

Review

A Practical Overview of Methodologies for Sampling and Analysis of Microplastics in Riverine Environments

Claudia Campanale ^{1,*}, Ilaria Savino ¹, Iulian Pojar ²,
Carmine Massarelli ¹ and Vito Felice Uricchio ¹

¹ Water Research Institute-Italian National Research Council (IRSA-CNR), V.le F. De Blasio 5, 70132 Bari, Italy; ilaria.savino@ba.irs.cnr.it (I.S.); carmine.massarelli@ba.irs.cnr.it (C.M.); vito.uricchio@ba.irs.cnr.it (V.F.U.)

² National Institute of Marine Geology and Geo-Ecology (GeoEcoMar), 23-25 Dimitrie Onciul St., 024053 Bucharest, Romania; iulianpojar@geoecomar.ro

* Correspondence: claudia.campanale@ba.irs.cnr.it

Received: 24 June 2020; Accepted: 5 August 2020; Published: 20 August 2020

Abstract: Microplastics have recently been stated as being remarkable contaminants of all environmental matrices. The lack of consistent and standardised methods and protocols used to evaluate and quantify microplastics present in riverine systems made a comparison among different studies a critical issue. Based on literature research and the practical expertise of the authors, this work presents a complete collection and analysis of procedures concerning the monitoring of microplastics in riverine environments, focusing on their sampling and analytical protocols to identify, quantify, and characterise them. Further details regarding the advantages and disadvantages of each analytical technique described, such as general recommendations and suggestions, are provided to give practical support for analytical procedures. In particular, microplastics studies consist firstly of their sampling from the aquatic compartment (aqueous and solid phase). Based on the goal of the research, specific devices can be used to collect particles from different matrices. It follows their quantification after extraction from the environmental matrix, adopting different protocols to isolate microplastics from a large amount of organic matter present in a riverine system. In the end, additional qualitative analyses (e.g., RAMAN and FTIR spectroscopy, GC-MS) are required to identify the chemical composition of particles for a better image regarding the abundance of polymer types, their origin, or other information related to manufacturing processes.

Keywords: microplastics; sampling; analytical methods; riverine environments

1. Introduction

The term microplastics (MPs), coined in 2004 [1], has been used to describe small plastic particles. However, a comprehensive definition has been recently given by [2]: “MPs are every solid-synthetic particle or polymeric matrix, with regular or irregular shape and dimensions between 1 µm and 5 mm, of primary or secondary manufacture origin, water-insoluble”.

In contrast to vast scientific studies describing the contamination of the marine environment with plastic and microplastic waste [3,4], MPs’ presence in freshwaters has only recently received attention. Rivers and lakes were mainly investigated with an increasing amount of publications released in the last few years [5–9], although other freshwater bodies (e.g., canals, volcanic lakes, deltaic lakes) are still under initial examination.

Among freshwater environments, rivers are considered the primary vector of transport of MPs from terrestrial environments to marine ecosystems, suggesting an overlooked and potentially significant component of the global microplastic life cycle [10].

The small dimensions of MPs complicate their collection and quantification in environmental samples compared to meso- and macroplastics. The existing methods adopted for the collection, extraction, and quantification of MPs from riverine systems are often time-consuming, require a high labour force, and demand elaborate techniques and expensive analytical instruments.

A reasonable investigation of MP contamination in these environments strongly depends on the appropriate use of suited sampling and analytical strategies. Difficulties in the methodological aspect concerning MPs research are due to the lack of harmonised and standardised protocols to which to refer for a monitoring study of MPs in riverine systems.

Several studies and reviews have been published about methodologies concerning MPs quantification and characterisation in the marine [11–13], freshwater [14–17], and terrestrial ecosystems [18–20]. However, few of these works aimed to give specific practical guidelines and workflow protocols for precise monitoring of MPs in riverine environments.

In light of this, the present work aimed to provide technical recommendations and suggestions for monitoring MPs in the river sector, focusing on sampling approaches and methodologies for their extraction, quantification, and characterisation, furnishing an in-depth analysis of advantages and disadvantages of each technique. Collecting and summarising existing methods, comparing at the same time advantages and disadvantages of each technique, is a useful tool to start a harmonisation process to make the results of studies all over the world comparable and to quantify the problem on a global level.

2. Sampling Strategies in Riverine Environments

Sampling MPs in a riverine system is different from collecting particles in the marine environment. Several factors, including hydrological conditions of the water body (e.g., water density, wind, currents, waves and tides), temporal and geographical factors determined by the shape of the river, the morphology, and the meteorological situation will influence the pathway of microlitter in the catchment area. These natural elements should be considered when developing a sampling strategy and monitoring of MPs [21].

Moreover, the distribution, fate, abundance, and type of MPs in the aquatic compartment are also influenced, on the one hand, by their physicochemical properties (shape, size and density) and, on the other hand, by different variabilities of the riverine environment such as the river length, the catchment size, and population density. All these factors, singularly or together, contribute to affect the transport and behaviour of MPs in the water compartment [14,22,23].

It follows that the choice of the sampling methodology is an extremely complex and delicate phase that has a significant impact on the total uncertainty of the analysis results, the quality of which is closely related to that of the sampling.

As a result of the minimal size, weight, and relative density of the material investigated (MPs density usually range between 0.9 and 1.5 g·cm⁻³), MPs should float on the surface of the water and, secondly, in the basal area of the thermocline in case of marine waters. For this reason, sampling is, to date, preferably performed on the surface and, where possible, on the water column.

However, key processes like windage, hydrodynamic alterations, degradation, sedimentation, adsorption, aggregation, biofouling, resuspension, burial, ingestion, and excretion of MPs by biota play a crucial role in their transport and accumulation resulting in a complex and dynamic equilibrium that brings to a horizontal and vertical movement of particles, as well as sedimentation in deposition areas [24–27].

It follows that sampling should be differentiated between the collection of the aqueous phase (surface water, water column), the sediment, and the biota. This strategy is essential to achieve a full picture of the presence and distribution of MPs regarding the entire aquatic compartment and to consider their impact on the environment.

3. Collection of the Aqueous Phase

Applied sampling strategies differ depending on the targeted environmental compartment that needs to be monitored.

In riverine environments, a collection of MPs in water can be performed by a dynamic or stationary sampling method (Figure 1).



Figure 1. On the right, an example of a stationary sampling of microplastics with three plankton nets fixed in the middle of the river. On the left, a dynamic sampling using a manta net towed by a boat.

3.1. Dynamic Sampling

In dynamic sampling, trawls are towed by boats using a rope of 50–70 m, keeping the nets outside the wake or bow waves from the vessel to prevent disturbance of vertical dispersion of the particles. Where possible, it is appropriate to position the net laterally, by a suitable spinnaker pole installed on one side of the boat. In both cases, possible contamination from the ship, e.g., by paint particles can occur.

The speed of the boat must be constant and low to avoid friction during the sampling process. As the speed increases, friction rises, and the quantity of filtered water decreases.

In any case, towing speeds greater than 3–4 knots drastically reduce the filtration efficiency of the net, since they cause turbulence at the mouth of the net, as well as causing risks of damage the trawl itself, although this depends on the type of equipment and vessels [28–31]. For nets with mesh less than 20 μm , the recommended speed is 0.5 knots [32].

Tow runs must be made against water. Once the run is over, the transept must be resumed from the original starting position to collect a sample replicate.

To avoid obstruction problems, following clogging of nets, which can happen especially in the presence of eutrophic waters, it is recommended to check the filtration efficiency monitoring the clogging with two flowmeters, one inside and one outside the net [33].

To ensure more representative data, it would be preferable to have at least three spatial replicates for each sample, repeating the transects, always with the same length, starting at different points to covering the whole section of the river, from one bank to another. Moreover, the sampling should be repeated at various times to have temporal replicates because daily and seasonal alterations can influence the variation of MPs density [10,16].

3.2. Stationary Sampling

Smaller rivers with variable water regime, also streams and creeks, are often not entirely navigable throughout the year, so dynamic sampling through the use of boats is rather complicated if not impossible in some cases.

Therefore, in these conditions, the installation of floating nets fixed on the banks that filter water using the river current with their mouths skimming the surface can be employed as a strategy for stationary sampling. A weight is used to maintain a continuous and consistent submersion depth of

the nets throughout the sampling duration, for monitoring and recording the portion of the submerged net. Sampling duration is dependent on the flow of the river and the rate of clogging of nets. The nets have to be collocated in the opposite direction of the river water flow. As well as mentioned above for the dynamic sampling, sample replicates are much suggested to reduce temporal and spatial variability.

4. Water Surface Sampling

Based on the relatively low concentrations of MPs in the environment, their sampling on the water surface generally requires water filtration or collection of large sample volumes employing mostly neuston, plankton, and manta nets (Figure 2) of different mesh sizes, considered as volume-reduced or nondiscrete sampling devices in which the volume of water is reduced until only the particles of interest for further processing in a laboratory remain [34].

MPs' small sizes demand sampling devices that allow the collection of a large volume of water in order to reduce the spatial and temporal variations adequately [15]. Nets are the most used instruments; they may have a mesh of different size and are more suitable for sampling in quiet surface waters.

A metallic frame usually forms nets, from which is set a cone made by a filtering mesh connected to a final collector tube. Some nets are endowed with wings on each side to ensure stability and buoyancy in the water [35].

Freshwater studies have mainly been adopting methods from oceanic research that used, for decades, sampling strategies and devices dedicated to other fields of study (e.g., to collect plankton) [36,37]. These methods are based on the use of manta and neuston nets, usually with a mesh size of 300–500 μm [11,13,38], equal to the size limit of MPs suggested by National Oceanic and Atmospheric Administration of USA (NOAA) [39]. These nets do not sample particles smaller than 300 μm because the mesh cannot retain tiny particles, including microfibers, which make up a significant portion of all MPs present in the aquatic environment. The adoption of such sampling devices underestimates the real quantity of microfibres that, often ignored, are flushed through the pores of the filters of the nets, providing a rough estimation of their abundance [40].

Furthermore, MPs in environmental samples can currently be detected up to a size of 1 μm , but only a few studies identify particles smaller than 50 μm due to the methodological limitations [22].

Plankton nets may have smaller dimensions of the mesh size ($\sim 100 \mu\text{m}$), thus allowing the recovery of concentrations thirty times higher compared to manta ones. Nonetheless, plankton nets are usually towed at low speed because of small mesh size ($\sim 100 \mu\text{m}$), and this could lead to clogging. Besides the horizontal transport, these nets allow vertical or oblique sampling [23,41]. In a recent study [42], a new device has been developed, coupling different nets, one beside the other and one above the other to collect multi-point measurements in medium- and large-sized natural streams. Instead, MP traps are used for MPs sampling in small rivers, and periodically streams with shallow depths. MP traps are equipped with an aluminium frame, pickets for anchoring to the riverbed and a filtering nylon net. The traps can be placed in several points in order to cover the width of the water stream and to have a more representative sampling. Pump systems are used for water sampling through a filtering system, resulting in a more efficient selection and a more significant sampling of fibres compared to nets [43,44]. Autosamplers are among the newest, and most used, techniques that fractionate the dimension of the sample through a pumping system and filtering waterfalls [45]. All these instruments may carry risks such as high degree of contamination of the samples during their manipulation and relocation.

Alternatively, discrete sampling devices (Niskin bottles, rosettes, integrating water sampler (IWS), buckets, bottles, and steel samplers; KC Denmark A/S Research equipment, Silkeborg, Denmark) can be used to collect bulk samples [34]. In bulk sampling, a known and recorded volume of the sample is collected without reducing it. The main advantage of this method is that, theoretically, all the MPs present in the environmental matrix can be sampled without any size limitation, thus preventing any loss that can happen in volume-reduced sampling. Moreover, this type of sampling is rapid and can help to reduce any risk of contamination, given that the time of

handling of the sample and the time of exposition of it to the surrounding environment are shortened. However, disadvantages consist of a limited amount of samples that can be collected, stored, and processed [34].

