern reflects their relative level of industrialization and urbanization. Hence, the proximity to anthropogenic inputs has been found to be a fundamental determinant of microplastic abundance in this area.

4.3. Abundance and Characteristics of Microplastics in Green Mussels

Green mussels are filter feeders with a filtration rate of about 24–60 L/day; however, the filtration rate also depends on various factors such as body sizes and suspended particles in water [71]. Water is sieved into the body through relevant organs, which enhance the possibility of intaking or ingesting microplastics suspended in water. Thus came the assumption of relating microplastic quantities in the water column to the one found in mussels. Sri Racha Bay is one of the important green mussel (*Perna viridis*) farming areas using float rafting in Thailand. The average amount of microplastic in green mussels was 0.15 ± 0.41 (45.63 items/Kg wet weight) and 0.22 ± 0.57 particles/individual (70.16 items/Kg wet weight) in the dry and wet seasons, respectively. The level of microplastics in this study was relatively lower than those reported in other studies despite using different methods for analyzing microplastics, such as those for green mussel (*Perna viridis*) in Ban Don Bay [39]; blue mussel (*Mytilus edulis*) and Pacific oyster (*Crassostrea gigas*) from the French Atlantic coast; green mussel in France [72]; and green mussel (*Perna viridis*) from Phuket [40], as in Table 3. Interestingly, [73] found very high levels of contamination with 34 to 178 items/individual in mussels in Canada, but their work was based on visualization only.

Fiber formed the significant majority of microplastics found in mussel tissues. Consistently with this finding, fibers were the dominant shape category of microplastics in seawater and sediment at the same area. According to previous studies, fiber was the most common shape in mussels [39,42,74–76].

In this work, black plastic was the most abundant and was found in all stations in both seasons (Figure 2). The colorful microplastics were about half of them; red ones were predominant (23.08%), followed by green (15.93%), and blue (8.79%). This provides strong evidence of the anthropogenic origin of synthetic materials. The colorant particles observed in this study can have principal sources from the degradation of farming material, such as rope and gallon bottles, that serve as supports for green mussel cultivation [77]. Moreover, colorful microplastics were simply observed by visual inspection during the analysis. According to the color and size of microplastics, it is potentially selected as food by filter-feeding bivalves. Due to varying in microplastic size, i.e., nano- to microplastics, they can be mistaken as prey by various pelagic and benthic marine organisms, including copepods [78], fish [79], and mussels [12]. The abundance of microplastics can be attributed to fishery activities and plastic materials widely used in mussel farms [73].

Considering the plastic types, PET was the dominant polymer type for all locations except S5, where nylon was the most abundant. This observation is more convincing in using different materials in green mussel farms along the coastal area. Additionally, no evident influence on microplastic contents could be observed regarding the sampling period.

Table 2. Microplastic abundance in sea water and sediments in other studies.

Study Area	Mean Density (n/m^3) in Water	Mean Density (n/Kg D.W) in Sediment	References
East China Sea	0.167		Zhao et al., 2014 [18]
Goiana Estuary, Brazil	0.026		Lima et al., 2014 [50]
Tamar Estuary, Southwest England	0.028		Sadri and Thompson, 2014 [51]
Sea of Japan	0.004		Isobe et al., 2015 [52]
Southern Sea of Korea	1.92–5.51		Kang et al., 2015 [63]
Northeastern of Qatar	0.71		Castillo et al., 2016 [80]
Tolo Harbor, Hongkong	0.65–13.08		Tsang et al., 2017 [48]
Coastal waters of Tuscany, Italy	0.26		Baini et al., 2018 [81]
Chaopraya River, Thailand	0.348 ± 0.16 0.610 ± 0.36		Sukhsangchan et al., 2020 [35]
Hainan, China	523		Lin et al., 2022 [49]
Tokyo Bay, Japan		1900	Matsuguma et al., 2017 [67]
Tunisian coast		130.55 ± 65.61	Jaouani et al., 2022 [66]
Gulf of Thailand		150.4 ± 86.2	Wang et al., 2020 [65]
Ban Don Bay, Thailand	$0.63 \pm 0.13 \times 10^3$ 0.28 ± 0.07	15–35	Chinfak et al., 2021 [39]
Phuket (dry season) (rainy season)		300–900 33–400	Jiwarungrueangkul et al., 2021 [38]
Sri Racha Bay, Thailand (dry season) (rainy season)	0.85 ± 0.25 2.06 ± 1.78	48.30 ± 28.17 69.35 ± 22.29	This study

The statistical tests showed no significant differences between mussels related to the sampling location, and the season. Without statistical basis, some light effects of sampling locations could be evidenced. The anthropogenic pressures of the different sites did not lead to different microplastic contaminations. Though, there is a tendency for a higher number of particles in mussels at locations S4 and S5. Moreover, the contamination of cultivated mussels could be explained by proximity to microplastics from the degradation of plastic materials (PET, PE, PP, Nylon) used in aquaculture as collectors, ropes, nets, and pipes used from the spat collection to on growing steps. However, there were no plastic pellets (primary microplastic) in green mussels but some plastic pallets were found in mussels [42,82].

The correlation between biodata (mussel wet weight, shell length, width length, and gender) and quantities of microplastics had been investigated. There were only the correlation between both the shell length and gender with the microplastic quantities even if with low correlation ($r = -0.009$ $p > 0.05$ for weight; $r = -0.062$ $p < 0.05$ for length; $r = -0.011$ $p > 0.05$ for width; $r = 0.154$ $p < 0.01$ for gender). However, the shell length and weight, both are not good indication of age because the growth of the organisms is depended on the local environmental conditions, especially the water quality and the nutrient contents [83]. Many studies suggested no influence of mussel size on microplastic accumulation, such as in mussel in French Atlantic coast [72]; Mediterranean Sea [74], North Sea coasts [24], and blue mussels (Mytilus edulis) on the southwest coast of UK [84]. Otherwise, some previous studies reported sizes of mussels related with microplastic accumulation in farmed and wild mussels along coastal waters of China [42], and green mussels in Thailand [39]. This contrast results might imply that the filtration and feeding rate are not only depended on mussel sizes but also environment factors. Some evidence revealed the efficiency filtration rate of mussels on its size, however filtration, ingestion and absorption also based on the quantities of food in the water and organic contents in water [85]. Higher contents of food (phytoplankton) and organic materials in the water will require lower filtration rates to intake the maximum volume of food, and thus generates faster growth rates [86]. Low salinities influenced from freshwater discharge into the sea sometimes caused a rapid high nutrient loading and then mussel mortality. Salinity and bathymetry are considered to be the important factors affecting the green mussel growth. Hence, our study suggests that mussel shell length may be considered a more reliable indicator of filtration rate and similar suggestion by [87].

Table 3. Comparison of microplastics in aquatic organisms.

Study Area	Density (Items/Ind.)	Species	References
Nova Scotia, Canada	34–178	*Mytilus edulis*	Mathalon and Hill, 2014 [73]
New Zealand	0–0.48	*Perna canaliculus*	Webb et al., 2019 [88]
China	1.5–7.6	*Mytilus edulis*	Li et al., 2016 [42]
Norway	1.5 (±2.3)	*Mytilus* spp.	Bråte et al., 2018 [89]
Mediterranean Sea	1.7–2	*Mytilus galloprovincialis*	Digka et al., 2018 [90]
South Africa	3.4	*Mytilus galloprovincialis*	Sparks, 2020 [76]
	5.6	*Choromytilus meridionalis*	
	2.9	*Aulyacoma ater*	
Ban Don Bay, Thailand	0.50 ± 0.06	*Perna viridis*	Chinfak et al., 2021 [39]
	0.13 ± 0.03	*Meretrix lyrata*	Chinfak et al., 2021 [39]
Eastern Coast, Thailand	0.14	Fish	Phaksopa et al., 2021 [31]
Phuket, Thailand	2.7–5.8	*Perna viridis*	Cherdsukjai et al., 2022 [40]
Sri Racha Bay, Thailand	0.15 ± 0.41	*Perna viridis*	This study
	0.22 ± 0.57	*Perna viridis*	This study

4.4. Dynamics of Microplastics in Sri Racha Bay

The correlation and the transfer path between microplastics in organisms and their living environment are still not well understand. Therefore, we investigated microplastic pollution in the water, sediments, and the mussels in Sri Racha Bay in this study. Some examples of microplastic under the stereo-microscope are shown in Figure 3. Around Sri Racha Bay, there are two types of green mussel culture, namely bamboo-pole and raft culture. This area hosts a relatively moderate human population and has industrial sources, unlike other coastal areas where microplastic abundance has been reported. Sources of microplastics here included those transported through river discharge (agriculture and municipal wastes) and sea-based activities (fisheries, aquaculture, and tourism).

Microplastics were ubiquitous in all samples collected within our study area, reflecting the microplastic pollution of the water and sediment of Sri Racha Bay. The results indicate a low positive correlation between the microplastic abundance of water and sediments with no statistical significance ($r = 0.45$, $p > 0.05$). This result is comparable with the study by [91], which suggested no correlation between sediments and water in Poyang Lake. However, the positive correlation in the present study suggests the higher flow rate or sinking rate of microplastics in water and the tremendous potential of sediments holding microplastics in the study area. A snapshot of microplastic pollution level was not representative enough to reflect the overall situation of a whole season. Repeated sampling for at least two years in the same spatial and temporal trends might be needed to corroborate the conclusion.

The pollution levels of microplastics in the sea surface water, sediment, and biota differed significantly. We found no significant correlation between the number of particles in the sediments (waters) and those found within the mussels at our given sites and periods, but did observe a tendency of positive correlation between number of particles in mussels and particles in their surrounding sediment (water) samples (Figure 4.). Our findings were comparable with those described by [92], who found significant correlations between the abundance of microplastics in surface seawater and microplastics with mussel at sampling sites on the coast of China. Therefore, we believe that microplastics may primarily originate from land-based sources, including inputs from residential areas (e.g., garbage from food packaging, fibers in clothing, and household waste) and farming activities (rope and gallon bottles).

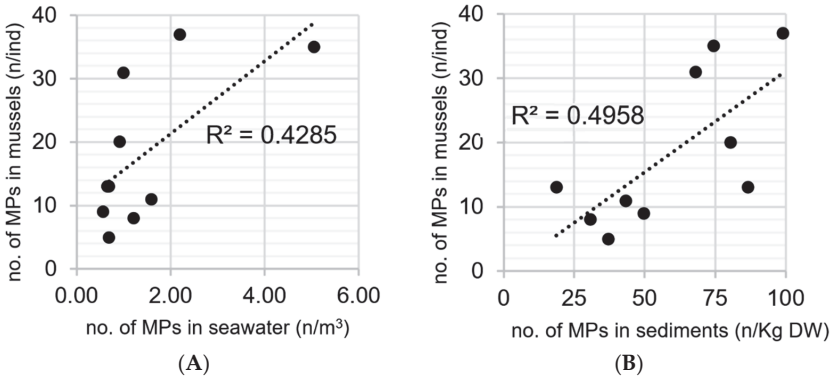


Figure 4. The number of anthropogenic particles in green mussels compared to those in (**A**) the overlying seawater (**B**) the sediment at coastal sites around Sri Racha Bay.

Fibers were the most widespread microplastics found across all environmental media (water, sediment, and soft tissue of green mussels) in our study. Fishery and aquaculture activities are considered potential sources of microplastics. Fishing gear and aquaculture materials, such as plastic ropes, nets, and plastic gallon bottles, are commonly used in this area. Fibers found in the bay may originate from the weathering or fragmentation of these farming materials [2,18,93] or hygiene and cosmetics products and textiles. The bay also received land-based microplastics via discharge, and we theorized that these might be the main contributor to microplastic contamination. Moreover, tidal forcing controlled the amount of microplastics in estuaries [39,93]. As shown in Figure 2, PE, PP, and nylon were found in all surrounding living environments with different proportions. Otherwise, PET, PP, and nylon were the majority of polymer types in green mussel. PE, PP, and nylon are frequently used in aquaculture and fishery in this area. Nylon is usually used in ropes, fishing nets, and cloth. PET and PE are widely used in food packaging, pipes, and plastic containers. The similarity of microplastic compositions (shape and polymer types) between surrounding water and mussels show evidence of the seawater–mussel transfer of microplastics. Therefore, green mussel (*Perna viridis*) can be used as a good indicator for microplastic pollution around this area.

The levels of microplastic in green mussels tend to be relate with their growing area, considering with microplastic types in water and sediments, as pointed out in other studies [42,82,94]. Most microplastics are less dense than seawater and tend to float at the sea surface. One of the most common types of fiber is nylon, which is used for cultivation materials and has negative or neutral buoyancy. Some pieces are taken up by mussels and some sink into the mid-water column or towards the sediment [3,95]. The study area is a coastal area, which is dominated by tide. Therefore, the tidal current causes fine marine debris on the tidal flat to be constantly suspended in the water column, thereby increasing microplastic levels in seawater [96]. The suspended microplastics in seawater may cause mussels to ingest and accumulate microplastics. Although the level of microplastic contamination is comparable low, it is not claimed to undervalue the potential health risks associated with the consumption of MP-contaminated mussels. This provides basic information for a better understanding of the fate of microplastics within an aquaculture area, and for management actions to address microplastics in this area. Generally, an annual survey should be continued in order to monitor the level of microplastic contamination.

5. Conclusions

To the best of our knowledge, this is the first study of microplastic distribution in the surface water, sediment, and green mussels of the Sri Racha Bay, Thailand. Microplastics

were found in all of the water, sediment, and shellfish samples. An abundance of microplastics was found widely in the surface waters and ranged from 0.69 to 1.22 (with average of 0.85 ± 0.25 particles/m^3) during the dry season and 0.57 to 5.04 items/m^3 (with average of 2.06 ± 1.78 particles/m^3) during the wet season, indicating a medium pollution level compared with other coastal area. In the sediments, the abundance ranged from 18.58 to 86.69 (with the average of 48.30 ± 28.17) during the dry season and 43.34 to 99.07 (with the average of 69.35 ± 22.29) items/kg D.W. during the wet season. The concentration of microplastics in green mussels varied from 0.15 ± 0.41 to 0.22 ± 0.57 items/individual, during the dry and wet seasons, respectively, with an average of 0.19 ± 0.50 items/individual. Microplastic abundance in green mussels is negatively correlated with shell length, implying that species with lower length are more likely to have a higher number of microplastics. The microplastic abundance in water and sediments is not influenced by spatial and seasonal variations, whereas the microplastic abundance is lower in the dry season. The spatial trends suggest that microplastic abundance tends to be higher in the northern part. Microplastics in the form of fibers and sheet were the dominant polymer shapes, originating from the secondary microplastics. This study is the first report of microplastic pollution in mussels, seawater, and sediments at the same location in this area. The microplastic abundance in water and sediment is low compared to that which was reported for areas with fewer activities. This should be an incentive for early action to prevent microplastic contamination in Sri Racha Bay from becoming more severe.

Author Contributions: Conceptualization, methodology, data curation, J.P. and R.S.; sample collection, R.S., R.K., K.T. and B.A.; investigation, R.S., R.K. and K.T.; writing—original draft, writing—review and editing, J.P., R.S., S.W. and T.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data sharing is not applicable.

Acknowledgments: The authors would like to thank Sriracha Fisheries Research Station staff for green mussel samples. This work was kindly supported by PTT Global Chemical. We appreciate the provision of Department of Marine Science, Faculty of Fisheries, Kasetsart University. The authors also would like to acknowledge Combating Marine Debris Laboratory members from the Department of Marine Science, Faculty of Fisheries, Kasetsart University.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ryan, P.G.; Moore, C.J.; Van Franeker, J.A.; Moloney, C.L. Monitoring the abundance of plastic debris in the marine environment. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 1999–2012. [CrossRef] [PubMed]
2. Andrady, A.L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62*, 1596–1605. [CrossRef] [PubMed]
3. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. Microplastics as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* **2011**, *62*, 2588–2597. [CrossRef] [PubMed]
4. Gewert, B.; Ogonowski, M.; Barth, A.; MacLeod, M. Abundance and composition of near surface microplastics and plastic debris in the Stockholm Archipelago, Baltic Sea. *Mar. Pollut. Bull.* **2017**, *120*, 292–302. [CrossRef]
5. Naji, A.; Esmaili, Z.; Khan, F.R. Plastic debris and microplastics along the beaches of the Strait of Hormuz, Persian Gulf. *Mar. Pollut. Bull.* **2017**, *114*, 1057–1062. [CrossRef]
6. Robin, R.S.; Karthik, R.; Purvaja, R.; Ganguly, D.; Anandavelu, I.; Mugilarasan, M.; Ramesh, R. Holistic assessment of microplastics in various coastal environmental matrices, southwest coast of India. *Sci. Total Env.* **2020**, *703*, 134947. [CrossRef]
7. Moore, C.J. Synthetic polymers in the marine environment: A rapidly increasing, long-term threat. *Environ. Res.* **2008**, *108*, 131–139. [CrossRef]
8. Andrady, A.L. The plastic in microplastics: A review. *Mar. Pollut. Bull.* **2017**, *119*, 12–22. [CrossRef]
9. Prunier, J.; Maurice, L.; Perez, E.; Gigault, J.; Pierson-Wickmann, A.; Davranche, M.; Ter Halle, A. Trace metals in polyethylene debris from the North Atlantic subtropical gyre. *Environ. Pollut.* **2019**, *245*, 371–379. [CrossRef]
10. Massos, A.; Turner, A. Cadmium, lead and bromine in beached microplastics. *Environ. Pollut.* **2017**, *227*, 139–145. [CrossRef]

11. Li, H.; Wang, F.; Li, J.; Deng, S.; Zhang, S. Adsorption of three pesticides on polyethylene microplastics in aqueous solutions: Kinetics, isotherms, thermodynamics, and molecular dynamics simulation. *Chemosphere* **2021**, *264*, 128556. [CrossRef] [PubMed]
12. Browne, M.A.; Dissanayake, A.; Galloway, T.S.; Lowe, D.M.; Thompson, R.C. Ingested microscopic plastic translocate to the circulatory system of the mussel, *Mytilus edulis* (L.). *Env. Sci. Tech.* **2008**, *42*, 5026–5031. [CrossRef] [PubMed]
13. Browne, M.A.; Niven, S.J.; Galloway, T.S.; Rowland, S.J.; Thompson, R.C. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. *Curr. Biol.* **2013**, *23*, 2388–2392. [CrossRef] [PubMed]
14. Rochman, C.M.; Hoh, E.; Kurobe, T.; Teh, S.J. The Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Sci. Rep.* **2013**, *3*, 3263. [CrossRef]
15. Wright, S.L.; Frank, J.; Kelly, F.J. Plastic and human health: A micro issue? *Environ. Sci. Technol.* **2017**, *51*, 6634–6647. [CrossRef] [PubMed]
16. Prinz, N.; Korez, Š. Understanding How microplastics affect marine biota on the cellular level is important for assessing ecosystem function: A review. In *YOUMARES 9-The Oceans: Our Research, Our Future*; Jungblut, S., Liebich, V., Bode-Dalby, M., Eds.; Springer: Cham, Switzerland, 2020; pp. 101–120.
17. Doyle, M.; Watson, W.; Bowlin, N.; Sheavly, S. Plastic particles in coastal pelagic ecosystems of the Northeast Pacific ocean. *Mar. Environ. Res.* **2011**, *71*, 41–52. [CrossRef]
18. Zhao, S.; Zhu, L.; Wang, T.; Li, D. Suspended microplastics in the surface water of the Yangtze Estuary System, China: First observations on occurrence, distribution. *Mar. Pollut. Bull.* **2014**, *86*, 562–568. [CrossRef]
19. Aytan, U.; Valente, A.; Senturk, Y.; Usta, R.; Sahin, F.B.E.; Mazlum, R.E.; Agirbas, E. First evaluation of neustonic microplastics in Black Sea waters. *Mar. Env. Res.* **2016**, *119*, 22–30. [CrossRef]
20. Jiang, Y.; Zhao, Y.; Wang, X.; Yang, F.; Chen, M.; Wang, J. Characterization of microplastics in the surface seawater of the South Yellow Sea as affected by season. *Sci. Total Env.* **2020**, *724*, 138375. [CrossRef]
21. Zainuddin, A.H.; Aris, A.Z.; Zaki, M.R.M.; Yusoff, F.M.; Wee, S.Y. Occurrence, potential sources and ecological risk estimation of microplastic towards coastal and estuarine zones in Malaysia. *Mar. Pollut. Bull.* **2022**, *174*, 113282. [CrossRef]
22. Jiang, Y.; Yang, F.; Ul Hassan Kazmi, S.S.; Zhao, Y.; Chen, M.; Wang, J. A review of microplastic pollution in seawater, sediments and organisms of the Chinese coastal and marginal seas. *Chemosphere* **2022**, *286 Pt 1*, 131677. [CrossRef] [PubMed]
23. Vianello, A.; Boldrin, A.; Guerriero, P.; Moschino, V.; Rella, R.; Sturaro, A.; Da Ros, L. Microplastic particles in sediments of lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. *Estuar. Coast. Shelf Sci.* **2013**, *130*, 54–61. [CrossRef]
24. Vandermeersch, G.; van Cauwenberghe, L.; Janssen, C.R.; Marques, A.; Granby, K.; Fait, G.; Kotterman, M.J.; Diogène, J.; Bekaert, K.; Robbens, J.; et al. A critical view on microplastic quantification in aquatic organisms. *Environ. Res.* **2015**, *143 Pt B*, 46–55. [CrossRef] [PubMed]
25. Khoironi, A.; Hadiyanto, H.; Anggoro, S.; Sudarno, S. Evaluation of polypropylene plastic degradation and microplastic identification in sediments at tambak lorok coastal area, Semarang Indonesia. *Mar. Pollut. Bull.* **2020**, *151*, 110868. [CrossRef] [PubMed]
26. Wright, S.L.; Thompson, R.C.; Galloway, T.S. The physical impacts of microplastics on marine organisms: A review. *Environ. Pollut.* **2013**, *178*, 483–492. [CrossRef] [PubMed]
27. Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Goodhead, R.; Moger, J.; Galloway, T.S. Microplastic ingestion by zooplankton. *Environ. Sci. Technol.* **2013**, *47*, 6646–6655. [CrossRef]
28. Van Cauwenberghe, L.; Janssen, C.R. Microplastics in bivalves cultured for human consumption. *Environ. Pollut.* **2014**, *193*, 65–70. [CrossRef]
29. Jabeen, K.; Su, L.; Li, J.; Yang, D.; Tong, C.; Mu, J.; Shi, H. Microplastics and mesoplastics in fish from coastal and fresh waters of China. *Environ. Pollut.* **2017**, *221*, 141–149. [CrossRef]
30. Costa, E.; Piazza, V.; Lavorano, S.; Faimali, M.; Garaventa, F.; Gambardella, C. Trophic transfer of microplastics from copepods to jellyfish in the marine environment. *Front. Environ. Sci.* **2020**, *8*, 158. [CrossRef]
31. Phaksopa, J.; Sukhsangchan, R.; Keawsang, R.; Tanapivattanakul, K.; Thamrongnawasawat, T.; Worachananant, S.; Sreesamran, P. Presence and Characterization of Microplastics in Coastal Fish around the Eastern Coast of Thailand. *Sustainability* **2021**, *13*, 13110. [CrossRef]
32. Kibria, G.; Nugegoda, D.; Haroon, A.K.Y. Microplastic pollution and contamination of seafood (including fish, sharks, mussels, oysters, shrimps and seaweeds): A global overview. In *Microplastic Pollution Emerging Contaminants and Associated Treatment Technologies*; Hashmi, M.Z., Ed.; Springer Nature: Cham, Switzerland, 2022; pp. 277–322.
33. Farrell, P.; Nelson, K. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). *Environ. Pollut.* **2013**, *177*, 1–3. [CrossRef] [PubMed]
34. Setälä, O.; Fleming-Lehtinen, V.; Lehtiniemi, M. Ingestion and transfer of microplastics in the planktonic food web. *Environ. Pollut.* **2014**, *185*, 77–83. [CrossRef] [PubMed]
35. Sukhsangchan, R.; Keawsang, R.; Worachananant, S.; Thamrongnawasawat, T.; Phaksopa, J. Suspended microplastics during a tidal cycle in sea-surface waters around Chao Phraya River mouth, Thailand. *ScienceAsia* **2020**, *46*, 724–733. [CrossRef]
36. Pradit, S.; Towatana, P.; Nitiratsuwan, T.; Jualaong, S.; Jirajarus, M.; Sornplang, K.; Noppradit, P.; Darakai, Y. Occurrence of microplastics on beach sediment at Libong, a pristine island in Andaman Sea, Thailand. *ScienceAsia* **2020**, *46*, 336–343. [CrossRef]
37. Jualaong, S.; Pransilpa, M.; Pradit, S.; Towatana, P. Type and Distribution of Microplastics in Beach Sediment along the Coast of the Eastern Gulf of Thailand. *J. Mar. Sci. Eng.* **2021**, *9*, 1405. [CrossRef]
38. Jiwarungrueangkul, T.; Phaksopa, J.; Sompongchaiyakul, P.; Tipmanee, D. Seasonal microplastic variations in estuarine sediments from urban canal on the west coast of Thailand: A case study in Phuket province. *Mar. Pollut. Bull.* **2021**, *68*, 112452. [CrossRef] [PubMed]

39. Chinfak, N.; Sompongchaiyakul, P.; Charoenpong, C.; Shi, H.; Yeemin, T.; Zhang, J. Abundance, composition, and fate of microplastics in water, sediment, and shellfish in the Tapi-Phumduang River system and Bandon Bay, Thailand. *Sci. Total Env.* **2021**, *781*, 146700. [CrossRef] [PubMed]
40. Cherdsukjai, P.; Vongpanich, V.; Akkajit, P. Preliminary Study and First Evidence of Presence of Microplastics in Green Mussel, Perna viridis from Phuket. *App. Env. Res.* **2022**, *44*, 28–41. [CrossRef]
41. Argamino, C.R.; Janairo, J.I.B. Qualitative assessment and management of micro-plastics in Asian green mussels (Perna viridis) cultured in Bacoor Bay, Cavite, Phillipine. *EnvironmentAsia* **2016**, *9*, 48–54.
42. Li, J.; Qu, X.; Su, L.; Zhang, W.; Yang, D.; Kolandhasamy, P.; Li, D.; Shi, H. Microplastics in mussels along the coastal waters of China. *Environ. Pollut.* **2016**, *214*, 177–184. [CrossRef]
43. Li, J.; Lusher, A.L.; Rotchell, J.M.; Deudero, S.; Turra, A.; Bråte, I.L.N.; Sun, C.; Hossain, M.S.; Li, Q.; Kolandhasamy, P.; et al. Using mussel as a global bioindicator of coastal microplastic pollution. *Environ. Pollut.* **2019**, *244*, 522–533. [CrossRef] [PubMed]
44. Cholumpai, V.; Kachanopas-Barnette, P.; Tubmeka, S.; Wu, R.S.S. Contamination of Cadmium and Mercury along the East Coast of the Gulf of Thailand: A Comparative Study Using the Green Mussel (*Perna viridis*), Artificial Mussel, Water and Sediment. *Am. Acad. Sci. Res. J. Eng. Tech. Sci.* **2020**, *73*, 61–75.
45. Peng-ngeiw, P.; Jaritkhuan, S.; Tunkijjanukij, S. Effect of Green Mussel Raft Culture to Total Organic Matter (TOM) and Flux of Dissolved Inorganic Nitrogen (DIN) in Sriracha Bay, Chon Buri Province. *Burapha Sci. J.* **2021**, *26*, 270–284.
46. Lamsawat, P.; Phaksopa, J.; Sojisuporn, P. Tracking of Floating Objects in the Area of Chao Phraya River Mouth by Using Numerical Model. *Burapha Sci. J.* **2022**, *25*, 953–967.
47. Masura, J.; Baker, J.; Foster, G.; Arthur, C. *Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommendations for Quantifying Synthetic Particles in Waters and Sediments*; NOAA Technical Memorandum NOS-OR&R-48; NOAA: Silver Spring, MD, USA, 2015; pp. 1–39.
48. Tsang, Y.Y.; Mak, C.W.; Liebich, C.; Lam, S.W.; Sze, E.T.-P.; Chan, K.M. Microplastic pollution in the marine waters and sediments of Hong Kong. *Mar. Pollut. Bull.* **2017**, *115*, 20–28. [CrossRef]
49. Lin, F.; Zhang, Q.; Xie, J.; Lin, Y.; Chen, Y.; Mao, K.; Qin, Y.; Diao, X. Microplastics in biota and surface seawater from tropical aquaculture area in Hainan, China. *Gondwana Res.* **2022**, *108*, 41–48. [CrossRef]
50. Lima, A.R.A.; Costa, M.F.; Barletta, M. Distribution patterns of microplastics within the plankton of a tropical estuary. *Environ. Res.* **2014**, *132*, 146–155. [CrossRef]
51. Sadri, S.S.; Thompson, R.C. On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England. *Mar. Pollut. Bull.* **2014**, *81*, 55–60. [CrossRef]
52. Isobe, A.; Uchida, K.; Tokai, T.; Iwasaki, S. East Asian seas: A hot spot of pelagic microplastics. *Mar. Pollut. Bull.* **2015**, *101*, 618–623. [CrossRef]
53. Campanale, C.; Stock, F.; Massarelli, C.; Kochleus, C.; Bagnuolo, G.; Reifferscheid, G.; Uricchio, V.F. Microplastics and their possible sources: The example of Ofanto river in southeast Italy. *Environ. Pollut.* **2020**, *258*, 113284. [CrossRef]
54. Malli, A.; Corella-Puertas, E.; Hajjar, C.; Boulay, A.M. Transport mechanisms and fate of microplastics in estuarine compartments: A review. *Mar. Pollut. Bull.* **2022**, *177*, 113553. [CrossRef] [PubMed]
55. Barnes, D.K.; Galgani, F.; Thompson, R.C.; Barlaz, M. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 1985–1998. [CrossRef] [PubMed]
56. Eerkes-Medrano, D.; Thompson, R.C.; Aldridge, D.C. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res.* **2015**, *75*, 63–82. [CrossRef] [PubMed]
57. Higuchi, M.; Anongponyoskun, M.; Phaksopa, J.; Onishi, H. Influence of monsoon-forced Ekman transport on sea surface height in the Gulf of Thailand. *Agri. Nat. Res.* **2022**, *54*, 205–210.
58. Kim, I.S.; Chae, D.H.; Kim, S.K.; Choi, S.; Woo, S.B. Factors influencing the spatial variation of microplastics on high-tidal coastal beaches in Korea. *Arch. Environ. Contam. Toxicol.* **2015**, *69*, 299–309. [CrossRef]
59. Aliabad, M.K.; Nassiri, M.; Kor, K. Microplastics in the surface seawaters of Chabahar Bay, Gulf of Oman (Makran coasts). *Mar. Pollut. Bull.* **2019**, *143*, 125–133. [CrossRef]
60. Zheng, Y.; Li, J.; Cao, W.; Liu, X.; Jiang, F.; Ding, J.; Yin, X.; Sun, C. Distribution characteristics of microplastics in the seawater and sediment: A case study in Jiaozhou Bay, China. *Sci. Total Environ.* **2019**, *674*, 27–35. [CrossRef] [PubMed]
61. Wei, Y.; Ma, W.; Xu, Q.; Sun, C.; Wang, X.; Gao, F. Microplastic Distribution and Influence Factor Analysis of Seawater and Surface Sediments in a Typical Bay with Diverse Functional Areas: A Case Study in Xincun Lagoon, China. *Front. Env. Sci.* **2022**, *10*, 1–10. [CrossRef]
62. Su, L.; Xue, Y.; Li, L.; Yang, D.; Kolandhasamy, P.; Li, D.; Shi, H. Microplastics in Taihu lake, China. *Environ. Pollut.* **2016**, *216*, 711–719. [CrossRef]
63. Kang, J.H.; Kwon, O.Y.; Lee, K.W.; Song, Y.K.; Shim, W.J. Marine neustonic microplastics around the southeastern coast of Korea. *Mar. Pollut. Bull.* **2015**, *96*, 304–312. [CrossRef]
64. Krelling, A.P.; Souza, M.M.; Williams, A.T.; Turra, A. Transboundary movement of marine litter in an estuarine gradient: Evaluating sources and sinks using hydrodynamic modelling and ground truthing estimates. *Mar. Pollut. Bull.* **2017**, *119*, 48–63. [CrossRef] [PubMed]
65. Wang, Y.; Zou, X.; Peng, C.; Qiao, S.; Wang, T.; Yu, W.; Khokiattiwong, S.; Kornkanitnan, N. Occurrence and distribution of microplastics in surface sediments from the Gulf of Thailand. *Mar. Pollut. Bull.* **2020**, *152*, 110916. [CrossRef] [PubMed]

