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Review

An Overall Perspective for the Study of Emerging Contaminants in Karst Aquifers

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Abstract: Karst aquifers are essential drinking water sources, representing about 25% of the total available sources globally. Groundwater ecosystems consist of fissured carbonate rocks commonly covered with canopy collapse sinkholes. The open nature of karst aquifers makes them susceptible to rapidly transporting contaminants from the surface in dissolved and particulate forms. The principal aim of this review is to contribute to filling the gap in knowledge regarding major concerns affecting karst aquifers and understanding their vulnerabilities and dynamics. The principal groundwater pollutants of relevance are detailed in the present work, including well-known issues, such as the input of agriculture and its role in water quality. Emerging pollutants such as microplastics, still poorly studied in the groundwater systems, were also considered. Case studies for each typology of pollutant were highlighted, as their relative concerns for karst environments. Final considerations underlined an approach for studying karst environments more focused on understanding dynamics and links among different pollutants inputs and their drivers than on individual sources and impacts.

Keywords: groundwater; microplastics; fertilisers; plant protection products; pharmaceutical and personal care products; per- and polyfluoroalkyl substances; microbial biodiversity; climate change; human health

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1. Introduction

Groundwater in karst aquifers is a significant source of drinking water worldwide [1]. Approximately 14 % of the earth's land surface is covered by karst, representing about 25% of drinking water sources globally [2]; one-fourth of the world's population uses groundwater from karst aquifers [3].

Karst aquifers are groundwater ecosystems made of fissured chemically soluble carbonate rocks with large passages and caves commonly covered with collapsed cover sinkholes; they do not have any protective cover from soil or sediments [4]. Most of the water in these aquifers flows through a network of karst conduits formed by the dissolution of the rocks following discontinuities positioned heterogeneously and independently of the terrain's topography [5].

Groundwater in karst aquifers is driven by a broad spectrum of porosities and permeability. The permeability can be illustrated by a triple porosity model, which includes: (i) intergranular permeability, which involves the spaces among the mineral particles, (ii) fracture permeability, consisting of significantly thinner voids, and (iii) conduit permeability, created through the dissolution of pre-existing fractures [6].

The open nature of karst systems, with their high permeability, complicated network of channels, caves, fractures, sinkholes, and sinking streams, leads to a direct recharge through the conduit porosity into the aquifers.

The rapid transport of contaminants from the surface in dissolved and particulate forms makes groundwater systems in carbonate areas the most vulnerable aquifers to anthropogenic pollution [1,2]. Moreover, in addition to being important sources of drinking water, karst ecosystems are vital aquatic habitats for the microbial population and rare and endemic troglobitic fauna species that can be sensitive to contamination [7]. Nonetheless, these environments' microbial biodiversity and structure remain poorly understood, as most karst groundwater research focuses on identifying microbial communities related to contamination rather than native microbial community structure [8].

From a sustainable development perspective, pursuing water resource protection objectives implies the development of complex policies to preserve water resources. It is necessary to affect a heterogeneous set of phenomena at the origin of the ecological status of the resource. Those who work in this area know that it is essential to rely on in-depth and adequate knowledge bases to proceed effectively; this allows understanding of the degradation, the sources, the diffusion phenomena, the evolution processes, the risks to health and the environment and the development of technologies to contain and remedy impacts.

We are in a period in which we have yet to win all the primary battles aimed at containing the anthropic pressures that impact water quality. However, we are aware of recent vast areas of problems, originating, for example, from alternative materials that enter the production cycles.

Some emerging pollutants that are not routinely monitored and for which specific regulations still need to be created have been recently detected in these systems due to progress in analytical methodologies. Microplastics are gaining an ever-growing interest, although a significant knowledge gap exists, especially for karst aquifers.

With particular reference to karst aquifers, this study provides a comprehensive knowledge framework of the primary threats and interesting alterations that develop in these types of environments when anthropic pressures occur, contributing to understanding significant vulnerabilities.

2. Environmental Vulnerabilities and Primary Emerging Pollutants in Karst Systems

Groundwater quality degradation is a well-recognised phenomenon that has received considerable attention since the Industrial Revolution. However, the vulnerability of an aquifer includes complex dynamics of the natural hydrological cycle combined with the anthropic alterations of the earth's surface, water resource exploitation and pollutants emissions [3]. It is necessary to affect a heterogeneous set of phenomena at the origin of the compromise of the ecological status of the resource.

Generally, the karst aquifers' hydrogeological properties make these systems particularly vulnerable to pollution, limiting the natural attenuation of contaminants due to the rapid infiltration of water via sinkholes and fractures [9].

Moreover, the triple porosity of aquifers makes the prediction of pollutant transport and interaction with the porous medium due to the potential migration of contaminants via different flow pathways challenging.

On the one hand, groundwater flow through preferential routes can reach speeds of up to several hundred m/h. Consequently, the transport of the contaminants can be high-speed and spread far from the source of contamination [10]. Moreover, the transfer of pathogens can be favoured at the same time.