The choice of which instrument to use depends on the available equipment, but also on the aim of the study, the type of environment, and the matrix that has to be collected. The adopted sampling devices will have an impact on the quantity, and thus, on the representativeness of the sampled material [23]. Therefore, there is not a unique, valid instrument for MPs sampling in surface waters; each one shows, indeed, advantages and disadvantages for MPs sampling (Table 1).

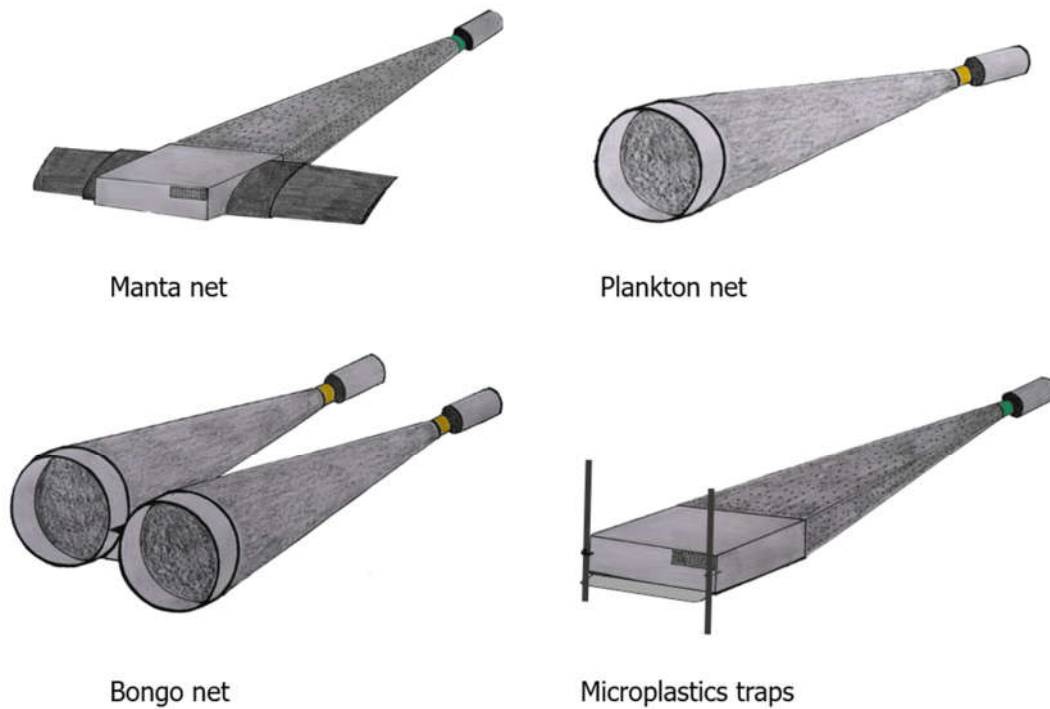


Figure 2. Examples of nets used for microplastics (MPs) sampling.

Table 1. Sampling devices used for the collection of MPs in surface waters.

Sampling Device	Advantages	Disadvantages	Costs \$	Time (Minutes)	References
Nondiscrete sampling devices	Manta net	-Sampling of large volumes of water; -The lateral wings allow the floating of the device and the sampling of the water surface.	~3500	15–240	[34,41,42,46–51]
	Neuston net	-Sampling of large volumes of water; -Widely used (useful for compare positions).	~2300	30	[34,52–55]
	Plankton net	-The lower limit of detection is 100 μm; -Sampling of medium volumes of water; -Possibility to sample the water column.	~2400	30	[26,34,56–58]
	MP traps	-Possibility to sample in several points of the water stream; -Possibility to choose mesh dimensions from 100 μm to 333 μm.	~1200	30	[35]
	Autosampler	-Well-known and precise volume of filtered water; -Minimises the risk of contamination; -Allows a dimensional separation of the particles directly in the field.	10,000–70,000	-	[45,59]

	Pumping systems	-Allows the user to sample smaller MPs and fibre loss is limited; -Well-known and precise volume of filtered water; -Allows standardisation of sampling.	-Sampling of a small volume of water; -Requires energy to work; -Requires boat; -It can be challenging to transport and apply. -Allows the sampling of a single point; -Requires the transport of bulky samples to the lab; -Sampling is less representative; -Risk of sample contamination.	300–1000	15–180	[30,34,43,44,51,60]
Discrete sampling devices	Niskin bottles/Jars/Bottles/Buckets/Rosette/Integrated water sampler (IWS)/Ruttner bottles/Friedinger bottles/Bernatowicz bottles	-Relatively quick and straightforward to use; -Rosette provides multi-point measurements; -Allows sampling at different depths; -Allows the user to sample smaller MPs and fibre loss is limited; -Well-known and precise volume of filtered water; -Allows standardisation of sampling.	-Requires boat; -Rosette can be challenging to transport; -Sampling of a small volume of water; -May be very fragile; -Requires the transport of bulky samples to the lab; -Sampling is less representative; -Risk of sample contamination.	Very variable (300–50,000)	15–30	[34,35,58,61–66]
Devices for surface microlayer	Stainless-steel sieves/Rotating Drum Sampler	-Does not require specialised equipment; -Quick and straightforward to use; -Well-known and precise volume of filtered water; -Allows choice of mesh size; -Allows a dimensional separation of the particles directly in the field.	-Sampling of medium/low volumes of water; -Requires the transport of significant volume of water to the lab; -Manual transfer of water with buckets; -Potential contamination by the apparatus.	From 50	Depending on mesh size	[34,60,67]

5. Water Column Sampling

Since the majority of synthetic polymers have a lower density than the seawater, in the past it was considered that microplastic particles could foremost float at surface level and only a small fraction could accumulate in the water column [11,58]. Nevertheless, several factors like flow turbulence, current conditions, the adsorption of a biofilm that increases MPs density and speeds up the sedimentation process, the different composition, density, and shape of polymers, move and homogenise the MPs' distribution throughout the water column in rivers, especially since freshwater density is lower than that of marine water [42,50,58]. Indeed, recent studies have pointed out a vertical distribution of MPs. High concentrations of them can also be found through the water column and at the sediment level in marine [44,68–70] and riverine environments [42,50,58,71], reinforcing that the common strategy of sampling only the surface layer of water is insufficient and can result in considerable bias, particularly in estuarine, harbour, and lake environments [50].

Sampling MPs in the water column is carried out using mostly plankton nets or bongo nets. These are usually equipped with a couple of circular aluminium frames linked to a central axis, to which a flowmeter and a pair of nylon plankton nets are fixed (Figure 2) [27].

Manta nets, due to the buoyancy of the instrument, cannot be used for sampling in the water column. The water column can be sampled both horizontally and vertically. In horizontal sampling, after placing a weight (about 10–20 kg) to the net, it can be slowly dropped to the maximum depth (avoiding contact with the bottom) and then immediately trawled obliquely at a speed of fewer than 2 knots in order to let the water pass through the net with a steady flow. In vertical sampling, on the other hand, the net is lifted towards the surface until a specific depth, thus sampling the entire water column. Bongo nets have the advantage of being able to sample at different depths and to locate MPs of different densities along the water column [35]. Some researchers [42,58,71] used different devices to measure MPs at various depths. Authors [71] have adopted a modified large Helley Smith sampler to collect mid-depth to bottom samples in the Los Angeles river, while others [58] have coupled a plankton net with a mesh size of 80 μm to a propeller-type current meter to collect microfibers at different depths in the Seine river. Also, [42] presented a newly developed homemade device applicable in medium and large streams to measure MPs distributed within different vertical profiles of the Austrian Danube river using different nets of different sizes, one above the other.

6. Expression of Results and Water Volume Calculation

Data comparison can be difficult due to the lack of standardised and harmonised protocols and approaches in used sampling methods, size ranges, and data reporting units employed for expressing results. Data reporting units will depend primarily on the adopted methodologies, on the goal, and on the research question. There is no consensus yet about whether MPs should be referred to as MPs mass per volume for filtered waters (g/m^3) or as particle numbers per volume for filtered water (number of particles $\cdot \text{m}^{-3}$) and this topic is still subject to research and discussion [16,17].

In both sampling strategies (dynamic and stationary), the concentration of MPs can be expressed as the number of particles or weight of material per m^3 of filtered water.

Recently, the Joint Programming Initiative Healthy and Productive Seas and Oceans (JPI Oceans) proposed reporting units to express the concentration of MPs referred to in water samples, encouraging at the same time researchers to report both the count and weight of MPs [72]:

1. no. MPs per area (no. particles/ km^2 or no. particles/ m^2);
2. no. MPs per volume (no. particles/ m^3);
3. mass of MPs per area (g MPs/ km^2 or g MPs/ m^2);
4. mass of MPs per volume (g MPs/ L^{-1} or g MPs/ m^3);

It follows that that filtration volume, tow length, or area sampled need to be measured.

The filtered volume is usually calculated by recording with a mechanical or electronic flow meter attached at the net opening that measures the accurate volume of water passing through the net. The volume filtered is then calculated as follows:

$$V = \text{area of the mouth of the net} \times n \times p \quad (1)$$

where: V : volume of filtered water; n : number of revolutions of the impeller recorded by the flow meter; p : hydraulic pitch.

In the case of dynamic sampling, in the absence of a flow meter, the total volume of water filtered through the net can be computed by recording the distance covered during the tow by the boat. In this case, the filtered volume can be calculated as follows:

$$V = \text{area of the mouth of the net} \times d \quad (2)$$

where: V : volume of filtered water; d : distance covered during the tow.

Alternatively, relating concentrations of MPs can also be referred to based on the sampled area by multiplying transect length (computed using GPS start and stop positions) by the width of the net opening.

Final results will be expressed as the number of particles or grams per Km^2 or m^{-2} .

However, GPS and flowmeter can give very different results, thus affecting the final value of MP concentration; therefore JPI Oceans highly suggested using both methods during net sampling to evaluate eventual differences between the two [72].

In stationary sampling, in the absence of a flow meter, the total volume of water filtered through the net can be computed by multiplying the surface of the submerged portion of the net, the sampling duration, and the average velocity of the river.

Moreover, the MPs' transport value for the cross-section of the river can be determined as a product of MP concentration and flow velocity of the river as in [42]:

$$q = C \times v \quad (3)$$

where: q = transport rate ($\text{g MPs/m}^2/\text{s}^{-1}$ or $\text{no. particles/m}^2/\text{s}^{-1}$); C = MPs concentration (g MPs/m^3 or no. particles/m^3); v = flow velocity (m/s^{-1}).

7. Sediment Sampling

MPs' occurrence in sediments was mentioned for the first time in 1977 [73]; however, the scientific literature regarding this type of pollution has seen an increase in the last two decades. In this period, the study areas expanded in terms of space covered and sediment type, from urban soil [74] to riverine alluvia [75] and beach sands [76] to deep-sea cores [77]. In the same manner, the methodology techniques used for microplastic evaluation have been diversified for each type of sediment sampling [17,78], to discriminate the plastic particles from the rest of the sample.

7.1. Collecting Sediments and Preservation

Coarse fractions usually represent fluvial sediments like sand and gravels; meanwhile, silts, muds, and clays are observed in some low current velocity areas (e.g., dams and deltas), as well in all different type of lakes. Anyhow, the sampling of any type of lithology from superficial freshwater sediments should not differ.

The accumulation of MPs on riverbanks depends on several factors regarding the land (deposition/erosion bank-area, abundance/lack of vegetation, finer/coarser sediments) and the hydrological conditions [17]. Therefore, all factors mentioned above should be observed, and the sampling must always be done in the same conditions for an accurate comparison among results, especially in fluvial environments collecting different sediment types and at least six samples per site [72].

Onshore sampling is best to be performed in the swash zone (Figure 3), where MPs tend to accumulate under waves action [79] or underwater, at different depths. Underwater sampling, for both fluvial and lacustrine environments, can be done at different depths, acknowledging the fact that a finer texture of the sediment usually accumulates a higher quantity of MPs that can be occasionally visually observed.