66. Jaouani, R.; Mouneyrac, C.; Châtel, A.; Amiard, F.; Dellali, M.; Beyrem, H.; Michelet, A.; Lagarde, F. Seasonal and spatial distribution of microplastics in sediments by FTIR imaging throughout a continuum lake-lagoon-beach from the Tunisian coast. *Sci. Total Env.* **2022**, *838 Pt 4*, 156519. [CrossRef] [PubMed]
67. Matsuguma, Y.; Takada, H.; Kumata, H.; Kanke, H.; Sakurai, S.; Suzuki, T.; Itoh, M.; Okazaki, Y.; Boonyatumanond, R.; Zakaria, M.P.; et al. Microplastics in sediment cores from Asia and Africa as indicators of temporal trends in plastic pollution. *Arch. Environ. Con. Tox.* **2017**, *73*, 230–239. [CrossRef] [PubMed]
68. Murray, F.; Cowie, P.R. Plastic contamination in the decapod crustacean *Nephrops Norvegicus* (Linnaeus, 1758). *Mar. Pollut. Bull.* **2011**, *62*, 1207–1217. [CrossRef] [PubMed]
69. Coppock, R.L.; Lindeque, P.K.; Cole, M.; Galloway, T.S.; Näkki, P.; Birgani, H.; Richards, S.; Queirós, A.M. Benthic fauna contribute to microplastic sequestration in coastal sediments. *J. Hazard. Mater.* **2021**, *415*, 125583. [CrossRef]
70. Vichkovitten, T.; Intarachart, A.; Khaodon, K. Impact of Green Mussel (*Perna Viridis*) Raft-Culture on Benthic Environment in Sriracha Coastal Water, Thailand. *GMSARN Int. J.* **2017**, *11*, 116–122.
71. Luesiri, M.; Boonsanit, P.; Lirdwitayaprasit, T.; Pairohakul, S. Filtration rates of the green-lipped mussel *Perna viridis* (Linnaeus, 1758) exposed to high concentration of suspended particles. *ScienceAsia* **2022**, *48*, 452–458. [CrossRef]
72. Phuong, N.N.; Poirier, L.; Pham, Q.T.; Lagarde, F.; Zalouk-Vergnoux, A. Factors influencing the microplastic contamination of bivalves from the French Atlantic coast: Location, season and/or mode of life? *Mar. Pollut. Bull.* **2018**, *129*, 664–674. [CrossRef]
73. Mathalon, A.; Hill, P. Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Mar. Pollut. Bull.* **2014**, *81*, 69–79. [CrossRef]
74. De Witte, B.; Devriese, L.; Bekaert, K.; Hoffman, S.; Vandermeersch, G.; Cooreman, K.; Robbens, J. Quality assessment of the blue mussel (Mytilus edulis): Comparison between commercial and wild types. *Mar. Pollut. Bull.* **2014**, *85*, 146–155. [CrossRef] [PubMed]
75. Naidu, S.A. Preliminary study and first evidence of presence of microplastics and colorants in green mussel, *Perna viridis* (Linnaeus, 1758), from southeast coast of India. *Mar. Pollut. Bull.* **2019**, *140*, 416–422.
76. Sparks, C. Microplastics in Mussels Along the Coast of Cape Town, South Africa. *Bull. Environ. Contam. Toxicol.* **2020**, *104*, 423–431. [CrossRef]
77. Castro, R.O.; Silva, M.L.; Marques, M.R.C.; de Araújo, F.V. Evaluation of microplastics in Jurujuba Cove, Niterói, RJ, Brazil, an area of mussels farming. *Mar. Pollut. Bull.* **2016**, *110*, 555–558. [CrossRef]
78. Lee, K.W.; Shim, W.J.; Kwon, O.Y.; Kang, J.H. Size-dependent effects of micro polystyrene particles in the marine copepod Tigriopus japonicus. *Env. Sci. Tech.* **2013**, *47*, 11278–11283. [CrossRef] [PubMed]
79. Ory, N.C.; Sobral, P.; Ferreira, J.L.; Thiel, M. Amberstripe scad Decapterus muroadsi (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. *Sci. Total Env.* **2017**, *586*, 430–437. [CrossRef]
80. Castillo, A.B.; Al-Maslamani, I.; Obbard, J.P. Prevalence of microplastics in the marine waters of Qatar. *Mar. Pollut. Bull.* **2016**, *111*, 260–267. [CrossRef]
81. Baini, M.; Fossi, M.C.; Galli, M.; Caliani, I.; Campani, T.; Finoia, M.G.; Panti, C. Abundance and characterization of microplastics in the coastal waters of Tuscany (Italy): The application of the MSFD monitoring protocol in the Mediterranean Sea. *Mar. Pollut. Bull.* **2018**, *133*, 543–552. [CrossRef]
82. Li, J.; Yang, D.; Li, L.; Shi, H. Microplastics in commercial bivalves from China. *Environ. Pollut.* **2015**, *207*, 190–195. [CrossRef]
83. Gosling, E.M. *Bivalve Molluscs: Biology, Ecology and Culture*; Fishing News Books: Oxford, UK, 2003; p. 443.
84. Scott, N.; Porter, A.; Santillo, D.; Simpson, H.; Lloyd-Williams, S.; Lewis, C. Particle characteristics of microplastics contaminating the mussel Mytilus edulis and their surrounding environments. *Mar. Pollut. Bull.* **2019**, *146*, 125–133. [CrossRef]
85. Wong, W.; Cheung, S. Seasonal variation in the feeding physiology and scope for growth of green mussels, *Perna viridis* in estuarine Ma Wan, Hong Kong. *J. Mar. Bio. Ass. UK* **2003**, *83*, 543–552. [CrossRef]
86. Hawkins, A.J.S.; Smith, R.F.M.; Tan, S.H.; Yasinm, Z.B. Suspension-feeding behaviour in tropical bivalve molluscs: *Perna viridis*, *Crassostrea belcheri*, *Crassostrea iradelei*, *Saccostrea cucculata* and *Pinctada margarifera*. *Mar. Ecol. Prog. Ser.* **1998**, *166*, 173–185. [CrossRef]
87. Riisgård, H.U.; Larsen, P.S.; Pleissner, D. Allometric equations for maximum filtration rate in blue mussels *Mytilus edulis* and importance of condition index. *Helgol. Mar. Res.* **2014**, *68*, 193–198. [CrossRef]
88. Webb, S.; Ruffell, H.; Marsden, I.; Pantos, O.; Gaw, S. Microplastics in the New Zealand green lipped mussel *Perna canaliculus*. *Mar. Pollut. Bull.* **2019**, *149*, 110641. [CrossRef]
89. Bråte, I.L.N.; Hurley, R.; Iversen, K.; Beyer, J.; Thomas, K.V.; Steindal, C.C.; Green, N.W.; Olsen, M.; Lusher, A. Mytilus spp. as sentinels for monitoring microplastic pollution in Norwegian coastal waters: A qualitative and quantitative study. *Env. Pollut.* **2018**, *243*, 383–393. [CrossRef]
90. Digka, N.; Tsangaris, C.; Torre, M.; Anastasopoulou, A.; Zeri, C. Microplastics in mussels and fish from the Northern Ionian Sea. *Mar. Pollut. Bull.* **2018**, *135*, 30–40. [CrossRef]
91. Yuan, W.; Liu, X.; Wang, W.; Di, M.; Wang, J. Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China. *Ecotoxicol. Environ. Saf.* **2019**, *170*, 180–187. [CrossRef]
92. Qu, X.; Su, L.; Li, H.; Liang, M.; Shi, H. Assessing the relationship between the abundance and properties of microplastics in water and in mussels. *Sci. Total Environ.* **2018**, *621*, 679–686. [CrossRef]
93. Browne, M.A.; Crump, P.; Niven, S.J.; Teuten, E.; Tonkin, A.; Galloway, T.; Thompson, R. Accumulation of microplastic on shorelines woldwide: Sources and sinks. *Env. Sci. Tech.* **2011**, *45*, 9175–9179. [CrossRef]

94. Van Cauwenberghe, L.; Devriese, L.; Galgani, F.; Robbens, J.; Janssen, C.R. Microplastics in sediments: A review of techniques, occurrence and effects. *Mar. Environ. Res.* **2015**, *111*, 5–17. [CrossRef]
95. Andrady, A.L. Persistence of plastic litter in the oceans. In *Marine Anthropogenic Litter*; Springer: Cham, Switzerland, 2015; pp. 57–72.
96. Cho, Y.; Shim, W.J.; Jang, M.; Han, G.M.; Hong, S.H. Abundance and characteristics of microplastics in market bivalves from South Korea. *Env. Poll.* **2019**, *245*, 1107–1116. [CrossRef] [PubMed]

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Experimental Investigation of Water-Retaining and Unsaturated Infiltration Characteristics of Loess Soils Imbued with Microplastics

Jiahui Gu [1,2], Liang Chen [1,2], Yu Wan [1,2,*], Yaozong Teng [1,2], Shufa Yan [1,2] and Liang Hu [1,2]

[1] School of Civil Engineering and Transportation, Hohai University, Nanjing 210098, China
[2] Key Laboratory of Geomechanics and Embankment Engineering, Hohai University, Nanjing 210098, China
* Correspondence: wy0209@hhu.edu.cn; Tel.: +86-183-0517-6096

Abstract: Microplastics are abundant in agricultural soils and have significant impacts on rainfall infiltration and soil water-retaining capacity. To explore the effect of microplastics on agricultural soil permeability by simulating the rainfall irrigation process, a one-dimensional vertical soil column rainfall infiltration test device was used to study the unsaturated infiltration characteristics of loess soil imbued with microplastics under rainfall conditions. The following conclusions could be obtained: the microplastic content (q), the microplastic particle size (p), and the soil density (γ) have effects on rainfall infiltration; the soil water-retaining capacity would be weakened owing to the existence of microplastics; and intermittent rainfall is preferred in agricultural irrigation. Finally, the permeability coefficient (k) and average flow rate (V) of the unsaturated soil are deduced together, and the relationship between the permeability coefficient (k) and the matrix suction (ψ) of the unsaturated loess soil containing microplastics is calculated by an example, proving good consistency between the experimental results and theoretical calculations. Microplastics represent negative effects on rainfall infiltration and soil water retention, so it is recommended to dispose of them.

Keywords: rainfall infiltration; water-retaining; loess soils; microplastics; volumetric moisture content; permeability coefficient

Citation: Gu, J.; Chen, L.; Wan, Y.; Teng, Y.; Yan, S.; Hu, L. Experimental Investigation of Water-Retaining and Unsaturated Infiltration Characteristics of Loess Soils Imbued with Microplastics. *Sustainability* **2023**, *15*, 62. https://doi.org/10.3390/su15010062

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

Received: 22 October 2022
Revised: 30 November 2022
Accepted: 13 December 2022
Published: 21 December 2022

1. Introduction

As a new type of pollutant found globally, microplastics are distributed from ocean to land, even at the North and South Poles [1]. Studies have shown that microplastics can change soil properties and impact plant growth [2]. Several studies have suggested that more microplastics exist on land than in the ocean [3,4] and that microplastics are imported from land to the ocean [5–7]. The sources of microplastics in agricultural soil are diverse and include the employment of plastic film and mulch, crop planting and fertilizing, irrigation water, the utilization of sludge, and atmospheric deposition [8–10]. The agricultural soil moisture cycle generally refers to the migration and transformation of rainwater, irrigation water, and steam water in unsaturated soil regions above groundwater. Microplastics are characterized by their small size, strong hydrophobicity, and relatively stable properties. Their enrichment in soil participates in the water cycle of agricultural soil and affects the water-retaining and unsaturated infiltration of agricultural soil, which directly affects the usage of water resources and plant growth, especially in areas affected by soil erosion [11–13]. As the soil type with the largest distribution area on the Loess Plateau, loess soil areas are experiencing the most serious soil erosion in China, and the change in their water-retaining ability and water permeability is of great importance for crop yields. Water resources are very significant in these areas, and it is of great necessity to make full use of rain.

Currently, research on microplastics has focused on microplastic traceability [14–17], microplastic distribution [18–20], microplastic transportation mechanisms [21–25], mi-

croplastic enrichment principles [14,17,26,27], and microplastic management methods [28–32]. There are few studies on the effects of microplastics on soil water-retaining ability and water permeability, and most have focused on measuring the coefficient of permeability by the hydrostatic head method, which reflects the change in soil water permeability in the presence or absence of microplastics [11]. However, the coefficient of permeability obtained by the hydrostatic head method represents the permeability coefficient of saturated soil; agricultural soil is normally in an unsaturated state, and the hydrostatic head method cannot reflect the changing water permeability and water-retaining ability of agricultural soil during the infiltration process [33,34]. Meanwhile, rainfall infiltration of soil is an unsaturated process, and the factors affecting rainfall infiltration embrace two aspects. One is rainfall conditions, such as rainfall type, intensity, and duration. The other is soil properties, such as soil type, infiltration characteristics, and initial moisture content [35,36]. With regard to the abundance of microplastics, studies on rainfall infiltration and the unsaturated permeability coefficient curves of soils containing microplastics are still lacking.

This study takes loess soils as the research object to explore the water-retaining and unsaturated infiltration characteristics of soil imbued with microplastics under rainfall conditions. To simulate the rainfall irrigation process, experiments based on the wetting front advancing method employ a one-dimensional vertical soil column rainfall infiltration test device to study the influence of the microplastic content (q), microplastic particle size (p), soil bulk density (γ), and intermittent rainfall ratio (i) on loess soil rainfall infiltration by analyzing the infiltration rate (λ), cumulative infiltration amount (Q), wetting peak depth (H), and average conductivity (C). The change law of volumetric moisture content (θ) at monitoring points could reflect soil water-retaining ability. Combined with the effective fitting of the power function of the wet peak bulk density advancing curve, the permeability coefficient (k) and average flow rate (V) of the unsaturated soil are deduced together and the relationship between the permeability coefficient (k) and the matrix suction (ψ) of the unsaturated loess soil with microplastics is calculated by an example.

2. Materials and Test Methods

2.1. Materials

Loess soils are weakly developed soils and easily damaged. Loess soils are typical soils of the Loess Plateau. Particle size distribution analyses carried out on the studied sample reveal the following composition: 75.15% sand (particle size range 0.02~2 mm), 19.32% silt (particle size range 0.002~0.02 mm), and 5.53% clay (particle size range less than 0.002 mm) according to the international classification system of soil texture. The grain-size distribution curve is in A1 of the Appendix A. The maximum dry density and the minimum dry density of selected loess soil in the Shanbei area were 1.8 g/cm^3 and 1.5 g/cm^3 as measured by experiments. White spherical polystyrene plastics with good sorting and stability were used as microplastics for testing. The density of the spherical polystyrene plastics was 1.06 g/cm^3, with a particle size deviation of less than 10%.

The one-dimensional vertical rainfall infiltration device consists of 4 parts, as shown in Figure 1a: a soil column, rainfall system (a peristaltic pump and a rainmaker), rainfall control system (a marsh flask), and data-acquisition equipment (EC-5 soil moisture sensors and a MIK-R9600 paperless recorder). A 140 mm inner diameter, 150 mm external diameter, 295 mm high plexiglass column was used as a container for the soil samples. A drainage port was set at the lower end of the one-dimensional vertical soil column to facilitate drainage. A peristaltic pump was used to regulate the amount of water entering the rainmaker to control rainfall intensity. A rainmaker was employed to create even rainfall. Four EC-5 soil moisture sensors were inserted into the soil column to monitor the volumetric water content θ or conductivity of the soil over time. The soil moisture sensors were numbered #4, #3, #2, and #1 from the top to the bottom of the soil column. The generation of surface runoff occurred in the later period of rainfall. The tensiometers were employed to measure the soil matric suction (ψ). All data were recorded by the MIK-R9600 paperless

recorder automatically. The apparatus structure diagram of the rainfall infiltration test device is shown in Figure 1b.

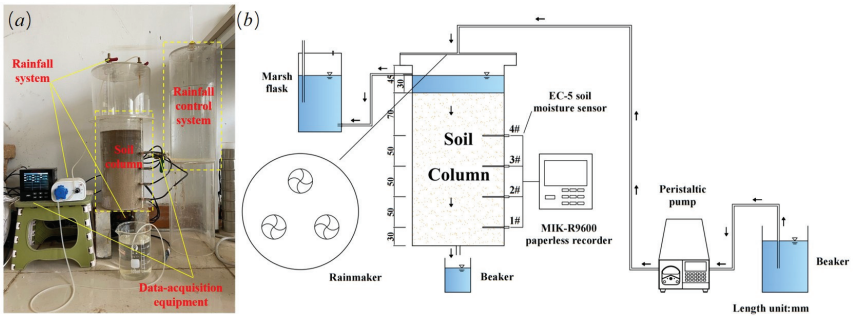

Figure 1. Experimental apparatus: (**a**) photograph; (**b**) schematic.

2.2. Test Scheme and Experiment Procedure

The study designed 13 sets of experiments with different microplastic content (q), microplastic particle size (p), soil bulk density (γ), and intermittent rainfall ratio (i) and the test scheme is shown below (Table 1). The rainfall intensity was set to 20 mm/h, which referred to the real rainfall situation in the local area, the same as the rain interval ratio (i). The i values were set to 1:2, 1:1, and 2:1, with 30 min for rain and 60 min for rest, 30 min for rain and 30 min for rest, and 60 min for rain and 30 min for rest, respectively.

Table 1. Test scheme.

No	Rain Interval Ratio i	Soil Bulk Density γ (g/cm^3)	Microplastic Content q (%)	Microplastic Particle Size p (μm)
1	\	1.57	0.00	5
2	\	1.57	0.05	5
3	\	1.57	0.10	5
4	\	1.57	0.25	5
5	\	1.57	0.50	5
6	\	1.57	0.25	3
7	\	1.57	0.25	8
8	\	1.61	0.25	5
9	\	1.65	0.25	5
10	\	1.73	0.25	5
11	1:2	1.57	0.25	5
12	1:1	1.57	0.25	5
13	2:1	1.57	0.25	5

The study assessed the influence of the 4 parameters above by analyzing the infiltration rate (λ), cumulative infiltration amount (Q), wetting peak height (H), and the change law of volumetric moisture content (θ) at monitoring points. The procedure includes 4 parts:

(1) Prepare the device and fill the column with loess.
(2) Ensure the rain interval ratio and adjust the rainfall system and the rainfall control system.
(3) Strat the experiment until soil enters the saturated infiltration stage.
(4) Collect data and undertake analysis.

2.3. Device Preparation

The density flotation method was used to wash the soil sample many times before the experiments to remove microplastics that had already been present in the soil sample began, and then the sample was dried. Invasive substances, such as litter and plant rhizomes in

the soil sample, were removed to avoid affecting. The washed soil particles were stored in a cool and dry place for later use. The structure and porosity of loess were not disturbed during experiments. Spherical microplastics of different particle sizes were prepared in advance for standby application.

The samples were divided into 6 parts after weighing the soil mass required for each test. The soil column was filled and compacted in layers. Each layer of soil sample was shaved to ensure uniform sample loading and consistent compaction over the entire height to ensure soil homogeneity. Before loading the sample, a filter was attached to the water outlet at the bottom of the soil column to prevent the soil sample from flowing out of the water outlet and damaging the soil sample. Each time a soil sample layer was loaded, a soil moisture sensor and tensiometer were inserted in a timely fashion, especially the tensiometer. If the tensiometer was left in the air for too long, vaporization occurred. All apparatuses were inserted slowly to prevent apparatus damage and soil disturbance during the process. The second layer was filled only when the data on the MIK-R9600 data paperless recorder were normal. In a similar fashion, the sample loading process was implemented until the soil reached the specified height.

During the test, the wetting peak height (H) was measured by a 50 cm steel ruler, which was measured every 5 min in the early stage, every 10 min in the middle stage, and every 20 min or 30 min in the later stage. In the later stage, when the accumulated water height reached the height required by the experiments, a Marsh flask was used to control the accumulated water height. The accumulated water height remained at 3 cm, which was used to simulate a state where the accumulated water height remained unchanged after the generation of surface runoff in the later period of rainfall. During the test, EC-5 moisture sensors and the MIK-R9600 paperless recorder collected data at a frequency of 1 data point per minute. The test environment temperature was relatively stable and remained at 26~27 °C.

3. Results and Discussions

3.1. Effect of Microplastic Contents q on Moisture Transportation

A parameter infiltration rate (λ) (unit: mm/min) is defined, which indicates the amount of water infiltrated per unit area of the soil surface per unit time. The cumulative infiltration amount (Q) (unit: mm) is the cumulative infiltration amount per unit area of the soil column from the beginning of rainfall to a certain time. The average conductivity (C) of soil is the variation in the wetting peak depth (Δh) in a certain period of time (Δt). An analysis of the test results from No. 1 to No. 5 are shown below.

The time–history curve of infiltration rate λ (Figure 2a) reflects the slope change in the cumulative infiltration amount (Q) time–history curve (Figure 2b), and the time–history curve of average conductivity (C) (Figure 2d) reflects the slope change in the wetting peak depth (H) time–history curve (Figure 2c) under conditions of different microplastic contents (q). The minimum infiltration rate (λ_{min}) with a q value of 0.05% is larger than that of the blank experimental control group, while λ_{min} with a q of 0.10%, 0.25%, and 0.50% is smaller than that of the blank experimental control group. Compared with the blank experiment control group, when q is 0.05%, the Q value is the largest, the time required for rainwater infiltration into the soil column bottom is the shortest, and the λ value continues to decline and fluctuates greatly. When q values are 0.10%, 0.25%, and 0.50%, the Q values gradually decrease with increasing q and all are smaller than the Q value of the blank experimental control group. With the increase in the content, the time required for rainwater infiltration into the bottom of the soil column gradually increased, and it was longer than that of the blank control group. Analyzing the minimum infiltration rate (λ_{min}) (Figure 2a) and the time for rainwater infiltration into the bottom of the soil column (Figure 2b,c) compared with the blank experimental control group, it could be speculated that microplastics with q values of 0.05% promote rainwater infiltration, while microplastics with q values of 0.10%, 0.25%, and 0.50% hinder rainwater infiltration, and the content change does not affect the

minimum infiltration ability. With increasing q values, the effect of blocking rainwater infiltration is stronger.

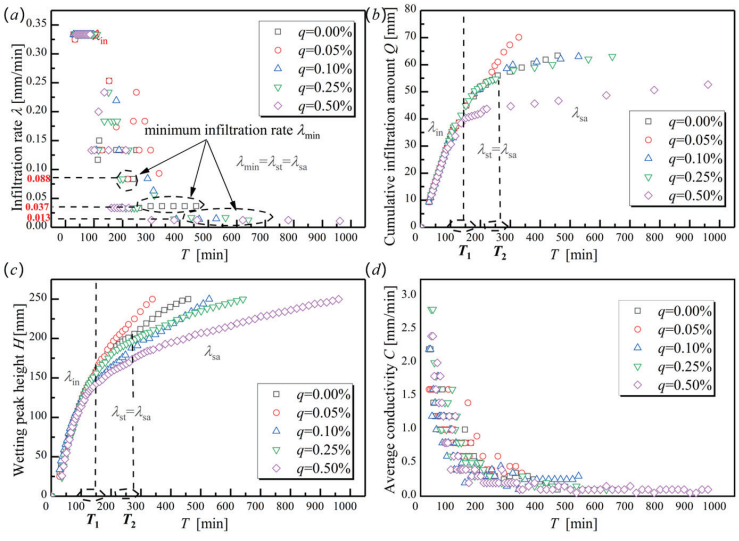

Figure 2. Time–history curve under different microplastic q: (**a**) infiltration rate λ; (**b**) cumulative infiltration amount Q; (**c**) wetting peak depth H; (**d**) average conductivity C.

The soil sample with q of 0.05% did not represent stable infiltration, and the rainfall infiltration process showed no pressure infiltration or pressure infiltration. The rainfall infiltration shows three stages: no pressure infiltration, pressure infiltration, and saturated infiltration with regard to the q values of 0%, 0.1%, 0.25%, and 0.5%. According to Figure 2a,b and Table 1, in the no-pressure infiltration stage, the initial infiltration rates (λ_{in}) are constant and equal to the rainfall intensity. The water begins to accumulate on the soil column surface, and the surface soil enters a transient saturation state. The soil moisture content is low, and the matrix suction is large. The time–history curves represent slanted straight lines, and the average conductivity (C) is large, which means that rainwater is quickly absorbed and continuously transmitted to the interior of the soil. The no-pressure infiltration stage ends at T_1 with the existence of the accumulation point as a symbol. In the pressure infiltration stage, the λ value gradually decays to a stable value with rainfall duration; that is, the infiltration rate (λ_{st}) is stable, and the water height on the soil column surface changes stepwise to a stable state. The soil mass on the surface transfers to a fully saturated state. The curves in Figure 2b show an upward convex shape. The increase in magnitude in wetting peak depth (H) also decreases gradually at the same time, and the C values begin to decrease rapidly with rainfall duration. The pressure stage ends at T_2 with the existence of the saturated point as a symbol. In the saturated infiltration stage, the time–history curve of cumulative infiltration is a straight line, and its slope λ tends to be constant, which is the saturated infiltration rate (λ_{sa}) and is equal to the permeability coefficient of stable soil infiltration ($\lambda_{st} = \lambda_{sa} = \lambda_{min}$). The soil column undergoes saturated infiltration, and the C value tends to be stable. According to Table 2, microplastics with a q of 0.05% promote rainfall infiltration, and the water accumulation time is much longer than that of the other test samples. For other microplastic content samples, the more q values there were, the earlier the water accumulation point and saturation point appeared.

Table 2. Time of water accumulation points and saturation points.

Microplastic Content q/%	Accumulation Points T_1/min	Saturation Points T_2/min	Time Difference $(T_2 - T_1)$/min
0.00	150	305	155
0.05	192	— —	— —
0.10	140	290	150
0.25	136	280	144
0.50	91	220	129

The existence of polystyrene microplastics in soils could fill the soil particle skeletons to bond and block water, and polystyrene microplastics themselves have strong hydrophobicity. Current research [37–40] shows that soil particles are often regarded as completely hydrophilic solids. The contact angle of soil increases and soil particles represent hydrophobic properties owing to the addition of hydrophobic materials. Meanwhile, microplastics would fill pores of soil particles and decrease the soil permeability coefficient, representing blocking effects. The two effects are contradictory and would play a dominant role in different situations with the change in soil compactness. When p is constant and q is relatively small, the strong hydrophobicity of microplastics plays a dominant role. Microplastics promote water infiltration. With q increasing, microplastics compact soil particles, representing blocking effects overall. The blocking effects enhance as q increases.

To explore the soil water-retaining capacity, the volume moisture content measured as θ by EC-5 moisture sensors with different q values are analyzed below.

As shown in Figure 3, there is little difference in the time required for water to reach sensors #3 and #4. The time taken for water to reach sensors #1 and #2 is the shortest when q is 0.05%, while the time for water to transfer to sensors #1 and #2 gradually increases with increasing q values compared with other microplastic contents (q). The θ_{max} values with different q values are all less than that of the blank experiment control group. With q values increasing, the peak soil moisture content (θ_{max}) slightly decreases. Microplastics weaken the water-retaining capacity of loess soil due to the hydrophobic properties of microplastics.

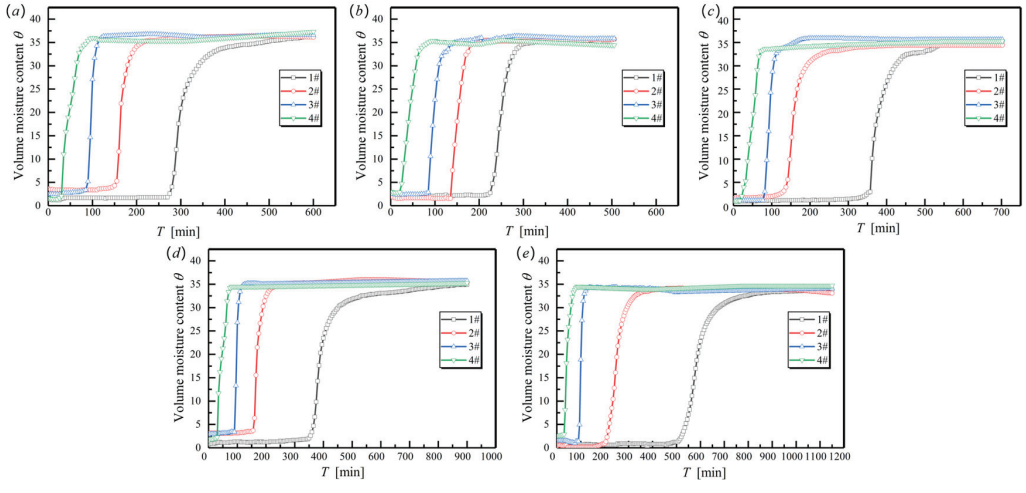

Figure 3. Time–history curve of volumetric moisture content θ at monitoring points with different microplastic contents q: (**a**) q = 0.00%; (**b**) q = 0.05%; (**c**) q = 0.10%; (**d**) q = 0.25%; (**e**) q = 0.50%.

3.2. *Effect of Microplastic Particle Size p on Moisture Transportation*

In this study, the microplastic content (q) is 0.25% and the soil bulk density (γ) is 1.57 g/cm^3. By analyzing the test results from No. 4, No. 6, and No. 7, the results are shown below.

Similar to the analysis above, microplastics with a p of 3 μm were able to promote rainfall infiltration compared with the blank experimental control group. By supplementing rainwater infiltration tests (A2 of the Appendix A) with a p of 3 μm and a q of 0.00%, 0.05%, 0.10%, 0.25%, and 0.50%, no water may accumulate on the soil column surface for q values of 0.05%, 0.10%, and 0.25%, and the water accumulates on the surface for a q of 0.50%, indicating that with the decrease in p values, the q that promotes water infiltration increases, and the hydrophobic effect is obviously enhanced. Microplastics hinder water infiltration with p values of 5 μm and 8 μm. The microplastic amount with a p of 8 μm is less than that with a p of 5 μm, and the soil with a p of 8 μm is looser. The saturated infiltration rate is larger with a p of 8 μm. Therefore, microplastics with a p of 8 μm promote rainfall infiltration compared with microplastics with a p of 5 μm.

The soil sample with a p of 3 μm represents the no-pressure infiltration stage, while the rainfall infiltration shows no-pressure infiltration, pressure infiltration, and saturated infiltration with p values of 5 μm and 8 μm. According to Figure 4, the time–history curves of the cumulative infiltration amount (Q) with p values of 5 μm and 8 μm have intersections in the pressure infiltration stage. This means that the smaller the particle size of microplastics, the more conducive they are to rainwater infiltration in the pressure infiltration stage, while the larger the particle size of microplastics, the more favorable the rainwater infiltration in the saturated infiltration stage. As shown in Figure 4, significant differences appear in the saturated infiltration stage for curves with p values of 5 μm and 8 μm, indicating that the saturated infiltration stage of rainfall infiltration was severely affected by microplastic particle sizes.