On the other hand, contaminants can be stored in sediments in low-flow areas, increasing natural attenuation processes with slow kinetics. However, in this case, pollutants are far from attenuation reactions involving oxygenation processes, and a constant level of pollutants can be released in the system [6].

The intrinsic vulnerability of an aquifer to pollution can be expressed as "the specific susceptibility of the aquifer system, in its various parts and the various geometric and hydrodynamic situations, to ingest and diffuse, even mitigating its effects, a fluid or waterborne pollutant such as to produce an impact on the quality of groundwater, in space and time" [11–13]. The intrinsic vulnerability is therefore configured as a characteristic of the aquifer system, whose evaluation would imply a correct vertical division of the system into its components. This estimate depends on the lithology, tectonics and hydraulic connections in the presence of karst. The vulnerability is represented as something dynamic and not static, that is, in close connection with the multiple phenomena that occur in the contamination mechanism or the phases of penetration of the pollutant into the aquifer system and its propagation, starting from the entry point [14].

Agricultural, industrial, residential and commercial activities are often responsible for leakage and spills of chemicals, waste and sewage discharge, contributing to the primary sources of groundwater pollution [1].

The most common contaminants found in karst aquifers include water-soluble compounds, both organic and inorganic (nitrates, chlorides); slightly soluble organic compounds, less dense than water (LNAPLs) and denser than water (DNAPLs); volatile organic compounds (VOCs); pathogens and different types of emerging contaminants (ECs) (pharmaceuticals; personal care products and hormones; flame retardants; perfluorinated and polyfluorinated alkyl compounds; and micro- and nanoplastics).

Contaminants of emerging concern (CEC, or emerging concern EC) refer to chemicals that are not yet regulated and whose traces are found in environmental matrices [15]. Identifying these substances has evolved mainly due to improving the analytical capabilities of detecting ever-lower concentrations [16,17]. At the same time as identification and measurement, the substances whose effects on human health and the environment are feared are included in the list of emerging compounds. Therefore, these compounds are not necessarily recently introduced, but their toxicity is currently under discussion to define a new environmental quality standard [18,19].

The most critical and frequent pollutants found in karst aquifers are reported below in Table 1.

Table 1. Overview of the most critical pollutants found in karst aquifers and their effects on aquatic ecosystems and human health.

Contaminant Type	Source of Contamination into Aquifers	Effects on Aquatic Ecosystems	Effects on Human Health
Nutrients, e.g., (NO_3^-, PO_4^{3-})	Old septic systems, landfills, leaks from cracks in sewer pipelines, acid mining waters, fertilisers used in agriculture, untreated industrial wastewater and urban sewage.	Eutrophication and hypoxia [20].	Methemoglobinemia. [21].
Pharmaceutical and Personal Care Products (PPCPs), e.g., Antibiotics, Anti-inflammatories, Lipid regulators, Psychiatric drugs, Stimulants, Insect Repellants and Sunscreen agents	Wastewater and contaminated surface water, landfills, septic systems and sewer leakages.	✓ Many PPCPs are toxic or even highly toxic to aquatic organisms; ✓ PPCPs may have an impact on aquatic organisms, even at the ng/L or μg/L levels; ✓ The potential effects of PPCPs include abnormal physiological processes, reproductive damage, mating behaviour changes, cytopathology damage, ✓ endocrine function effects, genotoxicity and mutagenic effects; ✓ There is increasing evidence that PPCPs have physiological toxicity to aquatic organisms and long-term bioaccumulation [22].	✓ Humans may be exposed to various PPCPs daily through exposure, inhalation, diet and the transformation of PPCPs through the water environment; ✓ Global rise of antibiotic resistance [22].