In the case of sampling the sediments from freshwater bodies, the sample collecting techniques are rarely detailed in the literature. Most of the authors usually collect sediments manually, establishing an area (e.g., from 0.04 m² [80] to 0.09 m² [81]) and a depth (e.g., 5 cm [80] to 32 cm [82]). Using a corer with a specific diameter (e.g., 50 mm diameter [83]), box corer [84], or a grab (e.g., Ekman or Van Veen, Figure 4) with a known area (e.g., 225 cm² [85]) can be referred easily to an exact volume of material, rather than using manual sampling.

Nonetheless, weight and volume measurements of the collected samples are essential [82] in order to compare the identified microplastic concentrations among samples or to other studies. The typical weight of the samples ranges between 0.5 and 10 Kg [16].

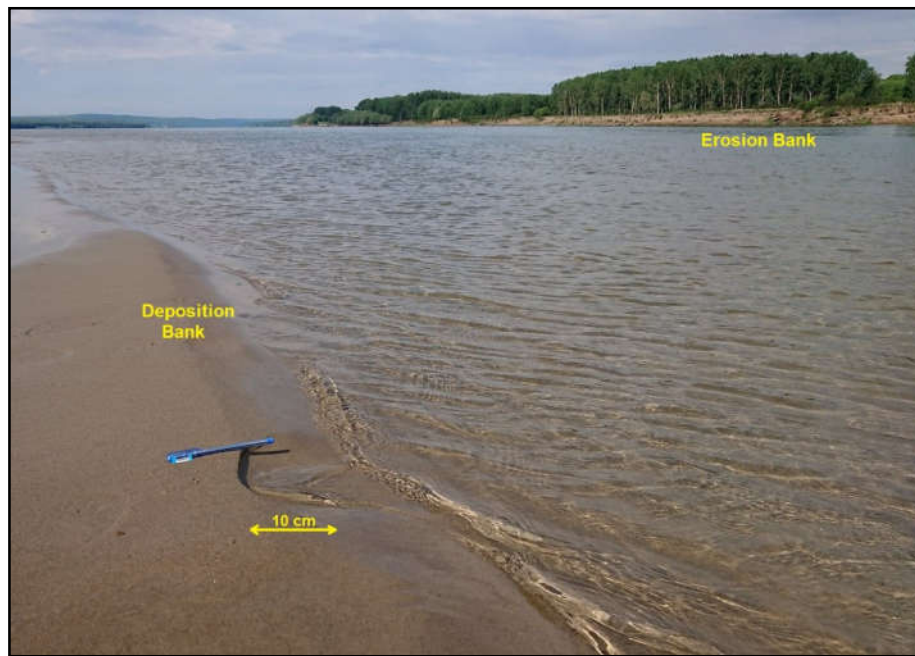


Figure 3. Riverbank sampling in the swash zone on the Danube River.

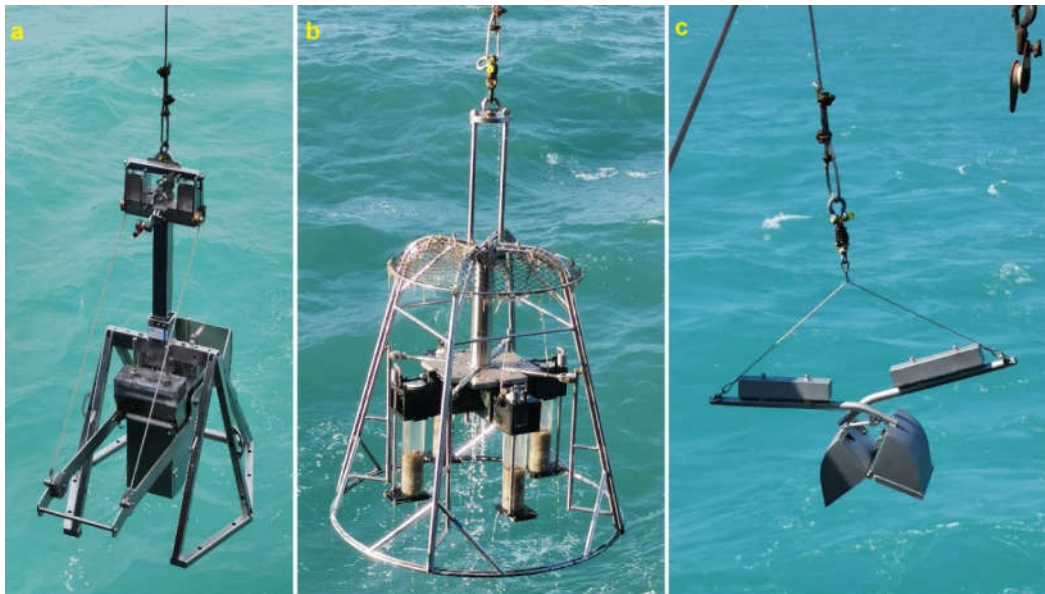


Figure 4. Sediment sampling instruments: (a) box corer; (b) multicorer; (c) Van Veen grab.

Plastic polymers are not described as a degrading material over time in a controlled environment; hence, the preservation step might not be necessary to protect plastic particles from degradation processes. However, [86] reported that incorrect storage of samples could cause changes in the MPs' size distribution due to partial destruction of smaller particles. Most of the authors suggested storing samples in darkness refrigerated (at 4 °C) or frozen (below −20 °C) until subsequent analysis if samples are not being processed immediately [72,78,86].

Nevertheless, preservation techniques are also employed to retard biological changes that inevitably continue after the sample is removed from the parent matrix [79,87]. In some cases, the identification and characterisation of the biological matter can be relevant to other aspects of the research [88,89] and the use of various fixing solutions (70%–80% ethanol, 4%–5% formalin) to preserve the biological material is generally reported [72,86,89,90].

7.2. Expression of Results for Sediment Samples

As mentioned above, sediment sampling is usually expressed in weight units. Therefore, after MP extraction and identification, the results are described as particles per Kg of sediment. For a better view of MP concentrations, some authors have used volume units as particles per L. Besides weight and volume, some authors have decided to associate the identified concentration of MPs with the estimated density of the sediment ($\text{g}\cdot\text{cm}^{-3}$) [91], considering the superficial sediment lithology a factor for microplastic accumulation.

As well as for water samples, JPI Oceans proposed reporting units also to express the concentration of MPs referred to sediment samples suggesting, whenever possible, to report results in all the following units [72]:

1. no. MPs per area (no. particles/ km^{-2} or no. particles/ m^{-2});
2. no. MPs per volume (no. particles/ m^{-3});
3. no. MPs per mass (no. particles/ kg^{-1} dry sediment);
4. mass of MPs per area ($\text{g MPs}/\text{km}^{-2}$ or $\text{g MPs}/\text{m}^{-2}$);
5. mass of MPs per volume ($\text{g MPs}/\text{L}^{-1}$ or $\text{g MPs}/\text{cm}^{-3}$),

8. Samples Processing

Analytical processes are required to achieve quantification data from collected samples and, further, qualitative description of the polymer assemblage. As part of the laboratory procedures, preparation of the samples is required for microplastic extraction.

8.1. Water Samples Preparation

At the end of the sampling step, the net used into the water body has to be washed from outside to direct retained material (MPs and organic particles) into the collector tube. The samples are then transferred in labelled glass jars and stored at 4 °C until analysis [72]. Firstly, wet sieving is carried out using two sieves, stacked up one on top of the other, the first with a mesh size of 5 mm and the second with a size $<300\text{ }\mu\text{m}$, depending on the net used to sample, washing the glass container several times with deionised water in order to retrieve all the MPs. This operation allows discarding the fraction of the sample larger than 5 mm [39]. Afterwards, the fraction retained by the sieve with the smaller mesh size shall be dried in a stove, then weighed, and finally the mass of total solids (natural material plus MPs and other anthropic material) determined in order to understand, once MPs have been extracted, how their mass varies compared to the mass of organic material sampled. The maximum temperature used for drying is about 60 °C because plastic fibres are known for having a weak thermoresistance [39,92]. Moreover, the sample recipients should be covered inside dry-ovens to prevent the contamination or the loss of particles.

8.2. Sediment Samples Preparation

As microplastic dimensions range lower than 5 mm, a sieving step might be required in case of coarse sediments or organic matter (e.g., gravel, vegetation), obviously after a drying process. As before, the maximum temperature used for drying is 60 °C.

Rather few studies regarding isolating microplastic from sediments have described a specific technique that uses the electrostatic properties of plastics to reduce the volume of the bulk sample [93]. The electrostatic separator (Figure 5) comprises a conveyor with programmable vibration speed that allows sediment to be carried by an earth drum under an electrical field. Plastic particles are, consequently, electrically charged, as well as other fine sediments. Therefore, these fractions can be separated from most of the sediment based on their electrostatic properties [16]. Usually, several runs over the same sample conduct to better results.



Figure 5. “Hamos” electroseparator and the resulting separation into 3 fractions: i. sediment, ii. mixture between sediment and MPs, and iii. fine sediment and MPs.

8.3. Sample Digestion

The analysis of complex riverine samples is very challenging due to high loads of interfering organic, biological, and lithogenic matrix particles (e.g., leaves, pieces of wood, flowers, pollen, sediment, minerals, etc.).

For a clearer sample, with as little material as possible, sample processing includes two steps that are essential: a. digestion of the organic matter and b. density separation.

Digestion of the organic matter is necessary to eliminate most of the floating material from the sample, leaving the MPs unharmed. One of the significant issues in MPs studies is how to isolate and extract MPs from complex matrices without destroying them.

The extraction of MPs needs to be performed based on the goal of the research and the type of samples in order to balance “cleanup” steps to time requirements looking for a good compromise. The removal of organic fraction needs to be performed thorough chemical or enzymatic digestion in order to extract MPs from the environmental matrix. Choosing the digestion protocol in terms of sample purification is a matter of concern, and it depends on the sample itself.

The reagents used to destroy labile organic fraction can be acidic, basic, oxidising, or enzymatic [16,94–97].

For acid digestion, hot nitric and hydrochloride acids were used, but this caused the degradation of some polymer types, especially polystyrene, polyamide, and polyethene [16,96,98,99]. On the one hand, the utilisation of nitric acid increased the destruction of organic matter between 93% and 98%. On the other hand, hydrochloric acid carried out incomplete digestion of the organic fraction. Indeed, in [96,100], the least effective treatment was hydrochloric acid, with 1 M HCl and 2 M HCl, resulting in a digestion efficacy between 82.6% and 72.1%.

Potassium hydroxide as a 10% solution used for 24 h at 60 °C, has a removal efficiency ranging from 99.6% to 99.8%, and it seems not to affect the integrity of all tested plastics (ca. 3 mm diameter) except for cellulose acetate (CA) [101].

The methods proposed by the NOAA suggest digestion of organic material with 30% hydrogen peroxide (H_2O_2) in the presence of 0.05 M Fe(II) solution (Fenton's reaction). Hydrogen peroxide can increase the polymer transparency and make it thinner; differently, at different concentrations, the hydrogen peroxide is less effective on removing the organic substance [16]. Nevertheless, hydrogen peroxide is still the most popular oxidant agent used for sample chemical degradation.

The enzymatic degradation, to remove interfering proteinaceous material from the surface of MPs and degrade durable cellulose fibres, requires the incubation of MPs with a mixture of several digestive enzymes, such as lipase, amylase, proteinase, chitinase, cellulase, corolase, trypsin, papain, and collagenase [15,96,97,102,103]. The first attempt of enzymatic purification of the samples was conducted by [96], which used the proteolytic enzyme (proteinase-K), gaining a degradation beyond the 97% (in weight) of the materials present in seawater samples. Subsequently, in 2017, ref. [97] developed an optimised universal enzymatic purification protocol (UEPP) appropriate for plankton, biota, and sediment samples.