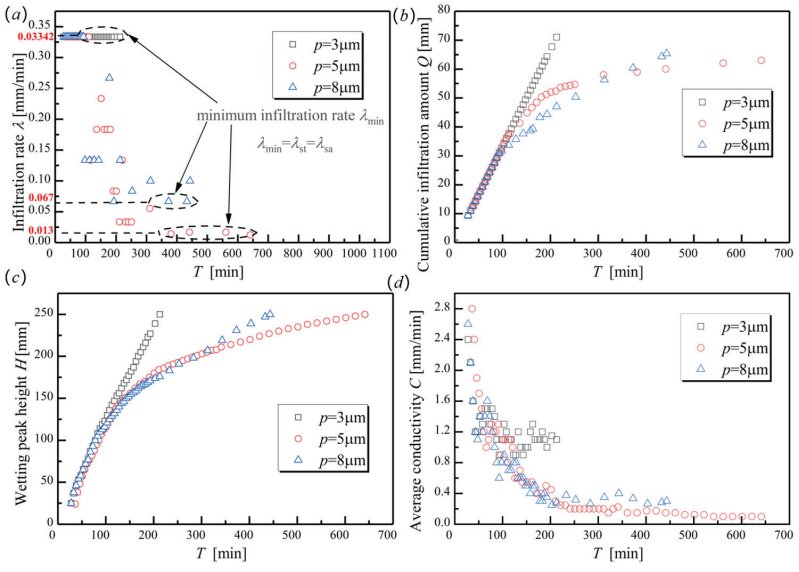

Figure 4. Time–history curve of under different microplastic particle sizes p: (**a**) infiltration rate λ; (**b**) cumulative infiltration amount Q; (**c**) wetting peak height H; (**d**) average conductivity C.

Current research [39] shows that there exist three types of soil particles: large, medium, and small. The soil permeability coefficient is greatly affected by large pores compared with medium and small pores. When q is constant and p is small, microplastics fill few large pores of soil particles and present hydrophobic properties to promote water infiltration.

Otherwise, with p increasing, more and more large pores are filled by microplastics, reducing the soil permeability coefficient. The existence of microplastics represents blocking effects. The blocking effects enhance as p increases. By controlling the mass of microplastics as constant, if p decreases, the amount of microplastics that compact soil particles should increase and blocking effects should increase. This explains the microplastic content range that promotes infiltration increases as p decreases.

To explore the soil water-retaining capacity, the volume moisture content measured θ by EC-5 moisture sensors with different p values are analyzed below.

As shown in Figure 5, the θ_{max} values with different p values are all less than that of the blank experiment control group. There are few differences in θ_{max} with different p values. This indicates that the existence of microplastics reduces soil water-retaining capacity. The effects on soil water-retaining capacity change slightly as p increases.

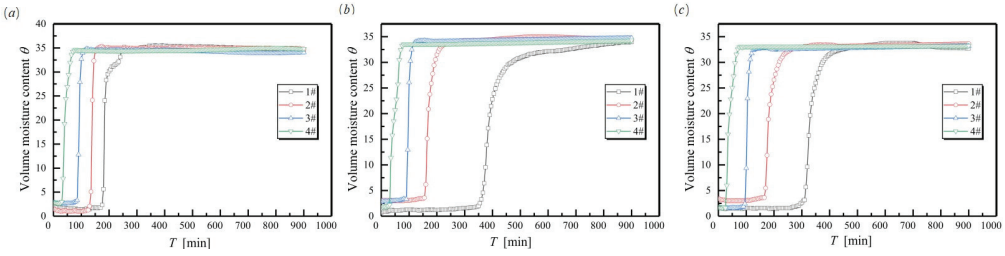

Figure 5. Time–history curve of volumetric moisture content θ at monitoring points with different microplastic contents p: (**a**) p = 3 μm; (**b**) p = 5 μm; (**c**) p = 8 μm.

3.3. Effect of Soil Bulk Density γ on Moisture Transportation

In this study, the microplastic content (q) is 0.25% and the microplastic particle size (p) is 5 μm. By analyzing the test results from No. 4, No. 8, No. 9, and No. 10, the results are shown below.

As shown in Figure 6, with increasing γ, the stable infiltration rate (λ_{st}) gradually decreases. This phenomenon is because the soil particles become more compact, the soil particle pores are smaller, and the soil infiltration rate (λ) is lower if the γ values increase. With the increase in γ, the cumulative infiltration amount (Q) gradually decreases. The rainfall infiltration shows three stages: no-pressure infiltration, pressure infiltration, and saturated infiltration. Meanwhile, with the increase in γ, the duration of the no-pressure stage gradually decreases, and the duration of the pressure stage also gradually shortens. The rainfall infiltration quickly transitions to the saturated infiltration stage. With the increase in γ, the time for rainwater to reach the soil column bottom gradually increases, and the saturated infiltration rate (λ_{sa}) gradually decreases. This is because as γ increases, the soil is denser, the porosity is smaller, and the amount of water passing through is less. It should also be noted that there is a good linear relationship between λ_{sa} and γ (A3 of the Appendix A).

To explore the soil water-retaining capacity, the volume moisture content measured as θ by EC-5 moisture sensors with different γ values are analyzed below.

As shown in Figure 7, at the beginning, the rising time of θ at #1 and #2 is roughly the same, but with the increase in soil depth and γ, the rising time of θ is slightly delayed, and the time difference between the increase in θ at #3 and #4 also gradually increases. The θ_{max} values with different γ values are all less than that of the blank experiment control group. The soil peak moisture content (θ_{max}) with a relatively large bulk density (γ) would decrease slightly. The existence of microplastics would weaken the soil water-retaining capacity.

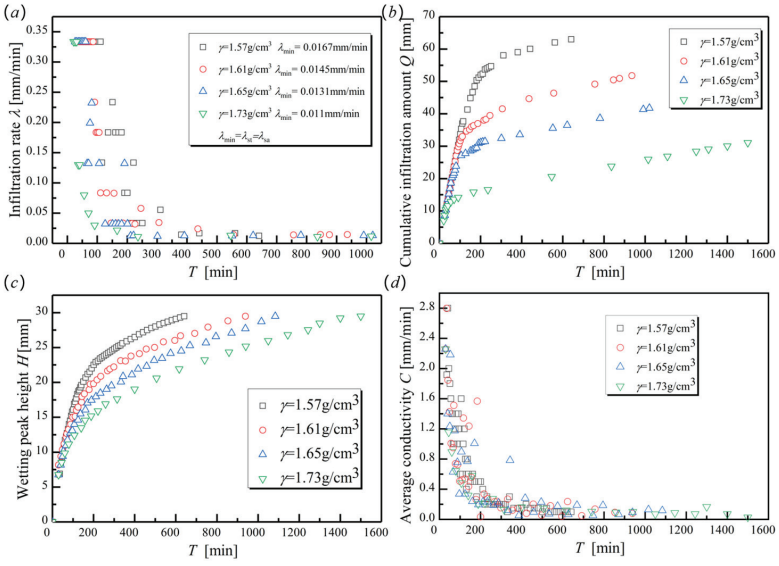

Figure 6. Time–history curve under different soil bulk density γ: (**a**) infiltration rate λ; (**b**) cumulative infiltration amount Q; (**c**) wetting peak height H; (**d**) average conductivity C.

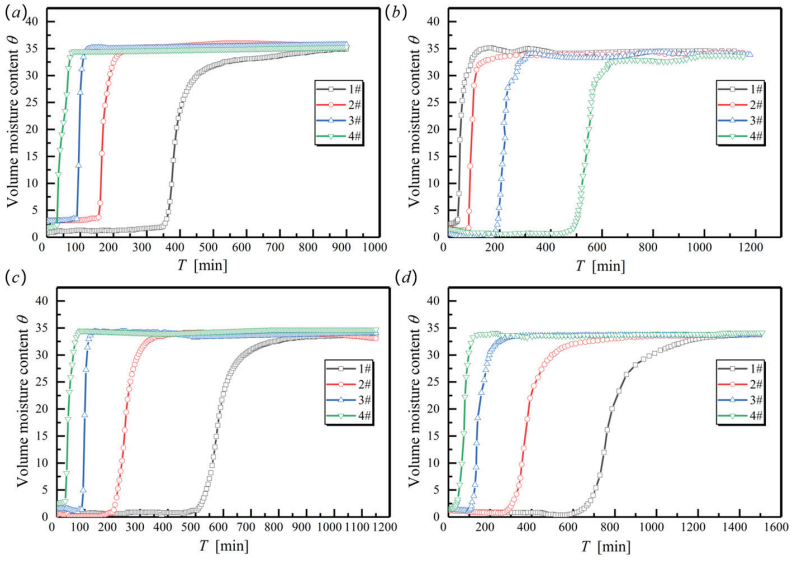

Figure 7. Time–history curve of volumetric moisture content θ at monitoring points with different soil bulk density γ: (**a**) γ = 1.57 g/cm^3; (**b**) γ = 1.61 g/cm^3; (**c**) γ = 1.65 g/cm^3; (**d**) γ = 1.73 g/cm^3.

The time–history curve of wetting peak height (H) with different soil bulk densities (γ) could be fitted by a power function, which shows significant scale symmetry:

$$H = at^b \tag{1}$$

Taking Equation (1) by the derivative of both sides with respect to time, the formula of wetting peak advancing velocity can be obtained:

$$\frac{dH}{dt} = abt^{b-1} \tag{2}$$

To explore the physical meanings of parameters a and b, we take the derivative of Equation (2) with respect to time and take the logarithm of both sides of this equation:

$$\log\left(\frac{dH}{dt}\right) = \log(ab) + (b-1)\log t \tag{3}$$

where dH/dt is the advancing speed of the wetting peak; $\log(ab)$ is related to the initial advancing speed of the wetting peak; and $b-1$ is the slope of Equation (3) and is related to the acceleration of the wetting peak advance. Combined with Equation (1), the time–history curves of the wetting peak advancing depth H and the wetting peak advancing speed dH/dt are shown in Figure 8.

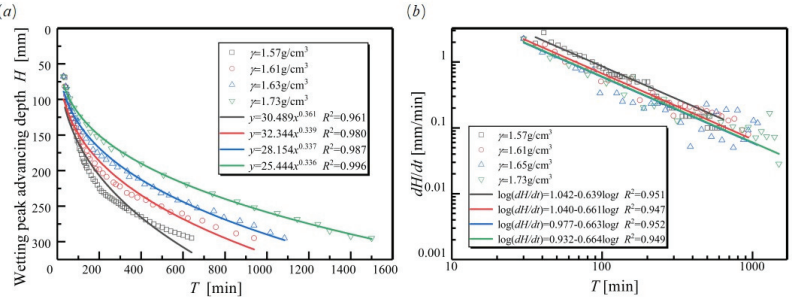

Figure 8. Fitting curve: (**a**) time-history curve of the wetting peak advancing depth H; (**b**) time-history curve of the wetting peak advancing speed dH/dt.

It could be speculated that there is a linear relationship between the wetting peak advancing speed and time, which could be used to calculate the unsaturated soil permeability coefficient proposed with microplastics in Section 4.2.

3.4. Effect of Rain Interval Ratio i on Moisture Transportation

This study investigates the soil infiltration rate (λ) when the microplastic content (q) is 0.25%, the soil bulk density (γ) is 1.57 g/cm^3 and the microplastic particle size (p) is 5 μm. By analyzing the test results from No. 11 to No. 13, the results are shown below.

The rainfall infiltration rate (λ) is barely unchanged for more than 600 min of rainfall duration, and the rainfall infiltration is stable under the circumstances of three different rain interval ratios (i). Therefore, the time–history curves only represent rainfall duration within 600 min. As shown in Figure 9, the rainfall infiltration process overall does not show no-pressure infiltration, pressure infiltration, or saturated infiltration. With time, the rainwater infiltration rate (λ) gradually decreases to approximately 0.013 mm/min regardless of the difference in i values. However, at the same time, the time–history curves of λ from high to low rain interval ratios (i) are 1:2, 1:1, and 2:1 in turn. It could be speculated that the rain interval ratio (i) has little influence on the saturated infiltration stage of the soil. It is easier for rainwater to infiltrate into the soil inside, and there is no significant loss of rainwater through surface runoff when i is equal to 1:2.

In the pressure infiltration stage, a water film begins to appear on the soil surface until ponding occurs and λ begins to decrease. Nonetheless, during the break time of rain, the water film and stagnant water disappear, and θ decreases. There appears to be a small rebound in λ. With changes in the rainfall cycle, the rate of decrease in λ and increase in

rebound gradually decrease, while the magnitude of decrease and increase in rebound gradually decrease. It is shown that the time of λ changes in the time–history curve of λ is consistent with the alternating time between rainfall continuation and rainfall pause, and there is no hysteresis phenomenon, indicating that the infiltration rate responds rapidly to the transition of rainfall types. Below are the comparison figures of the time–history curves of infiltration rate (λ) of loess soil containing microplastics during rainwater infiltration under continuous rainfall conditions and intermittent rainfall conditions.

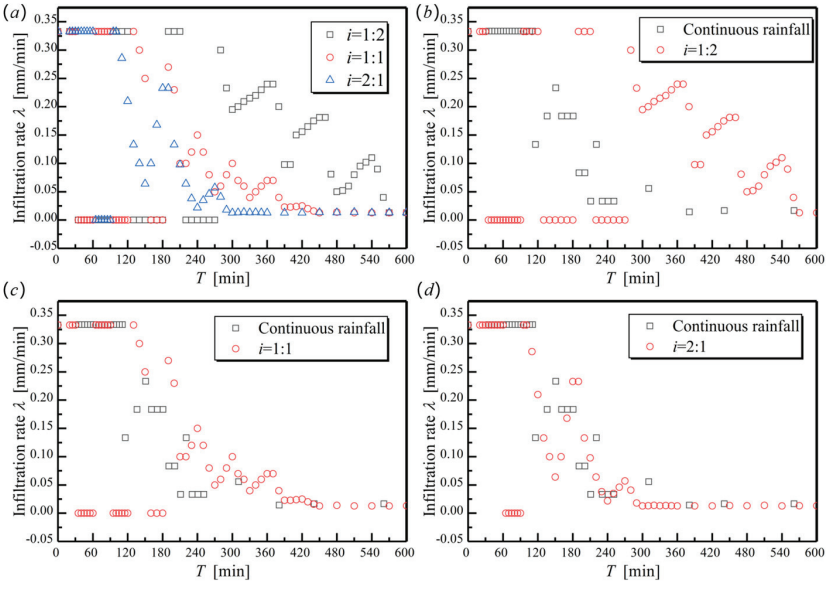

Figure 9. (**a**) Time-history curve of infiltration rate λ with different rain interval ratio i; comparative analysis of time-history curves of infiltration rate λ with different rain interval ratio I: (**b**) i = 1:2; (**c**) i = 1:1; (**d**) i = 2:1.

As shown in Figure 9, when rainfall conditions change from continuous rainfall to intermittent rainfall, the decline rate of λ in the early stage of rainfall changes from fast to slow, and λ under intermittent rainfall conditions is higher than that under continuous rainfall conditions at the same time. This shows that the rainwater infiltration speed is faster when rainfall changes to intermittent rainfall, and the Q value is larger in the middle and early stages. Owing to intermittent rainfall, rainwater needs to be redistributed, and the rainfall duration is long. There are few differences in λ when entering the saturated infiltration stage for continuous and intermittent rain, indicating that rain conditions have little effect on the saturated infiltration stage and that the rain interval ratio has a strong impact on the pressure infiltration stage owing to the rebound phenomenon above. For the soil column tests with rainfall durations longer than the rainfall intermittent time, the time–history curves are almost coincident, indicating that with the increase in rainfall duration, λ gradually became insensitive to the transition of rainfall type. Intermittent rainfall conditions are conducive to soil rainwater infiltration and do not generate excessive runoff on the surface. In agricultural irrigation, under the circumstances of the same irrigation water volume, the intermittent irrigation method is preferred, which is conducive to maximizing the use of water, and the infiltration time is long. Rainwater is beneficial to the absorption and utilization of plant rhizomes in the process of water redistribution in multiple rainfall cycles.

4. Coefficient of Permeability

4.1. Formula Derivation

The coefficient of permeability (θ) is an index that comprehensively reflects soil permeability and is the basis for seepage analysis and rainfall infiltration analysis, which has guiding significance for the seepage analysis of a project and for farmland irrigation. Below is the formula derivation process.

Assuming that the entire space of a soil flow area is filled with water flow, to make the flow in the soil flow model reflect the actual flow of the soil, the flow velocity of the water (V_w, unit: L/T) flow on any tiny area ΔA should be equal to the actual flow through the area ΔQ divided by ΔA, that is:

$$V_w = \Delta Q / \Delta A \tag{4}$$

Due to the volumetric water content of unsaturated soils at various cross-sectional locations and differences in the shape, width and direction of pores, the flow velocity here is also an average flow velocity that varies with location and time.

Assuming that the volumetric water content contour line and the matrix suction contour curve advance smoothly in a relatively short period of time, this requires that the volumetric water content distribution function $\theta(h, t)$ of different sections change the same with time, which can be converted into the following expression:

$$\theta(h, t + \Delta t) = \theta(h - \Delta h, t) \tag{5}$$

where Δh is the wetting peak infiltration depth during Δt.

As shown in Figure 10, within a short distance of the soil column between A and B, the development schematic diagram of the wetting peak when the rainfall duration is t_1 and t_2, respectively. The distance traveled by the wetting peak is Δh, so during the rainfall duration $\Delta t = t_2 - t_1$ (shorter time, generally less than five minutes), the water flow through section A of the soil column is:

$$Q_A = \Delta Q_{A-D} + Q_D \tag{6}$$

where Q_A is the water flow through section A, Q_D is the water flow through section D, and ΔQ_{A-D} is the amount of water stored in vertical section AD of the soil column during time Δt. When the wetting peak does not reach the cross section, Q_A and Q_D are equal to 0. As the test stops until the water infiltrates to the bottom of the soil column, Q_D is 0. It can be seen from the previous analysis that θ can be integrated to obtain the total water content in a vertical section of the soil column, so the above formula can be written as:

$$Q_A = \Delta Q_{A-D} = \int_{h_A}^{h_D} (\theta(h, t_2) - \theta(h, t_1)) A dh = \int_{h_A}^{h_D} \theta(h, t_2) A dh - \int_{h_A}^{h_D} \theta(h, t_1) A dh \tag{7}$$

where $\theta\ (h, t_2)$ and $\theta\ (h, t_1)$ are the distribution functions of the soil volumetric moisture content θ at times t_2 and t_1, respectively; A is the cross-sectional area of the vertical soil column; and h_A and h_D are the distances between section A and section D from the soil column surface. Assuming that the vertical soil column is within a small time period $\Delta t = t_2 - t_1$, the height difference of the wetting peak in the vertical direction is Δh. The equations below can be obtained from the previous assumptions:

$$\begin{aligned} Q_A = \Delta Q_{A-D} &= \int_{h_A}^{h_D} \theta(h,\ t_2) A dh - \int_{h_A}^{h_D} \theta(h, t_1) A dh = \int_{h_A}^{h_D} \theta(h - \Delta h, t_1) A dh - \int_{h_A}^{h_D} \theta(h, t_1) A dh \\ &= \int_{h_A - \Delta h}^{h_A} \theta(h, t_1) A dh - \int_{h_D - \Delta h}^{h_D} \theta(h, t_1) A dh \end{aligned} \tag{8}$$

Based on the assumption that the distribution function of soil volumetric moisture content θ is a smooth function, the equations can be simplified as:

$$\int_{h_A - \Delta h}^{h_A} \theta(h, t_1) A dh \approx \frac{[\theta(h_A, t_2) + \theta(h_A, t_1)] A \Delta h}{2} \tag{9}$$

$$\int_{h_D-\Delta h}^{h_D} \theta(h,t_1)\mathrm{A}dh \approx \frac{[\theta(h_D,t_2)+\theta(h_D,t_1)]\mathrm{A}\Delta h}{2} \approx \theta_0 \mathrm{A}h \tag{10}$$

where θ_0 is the initial volumetric moisture content of the vertical soil columns.

Inserting Equations (9) and (10) into Equations (4) and (8), the water flow Q_A and average flow rate of water flowing through section A V_A are:

$$Q_A = \frac{[\theta(h_A,t_2)+\theta(h_A,t_1)-2\theta_0]\mathrm{A}(h_B-h_A)}{2} \tag{11}$$

$$\begin{aligned} V_A &= \frac{\{0.5[\theta(h_A,t_2)+\theta(h_A,t_1)-2\theta_0]\mathrm{A}\}\Delta h}{t_2-t_1} \\ &= \frac{\{0.5[\theta(h_A,t_2)+\theta(h_A,t_1)-2\theta_0]\mathrm{A}\}(h_B-h_A)}{t_2-t_1} \\ &= \frac{\{0.5[\theta(h_A,t_2)+\theta(h_A,t_1)-2\theta_0]\mathrm{A}\}(h_2-h_1)}{t_2-t_1} \end{aligned} \tag{12}$$

where $(h_2 - h_1)/(t_2 - t_1)$ is the wetting peak advancing speed. In a short time t, let $t = (t_2 + t_1)/2$ be the wetting peak advancing speed formula derived earlier, and by substituting the previously obtained wetting peak advancing speed formula into it, Equation (13) can be obtained:

$$V_A = \frac{\{0.5[(h_A,t_2)+\theta(h_A,t_1)-2\theta_0]\mathrm{A}\}(h_2-h_1)}{t_2-t_1} = \{0.5[\theta(h_A,t_2)+\theta(h_A,t_1)-2\theta_0]\mathrm{A}\}ab\left(\frac{t_2+t_1}{2}\right)^{b-1} \tag{13}$$

At $(t_2 + t_1)/2$ time, the hydraulic gradient between sections A and B is approximately equal to the tangent of the angle α in Figure 10 and can be obtained by the following formula:

$$i = \frac{\varphi(h_A,t_1)-\varphi(h_A,t_2)}{\gamma_w(h_B-h_A)} - 1 = \frac{\varphi(h_A,t_1)-\varphi(h_A,t_2)}{\gamma_w(h_2-h_1)} - 1 \tag{14}$$

Assuming that the permeability coefficient of unsaturated soil remains unchanged in a small time period $\Delta t = t_2 - t_1$ according to Darcy's law, the following can be obtained:

$$Q_A = kiA\Delta t \tag{15}$$

$$\begin{aligned} k = \frac{Q_A}{iA\Delta t} &= \frac{0.5\{[\theta(h_A,t_2)+\theta(h_A,t_1)-2\theta_0]\mathrm{A}\Delta h\}\gamma_w\Delta h}{[\varphi(h_A,t_1)-\varphi(h_A,t_2)-\gamma_w\Delta h]A\Delta t} \\ &= \frac{0.5[\theta(h_A,t_2)+\theta(h_A,t_1)-2\theta_0]\Delta h\gamma_w}{[\varphi(h_A,t_1)-\varphi(h_A,t_2)-\gamma_w\Delta h]} ab\left(\frac{t_2+t_1}{2}\right)^{b-1} \end{aligned} \tag{16}$$

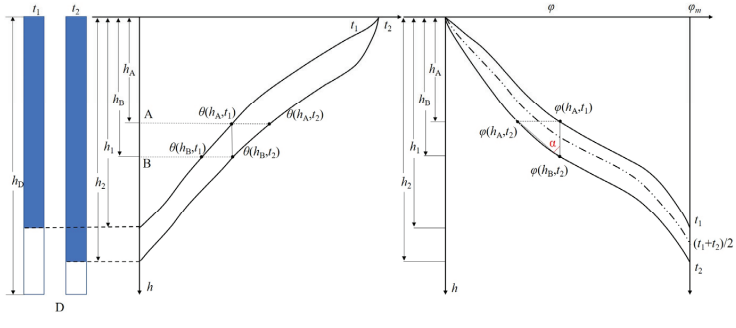

Figure 10. Moisture content profile and matrix suction profile at any two times during rainwater infiltration.

4.2. Calculation Example

In this paper, the section of the #2 moisture sensor of the test soil column with a microplastic content q of 0.25%, a microplastic particle size p of 5 μm, a uniform rainfall

intensity of 20 mm/h, and a soil bulk density γ of 1.57 g/cm^3 is selected to solve the problem of the permeability coefficient of unsaturated remodeled loess soils with microplastics.

From the study above, the wetting peak infiltration rate of loess soil with microplastics varies greatly in the early stage of rainfall and tends to be stable with increasing rainfall duration. The power exponent is used for fitting, and the fitting effect is better. Below is the fitting function with the same meaning as Equation (4):

$$v = abt^{b-1} = 11.01t^{-0.639}\ R^2 = 0.987 \tag{17}$$

The volumetric moisture content functions $\theta(h, t)$ and $\varphi(h, t)$ of the section where the #2 moisture sensor of the loess soil column is located are measured by the moisture sensor and the tensiometer, respectively. Taking $\theta(h, t)$, $\varphi(h, t)$, $v = 11.01t^{-0.639}$, $\theta_0 = 0.07$, $\gamma_w = 10$ KN/m^3, and $\Delta t = t_2 - t_1 = 5$ min into Equation (16), the permeability coefficient of unsaturated remolded loess soil with microplastics under different matrix suction values can be obtained, as shown in Figure 11.

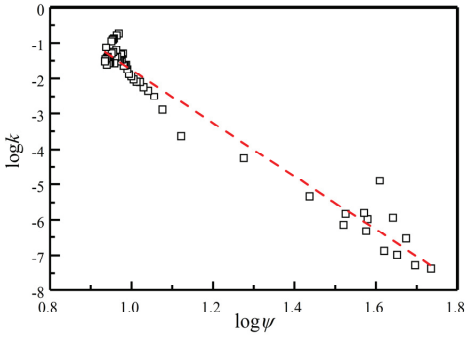

Figure 11. Permeability curve.

As shown in Figure 11, when the soil tends to be saturated, the matrix suction force ψ is small, and the permeability coefficient (k) is approximately equal to 0.0338 mm/min, which is in close proximity with the saturated permeability coefficient (λ) of 0.037 mm/min in Section 3.1. Therefore, the experimental results in this paper are consistent and reasonable with the theoretical calculation. Meanwhile, the distribution of the logarithm (lgk) and the matrix suction ($lg\varphi$) shows a linear distribution law, which can be fitted by a linear equation. The fitting curve under the double logarithmic coordinate is shown in Figure 11. The fitting equation is:

$$lgk = 5.77 - 7.53lg\varphi (R^2 = 0.987) \tag{18}$$

Therefore, according to the calculation method of the unsaturated soil permeability coefficient proposed with microplastics in this paper, combined with laboratory infiltration tests and soil–water characteristic curve tests, the permeability coefficient of unsaturated soil bodies can be obtained, which can be used for engineering seepage analysis and guidance for farmland soil irrigation.

5. Conclusions

Based on the wetting front advancing method, this paper employs a one-dimensional vertical soil column rainfall infiltration test device. By analyzing the experimental phenomena and data, the following conclusions are drawn:

(1) When the values of q and p are relatively small, microplastics reflect hydrophobic properties. With the increase in q, p, and γ, microplastics represent blocking effects owing to a significant increase in soil compactness. The rainfall infiltration process normally shows no-pressure infiltration, pressure infiltration, and saturated infiltration. When microplastics have the main effect of hydrophobic, saturated infiltration, even pressure infiltration would

not appear. When microplastics have the main effect of blocking, saturated infiltration begins sooner.

(2) The soil water-retaining capacity would be weakened due to the existence of microplastics.

(3) Compared with continuous rainfall, intermittent rainfall is preferred in agricultural irrigation, which would not cause a large amount of surface runoff loss and is conducive to the maximum utilization of water.

(4) Based on the assumption that the volumetric moisture content contour and the matrix suction contour curve advance smoothly in a relatively short period of time, combined with the effective fitting of the power function of the wet peak bulk density advancing curve, the permeability coefficient (k) and average flow rate (V) of unsaturated soils are jointly derived. The relationship between the permeability coefficient (k) and matrix suction (ψ) of unsaturated loess soil containing microplastics was calculated by an example.

Author Contributions: Conceptualization, J.G. and Y.W.; methodology, L.C.; validation, J.G., Y.W. and S.Y.; formal analysis, J.G.; investigation, J.G.; resources, Y.W.; data curation, J.G.; writing—original draft preparation, J.G.; writing—review and editing, Y.T.; visualization, L.H.; supervision, Y.T.; project administration, Y.W.; funding acquisition, L.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key Research and Development Program (No. 2019YFC1510802), the National Natural Science Foundation of China (Grant No. U2243244), the major science and technology project of the Ministry of Water Resources (SKR-2022072) and Open Project Funded by the Engineering And Technical Research Center for Dike Safety and Disease Control of the Ministry of Water Resources (Grant No. DFZX202004).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data used to support the findings of this study are available from the corresponding author upon request.

Acknowledgments: Thanks to all the reviewers and editors for the publication of this paper.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

A1: Grain-size distribution curve.

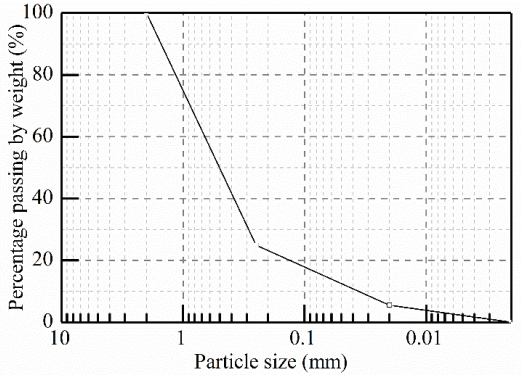

Figure A1. Grain-size distribution curve.

A2: The rainwater infiltration tests with a p of 3 μm and a q of 0.00%, 0.05%, 0.10%, 0.25%, and 0.50% are supplemented and the results are shown below.

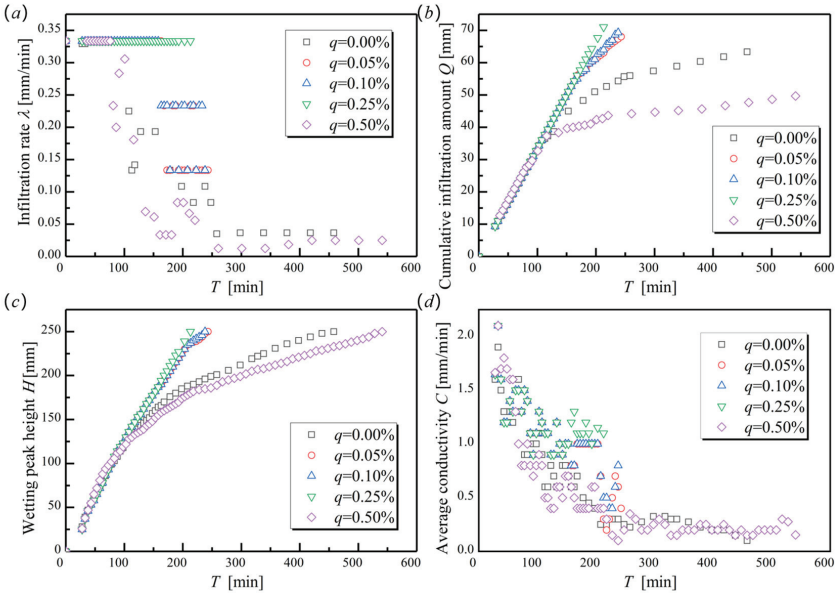

Figure A2. Time–history curve of under different microplastic *q*: (**a**) infiltration rate λ; (**b**) cumulative infiltration amount *Q*; (**c**) wetting peak depth *H*; (**d**) average conductivity *C*.

A3: Fitting curve of λ_{sa} and γ.