Table 1. *Cont.*

Contaminant Type	Source of Contamination into Aquifers	Effects on Aquatic Ecosystems	Effects on Human Health
Metals	Industrial activities and urban waste, urban surface runoff containing a high concentration of metals go through karst aquifers via sinkholes and conduit networks, natural leaching from rocks and soils within karst media and can be introduced with acidic deposition.	✓ Metal pollutants are conservative and often highly toxic to biota; ✓ They are an important group of toxic contaminants because of their high toxicity and persistence in all aquatic systems [23].	✓ As, Cd, Cr, Pb and Hg are classified as "known" or "probable" human carcinogens; ✓ Al, Sb, As, Ba, Cd, Cr (II), Co, Cu, Pb, Hg, Ni, Se, Sn and V are defined metal–estrogens showing high affinity to estrogen receptors because they can mimic estrogen activation; for this reason, they are considered harmful and potentially linked with breast cancer [24].
Volatile Organic Compounds (VOC) e.g., (e.g., trichloroethylene), fuel oxygenates (e.g., MTBE, ETBE), and by-products produced by chlorination during water treatment (e.g., chloroform)	Industrial activities, improper management of landfills, accidental spills, unidentified waste disposals, or residential septic systems.	✓ VOCs are a known causative agent of photochemical smog. Other environmental effects depend on the composition of the VOCs, the concentration and the length of exposure. ✓ Some VOCs can have severe effects on animals and plants. Effects may also occur due to secondary impacts, such as smog, ref. [25] Kotzias et al. 2017. ✓ Many VOCs commonly found in groundwater are toxic to various aquatic organisms [26].	✓ Various short-term and long-term diseases based on the concentration level in the air; ✓ Some carbonyl and aromatic compounds such as HCHO, CH_3CHO, benzene, toluene and xylene tend to produce cancer in the human body; ✓ They also irritate the nose, eyes, skin, etc. ✓ Some carbonyl compounds such as CH_3CHO, HCHO and acrolein are considered hazardous to human health. ✓ The lung functions become slow because of irritation in the nose, throat, etc.; ✓ Direct exposure can lead to fatal health problems such as carcinogenicity, teratogenicity, and mutagenicity [27].
Plant Protection Products (PPPs)	Point and non-point sources including runoff waters from agricultural and urban areas, deposition from the atmosphere, pesticide manufacturing plants, mixing-and-loading facilities, spills, wastewater recharge facilities (wells or basins), waste disposal sites and sewage treatment plants.	✓ Toxic, persistent, bioaccumulative, negatively impacting the soils' physical and chemical properties, extremely harmful to the whole ecosystem; ✓ They cause profound imbalances in the ecosystem due to their particular biochemical characteristics, such as high environmental persistence with direct ✓ Damage to aquatic ecosystems (fish, amphibians, etc.) and bioaccumulation in animal tissues [28].	✓ Adverse health effects associated with chemical pesticides include, among other effects, dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive and endocrine effects; ✓ High occupational, accidental, or intentional exposure to pesticides can result in hospitalisation and death [29].
Per- and polyfluoroalkyl substances (PFASs)	Wastewater treatment plants and resulting biosolids, domestic wastewater, landfills, fire training/fire response sites, industrial sites.	✓ The development time of destructive/delayed larvae is the most commonly observed effect of exposure to PFAS in many aquatic organisms; ✓ Possible adverse effects on the reproduction of fish due to high levels PFAS exposure; ✓ Possible genetic and transcriptomic responses after exposure to PFAS [30].	✓ High levels PFAS exposure with potential effects (results in laboratory rats, mice and common primates) linked to developmental neurotoxicity, carcinogenicity, genetic damage and cell membrane rupture; ✓ Probable relationship between PFAS exposure and increased liver weight; ✓ Histopathological changes in the lungs, hypertrophy of liver cells; ✓ Decreased reproductive outcomes, decreased hormone levels and developmental delays [30].

Table 1. *Cont.*

Contaminant Type	Source of Contamination into Aquifers	Effects on Aquatic Ecosystems	Effects on Human Health
Pathogens, e.g., viruses and bacteria and protozoa	Agricultural runoff, animal manure, compost, wastewater and sanitation systems; sources are intimately related to inadequate or absent sewage facilities and leaking from sewer pipes and septic tanks.	✓ The transport of pathogens from surface water to groundwater increases the vulnerability of groundwater; ✓ Pathogens such as viruses are much smaller than bacteria and protozoa, and many can potentially reach groundwater through porous soil matrices; ✓ Changes in microbial diversity [31].	✓ Water-borne diseases (i.e., gastrointestinal illness, such as diarrhoea, nausea, vomiting, fever, abdominal pain) caused by various bacteria, viruses and protozoa [31].
Micro- and nanoplastics, e.g., microbeads, pellets, microfibres, fragments	Wastewater, fragmentation of large plastic litter, and atmospheric deposition.	✓ Vectors of alien and pathogenic species; ✓ Alteration of the physicochemical characteristics of water and sediments; ✓ Interaction with aquatic organisms with consequent physical (locomotion, filtration, moult, injury), physiological (inflammation, liver stress, metabolism, life cycle) and chemical (release of additives, modified bioavailability, modified chemical toxicity, vector hypothesis) damages [32–34].	✓ Genotoxic and cytotoxic effects on pulmonary epithelial cells and macrophages of inhaled particles of 50 nm ✓ Immediate bronchial reactions (asthma-like), diffuse interstitial fibrosis and granulomas with fiber inclusions (extrinsic allergic alveolitis, chronic pneumonia), inflammatory and fibrotic changes in the bronchial and peribronchial tissue (chronic bronchitis) and interalveolar septa lesions (pneumothorax) depending on individual susceptibility [24].

2.1. Fertilisers

The sustainable management of water resources is a worldwide primary interest. Karst landforms constitute one-fifth of ice-free environments that provide drinking water for one-quarter of the global population [2]. Karst aquifers represent a necessary resource for human health, ecosystems, and industry [35,36]. In recent decades, agricultural, domestic, commercial and industrial progress has caused groundwater pollution [37,38] and decreased water quality.

Some authors [39] show that agricultural development, land cover change, irrational irrigation practices and the massive use of fertilisers constitute significant groundwater pollution [2].

Since the early 1970s, water bodies' widespread contamination due to the agricultural-nitrate intensification in the 20th century in industrialised regions of North America and Central and Western Europe has been a significant concern [40] as a direct consequence of the extensive use of fertilisers.