In contrast to the chemical digestion, the use of enzymes does not affect the polymer's structure and has an excellent removal efficiency of the natural organic fraction, but it takes more time and higher costs [15,16,97], especially for samples that contain a considerable amount of organic matter to digest and thus require significant quantities of enzymes [104].

Alternatively, to process high complex matrices (i.e., river samples, soil samples, biota), a viable option is to digest samples adopting a sequential combination of both chemical and enzymatic protocols.

The overall recommendation is the use of a digestion protocol tested on real samples each time before application and before decision considering digestion efficiency, polymer resistance, the dangerousness of reagents, and costs (Table 2).

Spike recoveries and blanks can be used to capture the entire processing procedure and are specific to the workflow for different sample types [105,106].

Table 2. Comparison of different reagents used for digestion of organic fraction necessary for MPs extraction from complex environmental matrices.

Reagents	Method	Costs	Hazard	Pros	Cons	References
HNO_3	20 mL of HNO_3 (22.5 M), 2 h heating (~100 °C), hot filtration (~80 °C)	\$38.00 for 1 L	Oxidiser, corrosive	Efficient in organic digestion	Possible degradation of PS, PA, and PE, makes the plastic yellow	[16,95,98,99]
HCl	4 mL of HCl at 20%	\$36.00 for 1 L	Corrosive, acute toxicity	Efficient in organic digestion (82.6%) of complex matrices (clams)	Degradation of polymers	[96,100,104]
NaOH	20 mL of NaOH (10 M) at 60 °C for 24 h	\$62.63 for 1 kg	Corrosive	Digestion efficiency up to 90%, stimulated by the rise of molarity and temperature	Degradation of PET and PVC	[16,96,104,107]
KOH	20 mL of KOH (1 M) at 18–21 °C for two days	\$85.72 for 1 kg	Corrosive, irritant	Good organic digestion efficiency	Requires lots of time, degradation of some polymers as cellulose acetate	[16,96,99,101]

					and some biodegradable plastics	
H ₂ O ₂	20 mL H ₂ O ₂ at 30% plus 20 mL of FeSO ₄ * 7H ₂ O (0.05 M) at 70 °C in stirring	\$27.67 for 1 L	Corrosive, harmful	Efficient in organic digestion	At high concentrations could degrade the polymers	[10,16,104,108]
Cellulase, lipase, chitinase, protease, proteinase-K	5 mL of Protease A-01 + 25 mL of Tris-HCl buffer, 1 mL of Lipase FE-01 + 25 mL of Tris-HCl buffer; 5 mL of Amylase TXL + 25 mL of NaOAc buffer, 1 mL of Cellulase TXL + 25 mL of NaOAc buffer; 1 mL of Chitinase + 25 mL of NaOAc buffer	Protease A-01 1 kg \$48.34; Lipase FE-01 1 kg \$48.34; Amylase TXL 1 Kg \$36.50; Cellulase TXL 1 kg \$43.95	No danger	Good inorganic and biological material digestion; does not affect the polymers	Expensive, requires lots of time	[15,16,96,97]

8.4. Density Separation and Filtration

MPs' density may vary according to the type of polymer and the manufacturing process ranging from 0.01 to 2.30 g cm⁻³ (Table 3) [109]. These values usually are lower than most of the minerals from the sediment, although organic matter and phyllosilicate minerals (e.g., clay minerals and micas) can be seen floating aside particles.

The density separation process is especially required for sediments to discriminate the microplastics from the rest of the sample.

A salinity-based density separation using a saturated solution of salt promotes the floatation of lower density microplastic particles on the supernatant of the solution and separation of higher density particles and inorganic material solids. In general, glass separating funnels are used for this process.

Differences among salts are due to different ability to separate and consequently extract MPs based on their density as well as on their costs and toxicity.

A list comprising commonly used reagents for density separation is presented in Table 4.

Sodium chloride (NaCl—density: 1.2 g cm⁻³) and sodium tungstate dihydrate (Na₂WO₄·2H₂O—density: 1.4 g cm⁻³), among salts, are both cost-effective and nontoxic. However, they do not allow the density separation of heavier polymers such as polycarbonate (PC), polyurethane (PU), polyethylene terephthalate (PET), polyvinyl chloride (PVC), or polytetrafluoroethylene (PTFE) [109]. Sodium iodide (NaI), even though is a high-density salt that allows the separation of most polymers, is quite expensive and it was used in low concentrations in support of a NaCl solution by [104]. The use of zinc chloride, despite being considered the most effective and least expensive method, is a highly dangerous and corrosive substance. Consequently, careful handling, disposal, and recycling of this reagent are required [109].

Moreover, riverine samples contain high loads of complex biological matrices that require a large volume of the oxidative or acid solution for digestion. Consequently, vast amounts of salts will also be necessary to obtain saturated heavy liquid solutions to suspend large sample volumes for density separation. Furthermore, it should be considered that salts like ZnCl₂, NaI, and ZnBr₂, even if allowing for the density separation of heavier polymers, are highly soluble in water, and therefore larger quantities are required respect to NaCl, Na₂WO₄·2H₂O, or NaBr (Table 4).

As the last step after the sample clean-up process, there is the filtration of nitrocellulose, silicon, polycarbonate, anodisc, or glass fibre filters. The filtration process needs to match the requirements of the following step of analysis (chemical characterisation) and the initial purpose of the research and could set the minimum size of MPs in the study.

In Figures 6 and 7, respectively, a workflow for MP analysis in water samples and the processing steps for sediments samples are represented.

Table 3. The most common types of polymers and their density.

Abbreviation	Polymer	Density (g/cm ³)
PS	Polystyrene	0.01–1.06
PP	Polypropylene	0.85–0.92
LDPE	Low-density polyethylene	0.89–0.93
HDPE	High-density polyethylene	0.94–0.98
Freshwater		1.00
Seawater		1.025
PA, PA 6,6	Polyamide, Nylon 6,6	1.12–1.15
PC	Polycarbonate	1.20–1.22
PU	Polyurethane	1.20–1.26
PET	Polyethylene terephthalate	1.38–1.41
PVC	Polyvinyl chloride	1.38–1.41
PTFE	Polytetrafluoroethylene	2.10–2.30

Table 4. Chemical reagents used for density separation, with their relative density, water solubility, toxicity, and costs.

Chemical Formula	Reagent Name	Water Solubility (g/L)	Density (g/cm ³)	Toxicity	Costs	References
NaCl	Sodium chloride	358 at 20 °C	1.0–1.2	Low	\$34.64 for 1 kg	[104,109–113]
Na ₂ WO ₄ ·2H ₂ O	Sodium tungstate dihydrate	742 at 25 °C	1.40	Low	\$224.92 for 500 g	[109,112]
NaBr	Sodium bromide	905 at 20 °C	1.37–1.40	Low	\$118.51 for 1 kg	[23,77,109]
3Na ₂ WO ₄ ·9WO ₃ ·H ₂ O	Sodium polytungstate	3100 at 20 °C	1.40	Low	\$235.27 for 100 g	[109,113]
ZnCl ₂	Zinc chloride	4320 at 20 °C	1.6–1.8	High	\$143.34 for 1 kg	[16,104,108,109,114]
ZnBr ₂	Zinc bromide	4470 at 20 °C	1.71	High	\$166 for 500 g	[23,77,109]
NaI	Sodium iodide	1793 at 20 °C	1.80	High	\$159.46 for 500 g	[16,109,114,115]

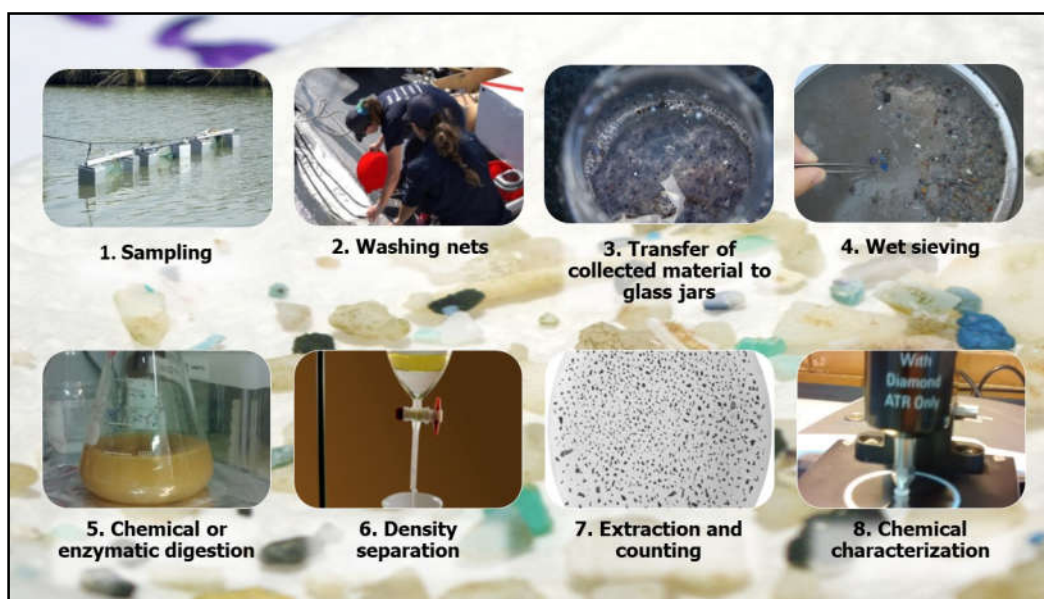


Figure 6. Workflow of MP analysis in water samples.

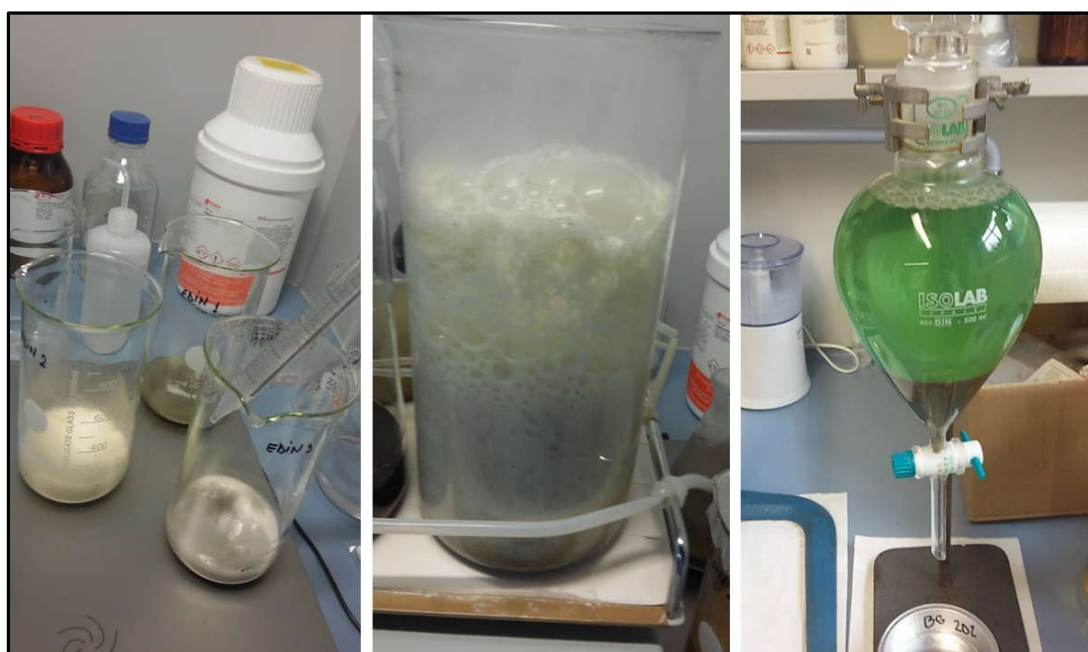


Figure 7. Digestion (left and centre image) and density separation (right image) processes performed on sediment samples (fluvial sand).