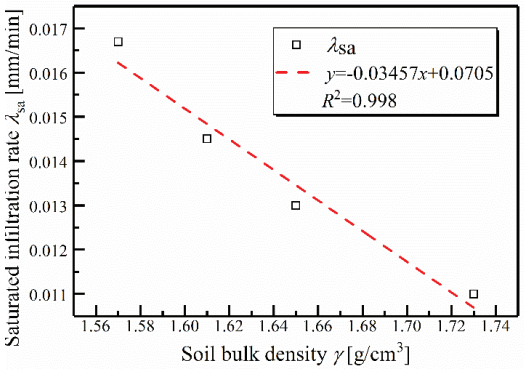

Figure A3. Fitting curve of λ_{sa} and γ.

References

1. Peeken, I.; Primpke, S.; Beyer, B.; Gütermann, J.; Katlein, G.; Krumpen, T.; Bergmann, M.; Hehemann, L.; Gerdts, G. Arctic sea ice is an important temporal sink and means of transport for microplastic. *Nat. Commun.* **2018**, *9*, 1505. [CrossRef] [PubMed]
2. Li, J.; Song, Y.; Cai, Y. Focus topics on microplastics in soil: Analytical methods, occurrence, transport, and ecological risks. *Environ. Pollut.* **2019**, *257*, 113570. [CrossRef] [PubMed]
3. Nizzetto, L.; Futter, M.; Langaas, S. Are Agricultural Soils Dumps for Microplastics of Urban Origin? *Environ. Sci. Technol.* **2016**, *50*, 10777–10779. [CrossRef]
4. Machado, S.A.; Lau, W.C.; Till, J.; Kloas, W.; Lehmann, A. Impacts of microplastics on the soil biophysical environment. *Environ. Sci. Technol.* **2018**, *52*, 9656–9665. [CrossRef] [PubMed]
5. Horton, A.A.; Walton, A.; Spurgeon, D.J.; Lahive, E.; Svendsen, C. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total Environ.* **2017**, *586*, 127–141. [CrossRef] [PubMed]

6. Zhao, J.M.; Ran, W.; Teng, J.; Liu, Y.L.; Liu, H.; Yin, X.N.; Cao, R.W.; Wang, Q. Microplastic pollution in sediments from the Bohai sea and the Yellow sea, China. *Sci. Total Environ.* **2018**, *640–641*, 637–645. [CrossRef] [PubMed]
7. Peng, G.Y.; Bellerby, R.; Zhang, F.; Sun, X.R.; Li, D.J. The ocean's ultimate trashcan: Hadal trenches as major depositories for plastic pollution. *Water Res.* **2020**, *168*, 115121.1–115121.8. [CrossRef]
8. Ramos, L.; Berenstein, G.; Hughes, E.A.; Zalts, A.; Montserrat, J.M. Polyethylene film incorporation into the horticultural soil of small periurban production units in Argentina. *Sci. Total Environ.* **2015**, *523*, 74–81. [CrossRef]
9. Bläsing, M.; Amelung, W. Plastics in soil: Analytical methods and possible sources. *Sci. Total Environ.* **2018**, *612*, 422–435. [CrossRef]
10. He, D.F.; Luo, Y.M.; Lu, S.B.; Liu, M.T.; Song, Y.; Lei, L.L. Microplastics in soils: Analytical methods, pollution characteristics and ecological risks. *TrAC Trends Anal. Chem.* **2018**, *109*, 163–172. [CrossRef]
11. Li, L.Z.; Luo, Y.M.; Li, R.J.; Zhou, Q.; Peijnenburg, W.J.G.M.; Yin, N.; Yang, J.; Tu, C.; Zhang, Y.C. Effective uptake of submicrometre plastics by crop plants via a crack-entry mode. *Nat. Sustain.* **2020**, *3*, 929–937. [CrossRef]
12. Ng, E.L.; Lwanga, E.H.; Eldridge, S.M.; Johnston, P.; Hu, H.W.; Geissen, V.; Chen, D. An overview of microplastic and nanoplastic pollution in agroecosystems. *Sci. Total Environ.* **2018**, *627*, 1377–1388. [CrossRef] [PubMed]
13. Rillig, M.C.; Lehmann, A. Microplastics in terestrial ecosystems. *Science* **2020**, *368*, 1430–1431. [CrossRef] [PubMed]
14. Auta, H.S.; Emenike, C.U.; Fauziah, S.H. Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environ. Int.* **2017**, *102*, 165–176. [CrossRef] [PubMed]
15. Mahon, A.M.; Connell, B.O.; Healy, M.G.; Connor, I.O.; Nash, R.; Morrison, L. Microplastics in Sewage Sludge: Effects of Treatment. *Environ. Sci. Technol.* **2017**, *51*, 810–818. [CrossRef] [PubMed]
16. Zhou, Y.F.; Liu, X.N.; Wang, J. Characterization of microplastics and the association of heavy metals with microplastics in suburban soil of central China. *Sci. Total Environ.* **2019**, *694*, 133798.1–133798.10. [CrossRef]
17. Georgia, G.; Arvaniti, O.S.; Stasinakis, A.S. Review on the occurrence and fate of microplastics in sewage treatment plants. *J. Hazard. Mater.* **2019**, *367*, 504–512.
18. Galloway, T.S.; Cole, M.; Lewis, C. Interactions of microplastic debris throughout the marine ecosystem. *Nat. Ecol. Evol.* **2017**, *1*, 116. [CrossRef]
19. Akdoan, Z.; Guven, B. Microplastics in the environment: A critical review of current understanding and identification of future research needs. *Environ. Pollut.* **2019**, *254*, 113011. [CrossRef]
20. Kane, I.A.; Clare, M.A. Dispersion, accumulation and the ultimate fate of microplastics in deep-marine environments: A review and future directions. *Front. Earth Sci.* **2019**, *7*, 1–27. [CrossRef]
21. Chrysikopoulos, C.V.; Syngouna, V.I. Effect of gravity on colloid transport through water-saturated columns packed with glass beads: Modeling and experiments. *Environ. Sci. Technol.* **2014**, *48*, 6805–6813. [CrossRef] [PubMed]
22. Syngouna, V.I.; Chrysikopoulos, C.V. Cotransport of clay colloids and viruses through water-saturated vertically oriented columns packed with glass beads: Gravity effects. *Sci. Total Environ.* **2016**, *545*, 210–218. [CrossRef] [PubMed]
23. Beckwith, V.K.; Fuentes, M.M.P.B. Microplastic at nesting grounds used by the northern Gulf of Mexico loggerhead recovery unit. *Mar. Pollut. Bull.* **2018**, *131*, 32–37. [CrossRef] [PubMed]
24. Tong, M.; He, L.; Rong, H.; Li, M.; Kim, H. Transport behaviors of plastic particles in saturated quartz sand without and with biochar/Fe3O4-biochar amendment. *Water Res.* **2020**, *169*, 115284. [CrossRef]
25. Alimi, O.S.; Farner, J.M.; Tufenkji, N. Exposure of nanoplastics to freeze-thaw leads to aggregation and reduced transport in model groundwater environments. *Water Res.* **2021**, *189*, 116533. [CrossRef]
26. Bakir, A.; Rowland, S.J.; Thompson, R.C. Transport of persistent organic pollutants by microplastics in estuarine conditions. *Estuar. Coast. Shelf Sci.* **2014**, *140*, 14–21. [CrossRef]
27. Gong, J.; Xie, P. Research progress in sources, analytical methods, eco-environmental effects, and control measures of microplastics. *Chemosphere* **2020**, *254*, 126790. [CrossRef]
28. Wu, M.; Tang, W.; Wu, S.; Liu, H.; Yang, C. Fate and effects of microplastics in wastewater treatment processes. *Sci. Total Environ.* **2020**, *757*, 143902. [CrossRef]
29. Edo, C.; González-Pleiter, M.; Leganés, F.; Fernández-Piñas, F.; Rosal, R. Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge. *Environ. Pollut.* **2020**, *259*, 113837. [CrossRef]
30. Scircle, A.; Cizdziel, J.V.; Tisinger, L.; Anumol, T.; Robey, D. Occurrence of Microplastic Pollution at Oyster Reefs and Other Coastal Sites in the Mississippi Sound, USA: Impacts of Freshwater Inflows from Flooding. *Toxics* **2020**, *8*, 35. [CrossRef]
31. Wolff, S.; Weber, F.; Kerpen, J.; Winklhofer, M.; Engelhart, M.; Backman, L. Elimination of Microplastics by Downstream Sand Filters in Wastewater Treatment. *Water* **2021**, *13*, 33. [CrossRef]
32. Lapointe, M.; Farner, J.M.; Hernandez, L.M.; Tufenkji, N. Understanding and Improving Microplastic Removal during Water Treatment: Impact of Coagulation and Flocculation. *Environ. Sci. Technol.* **2020**, *54*, 8719–8727. [CrossRef] [PubMed]
33. Wang, X.Z.; Wang, X.; Hu, M.J.; Zhu, C.Q.; Meng, Q.S.; Wang, R. Study of permeability of calcareous silty layer of foundation at an artificial reclamation island. *Rock Soil Mech.* **2017**, *38*, 3127–3135.
34. Wang, X.Z.; Wang, X.; Chen, J.W.; Wang, R.; Hu, M.J.; Meng, Q.S. Experimental study on permeability characteristics of calcareous soil. *Bull. Eng. Geol. Environ.* **2018**, *77*, 1753–1762. [CrossRef]
35. Lin, S.J.; Jian, W.B.; Dou, H.Q.; Chen, R.M.; Chen, R.M.; Nie, W. Rainfall Infiltration Mechanisms of Soil Columns Under Conductivity. *J. Eng. Geol.* **2022**, *30*, 394–406.

36. Wei, L.; Yang, M.Y.; Li, Z.; Shao, J.L.; Li, L.Q.; Chen, P.; Li, S.; Zhao, R.B. Experimental Investigation of Relationship between Infiltration Rate and Soil Moisture under Rainfall Conditions. *Water* **2022**, *14*, 1347. [CrossRef]
37. Yang, S.; Gong, A.M.; Wu, J.H.; Lu, T.H. Effect of contact angle on matric suction of unsaturated soil. *Rock Soil Mech.* **2015**, *36*, 674–678.
38. Yang, S.; Wu, J.H. Evaporation characteristics of liquid drops on silt soil surface and the influences on soil-water characteristic curve. *Rock Soil Mech.* **2020**, *S*, 1–9.
39. You, Y.; Xiao, H.L.; Tan, Y. Experimental research on permeability of hydrophobic material modified clay. *J. China Three Gorges Univ.* **2022**, *44*, 14–19.
40. Keatts, M.I.; Daniels, J.L.; Langley, W.G.; Pando, M.A.; Ogunro, V.O. Apparent contact angle and water entry head measurements for organo-silane modified sand and coal fly ash. *J. Geotech. Geoenviron. Eng.* **2018**, *144*, 04018030. [CrossRef]

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Adsorption Behavior of Nonylphenol on Polystyrene Microplastics and Their Cytotoxicity in Human Caco-2 Cells

Fangfang Ding, Qianqian Zhao, Luchen Wang, Juan Ma, Lingmin Song and Danfei Huang *

State Key Laboratory of Food Science and Technology, China-Canada Joint Lab of Food Science and Technology (Nanchang), Key Laboratory of Bioactive Polysaccharides of Jiangxi Province, Nanchang University, 235 Nanjing East Road, Nanchang 330047, China

* Correspondence: huangdanfei@ncu.edu.cn; Tel.: +86-791-8830-4447 (ext. 8315)

Abstract: As two environmental pollutants of great concern, polystyrene microplastics (PS-MPs) and nonylphenol (NP) often coexist in the environment and cause combined pollution. Batch adsorption experiments were carried out by varying parameters such as pH, the particle sizes of the PS-MPs, the initial concentration of NP, and metal ion content. The results showed that the particle size of the PS-MPs in the range tested (0.1, 1, 10, 50, and 100 μm) had a significant effect on their NP adsorption capacity. The NP adsorption process of the PS-MPs was best described by the pseudo-second-order kinetic model and the Langmuir isotherm model, while the intraparticle diffusion and Bangham models were also involved in determining the NP adsorption process of 0.1 μm PS-MPs. Both PS-MPs and NP significantly affected cell proliferation, which had been confirmed by reduced cell viability, a blocked cell cycle G1 phase, and elevated apoptosis by affecting the basic cell functions. Furthermore, the negative effects of 0.1 μm PS-MPs on cell proliferation and function were aggravated after the adsorption of NP. Further research on the potential health risks of PS-MPs combined with NP or other environmental contaminants is needed.

Keywords: microplastics; nonylphenol; adsorption; desorption; cytotoxicity

Citation: Ding, F.; Zhao, Q.; Wang, L.; Ma, J.; Song, L.; Huang, D. Adsorption Behavior of Nonylphenol on Polystyrene Microplastics and Their Cytotoxicity in Human Caco-2 Cells. *Water* **2022**, *14*, 3288. https://doi.org/10.3390/w14203288

Academic Editors: Grigorios L. Kyriakopoulos, Vassilis J. Inglezakis, Antonis A. Zorpas and María Rocío Rodríguez Barroso

Received: 29 September 2022
Accepted: 14 October 2022
Published: 18 October 2022

1. Introduction

The accumulation and fragmentation of plastic waste have always been the focus of environmental concerns. It is noted that trends in the accumulation rate of mega- and macro-plastic no longer uniformly increase, while the average size of plastic particles seems to decrease on a global scale. Microplastics, which are plastic fragments with diameters smaller than 5 mm, circulate in the system of the air/soil/ocean/living organisms and have been detected in aquatic organisms such as freshwater shrimp, seaweed laver, and edible fish [1–3]. The transmission and accumulation of microplastics in food chains make voluntary or involuntary ingestion almost inevitable. The detection rate of microplastics in human stool samples from Europe and Asia was 100%, and nine types of plastics were detected [4]. The potential health risk of environmentally released microplastics has become a topic of great concern.

Microplastics with particle sizes smaller than 150 μm are proven to pass through the intestinal mucosal barrier, accumulate in the intestine and other tissues, destroy the immune system, and cause inflammation and oxidative stress [5]. The accumulation of microplastics in the gills, liver, and gut of zebrafish was observed after seven days of exposure to 20 mg/L microplastics of 5 μm [6]. More specifically, some microplastics with smaller particle sizes passed through the blood-brain barrier, causing brain damage and behavioral disorder [7]. In addition, due to their high specific surface area and hydrophobic surface, microplastics can adsorb hydrophilic chemicals and act as significant vectors for pollutants [8]. After being exposed to 100 μg/L microplastics and organophosphorus flame retardants for up to 90 days, aggravated toxicity of organophosphorus flame retardants in mice were found [9]. Microplastics have been reported to change exposure routes, increase

the accumulation and share the common toxic mechanisms of di-(2-ethylhexyl) phthalate (DEHP) in mice [10].

Nonylphenol (NP), a widely researched phenolic endocrine disruptor, originates principally from the degradation of nonylphenol ethoxylates and is mainly used in the production of surfactants, lubricating oil additives, and pesticide emulsifiers [11]. Many studies have proven that NP is harmful to the immune system, the nervous system, and the reproductive system [12,13]. Mice exposed to NP had reduced oocyte quality, where the cytoskeletal dynamics and mitochondrial function were affected, which further induced oxidative stress and apoptosis in mouse oocytes [14]. NP in the intestinal tract after oral administration destroyed the intestinal barrier. Studies have shown that NP changed the permeability of Caco-2 monolayer cells by inhibiting the expression of tight junction protein and increasing intestinal permeability [15].

As two environmental pollutants of great concern, microplastics and NP coexist in the ocean, sludge, and sediments [16,17]. However, studies into the adsorption, desorption, and combined effects of microplastics and NP are still rare. This article aimed to systematically investigate the adsorption and desorption process of NP from polystyrene microplastics (PS-MPs) with different particle sizes. The mechanism of adsorption was investigated using kinetics and the isotherm model. The desorption experiments of NP from PS-MPs under water or simulated warm-blooded body gastrointestinal environments were also studied and provided support for the analysis of the single and combined toxicity of NP and PS-MPs on human intestinal epithelial Caco-2 cells. These results can provide new insights into the potential health risks of PS-MPs combined with NP or other environmental contaminants in humans.

2. Materials and Methods

2.1. Materials and Chemicals

NP (analysis standard, CAS: 104-40-5, Aladdin Bio-Chem Technology, Shanghai, China) was dissolved in acetonitrile to prepare a stock solution and stored at 4 °C. PS-MPs with diameters of 0.1, 1, 10, 50, and 100 μm were bought from Dongguan Zhangmutou Company (Dongguan, China). After the removal of surfactants using ethanol and water alternately, PS-MPs were dried using vacuum freeze-drying and subjected to the adsorption testing. High glucose Dulbecco's modified Eagles medium (DMEM), nonessential amino acid, 0.25% Trypsin-EDTA solution (without phenol red), and L-glutamine were all purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China), while fetal bovine serum (FBS) was brought from Biological Industries (Karmiel, Israel). Cell counting Kit-8 assay kit (CCK-8) was purchased from Dojindo Chemical Technology Co., Ltd. (Shanghai, China).

2.2. Characterization of PS-MPs and Detection of NP

The surface morphology of the PS-MPs was investigated by cold field emission scanning electron microscope (SU8100, Hitachi, Japan); The surface area of the PS-MPs was evaluated by N_2 adsorption-desorption using Brunauer-Emmett-Teller (JW-BK132F, JWGB Sci & Tech Ltd., Beijing, China). The surface functional groups of the PS-MPs were measured usin Fourier transform infrared spectroscopy (FTIR, Nicolet iS50, Thermo Fisher Scientific, Waltham, MA, USA) in the wavenumber range of 4000–400 cm^{-1} with KBr pellet.

Ultra-high performance liquid chromatography-fluorescence detector (excitation wavelength = 230 nm, emission wavelength = 310 nm) (Agilent technologies company, Santa Clara, CA, USA) was applied to the determination of NP [18,19], 10 μL of sample was loaded in Eclipse XDB-C18 column (5 μm, 4.6 × 150 mm, Agilent technologies company, Santa Clara, CA, USA), with 0.4 mL/min flow rate of acetonitrile: water (90:10) as mobile phase (isocratic elution). An external standard method was used for the construction of the peak area-concentration standard curve, and then the concentration of NP was calculated.

2.3. Adsorption and Desorption Experiments

An amount of 2 mg of PS-MPs with different particle sizes was mixed with 100 mL NP solution (4 mg/L and pH = 7). All samples were oscillated in a shaker at a temperature of 25 °C and at a constant speed of 150 r/min. Three samples were taken as parallel samples at each time point (0, 0.25, 0.5, 1, 2, 3, 6, 9, 12, 18, 24, 36, 48, 72 and 96 h). After centrifugation and filtration with a 0.22 μm PTFE filter membrane, the concentration of the residual NP in the filtrate was measured. The NP adsorption capacity of PS-MPs (q_e) at equilibrium (t = 96 h) or a selected time (q_t) were calculated, respectively, by Equations (1) and (2):

$$q_e = \frac{C_0 - C_e}{m} \times V \quad (1)$$

$$q_t = \frac{C_0 - C_t}{m} \times V \quad (2)$$

where C_0, C_e, and C_t are the solution concentration of NP at initial, equilibrium (t = 96 h), and selected times t (h), respectively; V (mL) is the volume of solution and m (g) represents the weight of the PS-MPs.

The desorption behavior of NP on the PS-MPs in a water environment and the warm-blood body gastrointestinal environment were investigated. The simulated gastrointestinal fluid was an acid solution (pH = 2.8) with 15.5 mmol/L sodium taurocholate and 4 g/L of pepsin. The mixtures were then agitated in a constant temperature shaker at the intestinal temperature of 35 ± 2 °C and a speed of 100 r/min [20]. A total of 50 mL of water (3.5% sodium chloride in ultrapure water, pH 7.0) was used as the control for comparison with the gastrointestinal fluids for the desorption experiments, which occurred at a constant temperature of 25 ± 2 °C and speed of 150 r/min [21].

After 120 h of desorption. The amount of NP desorbed (qdt, mg/g) was calculated by Equation (3):

$$q_{dt} = \frac{C_{dt} - C_{d0}}{m_d} \times V_d \quad (3)$$

where q_{dt} (mg/g) denoted the desorption capacity of the PS-MPs at the time t (h); C_{d0} and C_{dt} are the concentration of NP in the solution at an initial and selected time t (h); V_d (L) was the volume of solution added, and m_d (g) was the total weight of the PS-MPs and adsorbed NP used for the desorption experiment.

NP desorption percentage (A_{dt}, %) can be calculated based on Equation (4):

$$A_{dt} = \frac{q_{dt}}{q_t} \times 100 \quad (4)$$

2.4. The Factors That Influence the Adsorption Behavior of NP on PS-MPs

Separate sets of experiments were conducted to investigate the effects of pH, metal ions, and the diameter of the PS-MPs on NP adsorption. The influence of pH was studied by adjusting the reaction solutions to different pH values (3, 5, 7, 9, and 11.0, adjusted with 1 mol/L HCl solution and NaOH solution) and the effects of the metal ions on adsorption were carried out in reaction solution containing 0.2 mol/L metal ions (Na^+, Fe^{2+}, Ca^{2+}, and K^+ respectively) until the equilibrium of the NP solutions (4 mg/L) and the 0.1 μm PS-MPs (20 μg/mL) was established. In addition to the normal influencing factors, the initial concentration of NP and the particles sizes of the PS-MPs are non-negligible effects. The influence of the initial concentration of NP was investigated by agitating the NP solution at a series of gradients (1, 4, 10, 20, 30, and 40 mg/L) with 20 μg/mL 0.1 μm PS-MPs at 25 °C for 96 h. The effect of the diameter of the PS-MPs on NP adsorption was carried out in NP solutions (4 mg/L) with 20 μg/mL PS-MPs, with different particle sizes (0.1, 1, 10, 50, and 100 μm) at 25 °C until the adsorption reached equilibrium.

2.5. Adsorption Kinetic and Isotherm Equations

The adsorption kinetics of NP in the solution via the PS-MPs with different particle sizes were studied in terms of four kinetic models: pseudo-first-order, pseudo-second-order, intra-particle diffusion, and Bangham model. Three adsorption isotherms models, including Langmuir, Freundlich, and Dubinin-Radushkevich (D-R) model, were also constructed and used to examine the adsorption mechanism and corresponding rate control step. The models mentioned here can be found in Appendix A Table A1.

2.6. Cell Culture

The Caco-2 (Cat NO.: CL-0050) cell lines were provided by Procell Life Science & Technology Co., Ltd. Wuhan, Hebei, China. They were cultured in DMEM high-sugar medium containing 18% fetal bovine serum, 1% L-glutamine, and non-essential amino acids and were grown under 5% CO_2, 37 °C constant temperature culture conditions. When the cells grew to cover 80–90% of the bottom of the flask, they were digested with trypsin and passaged every three days.

Different particle sizes of PS-MPs (0.1, 1, 10, 50, and 100 μm) were dispersed in serum-free DMEM medium at a concentration of 500 mg/L; an NP solution with a concentration of 40 μmol/L, and PS-MPs synergistic with NP (1 h adsorption reaction product of 500 mg/L PS-MPs and 40 μmol/L NP) were added to the Caco-2 cells. Culture mediums without any contaminants were set as control groups.

2.7. Cytotoxicity Assays

2.7.1. Cell Viability

Cell viability was quantitatively evaluated using the Cell Counting Kit-8 (CCK-8). After 12 and 48 h of drug intervention, 10 μL of CCK-8 assay solution was added to each well of the 96-well plate, followed by incubation for 1.5 h. The absorbance at 450 nm was measured using a microplate reader (Varioskan Flash, Thermo Fisher Scientific, Waltham, MA, USA). Cell viability can be calculated according to the following formula:

$$\text{Cell viability}(\%) = \frac{(A_e - A_p)}{(A_c - A_0)} \times 100\% \quad (5)$$

where A_e and Ac represented the absorbance of the experimental group and the control group, A_0 and A_p were the absorbances of the culture medium and solution of the PS-MPs dispersed in the medium, respectively.

2.7.2. Cell Cycle

After treatment with drugs for 48 h, cells were harvested by centrifugation and resuspended to a final concentration of 1×10^6 cells/mL, and then fixed with 70% cold ethanol. Cells were incubated with a propidium iodide staining working solution in the dark for 1 h. The red fluorescence of 10,000 events of propiodium iodide-stained cells was countered using a Cytoflex-S flow cytometer (Beckman Coulter, Inc. in Brea, CA, USA). The percentage of cells in the different phases of the cell cycle was calculated using Kaluza Analysis Software (Beckman Coulter, Inc. in Brea, USA).

2.7.3. Apoptosis

After exposure to the PS-MPs and NP, 5 μL Annexin V-FITC dye solution and 10 μL propidium iodide were added to the cell suspension for a 15 min incubation in the dark at room temperature (20–25 °C). Afterward, the cells were measured in a Cytoflex-S flow cytometer (Beckman Coulter, Inc. in Brea, USA).

2.7.4. Mitochondrial Membrane Potential (MMP)

A total of 1 mL of JC-1 staining working solution was directly mixed with the treated cells for a 20 min of incubation at 37 °C. The supernatant was aspirated off and the cells

were washed twice with JC-1 staining buffer (1×). The red and green fluorescence values of the cells were measured using a fluorescence microscope (Leica DMi8, Leica Microsystems, Wetzlar, Germany), and the ratio of green/red JC-1 fluorescent intensity was calculated.

2.7.5. Reactive Oxygen Species (ROS)

After 48 and 72 h of drug intervention in the cells, an appropriate amount of 10 μmol/L DCFH-DA fluorescent probe dye was added and incubated for 20 min at 37 °C. Cells were then collected and washed three times with PBS to sufficiently remove the DCFH-DA that did not enter the cells. Subsequently, the average fluorescence intensity value detected by the flow cytometer (Cytoflex S, Beckman Coulter, Inc. in Brea, USA) at a 488 nm excitation wavelength and a 525 nm emission wavelength characterized the active oxygen levels.

2.8. Statistical Analysis

The fitting curve and fitting parameters of the adsorption kinetics and isotherm models were performed using the Origin 2019 software. All experimental data were expressed as a mean with standard deviations. One-way ANOVA and Tukey or Dunnett testing for multiple comparisons were performed using GraphPad Prism 8.0. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Characterization of PS-MPs

The PS-MPs used in this study were pure particles with particle sizes of 0.1, 1, 10, 50, and 100 μm. Uniform particle size can be observed using a scanning electron microscope (Figure 1A). As to the surface morphology observed, representative phenomena of different particle sizes were found. The surface of the 1 μm PS-MPs was not smooth and flat but was adhered to the unaggregated fragments with cracks. In the observation of the PS-MPs with a diameter of 50 μm, a surface that was either relatively smooth or attached with loose and porous foam-like substances was found. In addition, the shape of a particle of 100 μm in size was closer to an ellipse, with particles attached to its rough surface. The specific surface area and porosity tests proved that the PS-MPs had a porous structure, and the PS-MPs with smaller particle sizes had a larger specific surface and average pore diameters (Table 1).

The FT-IR spectrum of the PS-MPs is shown in Figure 1B as a representative example. The infrared absorption peak at 3000 cm^{-1} and 750 cm^{-1} was attributed to the C-H stretching vibration mode and the out-of-plane bending vibration mode on the benzene ring in the polystyrene molecule; The 2850 cm^{-1} frequency belongs to the CH_2 symmetric stretching vibration mode in the polystyrene molecule, and 960 cm^{-1} is attributed to the out-of-plane deformation vibration mode of the olefin in the polystyrene molecule; the prominent peak at around 1455 cm^{-1} and 1730 cm^{-1} was attributed to the vibration mode of the benzene ring in the polystyrene molecule. The composition and characteristic diffraction peaks of the PS-MPs corresponded to previous reports [22].

Table 1. Surface area, total pore volume, and average pore diameter of PS-MPs.

Particle Size (μm)	0.1	1	10	50	100
Surface area (m^2/g)	62.248	38.193	16.887	3.824	3.351
Total pore volume (cc/g)	0.336	0.476	0.043	0.007	0.004
Average pore diameter (nm)	19.970	9.818	10.013	7.082	5.096

3.2. Effect of Reaction Time on Adsorption and Desorption of NP on PS-MPs

In this study, Figure 2A,B are the individual plotted curves of the NP adsorption capacity of PS-MPs changing with time. It can be seen that the NP sorption by PS-MPs rose rapidly to the maximum and then gradually slowed until reaching the adsorption equilibrium, which was followed by it then becoming unchanging. The results showed

similar trends for the NP adsorption processes for different particle sizes of the PS-MPs. Figure 2C showed that the NP desorption capacity curve of PS-MPs with a diameter of 0.1 μm under the gastrointestinal tract reached a maximum of about 0.12% within the first 2 h and then remained stable, whereas the NP desorption capacity of the PS-MPs under a water environment kept growing and was about nine times higher than that of the warm-blooded biological gastrointestinal tract at 120 h.

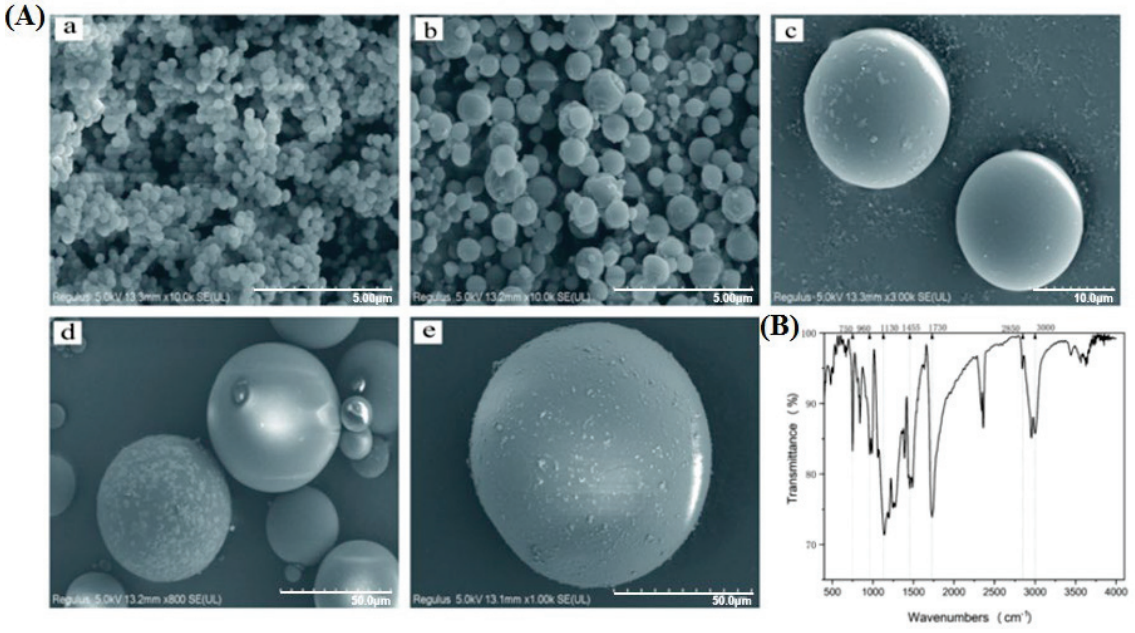

Figure 1. Characterization of PS−MPs used in this study. (**A**) SEM images of PS−MPs with different particle sizes: 0.1 μm (**a**); 1 μm (**b**); 10 μm (**c**); 50 μm (**d**); 100 μm (**e**). (**B**) FTIR spectra of PS−MPs.