Among fertilisers, nitrogen (N) is an essential nutrient for plant growth, correct performance of biological functions and crop yield, with a fundamental role in the growth of the world population [41].

As shown in Figure 1, N consumption increased worldwide from 2010 to 2020, except in East Asia, where the application of nitrogen-based products decreased from 35,432 to 32,740 thousand tonnes. In fact, after 2015, the use of N-based fertilisers in East Asia decreased due to the threat recognition related to their utilisation, introducing an action plan for protecting environmental matrices [42].

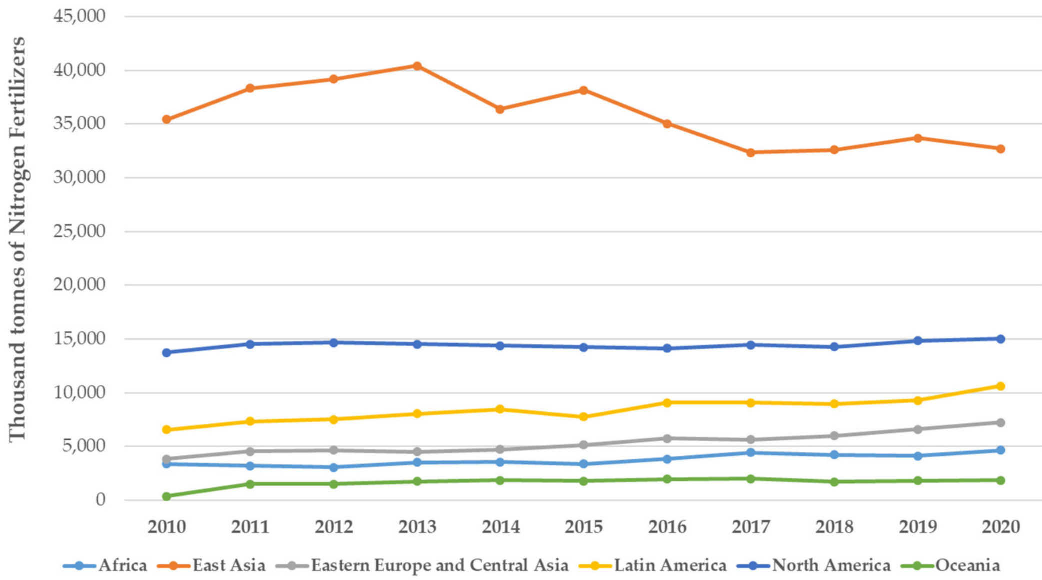

Figure 1. Nitrogen fertiliser intake over the last ten years in various world regions. Source data: http://ifadata.fertilizer.org/ucResult.aspx?temp=20220919013545; Accessed 19 September 2022.

In recent years, 10,639 million tonnes of agricultural products have been consumed. This has led to surface and groundwater nitrate pollution becoming a significant environmental problem in Asia, North America and Central Europe. The excessive use of chemical N fertilisers causes nitrate leaching phenomena and pollution of the karst water environment. Furthermore, a common environmental problem in karstic regions is associated with unreasonable irrigation practices, which increase the vulnerability of the karst water environment to agricultural contaminants [43–45].

High nitrate concentrations in waters can lead to a loss in water quality, with a consequent increase in acidification and eutrophication of water bodies. Excessive nitrate levels in drinking water may threaten human health, causing diseases such as infant methemoglobinemia, diabetes and stomach cancer [42,46].

The nitrate migration phenomenon to surface and groundwater is highly complex due to the heterogeneity and elevated karst conduits permeability [44,45]. For this reason, one of the scientific research objectives is to understand the origins of nitrate transformations in the karst systems. High application doses of fertiliser have caused nitrate pollution of the karst aquifer for the cultivations of pineapples [47], rice, corn, rapeseed [48], soybeans [49], beans, tubers, cotton, apple, pear, melons [50], olive trees [51] and other vegetables [52]. These studies indicated that sustainable nitrogen fertilisation practices and rational irrigation are the keys to mitigating nitrate pollution in the karst environment.

2.2. Plant Protection Products

The continuous large-scale use of plant protection products (PPPs) to safeguard the yield and quality of crops causes them to be constantly released into the environment; this leads to significant imbalances in ecosystems due to the particular biochemical characteristics of contaminants [28].

In recent decades, the demand for agriculture has proliferated, favouring intensive agriculture development. Pesticides, pharmaceutical and personal care compounds, lifestyle products, chemical and agricultural compounds, fall into the EC category. Many EC, such as pesticides, are among the most observed organic contaminants in urban deep water. They arise from anthropogenic activities, which subject the karst systems of groundwater to increasing contamination pressure. The high productivity of these systems favours agricultural, industrial and demographic development but also leads to chronic exposure of aquatic systems to many toxic pollutants [53]. Karst systems can undergo chemical changes that increase their porosity and permeability. Through macropores or soil fractures, pesticides and their metabolites can leach, escape and flow preferentially into groundwater, thereby deteriorating the quality of these ecosystems [54,55]. On the one hand, the massive use of the latest pesticides on the market, continuously updated with new formulations in pure form or in mixture with other substances, makes it challenging to assess their toxicity for human health and the environment, and it is even more difficult to establish specific reference legislation. On the other hand, with recent improvements in methodologies and instrumentation analysis, it is possible to identify, even at the µg/L or ng/L level, compounds in environmental matrices [9].