9. Quantification and Identification

After extraction and isolation of particles, what follows is the accurate quantification of MPs by counting them in order to express results as particle number, providing at the same time the evaluation of their size, colour, and shape distribution. Not all particles are visible to the naked eye; therefore, the use of a stereomicroscope is necessary for a preliminary visual sorting. This process requires considerable time and resources in terms of researchers involved in counting hundreds of particles per sample and a high risk of data overestimation for false positives [10,11,67,116,117]. To this purpose, recently, researchers tried to develop automatic image analysis approaches [118–121] for time-efficient, accurate and harmonised data analysis.

Free image analysis software (e.g., MPhunter/siMPle) [122,123] and computer vision-based systems (e.g., SMACC) [118,120] can be used, respectively, on the one hand for the systematic chemical identification of MPs, and on the other hand, for the automatic counting and classification of MPs in the environment, reducing the data calculation time and the human bias during manual data analysis.

Due to a large variety of polymers (more than 5000) [124], characterisation of their chemical composition is of primary importance to ensure the accuracy of collected pollution data and to determine their source and impact in the environment [10,125].

No technique can chemically identify MPs, but a broad range of complementary analytical methodologies have been applied regarding the detection and identification of MPs.

Depending on the goal and research question, mass-based or particle-based methods can be used. The first includes thermoanalytical techniques (pyrolysis–gas chromatography–mass spectrometry, Py–GC–MS, and thermal extraction desorption–gas chromatography–mass spectrometry, TED–GC–MS), the second includes spectroscopic ones (Raman and FTIR) [126].

The spectroscopic methods (FTIR and Raman) are the most common in the chemical identification of MPs. These techniques are nondestructive, and therefore, after sample acquisition it is possible, for example, to further process the bigger particles with other techniques (e.g., Py–GC–MS) to obtain additional and complementary information on the composition of plastic polymers [124]. Moreover, a minimal amount of sample is required, given that these methodologies are able to identify different types of polymers at a resolution from about 10 μm (FTIR) to 0.5 μm (Raman) by comparing the IR spectrum of an unknown plastic sample with spectra of known polymers provided by matching them to spectral libraries through database comparison algorithms [10,127]. FTIR and Raman spectroscopy identifies MP particles via their vibrational spectrum, which is unique for every polymer type. Coupling the spectrometer (FTIR or Raman) to a microscope, small particles are measurable (100 μm –1 μm) through the “micro”-spectroscopy (μ -FTIR and μ -Raman) [10].

The various types of instruments available on the market differ mainly by the type of microscope coupled to the spectrometer and the type of particles acquisition, being manual or automated.

A manual sample placement means there is a single-point acquisition of particles that have to be picked and positioned singularly.

More expensive instruments have the possibility of running in fully automated modes to measure multiple particles in a sample, and also to map or generate spatial chemical images of whole membrane filters through the motorised movement of the sample table of the microscope.

The main disadvantages of these methods are an extended processing time to map an entire filter, the measurement of irregularly shaped microplastic particles present in environmental samples resulting in refractive errors, the lack of information about associated organic additives to MPs, and the overlap of polymer bands given by organic and inorganic contaminations that can disturb identification of particles [10,13,128].

Thermoanalytical methods (Py–GC–MS and TED–GC–MS), less conventional than spectroscopic alternatives, are destructive techniques that produce, by pyrolysis, decomposition products characteristic of each polymer, trapped on a solid-phase adsorbent and thermally desorbed in a subsequent step (in the case of TED). The volatile marker compounds are then separated by gas chromatography and identified by mass spectrometry [129,130].

One of the main advantages of these techniques is the possibility of characterising the polymer itself and other compounds present in samples, such as additives or unique compounds that may be used for source profiling and toxicity calculations during the same analysis [131,132]. Contrarily to Raman or FTIR (in reflection and ATR mode), which only investigate the surface of a particle, Py–GC–MS allows the analysis of the whole particle [124,133].

Moreover, a concentration can be provided in $\mu\text{g/L}$ necessary for MPs' mass determination.

Among the disadvantages of Py–GC–MS, there is the inability to use the particles after analysis for further investigations, the long acquisition times compared to spectroscopy and limits in the selection of the particle size class ($\sim 50/100 \mu\text{m}$) [10,134].

In the following Table, a resume of the advantages and disadvantages of each technique is presented (Table 5).

Table 5. Methodologies used for the characterisation of MPs: advantages and disadvantages for each technique are described.

Methodology	Advantages	Disadvantages	Lower Size Limit	References
Fourier transform infrared coupled to microscopy (μ -FTIR)	-Easy to use -Many particles can be analysed simultaneously -Automatisation available -Short time of analysis for single particles -Evaluation of size and shape -Detecting the intensity of oxidation -Report particles with shape and size information -Transmission and reflection mode -Nondestructive -Less expensive than Raman and thermoanalytical techniques	-Difficulty in characterising black particles -Long time of analysis to measure multiple particles -Measures huge areas without particles -Detectors have to be cooled with liquid nitrogen -The analysis requires expert personnel -Huge data sets (several GB per filter) -No total mass determination -Expensive	~10–20 μ m	[10,123,126,127,134–137]
Raman spectroscopy	-Evaluation of size and shape -Many particles can be analysed simultaneously -Automatisation available -Detecting the intensity of oxidation -Staining possible -Thermoelectrically cooled (TEC) detectors obviate the necessity for liquid nitrogen cooling -Report particles with shape and size information -Nondestructive -Filter contributions can be subtracted out -It is possible to detect additives, pigments, and plasticisers	-More time-consuming measurements with respect to FTIR-spectroscopy -The analysis requires expert personnel -Interference of biological and inorganic contaminants -No total mass determination -Expensive	~1 μ m	[99,122,126,127,134,138]
Pyrolysis–Gas Chromatography–Mass spectrometry (Py–GC–MS)	-More holistic approach to characterise, in a single analysis, additives and plasticiser, in addition to polymer category -Powerful for mass determination	-No particle number information -No evaluation of size and shape -Particles can be analysed singularly -About 40 min of analysis for each particle determination -The analysis requires expert personnel -Destructive -Expensive	~50/100 μ m	[35,122,130,131,139–143]
Thermal Extraction Desorption–Gas	-More holistic approach to characterise, in a single	-No particle number information	~50/100 μ m	[123,139,142,144,145]

Chromatography–Mass Spectrometry (TED–GC–MS)	analysis, additives and plasticiser, in addition to polymer category -Powerful for mass determination	-No evaluation of size and shape -Particles can be analysed singularly -About 40 min of analysis for each particle determination -The analysis requires expert personnel -Destructive -Expensive
--	--	---

10. Quality Assurance and Quality Control of Analysis (QA/QC)

Contamination of samples is a risk to be seriously considered in all steps of MPs studies, and the quality of MPs research has been recently investigated [146–150]. Extensive precautions and a quality assurance perspective to mitigate contamination and validate the data are essential [116,135]. However, the use of blanks and blank correction is still not common in MPs studies; ref. [148] reported that 32 out of 50 studies did not run full procedural blanks. The lack of use of strict quality assurance criteria can often translate into misleading results [116,150].

Firstly, all the techniques performed in sampling and analysis should be done using non-plastic objects, as much as possible, considering the high degree of contamination rate. Therefore, metal as inox steel, aluminium, and glass recipients are considered a viable option for sediment sampling. In the same manner, metal utensils are recommended to use both in the fieldwork and during laboratory processes. To quantify any losses of MPs that may occur during sampling and processing of samples, positive controls are highly recommended. Experiments to verify a sufficiently high recovery of particles in all procedural steps of sampling and analyses, using targeted MPs of different size classes and polymer types should be performed [20,150,151].

Secondly, to identify and correct for potential contamination occurred during the preparation process and ensure thus accuracy of data, the use of daily replicated blank samples in all stages of the analysis is required, e.g., procedural blanks [96,150].

Adding the same reagents and amounts in the blank sample as in every ordinary sample can serve to provide knowledge about the contamination degree inside the laboratory and due to the reagents used.

Highly recommended precautions include rinsing work surfaces and glassware with Milli-Q and inspecting all apparatus before the analysis; processing the samples in a laminar flow cabinet; covering all materials used during the study with aluminium foils, washing them thoroughly between trials; washing the filters copiously before use; and analysing blank samples of Milli-Q concurrently with samples.

Particular attention should be made to microfibers contamination. Microfibres are ubiquitous (indoor and outdoor environments and environmental matrices) due to the presence of textile fibres in the environment that are most likely made from synthetic polymers, such as acrylic, rayon, polyester, and nylon, which have a high probability of ending up in collected samples, therefore making them potential sources of contamination [152,153].

To avoid fibre contamination, [153] developed strict quality control measures adapted from the field of forensic fibre examination that provided a reducing in fibres abundance of 90%. These control criteria require monitoring of the environment with control samples (air, water, and laboratory), minimising the contamination during processing of samples (e.g., wear natural fibre clothes and covering body and hair, limiting access to laboratory, cleaning deeply all surfaces, covering all vents with natural fibre cloths, ensuring that the processing laboratory does not open directly on to a corridor), and decreasing the contamination during sample collection (e.g., using items and preservation containers that are not plastic, using clean equipment, limiting air exposure of samples).

In order to reduce and avoid the airborne contamination, work in a microplastic-free laboratory as a cleanroom (ISO 7), with a particle filter air purger and compatible materials (e.g., glass, inox steel,

etc.), and employ plastic-free equipment and clothes, which can prevent such potential forms of contamination. Indeed, in this regard, [154] assessed aerial microfibres contamination of MPs samples during processing, demonstrating that standard QA/QC criteria like wearing only natural fibre clothes and using previously cleaned equipment and glassware are insufficient to ensure data quality. Authors highlighted that only suitable clean-air devices with particle filtration provide an effective method to reduce of 96.5% aerial microfibre contamination.

Other researchers [155] proposed using a further strategy based on hermetic enclosure devices (stereoscopic cover and glovebox) to reduce background contamination of microfibres during the analysis of biota samples.

11. Conclusions and Future Perspectives

The issue of pollution of ecosystems by plastic wastes has become a concern for the last fifty years. However, the, now evident, spread of macroplastic items into the environment represents only the tip of the iceberg of a growing and worrying environmental threat, invisible to the naked eye, which we now commonly call microplastics. Research in the field of microplastics has been growing a lot in recent years; nevertheless, the small dimensions of items in question involve several complexities regarding the sampling strategies adopted, the analytical methods for their quantification, and implications about their risks in the environment and for human health, which are not yet thoroughly understood.

Future studies in riverine environments should consider that conditions in rivers are often unfavourable due to high flow velocities, the turbulence of water, and an intense transport of suspended particulate and organic matter that imply a greater homogenisation of MPs in the water column, higher variability, and other complexities respect to marine ecosystems. Therefore, a sampling strategy providing a cross-section of the river, a multi-point collection of samples to consider the spatial and temporal distribution, and repetitiveness in the number of samples is highly recommended.

Improved techniques about the purification, quantification, and characterisation of MPs, for example, the use of more environmentally friendly and efficient reagents for sample digestion, are also significantly required. Considering the unavoidable increasing degradation of MPs in nanoplastic sizes, a consistently lower detection limit of available instruments of detection is also requested.

Finally, research and studies should also make a point to standardise and speed up the whole process of analysis of samples, focusing on the development of automatised systems using, for example, computer vision and machine learning approaches in order to avoid bias and harmonise data.

Author Contributions: Conceptualization: C.C.; writing—original draft preparation: C.C., I.S., I.P., and C.M.; providing case and idea: V.F.U., C.C., and C.M.; references collection: C.C., I.S., I.P., and C.M.; writing—review and editing: C.C., I.P., C.M., I.S., and C.M.; supervision: C.C.; funding acquisition: C.M., V.F.U., and C.C.

Funding: This work was supported by the project “MICROPLASMA- Micro and maCRO PLAStic pollution. Monitoring with Advanced technologies” POR Puglia FESR-ESF 2014–2020 INNOLABS “—Code P0XST06.