3.3. Intrinsic and Extrinsic Factors That Influence NP Adsorption to PS-MPs

There are many factors that affect the adsorption capacities of microplastics on a certain organic pollutant, such as the type, the physical characteristics (mainly particle size, specific surface area, and crystallinity) of the microplastics, the concentration of organic pollutant, and environmental conditions (pH and metal ions) [23–25]. Figure 3 showed the influence of four factors on the adsorption process. It was found that the adsorption of NP onto the PS-MPs was strongly dependent on the initial concentration of NP. As seen in Figure 3B, the adsorption capacity increased with the increasing initial concentration of NP, and reached the maximum when the initial concentration of NP was 40 mg/L. We also found that, under a certain concentration of NP, the increasing particle size of the PS-MPs would result in a decrease in the adsorption capacity.

Solution conditions, such as pH value and metal ion concentration, also significantly affected the adsorption capacity of the PS-MPs. Maximum NP adsorption occurred when the pH was 3 for the PS-MPs. NP adsorption capacity decreased as solution pH increased. Figure 3D shows the influence of various metal ions (0.2 M) on the adsorption of NP onto the PS-MPs at a pH of 7. The results showed that NP adsorption was strongly dependent on ions for the PS-MPs. An average increase of 6.5 mg/g for NP adsorption capacity for the PS-MPs was observed in the presence of other metal ions, among which Na^+ increased the most.

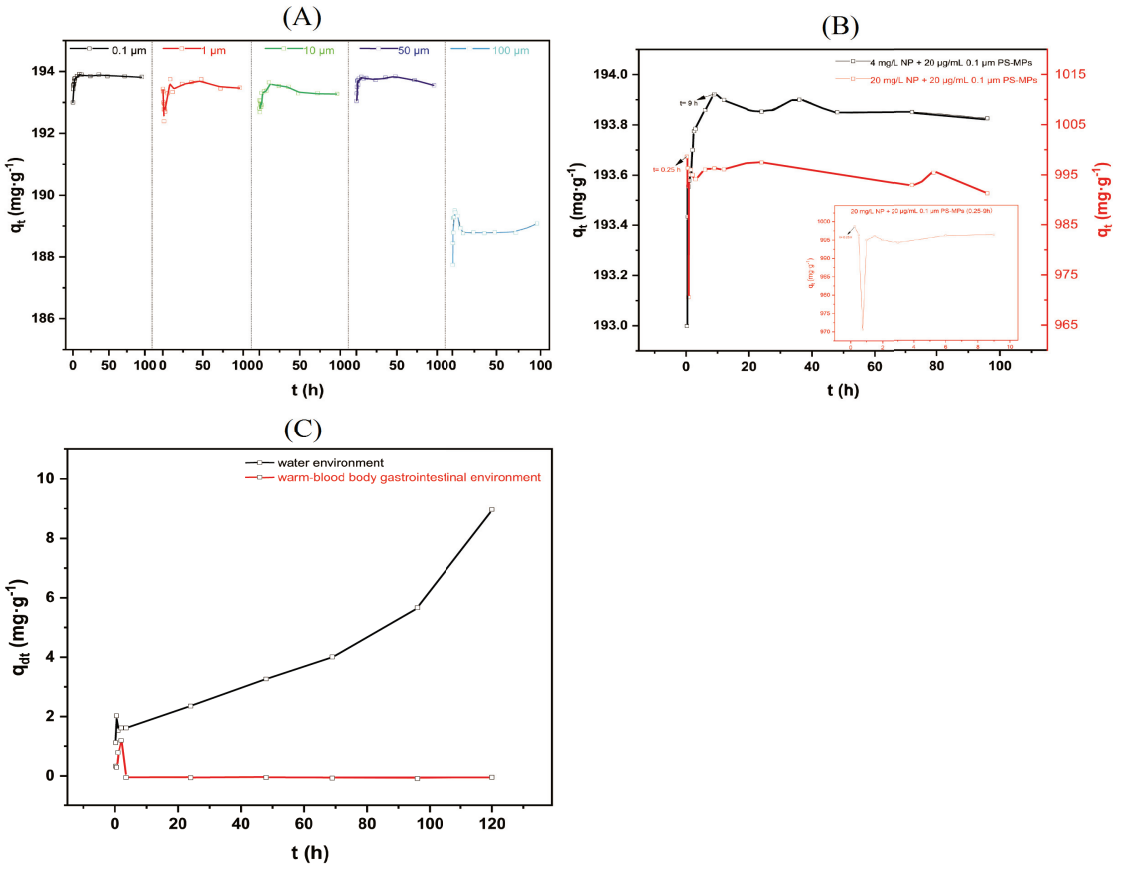

Figure 2. Effects of reaction time on the adsorption and desorption of NP in PS−MPs. (**A**) Adsorption process of NP solutions (4 mg/L) with 20 μg/mL PS−MPs with different particle size (0.1, 1, 10, 50, and 100 μm). (**B**) Effect of different initial NP concentrations (4 and 20 mg/L) on time-sorption capacity trends. (**C**) The desorption behavior of NP on the PS−MPs in the water environment (pH 7.0, 3.5% sodium chloride, 25 ± 2 °C and 150 r/min) and the warm-blood body gastrointestinal environment (pH = 2.8, 15.5 mmol/L sodium taurocholate, 10 g/L of pepsin, 35 ± 2 °C and 100 r/min) was investigated.

3.4. Adsorption Kinetics

The obtained data were analyzed with the pseudo-second-order, intra-particle diffusion, and Bangham models. As shown in Table 2, the NP adsorption process of the PS-MPs was best fitted with a pseudo-second order kinetic model with high correlation coefficient (R^2) values. The intra-particle diffusion kinetic model was more suitable for 0.1 μm PS-MPs (R^2 = 0.988) than for other particle sizes of the PS-MPs. The data from the intra-particle diffusion rate constant k_{1p} and the piece-wise fitting, C, of the 0.1 μm PS-MPs showed that the NP easily diffused inside the PS-MPs, whereas intra-particle diffusion was not the only rate-limiting step. Surprisingly, the Bangham kinetic model also gave good fittings for the adsorption of NP by 0.1 μm PS-MPs, indicating that intraparticle diffusion is crucial for NP adsorption by PS-MPs with small particle size. The kinetic model fitting curves mentioned here can be found in Appendix A Figure A1.

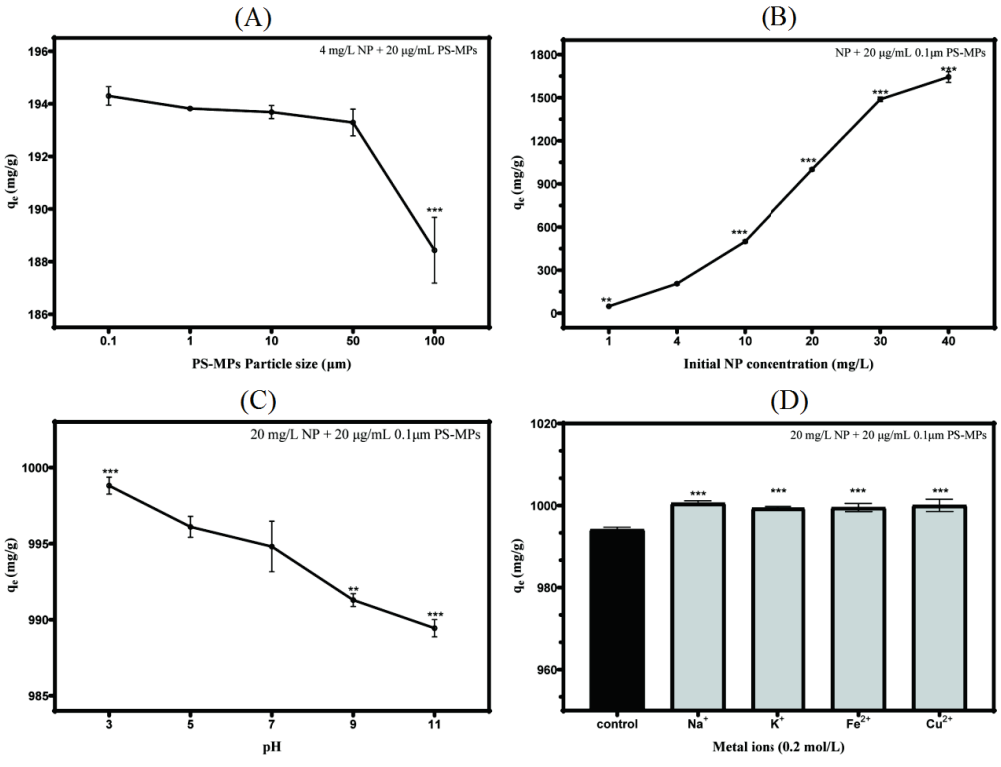

Figure 3. The effect of PS-MP particle size (**A**), initial NP concentration (**B**), solution pH (**C**), and metal ions contained in the solution (**D**) on the adsorption process of NP. * indicates the significant difference between the treatment group (0.1 μm PS-MPs, initial NP concentration of 4 mg/L, pH 7 and control without metal ions, respectively) and other groups (* $p < 0.05$, ** $p < 0.001$, *** $p < 0.0005$).

Table 2. Kinetic parameters of NP adsorption by the PS-MPs obtained from the pseudo-second-order, Intra-particle diffusion and Bangham models.

Kinetic Model		Particle Size (μm)				
		0.1	**1**	**10**	**50**	**100**
Pseudo-second-order model						
k_2 ($g \cdot mg^{-1} \cdot h^{-1}$)		127.567	27.779	18.979	66.961	13.285
q_e ($mg \cdot g^{-1}$)		193.923	193.870	193.859	193.851	189.560
R^2		0.9998	0.9998	0.9997	0.9982	0.9987
Intra-particle diffusion model						
k_{ip} ($mg \cdot g^{-1}$ min^{-1})	k_{1p}	2.10013	0.15685	0.26952	1.30454	0.15907
	k_{2p}	0.20992	0.04903	−0.11312	0.02179	−0.55142
	k_{3p}	−0.01188	−0.09727	−0.01576	−0.12874	0.19729
C ($mg \cdot g^{-1}$)	C_1	191.949	192.81716	192.65587	192.38299	188.71922
	C_2	193.385	193.36525	194.11567	193.68406	191.49437
	C_3	193.944	194.37526	193.43371	194.81593	181.1686
R^2		0.9875	0.4645	0.8277	0.9451	0.2302
Bangham model						
k		6.35439	5.47685	5.40951	6.52911	7.89394
z		0.11868	0.02838	0.03225	0.12528	0.3395
R^2		0.9519	0.2615	0.5853	0.7581	0.4534

3.5. Adsorption Isotherm

Under a given system with a constant temperature and adsorption equilibrium, the relationship between the concentration of the adsorbate and adsorbent can be described by different equilibrium sorption isotherm models. The Langmuir, Freundlich, and D-R isotherms are used frequently to describe the adsorption data. According to Table 3, the adsorption of NP onto the PS-MPs was found to follow the Langmuir isotherm model since the maximum adsorption capacity calculated by the Langmuir model is approximately equal to the actual test results, suggesting that the PS-MPs had homogeneous surface sites, and monolayer adsorption was occurring [26,27]. The value for the R_L of the D-R isotherm indicated the adsorption behavior of the PS-MPs was extremely favorable for NP. The adsorption isotherm model-fitting curve mentioned here can be found in Appendix A Figure A2.

Table 3. Langmuir and D-R parameters for NP sorption by PS-MPs with particle size of 0.1 μm.

Isotherm Model									
Langmuir Model									
q_{max} (mg·g^{-1})	K_L	R^2	R_L						
			1	4	10	20	30	40	50
1665.6118	3.9657	0.9880	0.2014	0.0593	0.0246	0.0125	0.0083	0.0067	0.0050
D-R model									
q_{max} (mg·g^{-1})	K_{D-R} (mol^2·KJ^{-2})	R^2	E						
19.5600	1.52938×10^8	0.8062	5.717×10^{-5}						

3.6. Cell Proliferation and Apoptosis

3.6.1. Cell Viability

Figure 4B shows that the toxicity of NP on Caco-2 cells seemed to be time- and concentration-dependent. As shown in Figure 4A, 500 mg/L of PS-MPs with all particle sizes tested except 0.1 μm had no significant cytotoxicity for 12 h, which suggested that smaller particle sizes may cause more rapid damage. When treatment time was extended to 48 h, those PS-MPs with a bigger diameter (50 and 100 μm) decreased cell viability by about 12%, indicating a time dimension to their cytotoxicity. When the 0.1 μm PS-MPs intervened in the Caco-2 cells synergistically with NP, the activity of the Caco-2 cells declined in a time-dependent manner. There was no significant difference in cell viability between the exposure to PS-MPs alone and PS-MP-adsorbed NP exposure (Figure 4C).

3.6.2. Cell Cycle

Problems with the cell cycle may also be a major reason for the interference in cell proliferation. Figure 5A,B showed that, in comparison with the control group, the NP, PS-MPs (with a diameter of 0.1, 10, 50, and 100 μm), or the 0.1 μm PS-MPs synergistic with NP groups showed similar trends in early DNA synthesis: a significantly higher proportion of cells in the G1 phase and a lower proportion of cells in the S and G2/M phases. Compared with the 0.1 μm PS-MPs group, the 0.1 μm PS-MPs synergistic with the NP group exhibited a higher proportion of cells in the G1 phase by about 6.5%.

3.6.3. Apoptosis

Our results showed that, compared with the control group, the NP, PS-MPs (with diameters tested in this study), or 0.1 μm PS-MPs synergistic with NP induced significant apoptosis in the Caco-2 cells, as shown in Figure 5C. The 48 h treatment of the larger PS-MPs (with a diameter of 50 and 100 μm) resulted in a significant increase in the population of both early and late apoptotic cells over the control group. The increase in the proportion of cells undergoing apoptosis was significantly higher following exposure to 0.1 μm PS-

MPs synergistic with NP treatment (17.7%) when compared to 0.1 μm PS-MP (10.5%) or NP-alone (8.94%) treatment, which was mainly attributed to the greater proportion of early apoptosis.

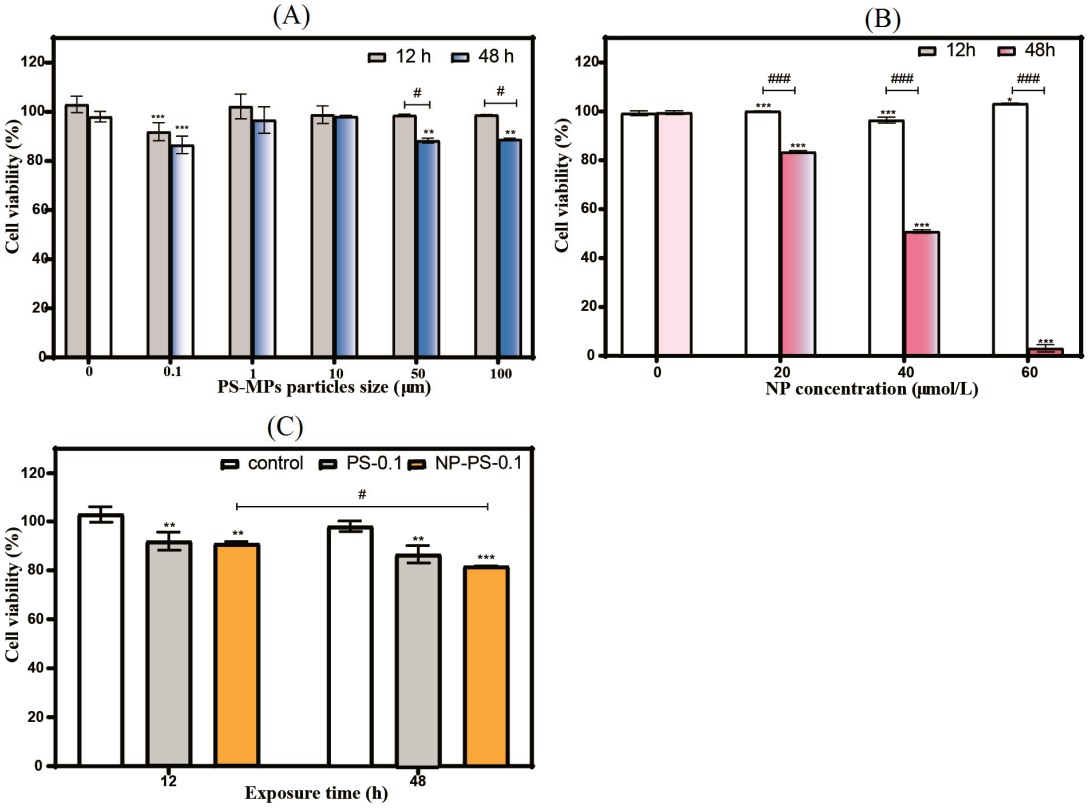

Figure 4. Effects of PS-MPs, NP, and synergistics on the cell viability of Caco-2 cells. Cells were incubated alone with different particle sizes of 500 mg/L PS-MPs (**A**) and different concentrations of NP (**B**) for 12 and 48 h. The effect of 0.1 μm PS-MPs alone and in combination with NP on Caco-2 cell viability at 12 h and 48 h (**C**). Cell viability was measured by CCK-8 assay. * indicates the significant difference between the treatment group and the control group (* $p < 0.05$, ** $p < 0.001$, *** $p < 0.0005$). # indicates the significant difference between the marked groups ($^{\#}$ $p < 0.05$, $^{\#\#\#}$ $p < 0.0005$).

3.7. Cell Function

3.7.1. Mitochondrial Depolarization

The destruction of mitochondrial membrane potential is considered to be one of the earliest events in the process of an apoptosis cascade. JC-1 fluorescent dye was used to detect the depolarization of cell mitochondrial membrane potential. Fluorescence images of the cells were recorded, and the relative levels of the intensities of green/red JC-1 fluorescence were quantified.

As shown in Figure 6 and Appendix A Figure A3, after 48 h of treatment, PS-MPs of various sizes caused a different degree of depolarization of the mitochondrial membrane potential. Further, in comparison to 0.1 μm PS-MPs alone or NP alone treatments, a statistical significance for MMP change was observed in the PS-MPs with adsorbed NP group, pointing to a synergistic effect once again.

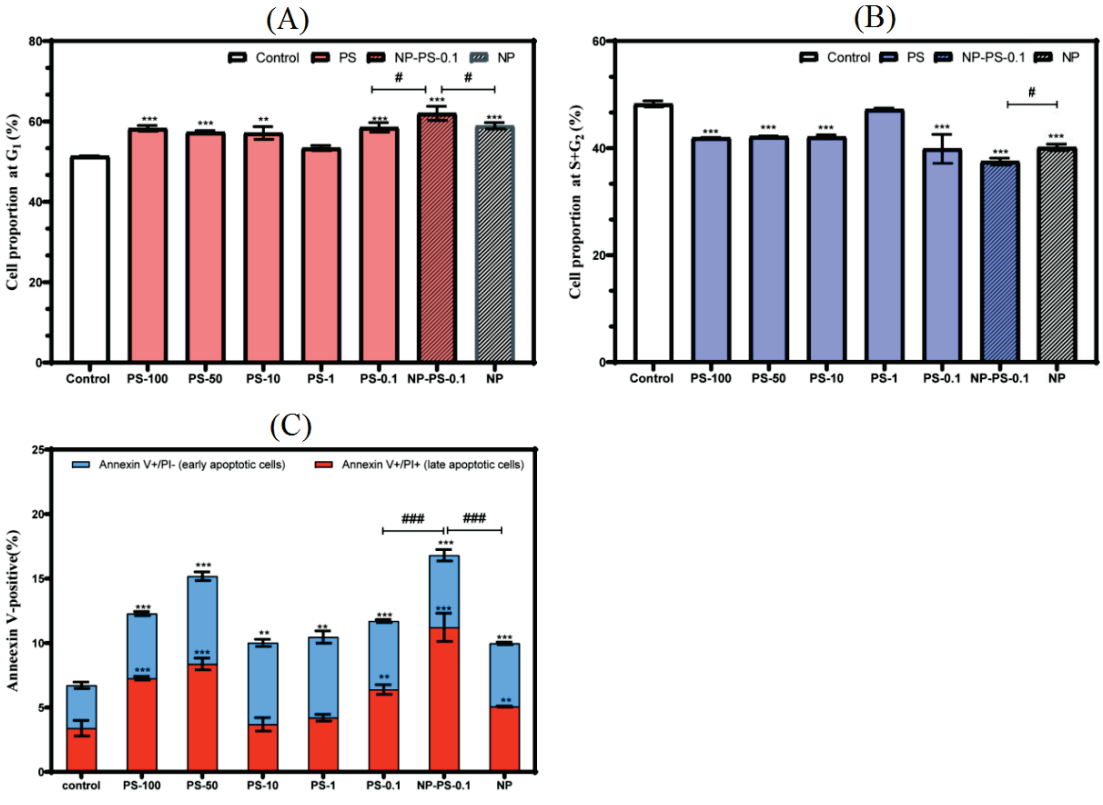

Figure 5. Effects of 48 h treatment of PS-MPs, NP and synergistics on the cell cycle and apoptosis of the Caco-2 cells. (**A**) Shows the proportion of cells in the G1 phase via different exposures. (**B**) Showed the sum of the cells in the S and G2 phases via different exposures. Early and late apoptotic Caco-2 cells were analyzed by flow cytometry after Annexin V and PI staining. A statistical graph of the percentage of cells in each phase was calculated (**C**). * indicates the significant difference between the treatment group and the control group (** $p < 0.001$, *** $p < 0.0005$). $^{\#}$ indicates the significant difference between the marked groups ($^{\#}$ $p < 0.05$, $^{\#\#\#}$ $p < 0.0005$).

3.7.2. Reactive Oxygen Species Production

Reactive oxygen species (ROS) are a group of chemically reactive chemical substances containing oxygen, which play important roles in cell signal transduction and homeostasis. In this study, the ROS change caused by the PS-MPs was roughly in accord with a time-dependent manner (Figure 7). In comparison with the control group, large PS-MP (with a particle size of 50 and 100 μm) treatment for 48 and 72 h resulted in a significant increase in ROS generation, which suggested that larger PS-MPs have a greater potential to break the oxidation–antioxidant balance of cells. In addition, compared with the NP group, 0.1 μm PS-MPs synergistic with NP treatment resulted in a significant increase in ROS generation, evidencing that the joint effect of the PS-MPs occurred.

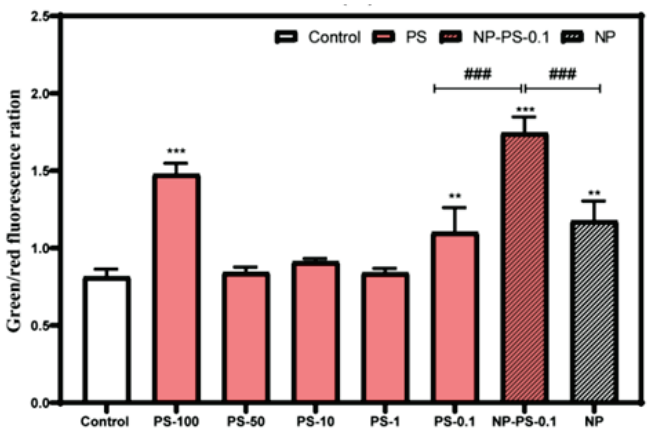

Figure 6. The mitochondrial membrane potential depolarization of Caco-2 cells treated with 40 μmol/L NP, 500 mg/L PS-MPs (with different particle sizes), and 0.1 μm PS-MPs that absorbed NP at 48 h; the fluorescence values were calculated by Image J software (National Institute of Mental Health, USA) and the ratio of red to green fluorescence reflected the degree of mitochondrial membrane depolarization (2). * indicates the significant difference between the treatment group and the control group (** $p < 0.001$, *** $p < 0.0005$). $^{\#}$ indicates the significant difference between the marked groups ($^{\#\#\#}$ $p < 0.0005$).

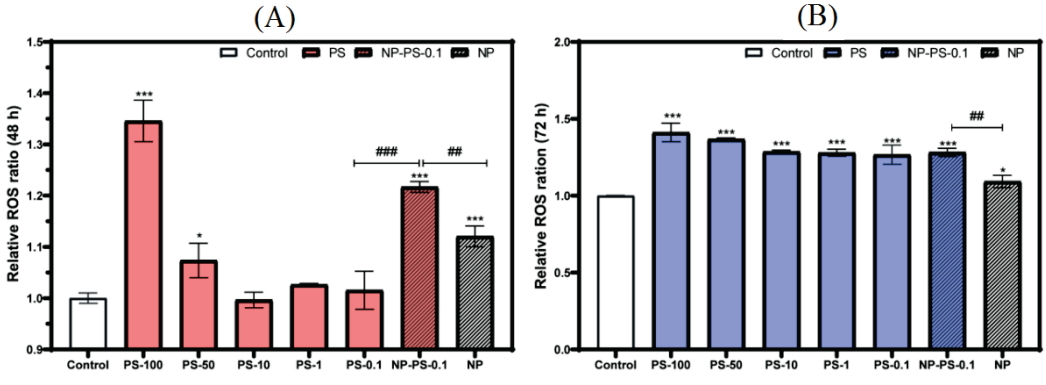

Figure 7. Effects of PS-MPs and NP on the generation of the intracellular ROS of Caco-2 cells at 48 (**A**) and 72 h (**B**), expressed as a ratio to the control group. * indicates the significant difference between the treatment group and the control group (* $p < 0.05$, *** $p < 0.0005$). $^{\#}$ indicates the significant difference between the marked groups ($^{\#\#}$ $p < 0.001$, $^{\#\#\#}$ $p < 0.0005$).

4. Discussion

Plastic polymers have been believed to be biochemically inert and not harmful to ecosystem health for many years. However, there is a growing consensus that more research is needed to explore the toxicological effects of MPs, especially when combined with other contaminants. Previous studies have shown that combined exposure to MPs with other contaminants may alter toxicokinetics [28]. Hence, this study aimed at systemically examining the adsorption/desorption behavior of NP on PS-MPs with different particle sizes and evaluating their cytotoxic effects on Caco-2 cells.

PS-MPs with different diameters caused various degrees of decline in intestinal cellar proliferation. PS-MPs with smaller particle sizes caused a quick decrease, whereas larger particle sizes (of PS-MPs) did not affect cell viability in the short term, which was consistent with the studies of Zhang et al. [29]. There is a combined toxicity of nanoscale and

micronscale plastic particles in the intestines [30]. Considering the accumulation of MPs in the intestine, their toxicity will be amplified; a total of 48 days chronic exposure to MPs triggered intestinal disorders [31]. As to the cell cycle, PS-MPs induced a G1 phase block, verifying a former study that showed that high concentrations of nanomaterials can adhere to the cell membrane to hinder the transport and adsorption of nutrients, which was related to cell cycle arrest [32]. Furthermore, we speculated that impaired cell function might be the main pathway of cell damage caused by PS-MPs, especially those of large particle sizes. They induced ROS generation and mitochondrial permeability transition (MPT), which correlates with the depolarization of the mitochondrial membrane potential and leads to an apoptotic cascade [33]. Similarly, there have been distinct pieces of evidence that long-term PS-MPs exposure may be a risk factor for kidney health and placental barriers [34,35].

In this study, PS-MPs were proven to have a high adsorbent of NP which may be due to their large specific surface area and rich microporous structure. The data of NP desorption from PS-MPs showed a linear increase in the aqueous environment, indicating that PS-MPs with absorbed NP may possess chronic toxic effects with the reduced metabolism efficiency of NP. Consistently, MPs acted as carriers and transport phthalate esters into organisms, as well as inducing combined health risks [10]. Microporous adsorption and intra-particle diffusion had a stronger influence on small-sized PS-MPs than on large particle sizes of PS-MPs, suggesting that the smaller the particle size of the microplastics, the higher the risk of synergistic toxicity [36]. Further studies were conducted with NP and smaller particle sizes of PS-MPs (0.1 μm) to elucidate a general mechanism concerning reactive oxygen species, mitochondrial membrane potential, apoptosis, and the cell cycle. Inversely, polycyclic aromatic hydrocarbons could decrease the toxicity of the nanoplastics by enhancing their stability, whereas metal oxide nanoparticles had the opposite effect [37]. Our results showed that oxidative stress and mitochondrial dysfunction were the main pathways through which PS-MPs destroy cell homeostasis. Similar results can be found in the study about the toxic effects of PS-MPs with bisphenol A, which showed that the synergistic toxicity of nano-scale polystyrene and bisphenol A was more obvious than that of polystyrene or bisphenol A alone [38]. Additionally, M Vagner et al. demonstrated that nanoplastics had a strong potential to cross the intestinal barrier [39]; thus, we speculate that the synergistic cytotoxicity of PS-MPs and NP also damages other organs and even creating whole systemic exposure risk, which requires further study. Furthermore, damage to the basic functions of cells caused by PS-MPs combined with NP should not be underestimated, especially considering that smaller particle-sized MPs can interact with the cellular components and increase the accumulation of hazards or change the distribution of hazards.

5. Conclusions

The current work focused on the environmental behavior and in vitro toxicity of NP on PS-MPs by using the results from desorption experiments in the intestinal or water environment as a bridge. The adsorption process of PS-MPs and NP can be better described by the pseudo-second-order kinetic model and Langmuir isotherm model, suggesting that the adsorption process was presumably chemisorption and spontaneous. An analysis of the NP adsorption of 0.1 μm PS-MPs using intra-particle diffusion and Bangham modeling confirmed that pore adsorption was also a possible adsorption mechanism. The desorption experiments showed that the biological environment significantly reduced the desorption capacity of NP, while the desorption rate was non-linearly increased in an aqueous environment. PS-MPs synergistic with NP can enhance the lethal toxicity of PS-MPs in Caco-2 cells. Both the PS-MPs and NP alone caused cellular oxidative stress, the depolarization of mitochondrial membrane potential, and cell cycle arrest, indicating that PS-MPs and NP may share the same mechanism, which can be seen as the main reason for the coordinated cell dysfunction caused by the combined group. These data can be used to comprehensively assess the environmental risks and human health threats from PS-MPs and NP. The next step is to focus on the damage caused to the function of specific organelles, such as mitochondria, lysosomes, and endoplasmic reticulum.

Author Contributions: Methodology and writing—original draft preparation, F.D.; validation, Q.Z.; data curation, L.W. and J.M.; writing—review and editing, L.S.; supervision, project administration and funding acquisition, D.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Natural Science Foundation of Jiangxi Province (No. 20212BAB206089), the National Natural Science Foundation of China (No. 31260364, and No. 32260563), the National Key R&D Program of China (No. 2017YFF0207805), and the Special Funds Project for Innovation of Postgraduates in Jianxi province (No. YC2019-S067).