Organophosphate pesticides (OPs), such as chlorpyrifos, glyphosate, malathion, parathion and dimethoate, are the most widely used PPP group in the world [56]. They have progressively replaced dangerous organochloride (OCP) pesticides, such as para-dichlorodiphenyltrichloroethane (DDT).

Many of these substances have a high leaching potential in groundwater (e.g., triclopyr, metribuzin, oxamil, 2,4-D, clopyralid, dicamba, etc.); in several cases, their presence has been detected in groundwater. Many examples were cited in work [57] where groundwater monitoring results for these and other pesticides were reported. In order to assess the potential hazards of these substances, it is necessary to collect information on their persistence, bioaccumulation, leaching power and use.

These requirements should be met for pesticides and their metabolites, for which even less information is often available. Some authors in 2004 [58] first referred to the complex issue of pesticide metabolites.

The researchers analysed the water samples from 86 municipal wells in Iowa (USA) and found higher levels of metolachlor and atrazine metabolites (48.8% and 29.1%, respectively) than parental compounds (9.3% and 15.3%, respectively). Among the analysed herbicides (15 in total), atrazine and metolachlor were the only herbicides detected in more than 5% of samples, but their degradation in their metabolites was observed in 53% of them [58]. Such metabolites are often more toxic than the parental compound and more complex to determine analytically [59].

Atrazine (6-chloro-N-ethyl-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine is the second most widely used pesticide worldwide. It is a herbicide used to combat weeds in different crops of food interest [60]. The United State Environmental Protection Agency (USEPA) has defined this herbicide as hazardous to living organisms due to its toxicity, even at low concentrations. It can have a neuroendocrine effect with repercussions on human development and reproduction.

The widespread use of atrazine has led to significant soil and water contamination. Even today, despite the prohibition of its use in various countries (in the European Union countries since the first years of the current century) [61], its presence in the environment is still observed. Atrazine can be catabolised by plants, animals and microorganisms, forming a series of metabolites. Three of these by-products (diaminochlorotrazine, dehsopropilatzine, and deetylatrazine) are soluble in the aqueous medium and show a half-life in water in rather long aerobic conditions. The half-life is about two times greater than the average half-life in the soil in the presence and absence of oxygen (20–146 and 58–547 days, respectively) [62]. This is a worrying issue if we consider that the three metabolites soluble in water, like the other atrazine metabolites, show potential toxic effects on humans and the environment [63].

A recent study showed that fractures in karst systems allow a direct passage of atrazine into groundwater. Therefore, the karst system's geomorphology affects the pesticide's fate [64]. Atrazine has also been seen to alter the natural processes of microorganisms capable of metabolising nitrogen in karst systems, leading to an accumulation of nitrate pollution [65].

Glyphosate in Karst Systems

Glyphosate is undoubtedly the most widespread herbicide in the world. Non-selective, broad-spectrum, post-emergency herbicide is applied for agricultural, urban and industrial purposes. The environmental fate of glyphosate is determined, as for other pesticides, by its mobility, persistence in soil, solubility in water and the microbial activity of the environment [66,67].

Studies have identified an inversely proportional relationship between the presence of glyphosate and its metabolite AMPA and groundwater depth; however, there may be exceptions due to weather conditions with climatic fluctuations. The application time and intensity of agricultural treatments may affect the leaching of these compounds in deep water [68].

In a recent study published in 2021 [69], the researchers monitored the presence of glyphosate and AMPA in the Swedish groundwater near railways to control vegetation growing on tracks. Authors detected their presence in 14–16% of samples and 4–6% of cases with concentrations exceeding the EU quality standard of 0.1 μg/L. The two compounds were prevalently found in samples directly below the railway, where the pesticide application was 1800 g/ha. A limited lateral transport of glyphosate and AMPA was also observed in the aquifers.

Another work conducted in Canada [70] investigated the occurrence of glyphosate and AMPA in shallow groundwater, evidencing their presence in riparian (surface seeps), upland (lower than 20 m below ground) and wetland settings (lower than 3 m below ground). The presence of pesticides was detected in 5–10.5% of samples enhancing seasonal differences in riparian samples, possibly linked to the climate conditions, period of application and degradation rate. The highest values were registered in upland groundwater samples with maximum concentrations of about 660–700 ng/L for both glyphosate and AMPA, suggesting that atmospheric transport and deposition can lead to contamination of these pollutants even in environments far from their source of application. Finally, most revelations of glyphosate and AMPA in wetlands were far (above 0.5 km) from possible application areas. Further positive detections revealed their presence in precipitation samples collected in the same watershed.