Conflicts of Interest: The authors declare no conflict 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.
2. Frias, J.P.; Nash, R. Microplastics: Finding a consensus on the definition. *Mar. Pollut. Bull.* **2019**, *138*, 145–147, doi:10.1016/j.marpolbul.2018.11.022.
3. Klein, S.; Dimzon, I.K.; Eubeler, J.; Knepper, T.P. Analysis, occurrence, and degradation of microplastics in the aqueous environment. In *Freshwater Microplastics: Emerging Environmental Contaminants?* Wagner, M., Lambert, S., Eds.; Springer: Berlin/Heidelberg, Germany, 2017; doi:10.1007/978-3-319-61615-5_3.

4. Lambert, S.; Wagner, M. The handbook of environmental chemistry. In *Freshwater microplastics. Emerging Environmental Contaminants?* Springer: Cham, Switzerland, 2018.
5. Lechner, A.; Keckeis, H.; Lumesberger-Loisl, F.; Zens, B.; Krusch, R.; Tritthart, M.; Glas, M.; Schludermann, E. The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environ. Pollut.* **2014**, *188*, 177–181, doi:10.1016/j.envpol.2014.02.006.
6. Van der Wal, M.; Van der Meulen, M.; Tweehuysen, G.; Peterlin, M.; Palatinus, A.; Virsek, M.K.; Coscia, L.; Krsan, A. SFRA0025: Identification and Assessment of Riverine Input of (Marine) Litter: Final Report for the European Commission DG Environment under Framework Contract No ENV.D2/FRA/2012/0025. 2015, Volume 25, p. 208. Available online: <http://ec.europa.eu/environment/marine/good-environmental-status/descriptor-10/pdf/iasFinal%20Report.pdf> (accessed on 10th July 2020).
7. Lebreton, L.C.M.; van der Zwet, J.; Damsteeg, J.-W.; Slat, B.; Andrady, A.; Reisser, J. River plastic emissions to the world's oceans. *Nat. Commun.* **2017**, *8*, 15611, doi:10.1038/ncomms15611.
8. Siegfried, M.; Koelmans, A.A.; Besseling, E.; Kroeze, C. Export of microplastics from land to sea. A modelling approach. *Water Res.* **2017**, *127*, 249–257, doi:10.1016/j.watres.2017.10.011.
9. Mani, T.; Burkhardt-Holm, P. Seasonal microplastics variation in nival and pluvial stretches of the Rhine River—From the Swiss catchment towards the North Sea. *Sci. Total Environ.* **2020**, *707*, 135579, doi:10.1016/j.scitotenv.2019.135579.
10. 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, doi:10.1016/j.envpol.2019.113284.
11. 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.
12. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. MPs as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* **2011**, *62*, 2588–2597.
13. Löder, M.G.J.; Gerdt, G. Methodology used for the detection and identification of MPs a critical appraisal. In *Marine Anthropogenic Litter*; Bergmann, M., Gutow, L., Klages, M., Eds.; Springer: Berlin/Heidelberg, Germany, 2015; p. 201, doi:10.1007/978-3-319-16510-3_8.
14. 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.
15. Li, J.; Liu, H.; Paul Chen, J. Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for MPs detection. *Water Res.* **2018**, *137*, 362–374.
16. Stock, F.; Kochleus, C.; Bansch-Baltruschat, B.; Brennholt, N.; Reifferscheid, G. Sampling techniques and preparation methods for microplastic analyses in the aquatic environment—A review. *TrAC Trend Anal. Chem.* **2019**, *113*, 84–92, doi:10.1016/j.trac.2019.01.014.
17. González-Fernández, D.; Hanke, G.; Tweehuysen, G.; Bellert, B.; Holzhauer, M.; Palatinus, A.; Hohenblum, P.; Oosterbaan, L. *Riverine Litter Monitoring-Options and Recommendations*; MSFD GES TG Marine Litter Thematic Report; JRC Technical Report; Joint Research Centre (JRC): Luxembourg 2016; EUR 28307.
18. Van Cauwenberghe, L.L.; Devriese, L.; Galgani, F.; Robbens, J.; Janssen, C.R. MPs in sediments: A review of techniques, occurrence and effects. *Mar. Environ. Res.* **2015**, *111*, 5–17.
19. Wright, S.L.; Thompson, R.C.; Galloway, T.S. The physical impacts of MPs on marine organisms: A review. *Environ. Pollut.* **2013**, *178*, 483–492.
20. Hermesen, 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.
21. González-Fernández, D.; Hanke, G.; Marin, J.V.; Cabañas, A.C. *Modelling Floating Macro Litter Loads from Rivers to the Marine Environment Based on Visual Observations*; European Geosciences Union General Assembly: Vienna, Austria, 2019.
22. Horton, A.; Walton, A.; Spurgeon, D.; 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*, doi:10.1016/j.scitotenv.2017.01.190.
23. 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, doi:10.1016/j.trac.2018.10.029.

24. Chubarenko, I.; Bagaev, A.; Zobkov, M.; Esiukova, E. On some physical and dynamical properties of microplastic particles in marine environment. *Mar. Pollut. Bull.* **2016**, *108*, 105–112.
25. Kukulka, T.; Proskurowski, G.; Mor  t-Ferguson, S.; Meyer, D.W.; Lawet, K.L. The effect of wind mixing on the vertical distribution of buoyant plastic debris. *Geophys. Res. Lett.* **2012**, *39*, doi:10.1029/2012GL051116.
26. Zhang, H. Transport of MPs in coastal seas. *Estuar. Coast. Shelf S.* **2017**, *199*, 74–86.
27. Rummel, C.D.; Jahnke, A.; Gorokhova, E.; K  hnel, D.; Schmitt-Jansen, M. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environ. Sci. Technol. Lett.* **2017**, *4*, 258–267.
28. Ogi, H.; Bab, N.; Ishihara, S.; Shibata, Y. Sampling of Plastic Pellets by Two Types of Neuston Net and Plastic Pollution in the Sea. *Bull. Fac. Fish. Hokkaido Univ.* **1999**, *50*, 77–91.
29. GESAMP. *Sources, Fate and Effects of Microplastics in the Marine Environment: Part Two of a Global Assessment*; Kershaw, P.J., Rochman, C.M., Eds.; Rep. Stud. GESAMP; IMO/FAO/ UNESCO- IOC/UNIDO/WMO/IAEA/UN/ UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection: London 2016; No. 93, 220p.
30. Set  l  , O.; Magnusson, K.; Lehtiniemi, M.; Nor  n, F. Distribution and abundance of surface water microlitter in the Baltic Sea: A comparison of two sampling methods. *Mar. Pollut. Bull.* **2016**, *110*, 177–183, doi:10.1016/j.marpolbul.2016.06.065.
31. Michida, Y.; Chavanich, S.; C  zar Caba  as, A.; Hagmann, P.; Hinata, H.; Isobe, A.; Kershaw, P.; Kozlovskii, N.; Li, D.; Lusher, A.L.; et al. *Guidelines for Harmonizing Ocean Surface Microplastic Monitoring Methods*; Ministry of the Environment Japan, 2019.
32. Basset, A.; Sangiorgio, F.; Sabetta, L. *Metodologie per la Determinazione Della Struttura Dimensionale di Fitoplancton e Macroinvertebrati Bentonici*; ISPRA-Istituto Superiore per la Protezione e la Ricerca Ambientale: Roma, Italy, 2009.
33. Tranter, D.J.; Smith, P.E. Filtration performance. In *Zooplankton Sampling*; Tranter, D.J., Fraser, J.H., Eds.; UNESCO Monographs on Oceanographic Methodology; UNESCO: Paris 1968; pp. 27–56.
34. Cutroneo, L.; Reboa, A.; Besio, G.; Borgogno, F.; Canesi, L.; Canuto, S.; Dara, D.; Enrile, F.; Forioso, I.; Greco, G.; et al. Microplastics in seawater: Sampling strategies, laboratory methodologies, and identification techniques applied to port environment. *Environ. Sci. Pollut. Res.* **2020**, *27*, 8938–8952, doi:10.1007/s11356-020-07783-8.
35. Crawford, C.B.; Quinn, B. Microplastic identification techniques. In *Microplastic Pollutants*, 1st ed.; Crawford, C.B., Quinn, B., Eds.; Elsevier Science: Oxford 2017; pp. 219–267.
36. Jacobs, F.; Grant, G.C. *Guidelines for Zooplankton Sampling in Quantitative Baseline and Monitoring Programs*; Special Scientific Report No. 83; Virginia Institute of Marine Science. US Environmental Protection Agency: Corvallis, OR, USA.
37. UNESCO. *Zooplankton Sampling. Monographs on Oceanographic Methodology*; UNESCO: Paris, France, 1979; ISBN 92-3-101194-4.
38. 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.
39. Arthur, C.; Baker, J.; Bamford, H. *Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Micro-Plastic Marine Debris*; NOAA Technical Memorandum NOS-OR&R-30; NOAA: Silver Spring USA, 2008; 49 p.
40. Ryan, P.G.; Suaria, G.; Perold, V.; Bornman, T.G.; Aliani, S. Sampling microfibr  s at the sea surface: The effects of mesh size and water depth. *Environ. Pollut.* **2020**, *258*, 113413.
41. Dris, R.; Gasperi, J.; Rocher, V.; Saad, M.; Renault, N.; Tassin, B. Microplastic contamination in an urban area: A case study in Greater Paris. *Environ. Chem.* **2015**, *12*, 592–599, doi:10.1071/EN14167.
42. Liedermann, M.; Gmeiner, P.; Pessenlehner, S.; Haimann, M.; Hohenblum, P.; Habersack, H. A methodology for measuring microplastic transport in large or medium rivers. *Water* **2018**, *10*, 414; doi:10.3390/w10040414.
43. 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* (Part A), 112970.
44. Zhang, L.; Liu, J.; Xie, Y.; Zhong, S.; Yang, B.; Lu, D.; Zhong, Q. Distribution of microplastics in surface water and sediments of Qin river in Beibu Gulf, China. *Sci. Total Environ.* **2020**, *708*, 135176, doi:10.1016/j.scitotenv.2019.135176.