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

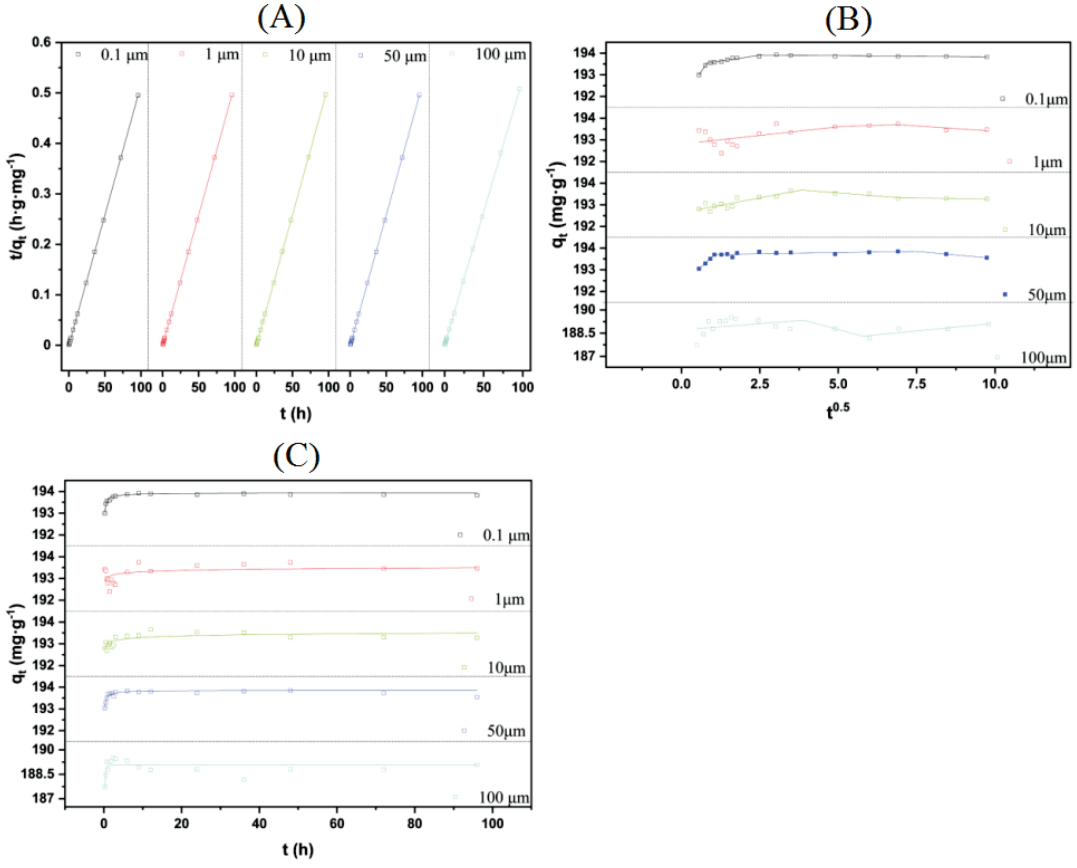

Figure A1. The plots of adsorption kinetics fitted by pseudo-second-order kinetic (**A**), intra−particle diffusion kinetics (**B**) and Bangham modes (**C**). The adsorption of NP (4 mg/L) and PS−MPs (20 μg/mL) of different particle sizes were studied.

Table A1. Adsorption kinetic and isotherm models used in this study.

Name	Equations	References
	kinetics	
Pseudo-first-order	$\ln(q_e - q_t) = \ln q_e - k_1 t$	[40]
Pseudo-second-order	$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q} t$	[41]
Intra-particle diffusion	$q_t = k_{ip} t^{0.5} + c$	[42]
Bangham	$q_t = q_e\left(1 - e^{-ktz}\right)$	[43]
	isotherm	
Langmuir	$\frac{c_e}{q_e} = \frac{1}{k_l q_m} + \frac{c_e}{q_{max}}$	[44]
Freundlich	$\ln q_e = \ln k_F + \frac{1}{n_f} \ln c_e$	[45]
D-R	$\ln q_e = \ln q_m - K_{DR}\varepsilon^2$ $\varepsilon = RT \ln\left(1 + \frac{1}{C_e}\right)$ $E = \frac{1}{\sqrt{2K_{DR}}}$	[46]

In adsorption kinetics, q_e and q_t denote the adsorption capacity of the PS-MPs at the condition of equilibrium and at the time t (h); k_1 (h^{-1}) is the rate invariable of the pseudo-first-order adsorption kinetic models; k_2 ($g{\cdot}mg^{-1}{\cdot}h^{-1}$) is the pseudo-second-order rate invariable; k_{ip} ($mg{\cdot}g^{-1}{\cdot}h^{-0.5}$) represents the rate invariable of intra-particle diffusion; C is a constant related to thickness and boundary layer. K is the adsorption rate constant of the Bangham model, and Z is the adsorption constant. q_e ($mg{\cdot}g^{-1}$) is the equilibrium adsorption capacity of NP per unit adsorbent; ce ($mg{\cdot}L^{-1}$) is the equilibrium concentration of NP insolution; k_L ($L{\cdot}mg^{-1}$) is the Langmuir adsorption constant; q_{max} ($mg{\cdot}g^{-1}$) represents the theoretical maximum adsorption capacity; n_f is the surface heterogeneity factor and k_F ($mg{\cdot}g^{-1}$) is the Freundlich partition co-efficient; K_{DR} is the constant related to adsorption energy ($mol^2{\cdot}KJ^{-2}$); ε is Polanyi potential energy ($KJ{\cdot}mol^{-1}$); R is the gas constant, T is the absolute temperature (K), and E is the average free energy of adsorption ($KJ{\cdot}mol^{-1}$), $|E| < 8$ ($KJ{\cdot}mol^{-1}$) is physical adsorption, $|E| > 16$ ($KJ{\cdot}mol^{-1}$) is chemisorption.

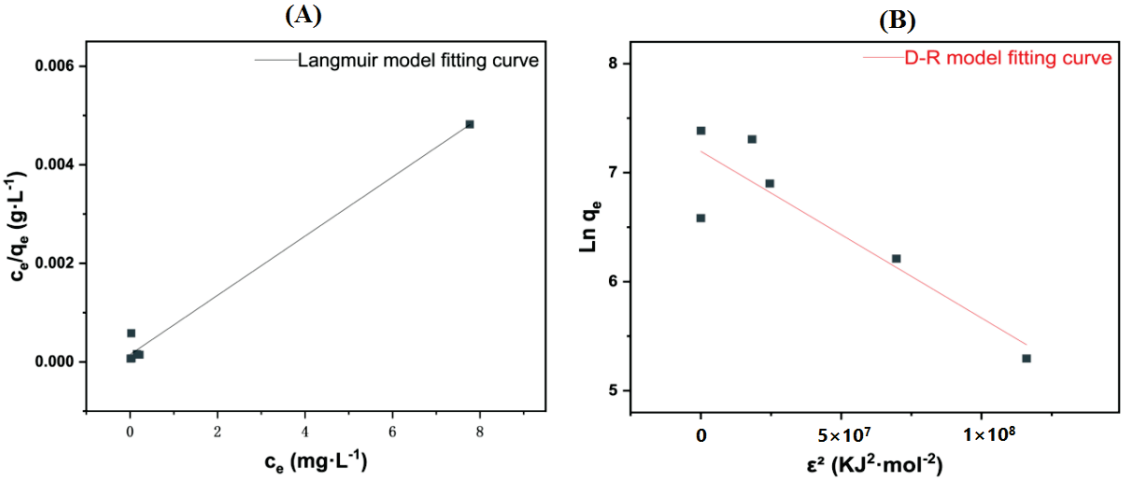

Figure A2. Adsorption isotherm model fitting of 20 μg/mL 0.1 μm of MPs−PS with different concentrations of NP. (**A**) Langmuir model fitting. (**B**) D−R model fitting.

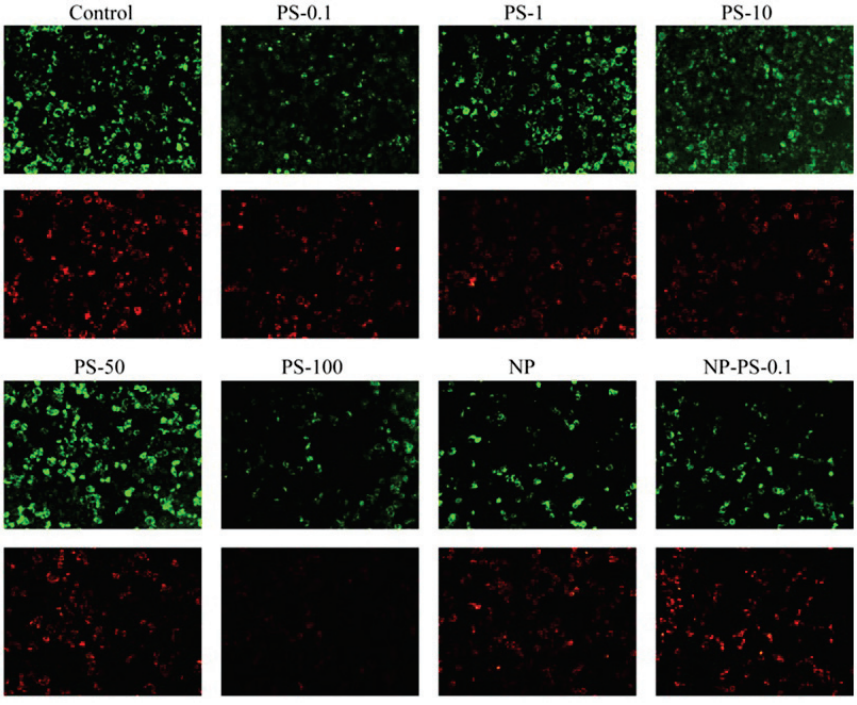

Figure A3. The mitochondrial membrane potential depolarization of Caco−2 cells treated by 40 μmol/L NP, 500 mg/L PS-MPs with different particle sizes, 0.1 μm PS−MPs that absorbed NP at 48 h. Representative photograph from an inverted fluorescence microscope.

References

1. Daniel, D.B.; Ashraf, P.M.; Thomas, S.N.; Thomson, K.T. Microplastics in the edible tissues of shellfishes sold for human consumption. *Chemosphere* **2021**, *264*, 128554. [CrossRef] [PubMed]
2. Li, Q.; Feng, Z.; Zhang, T.; Ma, C.; Shi, H. Microplastics in the commercial seaweed nori. *J. Hazard. Mater.* **2020**, *388*, 122060. [CrossRef] [PubMed]
3. Nan, B.; Su, L.; Kellar, C.; Craig, N.J.; Keough, M.J.; Pettigrove, V. Identification of microplastics in surface water and Australian freshwater shrimp Paratya australiensis in Victoria, Australia. *Environ. Pollut.* **2020**, *259*, 113865. [CrossRef] [PubMed]
4. Schwabl, P.; Koppel, S.; Konigshofer, P.; Bucsics, T.; Trauner, M.; Reiberger, T.; Liebmann, B. Detection of various microplastics in human stool: A prospective case series. *Ann. Intern. Med.* **2019**, *171*, 453–457. [CrossRef] [PubMed]
5. Hirt, N.; Body-Malapel, M. Immunotoxicity and intestinal effects of nano- and microplastics: A review of the literature. *Part. Fibre Toxicol.* **2020**, *17*, 57. [CrossRef] [PubMed]
6. Lu, Y.; Zhang, Y.; Deng, Y.; Jiang, W.; Zhao, Y.; Geng, J.; Ding, L.; Ren, H. Uptake and accumulation of polystyrene microplastics in Zebrafish (Danio rerio) and toxic effects in liver. *Environ. Sci. Technol.* **2016**, *50*, 4054–4060. [CrossRef] [PubMed]
7. Mattsson, K.; Johnson, E.V.; Malmendal, A.; Linse, S.; Hansson, L.; Cedervall, T. Brain damage and behavioural disorders in fish induced by plastic nanoparticles delivered through the food chain. *Sci. Rep.* **2017**, *7*, 11452. [CrossRef]
8. Vieira, Y.; Lima, E.C.; Foletto, E.L.; Dotto, G.L. Microplastics physicochemical properties, specific adsorption modeling and their interaction with pharmaceuticals and other emerging contaminants. *Sci. Total Environ.* **2021**, *753*, 141981. [CrossRef]
9. Deng, Y.; Zhang, Y.; Qiao, R.; Bonilla, M.M.; Yang, X.; Ren, H.; Lemos, B. Evidence that microplastics aggravate the toxicity of organophosphorus flame retardants in mice (Mus musculus). *J. Hazard. Mater.* **2018**, *357*, 348–354. [CrossRef]
10. Deng, Y.F.; Yan, Z.H.; Shen, R.Q.; Wang, M.; Huang, Y.C.; Ren, H.Q.; Zhang, Y.; Lemos, B. Microplastics release phthalate esters and cause aggravated adverse effects in the mouse gut. *Environ. Int.* **2020**, *143*, 105916. [CrossRef]
11. Soares, A.; Guieysse, B.; Jefferson, B.; Cartmell, E.; Lester, J.N. Nonylphenol in the environment: A critical review on occurrence, fate, toxicity and treatment in wastewaters. *Environ. Int.* **2008**, *34*, 1033–1049. [CrossRef]
12. Sun, D.; Chen, Q.; Zhu, B.; Zhao, H.; Duan, S. Multigenerational reproduction and developmental toxicity, and HPG axis gene expression study on environmentally-relevant concentrations of nonylphenol in zebrafish. *Sci. Total Environ.* **2021**, *764*, 144259. [CrossRef]

13. Li, M.; You, M.; Li, S.; Qiu, Z.; Wang, Y. Effects of maternal exposure to nonylphenol on learning and memory in offspring involve inhibition of BDNF-PI3K/Akt signaling. *Brain Res. Bull.* **2019**, *146*, 270–278. [CrossRef]
14. Xu, Y.; Sun, M.; Xu, Y.; Ju, J.; Pan, M.; Pan, Z.; Li, X.; Sun, S. Nonylphenol exposure affects mouse oocyte quality by inducing spindle defects and mitochondria dysfunction. *Environ. Pollut.* **2020**, *266*, 114967. [CrossRef]
15. Li, F.F.; Du, P.C.; Yang, W.Y.; Huang, D.F.; Nie, S.P.; Xie, M.Y. Polysaccharide from the seeds of *Plantago asiatica* L. alleviates nonylphenol induced intestinal barrier injury by regulating tight junctions in human Caco-2 cell line. *Int. J. Biol. Macromol.* **2020**, *164*, 2134–2140. [CrossRef]
16. Uzun, P.; Farazande, S.; Guven, B. Mathematical modeling of microplastic abundance, distribution, and transport in water environments: A review. *Chemosphere* **2022**, *288*, 132517. [CrossRef]
17. Bhandari, G.; Bagheri, A.R.; Bhatt, P.; Bilal, M. Occurrence, potential ecological risks, and degradation of endocrine disrupter, nonylphenol, from the aqueous environment. *Chemosphere* **2021**, *275*, 130013. [CrossRef]
18. Stenholm, Å.; Holmström, S.; Hjärthag, S.; Lind, O. Development of a high-performance liquid chromatography-fluorescence detection method for analyzing nonylphenol/dinonylphenol-polyethoxylate-based phosphate esters. *J. Chromatogr.* **2009**, *1216*, 6974–6977. [CrossRef]
19. Tsuda, T.; Suga, K.; Kaneda, E.; Ohsuga, M. Determination of 4-nonylphenol, nonylphenol monoethoxylate, nonylphenol diethoxylate and other alkylphenols in fish and shellfish by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr.* **2000**, *746*, 305–309. [CrossRef]
20. Liu, P.; Wu, X.; Liu, H.; Wang, H.; Lu, K.; Gao, S. Desorption of pharmaceuticals from pristine and aged polystyrene microplastics under simulated gastrointestinal conditions. *J. Hazard. Mater.* **2020**, *392*, 122346. [CrossRef]
21. Zuo, L.; Li, H.; Lin, L.; Sun, Y.; Diao, Z.; Liu, S.; Zhang, Z.; Xu, X. Sorption and desorption of phenanthrene on biodegradable poly(butylene adipate co-terephtalate) microplastics. *Chemosphere* **2019**, *215*, 25–32. [CrossRef]
22. Ziino, G.; Nalbone, L.; Giarratana, F.; Romano, B.; Cincotta, F.; Panebianco, A. Microplastics in vacuum packages of frozen and glazed icefish (*Neosalanx* spp.): A freshwater fish intended for human consumption. *Ital. J. Food Saf.* **2021**, *10*, 9974. [CrossRef]
23. Tang, S.; Lin, L.; Wang, X.; Feng, A.; Yu, A. Pb(II) uptake onto nylon microplastics: Interaction mechanism and adsorption performance. *J. Hazard. Mater.* **2020**, *386*, 121960. [CrossRef]
24. Yu, F.; Yang, C.; Huang, G.; Zhou, T.; Zhao, Y.; Ma, J. Interfacial interaction between diverse microplastics and tetracycline by adsorption in an aqueous solution. *Sci. Total Environ.* **2020**, *721*, 137729. [CrossRef]
25. Zhang, J.; Chen, H.; He, H.; Cheng, X.; Ma, T.; Hu, J.; Yang, S.; Li, S.; Zhang, L. Adsorption behavior and mechanism of 9-Nitroanthracene on typical microplastics in aqueous solutions. *Chemosphere* **2020**, *245*, 125628. [CrossRef]
26. Sun, M.; Yang, Y.K.; Huang, M.L.; Fu, S.K.; Hao, Y.Y.; Hu, S.Y.; Lai, D.L.; Zhao, L. Adsorption behaviors and mechanisms of antibiotic norfloxacin on degradable and nondegradable microplastics. *Sci. Total Environ.* **2022**, *807 Pt 3*, 151042. [CrossRef]
27. Calisto, V.; Jaria, G.; Silva, C.P.; Ferreira, C.I.A.; Otero, M.; Esteves, V.I. Single and multi-component adsorption of psychiatric pharmaceuticals onto alternative and commercial carbons. *J. Env. Manag.* **2017**, *192*, 15–24. [CrossRef]
28. Zhou, Y.; Liu, X.; Wang, J. Ecotoxicological effects of microplastics and cadmium on the earthworm Eisenia foetida. *J. Hazard. Mater.* **2020**, *392*, 122273. [CrossRef]
29. Zhang, X.; Wen, K.; Ding, D.X.; Liu, J.T.; Lei, Z.; Chen, X.X.; Ye, G.Z.; Zhang, J.; Shen, H.Q.; Yan, C.Z.; et al. Size-dependent adverse effects of microplastics on intestinal microbiota and metabolic homeostasis in the marine medaka (*Oryzias melastigma*). *Environ. Int.* **2021**, *151*, 106452. [CrossRef]
30. Liang, B.; Zhong, Y.; Huang, Y.J.; Lin, X.; Liu, J.; Lin, L.; Hu, M.J.; Jiang, J.Y.; Dai, M.Z.; Wang, B.; et al. Underestimated health risks: Polystyrene micro- and nanoplastics jointly induce intestinal barrier dysfunction by ROS-mediated epithelial cell apoptosis. *Part. Fibre Toxicol.* **2021**, *18*, 20. [CrossRef]
31. Visalli, G.; Facciolà, A.; Pruiti Ciarello, M.; De Marco, G.; Maisano, M.; Di Pietro, A. Acute and Sub-Chronic Effects of Microplastics (3 and 10 microm) on the Human Intestinal Cells HT-29. *Int. J. Environ. Res. Public Health* **2021**, *18*, 15833. [CrossRef] [PubMed]
32. Richter, J.W.; Shull, G.M.; Fountain, J.H.; Guo, Z.; Musselman, L.P.; Fiumera, A.C.; Mahler, G.J. Titanium dioxide nanoparticle exposure alters metabolic homeostasis in a cell culture model of the intestinal epithelium and Drosophila melanogaster. *Nanotoxicology* **2018**, *12*, 390–406. [CrossRef] [PubMed]
33. Fernández-Blanco, C.; Juan-García, A.; Juan, C.; Font, G.; Ruiz, M. Alternariol induce toxicity via cell death and mitochondrial damage on Caco-2 cells. *Food Chem. Toxicol.* **2016**, *88*, 32–39. [CrossRef] [PubMed]
34. Wang, Y.L.; Lee, Y.H.; Hsu, Y.H.; Chiu, I.J.; Huang, C.C.Y.; Huang, C.C.; Chia, Z.C.; Lee, C.P.; Lin, Y.F.; Chiu, H.W. The kidney-related effects of polystyrene microplastics on human kidney proximal tubular epithelial cells HK-2 and male C57BL/6 mice. *Environ. Health Perspect.* **2021**, *129*, 57003. [CrossRef]
35. Hesler, M.; Aengenheister, L.; Ellinger, B.; Drexel, R.; Straskraba, S.; Jost, C.; Wagner, S.; Meier, F.; von Briesen, H.; Büchel, C.; et al. Multi-endpoint toxicological assessment of polystyrene nano- and microparticles in different biological models in vitro. *Toxicol. Vitr.* **2019**, *61*, 104610. [CrossRef]
36. Yu, Y.; Mo, W.Y.; Luukkonen, T. Adsorption behaviour and interaction of organic micropollutants with nano and microplastics—A review. *Sci. Total Environ.* **2021**, *797*, 149140. [CrossRef]
37. Singh, N.; Bhagat, J.; Tiwari, E.; Khandelwal, N.; Darbha, G.K.; Shyama, S.K. Metal oxide nanoparticles and polycyclic aromatic hydrocarbons alter nanoplastic's stability and toxicity to zebrafish. *J. Hazard. Mater.* **2021**, *407*, 124382. [CrossRef]

38. Wang, Q.Q.; Bai, J.L.; Ning, B.; Fan, L.X.; Sun, T.Q.; Fang, Y.J.; Wu, J.; Li, S.; Duan, C.H.; Zhang, Y.C.; et al. Effects of bisphenol A and nanoscale and microscale polystyrene plastic exposure on particle uptake and toxicity in human Caco-2 cells. *Chemosphere* **2020**, *254*, 126788. [CrossRef]
39. Vagner, M.; Boudry, G.; Courcot, L.; Vincent, D.; Dehaut, A.; Duflos, G.; Huveta, A.; Tallec, K.; Zambonino-Infante, J.L. Experimental evidence that polystyrene nanoplastics cross the intestinal barrier of European seabass. *Environ. Int.* **2022**, *166*, 107340. [CrossRef]
40. Lagergren, S. About the theory of so-called adsorption of soluble substances. *K. Sven. Vetensk. Handl. Band* **1898**, *24*, 1–39.
41. Ho, Y.S.; Ng, J.C.Y.; McKay, G. Kinetics of pollutant sorption by biosorbents. *Separ. Purif. Method.* **2000**, *29*, 189–232. [CrossRef]
42. Morris, J.C.; Weber, W.J., Jr. Removal of biologically-resistant pollutants from waste waters by adsorption. *Adv. Water Poll. Res.* **1964**, *269*, 231–266.
43. Bangham, A.D.; Standish, M.M.; Watkins, J.C. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J. Mol. Biol.* **1965**, *13*, 238–252. [CrossRef]
44. Langmuir, I. The adsorption of gases on plane surfaces of glass, mica and platinum. *J. Am. Chem. Soc.* **1918**, *40*, 1361–1403. [CrossRef]
45. Freundlich, H. Ueber die adsorption in loesungen. *Z. Phys. Chem.* **1996**, *57*, 385–470.
46. Rey, F.; Calle, E.; Casado, J. Study of the effects of concentration and pH on the dissociation kinetics of Fe(II)-fulvic acid complexes. *Int. J. Chem. Kinet.* **1998**, *30*, 63–67. [CrossRef]

microplastics

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Investigating the Epigenetic Effects of Polystyrene Nanoplastic Exposure in Bluegill (*Lepomis macrochirus*) Epithelial Cells Using Methylation-Sensitive AFLPs

Sheridan M. Wilkinson [1,2], Justine M. Whitaker [3] and Alexis M. Janosik [1,*]

[1] Department of Biology, University of West Florida, Pensacola, FL 32514, USA; shwilkinson1234@gmail.com
[2] Department of Physiological Sciences, University of Florida, Gainesville, FL 32608, USA
[3] Department of Natural Science, University of Maryland Eastern Shore, Princess Anne, MD 21853, USA; jwhitaker@umes.edu
* Correspondence: ajanosik@uwf.edu

Abstract: Microplastics, remnants of macroplastics that have broken down to fragments smaller than 5 mm, and nanoplastics, broken down even further to sizes < 1 µm, are pervasive in aquatic ecosystems. These plastic particles are consumed by microscopic organisms, leading to bioaccumulation up trophic levels. The accumulation of plastic in the organismal gut can result in various repercussions, including cellular contamination and genomic modifications such as DNA methylation. While methylation has been studied in teleost fishes, the impact of nanoplastic exposure on this process in any species remains largely unexplored. This study delves into this largely uncharted territory, investigating the accumulation of methylation due to nanoplastic exposure within the genome of cultured bluegill BF-2 cells (*Lepomis macrochirus*) using methylation-sensitive AFLPs. The methylation state was analyzed through capillary gel analysis and electropherograms. Differential methylation occurred between several control and experimental groups due to nanoplastic exposure; however, these differences were not dose- or time-dependent. These results could suggest that higher dosages and exposure times to nanoplastics do not result in increased methylation levels in congruence with the dosage and exposure time; rather, only the presence of nanoplastics is enough to cause DNA methylation changes.

Keywords: microplastics; nanoplastics; marine plastic; epigenetics; methylation; bluegill sunfish; MS-AFLPs

Academic Editor: Nicolas Kalogerakis

Received: 31 October 2024
Revised: 19 January 2025
Accepted: 24 February 2025
Published: 27 February 2025

Citation: Wilkinson, S.M.; Whitaker, J.M.; Janosik, A.M. Investigating the Epigenetic Effects of Polystyrene Nanoplastic Exposure in Bluegill (*Lepomis macrochirus*) Epithelial Cells Using Methylation-Sensitive AFLPs. *Microplastics* **2025**, *4*, 10. https://doi.org/10.3390/microplastics4010010

1. Introduction

1.1. Microplastics

Since the first reports of marine plastic debris in the early 1970s [1], the demand for plastic has increased dramatically. Plastics are made from several different polymers, including polyethylene, polypropylene, polystyrene, polyethylene terephthalate, and polyvinyl chloride [2]. These polymers create a highly durable, lightweight, and cost-efficient material with many applications, which has led to an explosion in the manufacturing and usage of plastics in the decades since its invention. Due to its high durability and ubiquitous uses, plastics are also a pervasive pollutant with a long residence time in the environment. For instance, Chamas et al. (2020) reported that high-density polyethylene degraded at 0 to 11 µm/year, translating to an estimated half-life of 58 years for bottles and 1200 years for pipes [3].

Much research has been conducted studying the pervasiveness of plastics in marine ecosystems, with surveys indicating that 60–90% of marine debris is composed of plastics;

however, many of these surveys focus primarily on macroplastics [4–8]. Microplastics, however, are much more difficult to quantify. Unfortunately, research into the abundance of microplastics in freshwater ecosystems has received less attention than in marine ecosystems. Data on surface water abundance are deficient in larger water bodies and practically non-existent in smaller waterways [9]. However, several recent studies have shown the occurrence of microplastics in surface waters [10], sediment, and biota [11] of even rural freshwater systems. In 2020, Gopinath et al. reported on the presence of microplastics in both the sediment and surface water of the Red Hills Lake in Chennai City, India [12]. Microplastics were also reported in the surface water of the River Thames, United Kingdom [13]. Additional reports of microplastic pollution have also come from Lake Victoria, East Africa [14]; Surabaya River, Indonesia [15]; Haihe River, China [16]; Ofanto River, Italy [17]; and several other freshwater systems around the world [18]. This is unsurprising as freshwater ecosystems are likely subject to the same pollution mechanisms as their marine counterparts. Freshwater species are also expected to suffer the same or similar impacts of microplastic consumption as marine species.

Microscopic aquatic organisms, such as zooplankton, have been documented to consume microplastics, which bioaccumulate up trophic levels [19,20]. A study by Mattsson et al. (2016) tracked the transfer of plastic particles from dosed algae (*Scenedesmus* sp.) to zooplankton (*Daphnia magna*) to crucian carp (*Carassius carassius*) [20]. While plastic fragments can be excreted or egested out of an organism's system, bioaccumulation occurs when the egestion mass is less than ingestion [19]. However, ingestion is not the only way organisms can uptake plastic fragments. Plastic fragments can also be absorbed through the gills, as was demonstrated by Watts et al. (2014) when crabs were exposed to 8–10 μm polystyrene microplastics [21]. Absorption of microplastics through the gills had a noticeably longer residence time within the body of crabs treated than those that ingested them [21]. This is likely because there is no distinct route of excretion when plastic fragments are taken in through the gills.

The accumulation of plastics in organisms can have severe consequences, including reduced food uptake and starvation due to blockage in the digestive tract. Chemicals used in producing plastics can also leach into the cells of organisms, and plastic fragments can be transposed from the gut to adjacent tissues. Furthermore, microplastic fragments can act as vectors for other contaminants, such as hydrophobic pollutants, significantly amplifying the ecological impact. For instance, Tanaka et al. (2013) found polybrominated diphenyl ethers in the abdominal adipose tissue of short-tailed shearwaters [22]. This compound was only detected in plastic particles found in the stomachs of birds that tested positive for the said chemical. Microplastic fragments can also hinder physical movement through ingestion [23].

Microplastics can also be further broken down both in the environment and inside the organism into nano-sized particles (10^{-9}–10^{-7} m) [24–27]. These nano-sized particles are believed to pose an even more significant threat than microplastics, as they can pass through biological barriers [20], penetrating tissues [28], accumulating in organs [29], and affecting the behavior and metabolism of organisms [20,30,31]. This highlights the urgent need for further research and action to address this escalating issue.

Microplastic exposure has also been found to affect gene expression in model organisms (*Danio rerio*) [32,33]. For instance, Karami et al. (2017) investigated how exposure to pristine low-density polyethylene fragments at 5, 50, and 500 μg/L for 10 or 20 days altered the expression of several biomarkers in zebrafish larvae [32]. While significant changes were not observed in the selected biomarkers on days 10 or 20, transcription of several critical biomarkers was markedly lower in the larvae on day 20 compared to day 10. LeMoine et al. (2018) examined how exposure for 14 days to 5 and 20 mg/L concentrations

of fluorescently labeled polyethylene particles affected the development, growth, and metabolic rates of embryonic and larval zebrafish, as well as effects on the transcriptome of the fish [33]. Again, transcriptomics via RNA sequencing revealed that widespread, yet temporary, changes in gene expression occurred within 48 h of exposure during a critical development period [33].

Differences in expression may also be linked to epigenetics, but little work has been carried out to examine how microplastic exposure affects epigenomes via global methylation changes. Exposure to bisphenol A—an essential component in the production of plastics—has shown differentially methylated genes essential in axon targeting, synaptic development, and neuronal survival in zebrafish embryos via whole-genome bisulfite sequencing [34]. Given that most aquatic organisms intentionally or accidentally ingest micro-/nanoplastics [21,35–40], it is entirely possible that exposure could alter the global methylation state of the affected organisms. Alteration of global methylation can be inferred from the studies conducted by LeMoine et al. (2018) and Olsvik et al. (2019) [33,34]; however, it has not been directly studied how whole microplastics affect the global methylation in the fish genome.

1.2. Epigenetics and DNA Methylation

Epigenetics studies genomic modification in organisms due to environmental conditions [41]. These genome modifications are not changes to the DNA sequence itself but, instead, affect how genes are regulated by the cell [42] and can, in turn, cause variation in morphology [43–45]. Examples of epigenetic changes include chromatin remodeling, deacetylation and modification of histones, position effects, and interference by small RNAs [46]. However, the epigenetic mechanism that has been studied the most is DNA methylation. Methylation is most commonly seen when a methyl group is attached to a cytosine followed by a guanine, known as CpG sites [47], and is often associated with decreased gene activity [47,48].

Methylation is naturally seen in the genome through the removal and addition of methyl groups via demethylases and methyltransferases, which alter the structure and function of the chromatin and, therefore, promote and silence gene transcription, respectively [49]. Specific methylation patterns can naturally be seen in response to development and cellular differentiation [50,51] and sex determination [52]. External factors, like temperature [53,54] and environmental contaminants [55,56], can also alter the methylation state of impacted organisms. As such, unnatural methylation could potentially repress the transcription of essential genes, hindering important cell functions, such as hormone production [54]. Changes in patterns of whole-genome DNA methylation can also be significant in development and cellular differentiation, as has been well documented in mammals [57]. These changes have also been studied in fungi, plants, and invertebrate animals [58–60].

All organisms naturally accumulate methylation in their genomes throughout their lives, and a certain degree of methylation is necessary for natural processes, such as development and growth [50,51]. For instance, DNA methylation is related to cell morphology and metabolism through gene expression regulation and epigenetic stability, and deviant DNA methylation can modify cytoskeletal organization genes (i.e., genes like *RhoA* and *Rac1*, critical regulators of actin cytoskeleton remodeling), affecting cell shape, adhesion, and migration [61–63]. However, the presence of pollutants, such as plastics, in the environment could exacerbate the methylation process and affect cell function. An increasing number of studies have begun to look at the effects of environmental pollutants, like microplastics or chemicals, and how these toxicants change the methylation patterns in exposed organisms, especially in marine fishes.