Other authors [54] focused on underestimating groundwater contamination due to the fast flow into carbonate aquifers. Authors considered in their continental-scale model three types of degradable pollutants, including glyphosate, to quantify the risk of groundwater contamination in the carbonate rock regions of Europe, North Africa and the Middle East. Concerning glyphosate, assuming realistic applications of the herbicide, the author's model exceeded pollutant values by 3 to 19 times once it reached the groundwater, above all in karst Mediterranean regions. Here, thin soil layers or direct outcrops of bare rocks at the surface favour rapid transit of pollutants to the aquifer underestimating the risk of contamination.

In order to assess the risk of groundwater contamination, it would be essential to evaluate the mobility of this pesticide in the soils covering the karst systems [71] and conduct studies on the absorption and desorption of this pesticide in calcareous soils [72].

2.3. PFASs

Perfluoroalkylated organic substances (PFASs) are synthetic chemicals used to produce various consumables and industrial products due to their water- and grease-impervious properties and enhanced resistance to high temperatures [73].

The primary sources of PFAS in groundwater are domestic and industrial wastewater, but fire training areas and airports also play an important role as sources of pollution [74–78].

They are very persistent pollutants in the environment and can harm human health. Their carcinogenicity has been studied by the US Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC). In addition, these emerging contaminants have shown low absorption and reactivity in transport under the soil surface.

A study conducted in the Edwards aquifer (located in the United States in Texas) assessed the vulnerability of a karst system to these contaminants. The vulnerability of the Edwards aquifer is conditioned by the sources of emerging contaminants, the karst system characteristics and the increasing urbanisation in the Texas centre. The study showed that the Edwards aquifer could be considered a sound system for evaluating the presence, fate and transport of these emerging contaminants in an urban karst context [73].

Many groundwater monitoring studies have focused more on perfluorooctanosolophonic acid (PFOS) and perfluorooctanoic acid (PFOA).

In 2014, Kuroda et al., evaluated concentrations of PFASs in 53 Tokyo urban groundwater bodies using a multi-tracer approach based on carbamazepine and crotamiton. They found several ng/L of PFAS and considerably significant values of PFOS (990 ng/L), PFOA (1800 ng/L) and perfluorononanoate (PFNA, 620 ng/L). These values were remarkably higher than those revealed in wastewater and urban runoff reported in the literature [79].

In another European study (Pan-European survey) in which groundwater was evaluated concerning the presence of PFAS, PFOA and PFOS, the compounds were found in 66% and 48% of the samples examined, with maximum concentrations of 39 and 135 ng/L, respectively [80].

Nevertheless, lower concentrations of PFOS and PFOA contaminants in groundwater have emerged in the USA, with mean values of PFOA and PFOS equal to 22 ng/L and 97 ng/L, respectively [76,81,82].

Other authors have verified the presence of other types of PFAS, e.g., perfluorodeccanic acid (PDFA), perfluorobutanosulfonic acid (PFBS) and others with less fluorinated carbon atoms such as the first two. Average concentrations below ten ng/L were found [79,80,82].

Hepburn et al., in 2019, observed concentrations of PFAS in Australia, showing quantities ranging from 26 to 5200 ng/L near an industrial point source [83].

2.4. PPCPs

Pharmaceutical and personal care products (PPCPs) represent a broad class of different organic chemicals, including pharmaceutical compounds (e.g., antibiotics, anti-inflammatories, insect repellents, lipid regulators, psychiatric drugs and stimulants) and personal care products (e.g., soaps, lotions, toothpaste, sunscreens, etc.). They are composed of many ingredients with complex structures which differ in chemical properties, behaviours and functions [84,85]. Their presence can reach the aquatic environment, such as groundwater associated with wastewater disposal, industrial waste and hospital discharges and aquaculture. Furthermore, karst groundwater systems show an increased vulnerability to these pollutants due to their geology, favouring significant hydraulic conductivities and groundwater velocities even under limited soil pollutants adsorption [84].

A vast range of PPCPs has been detected in worldwide groundwater bodies.

In the karst aquifers of Illinois (USA), researchers discovered pharmaceuticals and hormones in 89% and 23% of aquifers, respectively. In particular, they identified the antimicrobial triclocarban and the cardiovascular drug gemfibrozil [86].

Pharmaceuticals and personal care products were also monitored in Chinese water resources detecting 106 PPCPs among 432 investigated in groundwater; 75 were also found in surface waters, and 31 were peculiar to the aquifers [87].

Other Chinese researchers examined the presence and distribution of nine PPCPs at four aquifers in North China. The aquifers differed in lithology and permeability.

The authors detected N, N-diethyl-meta-toluamide (DEET) (128 ng/L), carbamazepine and caffeine with a detection frequency above 90%. Moreover, the spatial distribution of the compounds was distinct at each site, suggesting that sandy aquifers had a lower ability to attenuate PPCPs with respect to the fine sand. Correlations were also evidenced between PPCPS and physicochemical parameters of the aquifer (e.g., nitrate, potassium, manganese) [88].