45. Lenz, R.; Labrenz, M. Small microplastic sampling in water: Development of an encapsulated filtration device. *Water* **2018**, *10*, 1055, doi:10.3390/w10081055.
46. 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, doi:10.1016/j.marpolbul.2015.04.054.
47. Guven, O.; Gokdag, K.; Jovanovic, B.; Kideys, A.E. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. *Environ. Pollut.* **2017**, *223*, 286–294.
48. Anderson, P.J.; Warrack, S.; Langen, V.; Challis, J.K.; Hanson, M.L.; Rennie, M.D. Microplastic contamination in lake winnipeg, Canada. *Environ. Pollut.* **2017**, *225*, 223–231.
49. Kooi, M.; Reisser, J.; Slat, B.; Ferrari, F.F.; Schmid, M.S.; Cunsolo, S.; Brambini, R.; Noble, K.; Sirks, L.-A.; Linders, T.E.W.; et al. The effect of particle properties on the depth profile of buoyant plastics in the ocean. *Sci. Rep.* **2016**, *6*, 33882.
50. 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.
51. Karlsson, T.M.; Kärrman, A.; Rotander, A.; Hassellöv, M. Comparison between manta trawl and in situ pump filtration methods, and guidance for visual identification of microplastics in surface waters. *Environ. Sci. Pollut. Res.* **2020**, *27*, 5559–5571, doi:10.1007/s11356-019-07274-5.
52. Collignon, A.; Hecq, J.H.; Glagani, F.; Voisin, P.; Collard, F.; Goffart, A. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. *Mar. Pollut. Bull.* **2012**, *64*, 861–864.
53. McCormick, A.R.; Hoellein, T.J.; London, M.G.; Hittie, J.; Scott, J.W.; Kelly, J.J. Microplastic in surface waters of urban rivers: Concentration, sources, and associated bacterial assemblages. *Ecosphere* **2016**, *7*, e01556.
54. Barrows, A.; Neumann, C.; Berger, M.; Shaw, S. Grab vs. neuston tow net: A microplastic sampling performance comparison and possible advances in the field. *Anal. Methods* **2017**, *9*, 1446.
55. Syakti, A.; Hidayati, N.; Jaya, Y.; Yude, R.; Suhendy, S.; Asia, L.; Wong-Wah-Chung, P.; Doumenq, P. Simultaneous grading of microplastic size sampling in the Small Islands of Bintan water, Indonesia. *Mar. Pollut. Bull.* **2018**, *137*, 593–600.
56. Amélineau, F.; Bonnet, D.; Heitz, O.; Mortreux, V.; Harding, A.M.A.; Karnovsky, N.; Walkusz, W.; Fort, J.; Grémillet, D. Microplastic pollution in the Greenland Sea: Background levels and selective contamination of planktivorous diving seabirds. *Environ. Pollut.* **2016**, *219*, 1131–1139.
57. Tsang, Y.Y.; Mak, C.W.; Liebich, C.; Lam, S.W.; Sze, E.T.; Chan, K.M. Microplastic pollution in the marine waters and sediments of Hong Kong. *Mar. Pollut. Bull.* **2017**, *115*, 20–28.
58. Dris, R.; Gasperi, J.; Tassin, B. Sources and fate of microplastics in urban areas: A focus on Paris megacity. In *Freshwater Microplastics. The Handbook of Environmental Chemistry*; Wagner, M., Lambert, S., Eds.; Springer, Cham, Switzerland, 2018; Volume 58, doi:10.1007/978-3-319-61615-5_4.
59. Campanale, C.; De Palma, C.P.; Bollino, B.; Massarelli, C.; Uricchio, V.F. Innovativo sistema di campionamento automatizzato per il monitoraggio di microplastiche in ambienti fluviali. In Proceedings of the Proceedings of RemTech Expo–Hub tecnologica Campania, Napoli, Italy, 26–28 March 2020.
60. Ng, K.L.; Obbard, J.P. Prevalence of microplastics in Singapore’s coastal marine environment. *Mar. Pollut. Bull.* **2006**, *52*, 761–767.
61. Bagaev, A.; Khatmullina, L.; Chubarenko, I. Anthropogenic microlitter in the Baltic Sea water column. *Mar. Pollut. Bull.* **2018**, *129*, 918–923, doi:10.1016/j.marpolbul.2017.10.049.
62. Dai, Z.; Zhang, H.; Zhou, Q.; Tian, Y.; Chen, T.; Tu, C.; Fu, C.; Luo, Y. Occurrence of microplastics in the water column and sediment in an inland sea affected by intensive anthropogenic activities. *Environ. Pollut.* **2018**, *242*, 1557–1565, doi:10.1016/j.envpol.2018.07.131.
63. Kanhai, L.D.K.; Gårdfeldt, K.; Lyashevskaya, O.; Hassellöv, M.; Thompson, R.C.; O’Connor, I. Microplastics in sub-surface waters of the Arctic Central Basin. *Mar. Pollut. Bull.* **2018**, *130*, 8–18, doi:10.1016/j.marpolbul.2018.03.011.
64. 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, doi:10.1016/j.marpolbul.2018.01.066.
65. Covernton, G.A.; Pearce, C.M.; Gurney-Smith, H.J.; Chastain, S.G.; Ross, P.S.; Dower, J.F.; Dudas, S.E. Size and shape matter: A preliminary analysis of microplastic sampling technique in seawater studies with

- implications for ecological risk assessment. *Sci. Total. Environ.* **2019**, *667*, 124–132, doi:10.1016/j.scitotenv.2019.02.346.
66. Courteney-Jones, W.; Quinn, B.; Gary, S.F.; Mogg, A.O.; Narayanaswamy, B.E. Microplastic pollution identified in deep-sea water and ingested by benthic invertebrates in the Rockall Trough, North Atlantic Ocean. *Environ. Pollut.* **2017**, *231*, 271–280.
 67. Song, Y.K.; Hong, S.H.; Jang, M.; Han, G.M.; Rani, M.; Lee, J.; Shim, W.J. A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples. *Mar. Pollut. Bull.* **2015**, *93*, 202–209, doi:10.1016/j.marpolbul.2015.01.015.
 68. Van Cauwenberghe, L.; Vanreusel, A.; Mees, J.; Janssen, C.R. Microplastic pollution in deep-sea sediments. *Environ. Pollut.* **2013**, *182*, 495–499, doi:10.1016/j.envpol.2013.08.013.
 69. Fazey, F.M.; Ryan, P.G. Biofouling on buoyant marine plastics: An experimental study into the effect of size on surface longevity. *Environ. Pollut.* **2016**, *210*, 354–360, doi:10.1016/j.envpol.2016.01.026.
 70. Bagaev, A.; Mizyuk, A.; Khatmullina, L.; Isachenko, I.; Chubarenko, I. Anthropogenic fibres in the Baltic Sea water column: Field data, laboratory and numerical testing of their motion. *Sci. Total Environ.* **2017**, *599–600*, 560–571, doi:10.1016/j.scitotenv.2017.04.185.
 71. Moore, C.J.; Lattin, G.L.; Zellers, A.F. Quantity and type of plastic debris flowing from two urban rivers to coastal waters and beaches of Southern California. *Revista da Gestão Costeira Integrada. J. Integr. Coast. Zone Manag.* **2011**, *11*, 65–73.
 72. Gago, J.; Filgueiras, A.; Pedrotti, M.L.; Caetano, M.; Frias, J. *Standardised Protocol for Monitoring Microplastics in Seawater*; Deliverable 4.1. JPI-Oceans BASEMAN Project; JPI-Oceans: Brussels 2018; 33 p, doi: 10.13140/RG.2.2.36256.89601/1.
 73. Gregory, M.R. Plastic pellets on New Zealand beaches. *Mar. Pollut. Bull.* **1977**, *8*, 82–84.
 74. Nizetto, L.; Bussin, 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. Impact* **2016**, *18*, 1050–1059.
 75. 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.
 76. Stolte, A.; Forster, S.; Gerdt, G.; Schubert, H. Microplastic concentrations in beach sediments along the German Baltic coast. *Mar. Pollut. Bull.* **2015**, *99*, 216–229.
 77. Quinn, B.; Murphy, F.; Ewins, C. Validation of density separation for the rapid recovery of microplastics from sediment. *Anal. Methods* **2017**, *9*, 1491–1498, doi:10.1039/c6ay02542k.
 78. Miller, M.E.; Kroon, F.J.; Motti, C.A. Recovering microplastics from marine samples: A review of current practices. *Mar. Pollut. Bull.* **2017**, *123*, 6–18.
 79. 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.
 80. 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*, 867–868.
 81. Faure, F.; Demars, C.; Wieser, O.; Kunz, M.; Alencastro, L.F. Plastic pollution in Swiss surface waters: Nature and concentrations, interaction with pollutants. *Environ. Chem.* **2015**, *12*, 582, doi:10.1071/EN14218.
 82. Hidalgo-Ruz, V.; Thiel, M. Distribution and abundance of small plastic debris on beaches in the SE Pacific (Chile): A study supported by a citizen science project. *Mar. Environ. Res.* **2013**, *87–88*, 12–18.
 83. Naidoo, T.; Glassom, D.; Smit, A.J. Plastic pollution in five urban estuaries of KwaZulu-Natal, South Africa. *Mar. Pollut. Bull.* **2015**, *101*, 473–480.
 84. 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.
 85. Castañeda, R.A.; Avlijas, S.; Simard, M.A.; Ricciardi, A. Microplastic pollution in St. Lawrence River sediments. *Can. J. Fish. Aquat. Sci.* **2014**, *70*, 1767–1771, doi:10.1139/cjfas-2014-0281.
 86. Zobkov, M.B.; Esiukova, E.E. Microplastics in a marine environment: Review of methods for sampling, processing, and analyzing microplastics in water, bottom sediments, and coastal deposits. *Oceanology* **2018**, *58*, 137–143.

87. US Environmental Protection Agency. *Sample Preservation, Methods for Chemical Analysis of Water and Wastes*, EPA-600/4-79-020; U.S. Environmental Protection Agency: Cincinnati, OH, USA, 1983; pp. xv–xx.
88. 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, doi:10.1021/es400663f.
89. Frias, J.P.; Otero, V.; Sobral, P. Evidence of microplastics in samples of zooplankton from Portuguese coastal waters. *Mar. Environ. Res.* **2014**, *95*, 89–95, doi:10.1016/j.marenvres.2014.01.001.
90. Ivar do Sul, J.A.; Costa, M.F. The present and future of microplastic pollution in the marine environment. *Environ. Pollut.* **1983**, *185*, 352–364, doi:10.1016/j.envpol.2013.10.036.
91. 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.
92. Sanchez-Vidal, A.; Thompson, R.C.; Canals, M.; de Haan, W.P. The imprint of microfibrils in southern European deep seas. *PLoS ONE* **2018**, *13*, e0207033, doi:10.1371/journal.pone.0207033.
93. 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.
94. Liebezeit, G.; Dubaish, F. MPs in beaches of the East Frisian Islands Spiekeroog and Kachelotplate. *Bull. Environ. Contam. Toxicol.* **2012**, *89*, 213–217.
95. 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.
96. 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*, 4528.
97. Löder, M.G.J.; 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, doi:10.1021/acs.est.7b03055.
98. Avio, C.G.; Gorbi, S.; Regoli, F. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. *Mar. Environ. Res.* **2015**, *111*, 18–26.
99. Dehaut, A.; Cassone, A.-L.; Frère, L.; Hermabessiere, L.; Himber, C.; Rinnert, E.; Rivière, G.; Lambert, C.; Soudant, P.; Huvet, A.; et al. Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environ. Pollut.* **2016**, *215*, 223–233.
100. Thielea, C.J.; Hudson, M.D.; Russellb, A.E. Evaluation of existing methods to extract microplastics from bivalve tissue: Adapted KOH digestion protocol improves filtration at single-digit pore size. *Mar. Poll. Bull.* **2019**, *142*, 384–393.
101. Kühn, S.; van Werven, B.; van Oyen, A.; Meijboom, A.; Rebolledo, E.L.B.; van Franeker, J.A. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. *Mar. Pollut. Bull.* **2017**, *115*, 86–90, doi:10.1016/j.marpolbul.2016.11.034.
102. Catarino, A.I.; Thompson, R.; Sanderson, W.; Henry, T.B. Development and optimization of a standard method for extraction of microplastics in mussels by enzyme digestion of soft tissues. *Environ. Toxicol. Chem.* **2017**, *36*, 947–951.
103. Courtene-Jones, W.; Quinn, B.; Murphy, F.; Garya, S.F.; Narayanaswamy, B.E. Optimisation of enzymatic digestion and validation of specimen preservation methods for the analysis of ingested microplastics. *Anal. Methods* **2017**, *9*, 1437.
104. 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.
105. Wenfeng, W.; Jun, W. Investigation of microplastics in aquatic environments: An overview of the methods used, from field sampling to laboratory analysis. *TrAC Trends Anal. Chem.* **2018**, *108*, doi:10.1016/j.trac.2018.08.026.
106. Hanvey, J.; Lewis, P.; Lavers, J.; Crosbie, N.; Posa, K.; Clarke, B. A review of analytical techniques for quantifying microplastics in sediments. *Anal. Methods* **2016**, *9*, doi:10.1039/C6AY02707E.
107. 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.
108. Masura, J.; Baker, J.; Foster, G.; Courtney, A. *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 USA, 2015.