Nanoplastics have been shown to induce DNA methylation changes through several mechanisms at the molecular level. One key mechanism is oxidative stress, triggered by increased reactive oxygen species production due to nanoplastic exposure [64,65]. This oxidative stress can activate epigenetic regulatory mechanisms, such as DNA methyltransferases, which modify cytosine residues in CpG islands and potentially silence gene expression. For example, altered methylation patterns can affect stress-related and DNA repair genes and apoptotic pathways due to reactive oxygen species activity and associated damage responses [64]. Direct interaction of nanoplastics with cellular machinery is another mechanism that can induce methylation alterations, as nanoplastics have also been shown to disrupt mitochondrial function and impair autophagy, further escalating cellular stress responses. This cascade can lead to abnormal activation or repression of methylation-related pathways, affecting genes involved in inflammation, metabolism, and other critical processes [65]. Transgenerational effects are also possible, as studies have shown that nanoplastics can induce changes in germ cells, suggesting that epigenetic alterations may be heritable, which could be concerning for long-term health and development [66].

1.3. Bluegill Sunfish

Bluegill sunfish (*Lepomis macrochirus*), the focus species of this study, is a common fish native to the freshwater lakes, rivers and streams, ponds, reservoirs, and swamps of North America, ranging from Canada to northern Mexico [67]. *Lepomis macrochirus* has also been introduced to many countries worldwide, including Brazil, Iran, Japan, Korea, Madagascar, the Philippines, and Venezuela [67]. Generally, *L. macrochirus* inhabits silty, sandy, or gravel substrates near rooted aquatic plants in shallow waters up to a depth of 5 m [67].

Because *L. macrochirus* is widespread and prolific, it has been the focus of many ecotoxicological studies [68–71]. The use of *L. macrochirus* as a model organism is likely attributable to the ability to perform cell culture on BF-2 caudal trunk cell lines derived from bluegills, which have been suggested to be sensitive indicators of aquatic pollutants [71], negating the need for aquaculture of the whole animal.

As such, this study aimed to investigate how prolonged and increasing amounts of microplastic exposure can affect the genome of *L. macrochirus* BF-2 cell lines by looking at differential methylation of CpG sites using methylation-sensitive AFLPs (MS-AFLPs). Downregulation of proteins due to methylation could have consequences on reproduction, metabolism, growth, and development, as well as other biological processes of bluegills and other aquatic organisms. The results of this study are beneficial for this field of research, as knowing the global ramifications of plastic pollution on the epigenome will help to inform more targeted studies in the future.

2. Materials and Methods

2.1. Culture of BF-2 Cells

Bluegill BF-2 cells (ATCC CCL-91) [72,73] were acquired from C. Lavelle (Environmental Protection Agency) and were stored in liquid nitrogen. When ready to begin culturing, the frozen BF-2 cells were resuscitated using standard methods [74]. The complete media used comprised 500 mL HyClone™ Minimum Essential Medium (MEM) (Cytiva®; Marlborough, MA, USA), 10% fetal bovine serum (FBS; 50 mL), and 1× Gibco™ Antibiotic-Antimycotic (100×; 5.5 mL; ThermoScientific®; Waltham, MA, USA). Upon completion of the resuscitation protocol, the cells were transferred to a sterile T-25 flask and incubated at 37 °C, 5% CO_2, until the flask was at least 90% confluent.

To obtain adequate cells for three 6-well plates, the cells were split and plated onto three T-75 flasks. All subsequent subculturing of the BF-2 cells was carried out using standard methods [74]. When the cultures in the T-75 flasks reached a 95% confluency,

cells were split evenly into each well of a 6-well plate, with the top left well serving as the control. The remaining five wells were then dosed with microplastics according to the experiment's parameters. Three plates were created for each experiment for three sampling time points (T_1 = 24 h, T_2 = 48 h, and T_3 = 72 h). Following the dosing period for each plate, the cells were gathered, and 2 mL of cells were transferred to labeled 2 mL Eppendorf tubes and pelleted at 9000 rpm for 3 min, and the supernatant was aspirated off. The media of each plate was also replaced every 24 h until the cells were ready to be sampled.

2.2. Nanoplastic Treatment

The experimental cultures were dosed with 0.04–0.06 µm Spherotech® (Lake Forest, IL, USA) nile red fluorescently dyed polystyrene nanoplastic particles with a base concentration of 2.84×10^{11} particles at time 0 for each experiment, which is a dosage much higher than environmentally relevant concentrations. However, bioaccumulation of nanoplastics within cells and tissues of wild populations is not well quantified, so nanoplastic concentrations within organisms could be much higher than in the surrounding environment. As such, this was the smallest concentration that could be reliably quantified.

Specifically, four different experiments were employed. Experimental cultures in experiment I were continually dosed at the base concentration for T_1, T_2, and T_3 (Table 1). Control cultures for each experiment were not dosed with microplastic particles. In experiment II, all experimental cultures were given an initial dose of 5.68×10^{11} particles, and plates 1, 2, and 3 were sampled at T_1, T_2, and T_3, respectively (Table 1). For experiment III, all experimental cultures were given an initial dose of 8.52×10^{11} particles, and plates 1, 2, and 3 were sampled at their respective time points, T_1, T_2, and T_3 (Table 1). Lastly, for experiment IV, all the experimental cultures were dosed with the base concentration, and plate 1 was sampled at T_1; at this time, plates 2 and 3 were dosed with 5.68×10^{11} particles. At T_2, plate 2 was sampled, and plate 3 was dosed with a final concentration of 8.52×10^{11} particles and then sampled at T_3 (Table 1). This strategy for experiment IV was used to mimic bioaccumulation.

Table 1. Total concentrations of nanoplastic particles for each plate in the three time periods for experiments I, II, III, and IV.

Experiment	Time Period	Total Concentration (Particles)
I (Low Concentration)	T_1 (24 h)	2.84×10^{11}
	T_2 (48 h)	2.84×10^{11}
	T_3 (72 h)	2.84×10^{11}
II (Medium Concentration)	T_1 (24 h)	5.68×10^{11}
	T_2 (48 h)	5.68×10^{11}
	T_3 (72 h)	5.68×10^{11}
III (High Concentration)	T_1 (24 h)	8.52×10^{11}
	T_2 (48 h)	8.52×10^{11}
	T_3 (72 h)	8.52×10^{11}
IV (Bioaccumulation)	T_1 (24 h)	2.84×10^{11}
	T_2 (48 h)	5.68×10^{11}
	T_3 (72 h)	8.52×10^{11}

2.3. MS-AFLP Analysis of the Methylation State

The following protocol was derived from protocols published by Vos et al. (1995) [75], who described one of the original AFLP protocols, and Xiong et al. (1999) [76], with modifications from personal communication (J.M. Whitaker).

DNA was extracted from the preserved culture samples using the DNeasy Blood and Tissue Kit (Qiagen®; Hilden, Germany). Extracted DNA samples were then run through the

following MS-AFLP protocol. DNA concentration was determined using a Nanodrop 2000 Spectrophotometer (ThermoScientific®). The final DNA concentration was 40–60 ng/μL.

The digestion reaction master mix added to 4 μL of sample DNA consisted of 4.5 U *Eco*RI enzyme (0.45 μL), 3 U *Hpa*II/*Msp*I enzyme (0.3 μL), 3.5 μL 10× NEB buffer, 0.2 μL 0.1% BSA, and 11.55 μL ddH_2O to reach 20 μL [77]. See Figure S1 in Supplementary Materials for information on when the *Hpa*II and *Msp*I enzymes will cut CpG sites. The reaction was incubated for 4 h at 37 °C and then inactivated at 65 °C for 10 min. The restriction enzymes and buffer were purchased from New England Biolabs® (Ipswich, MA, USA).

Following digestion, double-stranded adaptors were ligated to the ends of the restriction fragments. Ligation was performed at 16 °C for 17 h using 10 μL digested DNA, 350 U T4 DNA ligase (0.875 μL), 2.0 μL 10× T4 DNA ligase buffer, 2.0 μL 5 μM *Eco*RI adaptors (Table S1 in Supplementary Materials), 5.0 μL 50 μM *Hpa*II/*Msp*I adaptors (Table S1 in Supplementary Materials), and 0.125 μL ddH_2O to reach 20 μL [77]. The DNA ligase and buffer were purchased from New England Biolabs® (Ipswich, MA, USA).

Pre-selective PCR of the ligated DNA product was completed with the following reaction master mix: 2.0 μL DreamTaq (ThermoScientific®) PCR buffer, 0.5 μL 10 mM dNTPs, 2.0 μL 10 μM *Eco*RI primer (Table S1 in Supplementary Materials), 2.0 μL 10 μM *Hpa*II/*Msp*I primer (Table S1 in Supplementary Materials), 0.6 μL 5 U/μL DreamTaq polymerase, and 11.9 μL ddH_2O. This master mix results in 19.0 μL per reaction that was aliquoted and added to 1.0 μL of ligated DNA for a total reaction volume of 20 μL. The reactions were run on the following thermocycler protocol with an initial denaturing step of 94 °C for 5 min, followed by 94 °C for 30 s, 56 °C for 1 min, and 72 °C for 1.5 min for 25 cycles. A final extension was performed at 72 °C for 10 min, then 60 °C for 30 min, and held at 4 °C indefinitely [68]. Once the PCR was completed, 10 μL of PCR product from each reaction was diluted in 90 μL of milli Q water and run on a 1–2% agarose gel to ensure amplification occurred.

The selective PCR master mix was created as follows: 2 μL DreamTaq PCR buffer, 0.5 μL 10 mM dNTPs, 1.5 μL 10 μM FAM labeled EcoRI primer (Table S1 in Supplementary Materials), 1.5 μL 10 μM HEX labeled EcoRI primer (Table S1 in Supplementary Materials), 1 μL 5 μM CAT/CAC primer (Table S1 in Supplementary Materials), 0.6 μL DreamTaq polymerase, and 7.9 μL ddH_2O. Two different primer combinations, master mix A (MMA) and master mix C (MMC), were used on all samples for selective PCR; the reagents and volumes used for MMA and MMC are summarized in Table S2 in Supplementary Materials. The 15 μL of the master mix was then aliquoted and added to 5.0 μL of the dilute pre-selective PCR product for a total reaction volume of 20 μL. The reaction mixtures were then run on the standard thermocycler protocol: (1) 94 °C for 5 min, (2) 94 °C for 30 s, (3) 64 °C for 30 s decreasing by 0.7 °C per cycle, (4) 72 °C for 1.5 min, (5) go to step 2 13×, (6) 94 °C for 30 s, (7) 56 °C for 30 s, (8) 72 °C for 2 min, (9) go to step 6 20×, (10) 72 °C for 10 min, (11) 60 °C for 30 min, and 12) 4 °C indefinitely [77]. Selective PCR products were then run through capillary electrophoresis at Yale's DNA Analysis Facility on Science Hill.

2.4. Fragment and Statistical Analysis

Fragment analysis was performed by S.M. Wilkinson using Geneious Prime 2020.1.2 (Auckland, New Zealand) and the Microsatellite plugin [78]. Fragments less than 70 bp and greater than 500 bp were excluded from the analysis, as they fell outside the size standard used. Fragments were scored in a binary fashion with uninformative states in which a peak absent from both reactions was scored as missing [79]. Using this binary data, the results of the restriction fragments were run through the *msap* package [80] in R [81].

For the determined methylation susceptible loci (MSL) in each grouping, the frequencies of unmethylated, hemimethylated, internal cytosine methylated, and fully methylated/target absent loci were ascertained. These frequencies were revealed by grouping the experimental cultures to determine the average methylation and comparing the average to the control culture for the respective time group. Experimental cultures were grouped by their time groups (T_1, T_2, and T_3) for each experiment. Statistically significant differences between the frequencies of methylated loci between the controls and experimental time groups, as well as between the time groups ($T_1 \times T_2$, $T_1 \times T_3$, and $T_2 \times T_3$), were determined via the χ^2 test (*chisq.test*()) function [82] in R [83]. Statistical significance was determined using a p-value < 0.05.

3. Results

The MS-AFLP protocol used in this study provided polymorphisms for data analysis. Specifically, two primer combinations (MMA and MMC) allowed many polymorphisms to be detected; 51–130 peaks out of the 232–306 scored peaks (21.5–46.4%) were polymorphic. The frequencies of MSL and non-methylated loci (NML) for each time group in each experiment as determined by *msap* are summarized in Table 2 for MMA and Table 3 for MMC. For MMA, most loci (64.1–78.6%) were shown to be methylation-susceptible, while only 21.4–35.9% of loci were not methylation-susceptible. Most of the polymorphisms also fell into the category of MSL, with 64–116 (33.2–52.7%) polymorphic loci, while few of the NML were polymorphic, 0–14 (0–23.3%). For MMC, most loci (59.5–70.6%) were also methylation-susceptible, and non-methylated loci comprised only 29.4–40.5% of the loci found. MMC also displayed many polymorphic MSL (32.2–43.2%) and a comparatively low frequency of NML (0–10%).

Table 2. Number of methylation-susceptible loci and non-methylated loci and the number of polymorphic methylation-susceptible loci (MSL) and non-methylated loci (NML) for each time group in each experiment for master mix A (MMA).

Experiment Number	Time	Number of MSL	Number of NML	Number of Polymorphic MSL	Number of Polymorphic NML
I	T_1	220 (78.6%)	60 (21.4%)	116	14
	T_2	211 (75.4%)	69 (24.6%)	81	4
	T_3	199 (71.1%)	81 (28.9%)	69	5
II	T_1	206 (73.8%)	73 (26.2%)	83	0
	T_2	198 (71.0%)	81 (29.0%)	80	1
	T_3	189 (67.7%)	90 (32.3%)	64	2
III	T_1	182 (65.5%)	96 (34.5%)	70	8
	T_2	181 (65.1%)	97 (34.9%)	64	1
	T_3	186 (66.9%)	92 (33.1%)	64	2
IV	T_1	203 (66.3%)	103 (33.7%)	91	4
	T_2	196 (64.1%)	110 (35.9%)	65	7
	T_3	216 (70.6%)	90 (29.4%)	77	2

Table 3. Number of methylation-susceptible loci and non-methylated loci and the number of polymorphic methylation-susceptible loci (MSL) and non-methylated loci (NML) for each time group in each experiment for master mix C (MMC).

Experiment Number	Time	Number of MSL	Number of NML	Number of Polymorphic MSL	Number of Polymorphic NML
I	T_1	151 (65.1%)	81 (34.9%)	55	2
	T_2	162 (69.8%)	70 (30.2%)	62	7
	T_3	139 (59.9%)	93 (40.1%)	48	3

Table 3. *Cont.*

Experiment Number	Time	Number of MSL	Number of NML	Number of Polymorphic MSL	Number of Polymorphic NML
II	T_1	149 (60.1%)	99 (39.9%)	59	0
	T_2	175 (70.6%)	73 (29.4%)	70	3
	T_3	168 (67.7%)	80 (32.3%)	67	2
III	T_1	144 (59.5%)	98 (40.5%)	58	2
	T_2	160 (66.1%)	82 (33.9%)	60	0
	T_3	162 (66.9%)	80 (33.1%)	61	7
IV	T_1	185 (67.3%)	90 (32.7%)	80	2
	T_2	171 (62.2%)	104 (37.8%)	55	4
	T_3	189 (68.7%)	86 (31.3%)	63	1

3.1. MMA

The percentage of no methylation, hemimethylation, internal cytosine methylation, and full methylation for each time group and experiment for MMA can be seen in Table 4 and Figure 1, along with the *p*-values from the χ^2 analysis between each time group and its respective control and between temporal groups in Table 5. There did not seem to be a pattern of a specific time group showing statistically significant differential methylation, as the only statistically significant time groups were T_3 in experiment I, T_2 and T_3 in experiment III, and T_1 in experiment IV. Interestingly, methylation patterns between the different time groups showed less variation with each other than each group displayed with its control, which might suggest exposure time is not a significant factor for any increased methylation, and only the presence of microplastics is enough to cause differential methylation (Tables 4 and 5).

Table 4. Percentage of loci of the experimental time groups T_1, T_2, and T_3 and their respective control groups that are either unmethylated, hemimethylated, internal cytosine methylated, or fully methylated using MMA; comparisons of the methylation state between controls and the respective experimental group that were statistically significant are in bold.

Experiment Number		C_1	T_1	C_2	T_2	C_3	T_3
I	No Methylation	17.73%	18.38%	21.33%	13.74%	**17.09%**	**13.15%**
	Hemimethylation	10%	10.97%	14.69%	10.16%	**10.55%**	**11.47%**
	Internal Cytosine Methylation	18.18%	23.96%	18.48%	25.8%	**56.28%**	**26.21%**
	Full Methylation	54.09%	46.69%	45.5%	50.3%	**16.08%**	**49.16%**
II	No Methylation	31.55%	18.12%	31.31%	17.53%	15.34%	13.68%
	Hemimethylation	7.77%	13.11%	12.63%	10.89%	13.23%	8.31%
	Internal Cytosine Methylation	28.16%	23.22%	16.67%	23.74%	20.11%	25.25%
	Full Methylation	32.52%	45.55%	39.39%	47.84%	51.32%	52.76%
III	No Methylation	21.15%	16.58%	**25.41%**	**14.36%**	**26.34%**	**14.58%**
	Hemimethylation	12.09%	9.43%	**7.74%**	**13.26%**	**5.38%**	**10.22%**
	Internal Cytosine Methylation	25.27%	23.90%	**35.91%**	**19.26%**	**29.03%**	**18.35%**
	Full Methylation	41.48%	50.09%	**30.94%**	**53.12%**	**39.25%**	**56.85%**
IV	No Methylation	**35.47%**	**18.37%**	15.82%	16.69%	16.67%	17.52%
	Hemimethylation	**6.90%**	**11.40%**	15.82%	9.55%	31.48%	16.59%
	Internal Cytosine Methylation	**30.05%**	**20.76%**	16.33%	24.85%	10.19%	12.96%
	Full Methylation	**27.59%**	**49.47%**	52.04%	48.91%	41.67%	52.93%

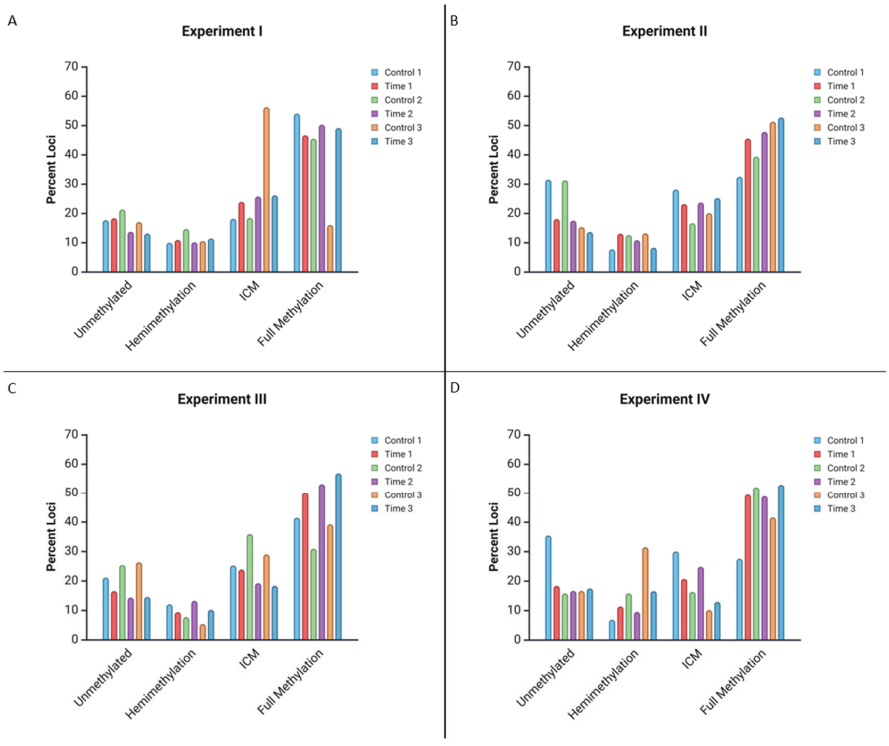

Figure 1. Bar graphs showing the percentage of loci that were either unmethylated, hemimethylated, internal cytosine methylated, or fully methylated and comparing time groups T_1 (24 h), T_2 (48 h), and T_3 (72 h) to their respective controls for MMA; (**A**) shows the results for experiment I (low concentration); (**B**) shows the results for experiment II (medium concentration); (**C**) shows the results for experiment III (high concentration); (**D**) shows the results for experiment IV (bioaccumulation).

Table 5. χ^2 values for experiments I, II, III, and IV comparing time groups T_1 (24 h), T_2 (48 h), and T_3 (72 h) to their respective controls, as well as the different time groups to each other for MMA; below the diagonal, the χ^2 statistic is provided for each comparison; above the diagonal, *p*-values for each comparison are provided, and bolded *p*-values are statistically significant.

Experiment Number		Control	T_1	T_2	T_3
I (Low Concentration)	Control		0.7141	0.2703	**3.162×10^{-6}**
	T_1	1.364		0.8245	0.7908
	T_2	3.919	0.904		0.9908
	T_3	28.286	1.043	0.109	
II (Medium Concentration)	Control		0.0539	0.1082	0.6104
	T_1	7.647		0.9643	0.5107
	T_2	6.072	0.277		0.775
	T_3	1.821	2.309	1.109	
III (High Concentration)	Control		0.6303	**0.0015**	**0.0147**
	T_1	1.730		0.707	0.7252
	T_2	15.398	1.394		0.9092
	T_3	10.512	1.317	0.544	
IV (Bioaccumulation)	Control		**0.0024**	0.3296	0.0977
	T_1	14.449		0.8933	0.4067
	T_2	3.433	0.614		0.121
	T_3	6.305	2.904	5.814	

3.2. MMC

As with MMA, the percentage of no methylation, hemimethylation, internal cytosine methylation, and full methylation for each time group and experiment for MMC can be seen in Table 6 and Figure 2, and the *p*-values from the χ^2 analysis between each time group and its respective control and between temporal groups in Table 7. Also, as with the experiments run through MMA, there was no clear pattern of statistically significant differential methylation, and $T_1 \times T_2$, $T_1 \times T_3$, and $T_2 \times T_3$ tended to show less variation with each other than the time groups showed with their controls (Tables 6 and 7). The only statistically significant time groups were T_3 in experiment I, T_1 and T_2 in experiment II, T_2 and T_3 in experiment III, and T_3 in experiment IV.

Table 6. Percentage of loci of the experimental time groups T_1, T_2, and T_3 and their respective control groups that are either unmethylated, hemimethylated, internal cytosine methylated, or fully methylated using MMC; comparisons of the methylation state between controls and the respective experimental group that were statistically significant are in bold.

Experiment Number		C_1	T_1	C_2	T_2	C_3	T_3
I	No Methylation	15.23%	15.14%	16.98%	16.68%	**20.86%**	**13.07%**
	Hemimethylation	17.22%	10.79%	22.22%	14.03%	**12.95%**	**16.91%**
	Internal Cytosine Methylation	19.87%	22.23%	14.81%	21.54%	**42.45%**	**24.22%**
	Full Methylation	47.68%	51.84%	45.99%	47.75%	**23.74%**	**42.80%**
II	No Methylation	**34.23%**	**20.02%**	**29.71%**	**19.59%**	17.26%	16.50%
	Hemimethylation	**16.11%**	**16.33%**	**13.14%**	**13.14%**	21.43%	12.16%
	Internal Cytosine Methylation	**30.87%**	**25.62%**	**29.71%**	**21.63%**	14.88%	19.98%
	Full Methylation	**18.79%**	**38.03%**	**27.43%**	**45.63%**	46.43%	51.36%
III	No Methylation	23.61%	18.29%	**28.75%**	**15.62%**	**28.40%**	**15.28%**
	Hemimethylation	15.97%	17.36%	**23.15%**	**22.86%**	**9.88%**	**14.89%**
	Internal Cytosine Methylation	24.31%	16.78%	**25.00%**	**14.46%**	**24.69%**	**16.82%**
	Full Methylation	36.11%	47.57%	**23.13%**	**47.05%**	**37.04%**	**53.01%**
IV	No Methylation	28.65%	18.30%	12.87%	16.21%	**8.47%**	**13.84%**
	Hemimethylation	14.05%	15.37%	24.56%	17.29%	**41.80%**	**20.46%**
	Internal Cytosine Methylation	23.24%	17.84%	16.37%	24.23%	**11.64%**	**16.40%**
	Full Methylation	34.05%	48.49%	46.20%	42.27%	**38.10%**	**49.29%**

Table 7. χ^2 values for experiments I, II, III, and IV comparing time groups T_1 (24 h), T_2 (48 h), and T_3 (72 h) to their respective controls, as well as the different time groups to each other for MMC; below the diagonal, the χ^2 statistic is provided for each comparison; above the diagonal, *p*-values for each comparison are provided, and bolded *p*-values are statistically significant.

Experiment Number		Control	T_1	T_2	T_3
I (Low Concentration)	Control		0.6187	0.3717	**0.0053**
	T_1	1.783		0.8788	0.4922
	T_2	3.132	0.676		0.7799
	T_3	12.715	2.408	1.088	
II (Medium Concentration)	Control		**0.0133**	**0.0485**	0.3118
	T_1	10.726		0.7109	0.3037
	T_2	7.883	1.377		0.8717
	T_3	3.570	3.635	0.706	
III (High Concentration)	Control		0.2978	**0.0019**	**0.0258**
	T_1	3.683		0.768	0.8606
	T_2	14.855	1.138		0.5281
	T_3	9.278	0.753	2.220	
IV (Bioaccumulation)	Control		0.1341	0.3417	**0.0126**
	T_1	5.577		0.6512	0.7034
	T_2	3.343	1.636		0.4752
	T_3	10.848	1.409	2.500	

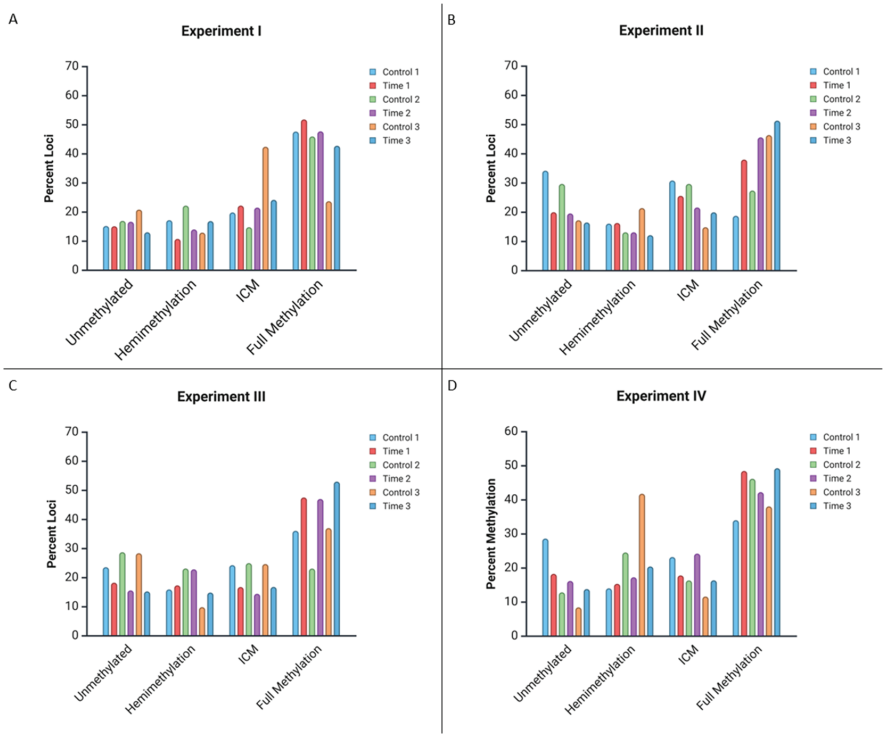

Figure 2. Bar graphs showing the percentage of loci that were either unmethylated, hemimethylated, internal cytosine methylated, or fully methylated and comparing time groups T_1 (24 h), T_2 (48 h), and T_3 (72 h) to their respective controls for MMC; (**A**) shows the results for experiment I (low concentration); (**B**) shows the results for experiment II (medium concentration); (**C**) shows the results for experiment III (high concentration); (**D**) shows the results for experiment IV (bioaccumulation).

4. Discussion

In this study, MS-AFLPs revealed that the majority of loci in bluegill BF-2 cell lines across both master mixes and all four experiments were methylation-susceptible and, therefore, vulnerable to epigenetic change as a result of nanoplastic exposure. MSL also displayed far more diversity in the form of more polymorphisms than their non-methylated counterparts. As for the estimated methylation patterns among the MSL, these results were unexpected. It did not appear that any one concentration or exposure time equated to significantly more methylation within the sampled genomes, but rather, the mere presence of nanoplastics could alter the methylation state. Perhaps the most interesting results were that the different time groups for each of the four experiments showed less differentiation with each other than they did with the controls. This suggests that exposure time was not a significant factor for increased methylation in the experimental cultures and that only the presence of nanoplastics is enough to cause methylation.

Full methylation of the experimental cultures never exceeded 56.85%, but generally, the frequency of full methylation fell well below this number. Perhaps this presents a threshold for the maximum amount of methylation for continued cell functionality in bluegills. It would be interesting to determine the maximum methylation allowed before cell death occurs, as this could be useful knowledge for future ecotoxicology studies and determining the bare minimum genes that must be expressed for continued cell functionality. However, a study conducted by Morán et al. (2013) found that full methylation in

brown trout (*Salmo trutta*) gill tissue was noticeably higher (57.5–63.6%) than the numbers reported in the current study, with the control group having a full methylation frequency of 61.8% [83]. However, gonad, kidney, and gill tissue in half-smooth tongue sole (*Cynoglossus semilaevis*) displayed full methylation frequencies of 15.97 ± 1.22%, 17.5 ± 4.11%, and 16.68 ± 5.34%, respectively, in females and 17.47 ± 7.04%, 18.64 ± 5.60%, and 12.06 ± 7.21%, respectively, in males [77]. As brown trout and bluegill are both freshwater species, perhaps this suggests that freshwater fish have higher frequencies of full methylation than their marine counterparts, like the half-smooth tongue sole. However, differences in methylation patterns between marine and freshwater fish warrant further investigation.

This is an emerging field of study, as most studies in a similar vein have not looked at the global methylation response to plastic exposure, and very little research involving micro- or nanoplastics is available to compare with our results directly; therefore, it is difficult to say whether the results of this study fit a pattern seen in response to plastic exposure. However, bluegill BF-2 cells have been used in other ecotoxicological studies. Srikanth et al. (2018) used bluegill cells to investigate how graphene oxide exposure induced cytotoxicity and oxidative stress [70], and Poornavaishnavi et al. (2019) used bluegill BF-2 cells to assess oxidative stress, cytotoxicity, and morphological changes in response to nickel nanoparticles (Ni NPs) [71]. Both studies showed that cell toxicity was both dose- and time-dependent. An organismal study involving exposure of Mozambique tilapia (*Oreochromis mossambicus*) to Ni NPs also confirmed the results of Poornavaishnavi et al. (2019) [71] in the whole animal [84], suggesting that results obtained in vitro can be extrapolated to entire organisms and, therefore, natural populations.

Though the current study employs different methods to determine a different outcome, the effects of Ni NPs and graphene oxide show that increases in cell damage can be linked to toxicant dosage and length of exposure. Similar results were expected for the current study, as research into the impacts of nanoparticles within the cell, such as the Srikanth et al. (2018) study [70], showed adverse effects across a wide range of contaminants and organisms. Given the mixed results of the current study, further investigation is required to gain a clearer picture of the toxicological effects of plastic nanoparticles. While no conclusive evidence showed that methylation was dose- or time-dependent, the presence of nanoplastics did cause significant increases in methylation in several of the experimental cultures and should be further investigated to determine if these increases could also be dose- and/or time-dependent.