In the Jazan area of Saudi Arabia, 46 wells were monitored in 2017 to examine the presence of eleven commonly used drugs, such as acetylsalicylic acid, paracetamol, ibuprofen, metronidazole, caffeine, olmesartan, omeprazole, nifedipine, diclofenac sodium, glibenclamide and loratidine. However, none of these compounds was detected in the analysed samples [89].

Poland groundwater bodies were recently investigated for the occurrence of PPCPs; the authors of 14 scientific papers detected frequently elevated concentrations of pharmaceuticals, such as diclofenac (2270 ng/L), sulfapyridine (177.1 ng/L), sulfamethoxazole (66 ng/L), ibuprofen, ketoprofen and naproxen. Among the drugs category, carbamazepine was also found in high concentrations (up to 869 ng/L) along with caffeine (873.3 ng/L).

Hormones were also considered for the groundwater quality, and the highest concentrations revealed were for estrone (up to 309 ng/L) and 17α-ethynyloestradiol (61 ng/L) [90].

2.5. Microplastics

The presence of macro- and microplastics is now ubiquitous, widely documented in ecosystems around the world and widespread in surface water environments and aquatic ecosystems, including oceans, inland waters (streams and lakes) [91] and terrestrial and agricultural environments [92,93].

Microplastics are considered EC of increasing concern and are defined as plastic particles smaller than five mm [94].

Significant sources of microplastics in waterways include wastewater, the fragmentation of macroplastic waste into smaller fragments and atmospheric deposition.

The major environmental concerns related to microplastics mainly concern their ability to absorb persistent organic pollutants (POPs) that can be transferred into animal tissues, influencing their bioaccumulation and irritation of digestive tissues after ingestion [34,95].

In 2019, a study entitled "Microplastic Contamination in Karst Groundwater Systems", edited by researchers from the Prairie Research Institute of the University of Illinois, reported the presence of microplastics and other anthropogenic pollutants in two aquifers of karst origin in Illinois [3].

However, to date, few studies have examined the presence, abundance or environmental factors related to microplastics in karst groundwater systems (Table 2).

Another recent study examined the presence of microplastics in the sediments of "Bossea Cave", a show cave in Italy (Piedmont region). The cave represents the terminal sector of an extensive karst system and is a protected nature reserve established in 2011. It is the first show cave in Italy, opened to the public in 1874, and it receives about 12,000 tourists/per year. Microplastics were found in all sediment samples, including the non-touristic traits explored for a mean microplastic abundance ranging from 1600 microplastics/kg to 4390 microplastics/kg of dry sediment.

Other studies, grouped in Table 1, investigated the presence of MPs in groundwater samples of different aquifers showing a variable amount of particles ranging from a minimum value of 0.48 MPs/L detected in an alluvial aquifer in Iran to a maximum concentration of 2103 MPs/L quantified in the Jiaodong Peninsula in China. A total of 15 papers divided into 10 research articles and 5 review studies concerning MPs in groundwater are presented in Table 1.

The main results from the review works regard the dominant shapes and polymer types of MPs detected in groundwater samples identified mainly as fibres, pellets and fragments of PE and PET. The primary sources of MPs identified refer to the terrestrial environment assuming a vertical migration, especially from agricultural soils where the presence of sewage sludge, plastic residues from agronomic practices, fertilisers with polymeric coating and various waste is well documented [96,97]. Moreover, the particles' age can influence their transport through the soil due to the alteration of their physiochemical properties, which could increase their mobility [98].

Microplastic's toxicological effects on ecosystems and human health are still seldom studied to regulate their concentration in environmental matrices [24]. Moreover, due to the heterogeneity of their physico-chemical characteristics concerning the size range, variety of morphologies, polymer types and degree of particle weathering [99], several difficult to reproduce variables affect ecotoxicological studies.

Table 2. Overview of research article and review studies concerning microplastics in groundwater environments. NA. = not available.