109. Frias, J.P.; Nash, R.; Pagter, E.; O'Connor, I. *Standardised Protocol for Monitoring Microplastics in Sediments*; Technical Report; BASEMAN Project; JPI-Oceans: Brussels 2018; doi:10.13140/RG.2.2.36256.89601/1.
110. 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-Stakeholders Role against Pollution*; Stock, F., Reifferscheid, G., Brennholt, N., Kostianaia, E., Eds.; Springer: Switzerland 2019.
111. Sánchez-Nieva, J.; Perales, J.A.; Gonzales-Leal, J.M.; Rojo-Nieto, R. A new analytical technique for extraction and quantification of microplastics in marine sediments focused on the easy implementation and repeatability. *Anal. Meth.* **2017**, *9*, 6371–6378, doi:10.1039/C7AY01800B.
112. Pagter, E.; Frias, J.; Nash, R. Microplastics in Galway Bay: A comparison of sampling and separation methods. *Mar. Pollut. Bull.* **2018**, *135*, 932–940.
113. Corcoran, P.L.; Biesinger, M.C.; Grifi, M. Plastics and beaches: A degrading relationship. *Mar. Pollut. Bull.* **2009**, *58*, 80–84.
114. Coppock, R.L.; Cole, M.; Lindeque, P.K.; Queirós, A.M.; Galloway, T.S. A small-scale, portable method for extracting microplastics from marine sediments. *Environ. Pollut.* **2017**, *230*, 829–837.
115. Kedzierski, M.; Tilly, V.L.; Bourseau, P.; Bellegou, H.; César, G.; Sire, O.; Bruzaud, S. Microplastic elutriation system. Part A: Numerical modeling. *Mar. Pollut. Bull.* **2017**, *119*, 151–161.
116. 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.
117. Shim, W.J.; Hongab, S.H.; Eoab, S.E. Identification methods in microplastic analysis: A review. *Anal. Methods* **2017**, *9*, doi:10.1039/C6AY02558G.
118. Lorenzo-Navarro, J.; Castrillón-Santana, M.; Gómez, M.; Herrera, A.; Marín-Reyes, P.A. Automatic counting and classification of microplastic particles. In *Proceedings of the 7th International Conference on Pattern Recognition Applications and Methods*, Funchal, Portugal, 16th January 2018; pp. 646–652.
119. Chaczko, Z.; Wajs-Chaczko, P.; Tien, D.; Haidar, Y. Detection of microplastics using machine learning. In *Proceedings of the International Conference on Machine Learning and Cybernetics (ICMLC)*, Kobe, Japan, 7–10 July 2019; pp. 1–8.
120. Lorenzo-Navarro, J.; Castrillón-Santana, M.; Santesarti, E.; De Marsico, M.; Martínez, I.; Raymond, E.; Gómez, M.; Herrera, A. SMACC: A system for microplastics automatic counting and classification. *IEEE Access* **2020**, *8*, 25249–25261, ISSN 2169-3536-8.
121. Wegmayr, V.; Sahin, A.; Sæmundsson, B.; Buhmann, J.M. Instance segmentation for the quantification of microplastic fiber images. In *Proceedings of the Proceedings of WACV 2020*, 1–5 March, Aspen 2020; Available online: IEEE Xplore (accessed on 5th July 2020).
122. Primpke, S.; Dias, P.A.; Gerdts, G. Automated identification and quantification of microfibrils and MPs. *Anal. Methods* **2019**, *11*, 2138–2147.
123. Primpke, S.; Cross, R.K.; Mintenig, S.M.; Simon, M.; Vianello, A.; Gerdts, G.; Vollertsen, J. Toward the systematic identification of MPs in the environment: Evaluation of a new independent software tool (simple) for spectroscopic analysis. *Appl. Spectrosc.* **2020**, *74*, doi:10.1177/0003702820917760.
124. Hermabessiere, L.; Himber, C.; Boricaud, B.; Kazour, M.; Amara, R.; Cassone, A.-L.; Laurentie, M.; Paul-Pont, I.; Soudant, P.; Dehaut, A.; et al. Optimization, performance, and application of a pyrolysis-GC/MS method for the identification of microplastics. *Anal. Bioanal. Chem.* **2018**, *410*, 6663–6676, doi:10.1007/s00216-018-1279-0.
125. Dekiff, J.H.; Remy, D.; Klasmeier, J.; Fries, E. Occurrence and spatial distribution of microplastics in sediments from Norderney. *Environ. Pollut.* **2014**, *186*, 248–256.
126. Xu, J.L.; Thomas, K.V.; Luo, Z.; Gowen, A.A. FTIR and Raman imaging for microplastics analysis: State of the art, challenges and prospects. *Trac Trend Anal. Chem.* **2019**, *119*, 115629, doi:10.1016/j.trac.2019.115629.
127. Renner, G.; Schmidt, T.C.; Schram, J. Analytical methodologies for monitoring micro(nano)plastics: Which are fit for purpose? *Curr. Opin. Environ. Sci. Health* **2018**, *1*, 55–61.
128. Käßler, A.; Fischer, M.; Scholz-Böttcher, B.M.; Oberbeckmann, S.; Labrenz, M.; Fischer, D.; Eichhorn, K.J.; Voit, B. Comparison of μ -ATR-FTIR spectroscopy and py-GCMS as identification tools for microplastic particles and fibers isolated from river sediments. *Anal. Bioanal. Chem.* **2018**, *410*, 5313–5327.
129. Dümichen, E.; Braun, U.; Senz, R.; Fabian, G.; Sturm, H. Assessment of a new method for the analysis of decomposition gases of polymers by a combining thermogravimetric solid-phase extraction and thermal desorption gas chromatography mass spectrometry. *J. Chrom. A* **2014**, *1354*, 117–128, doi:10.1016/j.chroma.2014.05.057.

130. Hendrickson, E.; Minor, E.C.; Schreiner, K. Microplastic abundance and composition in western Lake Superior as determined via microscopy, Pyr-GC/MS, and FTIR. *Environ. Sci. Technol.* **2018**, *52*, 1787–1796.
131. 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. Proc. Improv.* **2013**, *15*, 1949–1956.
132. Peñalver, R.; Arroyo-Manzanares, N.; López-García, I.; Hernández-Córdoba, M. An overview of microplastics characterization by thermal analysis. *Chemosphere* **2020**, *242*, 125170, doi:10.1016/j.chemosphere.2019.125170.
133. Schymanski, D.; Goldbeck, C.; Humpf, H.U.; Fürst, P. Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles from 691 different packaging into mineral water. *Water Res.* **2018**, doi:10.1016/j.watres.2017.11.011.
134. Käßler, 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, doi:10.1007/s00216-016-9956-3.
135. Corami, F.; Rosso, B.; Bravo, B.; Gambaro, A.; Barbante, C. A novel method for purification, quantitative analysis and characterisation of microplastic fibers using Micro-FTIR. *Chemosphere* **2020**, *238*, 124564, doi:10.1016/j.chemosphere.2019.124564.
136. Mason, S.A.; Welch, V.G.; Neratko, J. Synthetic polymer contamination in bottled water. *Front Chem.* **2018**, *6*, 407.
137. Comnea-Stancu, I.R.; Wieland, K.; Ramer, G.; Schwaighofer, A.; Lendl, B. On the identification of rayon/viscose as a major fraction of microplastics in the marine environment: Discrimination between natural and manmade cellulosic fibers using Fourier transform infrared spectroscopy. *Appl Spectrosc.* **2017**, *7*, 939–950.
138. Anger, P.M.; von der Esch, E.; Baumann, T.; Elsner, M.; Niessner, R.; Ivleva, N.P. Raman microspectroscopy as a tool for microplastic particle analysis. *Trend Anal. Chem.* **2018**, *109*, 214–226, doi:10.1016/j.trac.2018.10.010.
139. Dümichen, E.; Barthel, A.-K.; Braun, U.; Bannick, C.G.; Brand, K.; Jekel, M.; Senz, R. Analysis of polyethylene microplastics in environmental samples, using a thermal decomposition method. *Water Res.* **2015**, *85*, 451–457.
140. Fischer, M.; Scholz-Bottcher, B.M. Simultaneous trace identification and quantification of common types of microplastics in environmental samples by pyrolysis-gas chromatography–mass spectrometry. *Environ. Sci. Technol.* **2017**, *51*, 5052–5060.
141. Funck, M.; Yildirim, A.; Nickel, C.; Schram, J.; Schmidt, T.C.; Tuerk, J. Identification of microplastics in wastewater after cascade filtration using Pyrolysis-GC–MS. *Methods X* **2020**, *7*, 100778, doi:10.1016/j.mex.2019.100778.
142. Becker, R.; Altmann, K.; Sommerfeld, T.; Braun, U. Quantification of microplastics in a freshwater suspended organic matter using different thermoanalytical methods—Outcome of an interlaboratory comparison. *J. Anal. Appl. Pyrol.* **2020**, 104829, doi:10.1016/j.jaap.2020.104829.
143. Steinmetz, Z.; Kintzi, A.; Muñoz, K.; Schaumann, G.E. A simple method for the selective quantification of polyethylene, polypropylene, and polystyrene plastic debris in soil by pyrolysis-gas chromatography/mass spectrometry. *J. Anal. Appl. Pyrol.* **2020**, *147*, 104803, doi:10.1016/j.jaap.2020.104803.
144. Dümichen, E.; Eisentraut, P.; Bannick, C.G.; Barthel, A.K.; Senz, R.; Braun, U. Fast identification of microplastics in complex environmental samples by a thermal degradation method. *Chemosphere* **2017**, *174*, 572–584.
145. Eisentraut, P.; Dümichen, E.; Ruhl, A.S.; Jekel, M.; Albrecht, M.; Gehde, M.; Braun, U. Two birds with one stoned fast and simultaneous analysis of microplastics: Microparticles derived from thermoplastics and tire wear. *Environ. Sci. Technol. Lett.* **2018**, *5*, 608–613.
146. Burton, G.A. *Microplastics in Aquatic Systems: An Assessment of Risk (Summary of Critical Issues and Recommended Path Forward)*; Water Environment & Reuse Foundation (WE&RF): Alexandria VA 2017.
147. Connors, K.A.; Dyer, S.D.; Belanger, S.E. Advancing the quality of environmental microplastic research. *Environ. Toxicol. Chem.* **2017**, *36*, 1697–1703.
148. Koelmans, A.A.; Bakir, A.; Burton, G.A.; Janssen, C.R. Microplastic as a vector for chemicals in the aquatic environment: Critical review and model-supported reinterpretation of empirical studies. *Environ. Sci. Technol.* **2016**, *50*, 3315–3326.

149. Mintenig, S.M.; Kooi, M.; Erich, M.; Redondo-Hasselerharm, P.E.; Dekker, S.C.; Koelmans, A.A.; van Wezel, A.P. A systems approach to understand microplastics measured in riverine surface waters and sediments in prep. *Water Res.* **2019**, *176*, 115723. <https://doi.org/10.1016/j.watres.2020.115723>
150. Koelmans, A.A.; Nor, N.H.M.; Hermesen, 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.
151. Estahbanati, S.; Fahrenfeld, N.L. Influence of wastewater treatment plant discharges on microplastic concentrations in surface water. *Chemosphere* **2016**, *162*, 277–284.
152. Wang, T.; Zou, X.; Li, B.; Yao, Y.; Zang, Z.; Li, Y.; Yu, W.; Wang, W. Preliminary study of the source apportionment and diversity of microplastics: Taking floating microplastics in the South China Sea as an example. *Environ. Pollut.* **2019**, *245*, 965–974.
153. Woodall, L.C.; Gwinnett, C.; Packer, M.; Thompson, R.C.; Robinson, L.F.; Paterson, G.L.J. Using a forensic science approach to minimize environmental contamination and to identify microfibres in marine sediments. *Mar. Pollut. Bull.* **2015**, *95*, 40–46.
154. 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**, doi:10.1038/s41598-017-05838-4.
155. Torre, M.; Digka, N.; Anastasopoulou, A.; Tsangaris, C.; Mytilineou, C. Anthropogenic microfibres pollution in marine biota. A new and simple methodology to minimize airborne contamination. *Mar. Pollut. Bull.* **2016**, *113*, 55–61



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).