Transcriptional changes due to microplastic exposure have been studied in zebrafish [32,33], but the subject of how micro-/nanoplastics change methylation within the genome of teleost fish is decidedly lacking. However, it has been well documented that aquatic organisms of all kinds ingest microplastics at an alarming rate [38,85,86]. Bluegills in the Brazos River Basin, Texas, specifically—and their sister species, longear (*Lepomis megalotis*) sunfish—were documented ingesting micro- and macroplastics [85]. Peters and Bratton (2016) also found that sunfish in the two largest size classes (10.1–13.9 cm and ≥14 cm) had the highest ingestion frequency [85]. It is concerning that the larger fish seem to be ingesting plastics at a higher frequency, because egg production is exponentially linked to body length, with larger females producing the most eggs. Aberrant methylation levels have also been shown to cause serious damage in the form of changes in gene expression and/or genomic rearrangements [87]. Further, European seabass (*D. labrax*) larvae demonstrated a masculinization of the female gonadal tissue due to increased methylation in response to higher-than-normal temperatures [54]. It is unknown if a similar response would occur due to micro-/nanoplastic exposure; however, a similar reaction in fish larvae or even adult fish could have a detrimental effect on egg production or reproduction in general, which could lead to a trophic cascade.

Another notable finding within the current study was that nearly all the time groups across the four experiments did show increased methylation compared to their controls; although there was not always a significant difference, there was an increasing trend. The controls for each experiment also showed a trend of increased methylation as time progressed. DNA methylation is a sensitive process and can be altered by many natural and unnatural phenomena, such as temperature, improper nutrition, and pollutants. Though the media for each culture was changed daily, it is possible that with the cultures at full confluency at T_2 and T_3, there was a buildup of waste products that increased methylation within the controls and skewed methylation patterns in favor of a statistically insignificant result.

Fish at all trophic levels play an essential role within the ecosystem, and abnormally high methylation levels could hinder optimal cellular function. Physiological responses to microplastic exposure, such as diminished egg production, could further stress already burdened aquatic ecosystems, which could have far-reaching impacts even on human food supplies. It is also possible that problems encountered by aquatic organisms in response to the ingestion of microplastics could also manifest themselves in humans. In fact, Leslie et al. (2022) conducted a novel biomonitoring study looking for plastic particles $\geq$700 nm in whole human blood [88]. Between the 22 individuals sampled, the average concentration of quantifiable plastic particles was 1.6 μg/mL [88]. Another study by Wang et al. (2022) examined metabolic alterations in human cells in response to polystyrene nanoplastics, and it was found that 16.46% of the quantified proteins and 17% of the quantified metabolites displayed altered levels in response to the plastic particles [89]. As transcriptional changes leading to down-regulation of proteins can and do occur due to epigenetic alterations, it is entirely possible that epigenetic changes in response to the polystyrene nanoplastics could have led to the metabolic changes seen by Wang et al. (2022) [89]. A similar reaction to nanoplastic exposure would be expected in the bluegill epigenome; as this study explored only global methylation patterns and did not differentiate if any specific biomarkers were down-regulated, further investigation is warranted to determine if particular genes are also depressed in response to nanoplastic exposure.

Microplastics have a known anthropogenic impact on aquatic ecosystems and their inhabitants, so to ensure aquatic ecosystems remain healthy and functional and to understand the effects micro-/nanoplastics may even have on humans, it would be prudent to investigate how micro-/nanoplastics affect methylation in both fish and other organisms' genomes further. Given the current study's results, it seems that exposure to plastic nanoparticles does increase methylation within bluegill BF-2 cells. It cannot be said that this increase in methylation was dose- or time-dependent. Yet, there appeared to be an increase in methylation between nearly every experimental group and their controls, even though some were not significantly different. In future methylation micro-/nanoplastic studies, increasing the number of controls and experimental groups per time period would help negate the effects of any outlier cultures with increased methylation due to temperature fluctuations, waste product buildup, or other unforeseen factors. It would also be essential to look for physiological responses from the cells, such as cytotoxicity and oxidative stress, in response to plastic contamination.

However, as stated in the Materials and Methods Section, the dosages used in the present study were not environmentally relevant concentrations. Unfortunately, a decided lack of global standards exists for sampling and quantifying micro-/nanoplastics from environmental samples, making it difficult for researchers to use realistic dosages in experimental studies [90,91]. Even low concentrations of micro-/nanoplastics can cause an array of adverse effects [90]. As stated earlier, nanoplastics can cross biological barriers [20] and enter cells, where they can wreak havoc by creating reactive oxygen species to cause DNA

damage, DNA methylation, and oxidative stress [64,65]. They can also cause mechanical damage to cellular machinery and impede normal cellular functions [65]. All this could lead to the development of serious diseases, such as cancer [92]. Future work in this area of study and other micro-/nanoplastic research would benefit from standardized methods for micro-/nanoplastic collection and quantification from environmental samples, which would allow both in vivo and in vitro studies to look at the effects of current plastic levels rather than hypothetical, arbitrarily chosen levels. Greater clarity of the risks posed by micro-/nanoplastics through improved standardization of collection and quantification methods and risk assessment methods would help everyone better understand the breadth of the problem, thereby allowing scientific communities, industries, policymakers, and society at large to know how and why to mitigate plastic pollution and consumption.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/microplastics4010010/s1, Figure S1: Restriction sites of restriction enzymes *Msp*I and *Hpa*II based on methylation state. Table S1: Sequences of adaptors and pre- and selective primers used for MS-AFLP. Table S2: Reagents and volumes used for both primer combinations, master mix A (MMA) and master mix C (MMC).

Author Contributions: Conceptualization: S.M.W. and A.M.J.; methodology: S.M.W., A.M.J. and J.M.W.; software: S.M.W.; validation: S.M.W. and A.M.J.; formal analysis: S.M.W., A.M.J. and J.M.W.; investigation: S.M.W.; resources: A.M.J.; data curation: S.M.W. and A.M.J.; writing—original draft preparation: S.M.W. and A.M.J.; writing—review and editing: S.M.W., A.M.J. and J.M.W.; visualization: S.M.W.; supervision: A.M.J.; project administration: A.M.J.; funding acquisition: S.M.W. and A.M.J. The first author, S.M.W., hereby claims that she performed literature reviews, carried out the methods of this experiment, analyzed the results, and drew conclusions based on previous research and empirical data. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of West Florida Hal Marcus College of Science and Engineering Graduate Grant, University of West Florida Biology Department.

Institutional Review Board Statement: All research was conducted in accordance with the University of West Florida code of ethics.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article and Supplementary Material. Further inquiries can be directed to the corresponding author.

Acknowledgments: We thank Wayne Bennett (UWF) and Peter Cavnar (UWF) for their technical advice and the use of lab space for cell culture. We also thank the Hal Marcus College of Science and Engineering for providing funds to help support this study. We also thank Candice Lavelle (EPA) for her help in developing the methodology presented here and procuring the bluegill BF-2 cells.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Carpenter, E.J.; Smith, K.L., Jr. Plastics on the Sargasso Sea surface. *Science* **1972**, *175*, 1240–1241. [CrossRef]
2. Andrady, A.L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62*, 1596–1605. [CrossRef]
3. Chamas, A.; Moon, H.; Zheng, J.; Qiu, Y.; Tabassum, T.; Jang, J.H.; Abu-Omar, M.; Scott, S.L.; Suh, S. Degradation rates of plastics in the environment. *ACS Sustain. Chem. Eng.* **2020**, *8*, 3494–3511. [CrossRef]
4. Derraik, J.G. The pollution of the marine environment by plastic debris: A review. *Mar. Pollut. Bull.* **2002**, *44*, 842–852. [CrossRef] [PubMed]
5. Barnes, D.K.A.; Galgani, F.; Thompson, R.C.; Barlaz, M. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. Lond. Ser. B* **2009**, *364*, 1985–1998. [CrossRef]

6. Ryan, P.G.; Moore, C.J.; van Franeker, J.A.; Moloney, C.L. Monitoring the abundance of plastic debris in the marine environment. *Philos. Trans. R. Soc. Lond. Ser. B* **2009**, *364*, 1999–2012. [CrossRef]
7. Cózar, A.; Echevarría, F.; González-Gordillo, J.I.; Irigoien, X.; Úbeda, B.; Hernández-León, S.; Palma, Á.T.; Navarro, S.; García-de-Lomas, J.; Ruiz, A.; et al. Plastic debris in the open ocean. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 10239–10244. [CrossRef]
8. Eriksen, M.; Lebreton, L.C.M.; Carson, H.S.; Theil, S.; Moore, C.J.; Borerro, J.C.; Galgani, F.; Ryan, P.G.; Reisser, J. Plastic pollution in the world's oceans: More than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. *PLoS ONE* **2014**, *9*, e111913. [CrossRef]
9. Wagner, M.; Scherer, C.; Alvarez-Muñoz, D.; Brennholt, N.; Bourrain, X.; Buchinger, S.; Fries, E.; Grosbois, C.; Klasmeier, J.; Marti, T.; et al. Microplastics in freshwater ecosystems: What we know and what we need to know. *Environ. Sci. Eur.* **2014**, *26*, 12. [CrossRef] [PubMed]
10. Klein, S.; Dimzon, I.K.; Eubeler, J.; Knepper, T.P. Analysis, occurrence, and degradation of microplastics in the aqueous environment. In *Freshwater Microplastics: The Handbook of Environmental Chemistry*; Wagner, M., Lambert, S., Eds.; Springer: Cham, Switzerland, 2018; Volume 58, pp. 51–67.
11. Forrest, S.A.; Holman, L.; Murphy, M.; Vermaire, J.C. Citizen Science sampling programs as a technique for monitoring microplastic pollution: Results, lessons learned and recommendations for working with volunteers for monitoring plastic pollution in freshwater ecosystems. *Environ. Monit. Assess.* **2019**, *191*, 172. [CrossRef]
12. Gopinath, K.; Seshachalam, S.; Neelavannan, K.; Anburaj, V.; Rachel, M.; Ravi, S.; Bharath, M.; Achyuthan, H. Quantification of microplastic in Red Hills Lake of Chennai city, Tamil Nadu, India. *Environ. Sci. Pollut. Res.* **2020**, *27*, 33297–33306. [CrossRef]
13. Rowley, K.H.; Cucknell, A.C.; Smith, B.D.; Clark, F.P.; Morritt, D. London's river of plastic: High levels of microplastics in the Thames water column. *Sci. Total Environ.* **2020**, *740*, 140018. [CrossRef]
14. Egessa, R.; Nankabirwa, A.; Ocaya, H.; Pabire, W.G. Microplastic pollution in surface water of Lake Victoria. *Sci. Total Environ.* **2020**, *741*, 140201. [CrossRef]
15. Lestari, P.; Trihadiningrum, Y.; Wijaya, B.A.; Yunus, K.A.; Firdaus, M. Distribution of microplastics in Surabaya River, Indonesia. *Sci. Total Environ.* **2020**, *726*, 138560. [CrossRef] [PubMed]
16. Liu, Y.; Zhang, J.D.; Cai, C.Y.; He, Y.; Chen, L.; Xiong, X.; Huang, H.; Tao, S.; Liu, W. Occurrence and characteristics of microplastics in the Haihe River: An investigation of a seagoing river flowing through a megacity in northern China. *Environ. Pollut.* **2020**, *262*, 114261. [CrossRef]
17. Campanale, C.; Stock, F.; Massarelli, C.; Kochleus, C.; Bagnuolo, G.; Reifferscheid, G.; Uricchio, V.F. Microplastics and their possible sources: The example of Ofanto river in southeast Italy. *Environ. Pollut.* **2020**, *258*, 113284. [CrossRef]
18. Li, J.; Liu, H.; Chen, J.P. Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water Res.* **2018**, *137*, 362–374. [CrossRef] [PubMed]
19. Au, S.Y.; Lee, C.M.; Weinstein, J.E.; van den Hurk, P.; Klaine, S.J. Trophic transfer of microplastics in aquatic ecosystems: Identifying critical research needs. *Integr. Environ. Assess. Manag.* **2017**, *13*, 505–509. [CrossRef]
20. Mattsson, K.; Adolfsson, K.; Ekvall, M.T.; Hansson, L.A.; Linse, S.; Cedervall, T.; Prinz, C.N. Translocation of 40 nm diameter nanowires through the intestinal epithelium of *Daphnia magna*. *Nanotoxicology* **2016**, *10*, 1160–1167. [CrossRef]
21. Watts, A.J.R.; Lewis, C.; Goodhead, R.M.; Beckett, S.J.; Moger, J.; Tyler, C.R.; Galloway, T.S. Uptake and retention of microplastics by the shore crab *Carcinus maenas*. *Environ. Sci. Technol.* **2014**, *48*, 8823–8830. [CrossRef]
22. Tanaka, K.; Takada, H.; Yamashita, R.; Mizukawa, K.; Fukuwaka, M.; Watanuki, Y. Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. *Mar. Pollut. Bull.* **2013**, *69*, 219–222. [CrossRef]
23. Rehse, S.; Kloas, W.; Zarfl, C. Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of *Daphnia magna*. *Chemosphere* **2016**, *153*, 91–99. [CrossRef]
24. O'Brine, T.; Thompson, R.C. Degradation of plastic carrier bags in the marine environment. *Mar. Pollut. Bull.* **2010**, *60*, 2279–2283. [CrossRef] [PubMed]
25. Lambert, S.; Wagner, M. Characterisation of nanoplastics during the degradation of polystyrene. *Chemosphere* **2016**, *145*, 265–268. [CrossRef]
26. Gigault, J.; Pedrono, B.; Maxit, B.; Ter Halle, A. Marine plastic litter: The unanalyzed nano-fraction. *Environ. Sci. Nano* **2016**, *3*, 346–350. [CrossRef]
27. Dawson, A.L.; Kawaguchi, S.; King, C.K.; Townsend, K.A.; King, R.; Huston, W.M.; Bengtson Nash, S.M. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. *Nat. Commun.* **2018**, *9*, 1001. [CrossRef] [PubMed]
28. Kashiwada, S. Distribution of nanoparticles in the see-through medaka (*Oryazias latipes*). *Environ. Health Perspect.* **2006**, *114*, 1697–1702. [CrossRef] [PubMed]
29. von Moos, N.; Burkhardt-Holm, P.; Kohler, A. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. *Environ. Sci. Technol.* **2012**, *46*, 11327–11335. [CrossRef] [PubMed]
30. Cedervall, T.; Hansson, L.A.; Lard, M.; Frohm, B.; Linse, S. Food chain transport of nanoparticles affects behaviour and fat metabolism in fish. *PLoS ONE* **2012**, *7*, e32254. [CrossRef] [PubMed]

31. Mattsson, K.; Ekvall, M.T.; Borgstrom, M.T.; Linse, S.; Hansson, L.A.; Malmendal, A.; Cedervall, T. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. *Environ. Sci. Technol.* **2015**, *49*, 553–561. [CrossRef] [PubMed]
32. Karami, A.; Groman, D.B.; Wilson, S.P.; Ismail, P.; Neela, V.K. Biomarker response in zebrafish (*Danio rerio*) larvae exposed to pristine low-density polyethylene fragments. *Environ. Pollut.* **2017**, *223*, 466–475. [CrossRef] [PubMed]
33. LeMoine, C.M.R.; Kelleher, B.M.; Lagarde, R.; Northam, C.; Elebute, O.O.; Cassone, B.J. Transcriptional effects of polyethylene microplastics ingestion in developing zebrafish (*Danio rerio*). *Environ. Pollut.* **2018**, *243*, 591–600. [CrossRef]
34. Olsvik, P.A.; Whatmore, P.; Penglase, S.J.; Skjaerven, K.H.; d'Auriac, M.A.; Ellingsen, S. Associations between behavioral effects of bisphenol A and DNA methylation in zebrafish embryos. *Front. Genet.* **2019**, *10*, 184. [CrossRef]
35. Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Goodhead, R.; Moger, J.; Galloway, T.S. Microplastic ingestion by zooplankton. *Environ. Sci. Technol.* **2013**, *47*, 6646–6655. [CrossRef]
36. Fossi, M.C.; Coppola, D.; Baini, M.; Giannetti, M.; Guerranti, C.; Marsili, L.; Panti, C.; de Sabata, E.; Cló, S. Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: The case studies of the Mediterranean basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*). *Mar. Environ. Res.* **2014**, *100*, 17–24. [CrossRef]
37. Fossi, M.C.; Baini, M.; Panti, C.; Galli, M.; Jiménez, B.; Muñoz-Arnanz, J.; Marsili, L.; Finoia, M.G.; Ramírez-Macías, D. Are whale sharks exposed to persistent organic pollutants and plastic pollution in the Gulf of California (Mexico)? First ecotoxicological investigation using skin biopsies. *Comp. Biochem. Physiol. Part C* **2017**, *199*, 48–58. [CrossRef] [PubMed]
38. Murphy, F.; Russell, M.; Ewins, C.; Quinn, B. The uptake of macroplastic and microplastic by demersal and pelagic fish in the Northeast Atlantic around Scotland. *Mar. Pollut. Bull.* **2017**, *122*, 353–359. [CrossRef]
39. Kleinschmidt, J.M.; Janosik, A.M. Microplastics in Florida, United States: A case study of quantification and characterization with intertidal snails. *Front. Ecol. Evol.* **2021**, *9*, 645727. [CrossRef]
40. Prior, J.H.; Whitaker, J.M.; Janosik, A.M. Short-term microplastics exposure to the common mysid shrimp, *Americamysis bahia*: Effects on mortality and DNA methylation. *Microplastics* **2024**, *3*, 234–249. [CrossRef]
41. Metzger, D.C.H.; Schulte, P.M. Epigenomics in marine fishes. *Mar. Genom.* **2016**, *30*, 43–54. [CrossRef]
42. Richards, E.J. Inherited epigenetic variation—Revisiting soft inheritance. *Natl. Rev. Genet.* **2006**, *7*, 395–401. [CrossRef] [PubMed]
43. Cubas, P.; Vincent, C.; Coen, E. An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* **1999**, *401*, 157–161. [CrossRef]
44. Morgan, H.D.; Sutherland, H.E.; Martin, D.I.K.; Whitelaw, E. Epigenetic inheritance at the agouti locus in the mouse. *Nat. Genet.* **1999**, *23*, 314–318. [CrossRef]
45. Rakyan, V.K.; Chong, S.; Champ, M.E.; Cuthbert, P.C.; Morgan, H.D.; Luu, K.V.K.; Whitelaw, E. Transgenerational inheritance of epigenetic states at the murine Axin(Fu) allele occurs after maternal and paternal transmission. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 2538–2543. [CrossRef]
46. Schrey, A.W.; Alvarez, M.; Foust, C.M.; Kilvitis, H.J.; Lee, J.D.; Liebl, A.L.; Martin, L.B.; Richards, C.L.; Robertson, M. Ecological epigenetics: Beyond MS-AFLP. *Integr. Comp. Biol.* **2013**, *53*, 340–350. [CrossRef] [PubMed]
47. Bossdorf, O.; Richards, C.L.; Pigliucci, M. Epigenetics for ecologists. *Ecol. Lett.* **2008**, *11*, 106–115. [CrossRef] [PubMed]
48. Jablonka, E.; Lamb, M.J. The evolution of information in the major transitions. *J. Theor. Biol.* **2006**, *239*, 236–246. [CrossRef]
49. Li, E.; Zhang, Y. DNA methylation in mammals. *Cold Spring Harb. Perspect. Biol.* **2014**, *6*, a019133. [CrossRef] [PubMed]
50. Jiang, L.; Zhang, J.; Wang, J.-J.; Wang, L.; Zhang, L.; Li, G.; Yang, X.; Ma, X.; Sun, X.; Cai, J.; et al. Sperm, but not oocyte, DNA methylome is inherited by zebrafish early embryos. *Cell* **2013**, *153*, 773–784. [CrossRef]
51. Potok, M.E.; Nix, D.A.; Parnell, T.J.; Cairns, B.R. Reprogramming the maternal zebrafish genome after fertilization to match the paternal methylation pattern. *Cell* **2013**, *153*, 759–772. [CrossRef] [PubMed]
52. Devlin, R.H.; Nagahama, Y. Sex determination and sex differentiation in fish: An overview of genetic, physiological, and environmental influences. *Aquaculture* **2002**, *208*, 191–364.
53. Varriale, A.; Bernardi, G. DNA methylation and body temperature in fishes. *Gene* **2006**, *385*, 111–121. [CrossRef] [PubMed]
54. Navarro-Martin, L.; Viñas, J.; Ribas, L.; Díaz, N.; Gutiérrez, A.; Di Croce, L.; Piferrer, F. DNA methylation of the gonadal aromatase (*cyp19a*) promoter is involved in temperature-dependent sex ratio shifts in the European seabass. *PLoS Genet.* **2011**, *7*, e1002447. [CrossRef] [PubMed]
55. Timme-Laragy, A.R.; Meyer, J.N.; Waterland, R.A.; Di Giulio, R.T. Analysis of CpG methylation in the killifish CYP1A promoter. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2005**, *141*, 406–411. [CrossRef]
56. Pierron, F.; Baillon, L.; Sow, M.; Gotreau, S.; Gonzalez, P. Effect of low-dose cadmium exposure on DNA methylation in the endangered European eel. *Environ. Sci. Technol.* **2014**, *48*, 797–803. [CrossRef]
57. Ehrlich, M. Expression of various genes is controlled by DNA methylation during mammalian development. *J. Cell Biochem.* **2003**, *88*, 899–910. [CrossRef] [PubMed]
58. Li, W.; Wang, Y.; Zhu, J.; Wang, Z.; Tang, G.; Huang, B. Differential DNA methylation may contribute to temporal and spatial regulation of gene expression and the development of mycelia and conidia in entomopathogenic fungus *Metarhizium robertsii*. *Fungal Biol.* **2017**, *121*, 293–303. [CrossRef]

59. Kawakatsu, T.; Nery, J.R.; Castanon, R.; Ecker, J.R. Dynamic DNA methylation reconfiguration during seed development and germination. *Genome Biol.* **2017**, *18*, 171–183. [CrossRef] [PubMed]
60. Garcia-Fernandez, P.; Gestal, C.; Garcia-Souto, D.; Moran, P.; Almansa, E. Epigenetic DNA methylation mediating *Octopus vulgaris* early development: Effect of essential fatty acids enriched diet. *Front. Physiol.* **2017**, *8*, 292. [CrossRef]
61. Zhao, Y.; Wan, K.; Wang, J.; Wang, S.; Chang, Y.; Du, Z.; Zhang, L.; Dong, L.; Zhou, D.; Zhang, W.; et al. DNA methylation and gene expression profiling reveal the potential association of retinol metabolism-related genes with hepatocellular carcinoma development. *PeerJ* **2024**, *12*, e17916. [CrossRef]
62. Hughes, T.; Ture-Ozdemir, F.; Alibaz-Oner, F.; Coit, P.; Direskeneli, H.; Sawalha, A.H. Epigenome-wide scan identifies a treatment-responsive pattern of altered DNA methylation among cytoskeletal remodeling genes in monocytes and CD4+ T cells from patients with Behçet's disease. *Arthritis Rheumatol.* **2014**, *66*, 1648–1658. [CrossRef] [PubMed]
63. Katto, J.; Mahlknecht, U. Epigenetic regulation of cellular adhesion in cancer. *Carcinogenesis* **2011**, *32*, 1414–1418. [CrossRef]
64. Mahmud, F.; Sarker, D.B.; Jocelyn, J.A.; Sang, Q.-X.A. Molecular and cellular effects of microplastics and nanoplastics: Focus on inflammation and senescence. *Cells* **2024**, *13*, 1788. [CrossRef] [PubMed]
65. Martin-Folgar, R.; González-Caballero, M.C.; Torres-Ruiz, M.; Cañas-Portilla, A.I.; de Alba González, M.; Liste, I.; Morales, M. Molecular effects of polystyrene nanoplastics on human neural stem cells. *PLoS ONE* **2024**, *19*, e0295816. [CrossRef]
66. Poma, A.M.; Morciano, P.; Aloisi, M. Beyond genetics: Can micro and nanoplastics induce epigenetic and gene-expression modifications? *Front. Epigenet. Epigenom.* **2023**, *1*, 1241583. [CrossRef]
67. Espinosa-Pérez, H.; Lambarri-Martínez, C. NatureServe. *Lepomis macrochirus*. The IUCN Red List of Threatened Species. 2019. Available online: https://doi.org/10.2305/IUCN.UK.2019-2.RLTS.T61260A58309944.en (accessed on 11 October 2024).
68. Palchaudhuri, S.; Raymond, A.; Carlson, E.A.; Li, Y.; Zelikoff, J.T. Cytotoxic and cytoprotective effects of selenium on bluegill sunfish (*Lepomis macrochirus*) phagocytic cells in vitro. *Bull. Environ. Contam. Toxicol.* **2001**, *67*, 672–679. [CrossRef] [PubMed]
69. Connelly, H.; Means, J.C. Immunomodulatory effects of dietary exposure to selected polycyclic aromatic hydrocarbons in the bluegill (*Lepomis macrochirus*). *Int. J. Toxicol.* **2010**, *29*, 532–545. [CrossRef] [PubMed]
70. Srikanth, K.; Sundar, L.S.; Pereira, E.; Duarte, A.C. Graphene oxide induces cytotoxicity and oxidative stress in bluegill sunfish cells. *J. Appl. Toxicol.* **2018**, *38*, 504–513. [CrossRef]
71. Poornavaishnavi, C.; Gowthamia, R.; Srikantha, K.; Bramhacharic, P.V.; Venkatramaiahd, N. Nickel nanoparticles induces cytotoxicity, cell morphology and oxidative stress in bluegill sunfish (BF-2) cells. *Appl. Surf. Sci.* **2019**, *483*, 1174–1181. [CrossRef]
72. Browser, P.R.; Plumb, J.A. Channel catfish virus: Comparative replication and sensitivity of cell lines from channel catfish ovary and the brown bullhead. *J. Wildl. Dis.* **1980**, *16*, 451–454. [CrossRef]
73. Mekarunothai, A.; Bacher, M.; Buathong, R.; Intarasam, S.; Tayana, N.; Kongkiatpaiboon, S.; Charoenrat, T.; Napiroon, T. β-sitosterol isolated from the leaves of *Trema orientalis* (Cannabaceae) promotes viability and proliferation of BF-2 cells. *PeerJ* **2024**, *12*, e16774. [CrossRef] [PubMed]
74. MilliporeSigma; European Collection of Authenticated Cell Cultures. Subculture of adherent cell lines. In *Fundamental Techniques in Cell Culture Laboratory Handbook*, 4th ed.; Sigma-Aldrich: St. Louis, MO, USA, 2017; pp. 37–40.
75. Vos, P.; Hogers, R.; Bleeker, M.; Reijans, M.; van de Lee, T.; Hornes, M.; Frijters, A.; Pot, J.; Peleman, J.; Kuiper, M.; et al. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.* **1995**, *23*, 4407–4414. [CrossRef] [PubMed]
76. Xiong, L.Z.; Saghai Maroof, M.A.; Zhang, Q. Patterns of cytosine methylation in an elite rice hybrid and its parental lines, detected by a methylation-sensitive amplification polymorphism technique. *Mol. Gen. Genet.* **1999**, *261*, 439–446. [CrossRef]
77. Li, S.; He, F.; Wen, H.; Li, J.; Si, Y.; Liu, M.; He, H.; Huang, Z. Analysis of DNA methylation level by methylation-sensitive amplification polymorphism in half-smooth tongue sole (*Cynoglossus semilaevis*) subjected to salinity stress. *J. Ocean Univ. China* **2017**, *16*, 269–278. [CrossRef]
78. Kearse, M.; Moir, R.; Wilson, A.; Stones-Havas, S.; Cheung, M.; Sturrock, S.; Buxton, S.; Coope, A.; Markowitz, S.; Duran, C.; et al. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **2012**, *28*, 1647–1649. [CrossRef]
79. Morán, P.; Pérez-Figueroa, A. Methylation changes associated with early maturation stages in the Atlantic salmon. *BMC Genet.* **2011**, *12*, 86–93. [CrossRef] [PubMed]
80. Pérez-Figueroa, A. *msap*: A tool for the statistical analysis of methylation-sensitive amplified polymorphism data. *Mol. Ecol. Resour.* **2013**, *13*, 522–527. [CrossRef] [PubMed]
81. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2024. Available online: https://www.R-project.org/ (accessed on 5 October 2024).
82. Alberti, G.; Chisquare: Chi-Square and G-Square Test of Independence, Power and Residual Analysis, Measures of Categorical Association. R Package Version 1.0. 2024. Available online: https://CRAN.R-project.org/package=chisquare (accessed on 5 October 2024).
83. Morán, P.; Marco-Rius, F.; Megías, M.; Covelo-Soto, L.; Pérez-Figueroa, A. Environmental induced methylation changes associated with seawater adaptation in brown trout. *Aquaculture* **2013**, *392–395*, 77–83. [CrossRef]

84. Jayaseelan, C.; Rahuman, A.A.; Ramkumar, R.; Perumal, P.; Rajakumar, G.; Kirthi, A.V.; Santhoshkumar, T.; Marimuthu, S. Effects of sub-acute exposure to nickel nanoparticles on oxidative stress and histopathological changes in Mozambique tilapia, *Oreochromis mossambicus*. *Ecotox Environ. Saf.* **2014**, *107*, 220–228. [CrossRef]
85. Peters, C.A.; Bratton, S.P. Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos River Basin, Central Texas, USA. *Environ. Pollut.* **2016**, *210*, 380–387. [CrossRef]
86. Peters, C.A.; Thomas, P.A.; Rieper, K.B.; Bratton, S.P. Foraging preferences influence microplastic ingestion by six marine fish species from the Texas Gulf Coast. *Mar. Pollut. Bull.* **2017**, *124*, 82–88. [CrossRef] [PubMed]
87. Varriale, A. DNA methylation, epigenetics, and evolution in vertebrates: Facts and challenges. *Int. J. Evol. Biol.* **2014**, *2014*, 475981. [CrossRef]
88. Leslie, H.A.; van Velzen, M.J.M.; Brandsma, S.H.; Vethaak, A.D.; Garcia-Vallejo, J.J.; Lamoree, M.H. Discovery and quantification of plastic particle pollution in human blood. *Environ. Int.* **2022**, *163*, 107199. [CrossRef] [PubMed]
89. Wang, H.; Shi, X.; Gao, Y.; Zhang, X.; Zhao, H.; Wang, L.; Zhang, X.; Chen, R. Polystyrene nanoplastics induce profound metabolic shift in human cells as revealed by integrated proteomic and metabolomic analysis. *Environ. Int.* **2022**, *166*, 107349. [CrossRef]
90. Kyriakopoulos, G.L.; Zamparas, M.G.; Kapsalis, V.C. Investigating the human impacts and the environmental consequences of microplastics disposal into water resources. *Sustainability* **2022**, *14*, 828. [CrossRef]
91. Zantis, L.J.; Carroll, E.L.; Nelms, S.E.; Bosker, T. Marine mammals and microplastics: A systematic review and call for standardization. *Environ. Pollut.* **2021**, *269*, 116142. [CrossRef]
92. Goswami, S.; Adhikary, S.; Bhattacharya, S.; Agarwal, R.; Ganguly, A.; Nanda, S.; Rajak, P. The alarming link between environmental microplastics and health hazards with special emphasis on cancer. *Life Sci.* **2024**, *355*, 122937. [CrossRef]

MDPI AG
Grosspeteranlage 5
4052 Basel
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ISBN 978-3-7258-3754-0