Type of the Paper	Aim of the Work	Type of Aquifer/Depth	Country	Matrix Investigated	MPs Abundance (Mean)	Year of Publication	Ref.
Research Article	To provide modelling and simulations for a clear understanding of the transport phenomena of MPs	Saturated porous medium/NA.	NA.	NA.	NA.	2021	[100] Ryu et al., 2021
	To analyse microplastics in groundwater sampled from an alluvial sedimentary aquifer, using properly constructed monitoring bores that preclude atmospheric deposition as a major source of MPs	Alluvial sedimentary aquifer/10 to 25 m	Victoria, Australia	Water	38 microplastics/L	2022	[101] Samandra et al., 2022
	To investigate the occurrence of microplastics in groundwater sampled from five sites in Jiaodong Peninsula, China.	NA./4 to 8 m	The Jiaodong Peninsula, China	Water	2103 microplastics/L	2022	[102] Mu et al., 2022
	To investigate the presence of MPs in ten well samples obtained from an alluvial aquifer in a semi-arid region following filtration, digestion and inspection under a binocular microscope.	Alluvial aquifer with Quaternary deposits and surrounding karstic limestone/NA.	Shiraz, Iran	Water	0.48 microplastics/L	2022	[103] Esfandiari et al., 2022
	To simulate the transport of MP tracer particles compared to the solute conservative tracer uranine in a shallow alluvial aquifer over distances from 3.1 to 200 m using a natural gradient tracer test.	Shallow alluvial aquifer consisting of permeable sands and gravels/1.5 to 3 m	Upper Rhine Valley, Germany	NA.	NA.	2021	[104] Goeppert et al., 2021
	To investigate the sediments of a show cave in Italy, developing a methodology based on a cave-adapted version of the methods used in several studies to detect MPs from sediments of different environments and with various laboratory tests.	Karst aquifer/NA.	Piedmont, Italy	Sediment	1600 microplastics/kg 4390 microplastics/kg dry	2022	[105] Balestra et al., 2022
	To identify, characterise and quantify MPs in groundwater samples around Perungudi and Kodungaiyur municipal solid waste dumpsites in South India.	NA./3 to 34.48 m	Perungudi and Kodungaiyur, South India	Water	2 to 80 microplastics/L	2021	[106] Bharath et al., 2021
	To collect groundwater samples in mid-November under low-flow conditions from eight springs and three shallow (<65 m) wells to investigate MPs.	Karst aquifer/NA.	Illinois, USA	Water	15.2 microplastics/L	2019	[3] Panno et al., 2019
	To research MPs in groundwater and surface water from coastal south India (Tamil Nadu state) and to evaluate the heavy metal adsorption capacities of different polymers	NA./2–5 m	Tamil Nadu, South India	Water	4.2 microplastics/L	2021	[107] Selvam et al., 2021
Review Article	References	[96–98,108,109]					

The prevalent issue regarding groundwater safety in terms of MPs pollution mainly refers to human exposure to environmental pollutants and antibiotic resistance genes associated with the plastisphere. Knowledge concerning MPs in groundwater systems is needed to effectively support the promulgation of monitoring and control regulations.

3. Alterations of Microbial Biodiversity and Transport of Pathogens through Karst Systems

Karst's environment is characterised by different microorganisms, including bacteria and *Archea*, viruses, fungi and parasites (Figure 2). The aquifer microbial communities are constituted mainly of heterotroph bacteria able to grow in a groundwater environment [110], characterised by a typical hydrological, chemical and geological state [111]. Several biotic and abiotic factors can directly or indirectly control microbial diversity in ecosystems [112]. It has been shown how each aquifer is characterised by a specific bacterial community, generally stable in time and space. Groundwater systems offer opportune environments for microorganisms, with specific constant temperatures, a total absence of light and a scarcity of nutrients, minerals and colonising surfaces.

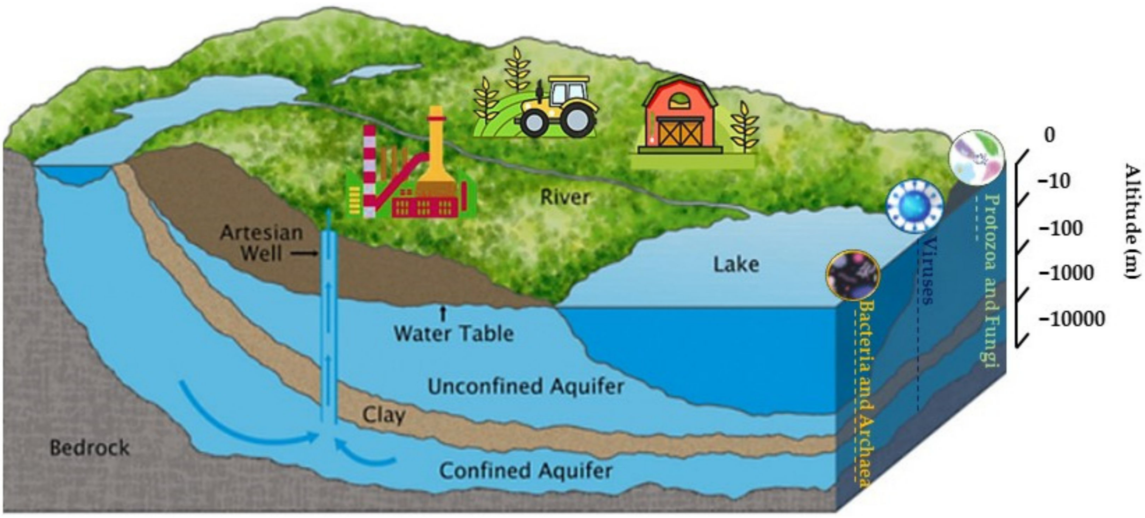

Figure 2. Groundwater microorganism distribution.

Simultaneously, microbial activity affects both the chemical composition and aquifer quality, influencing the drinking water supply for the world's population [113]. Moreover, groundwater microorganisms can become involved in the degradation of toxic substances. In the freshwater system, there is a dynamic balance between bacterial growth patterns and changes in environmental conditions, including nutrient availability or fluctuations in oxidation-reduction potential.

Microbial growth decreases with the increasing depth of groundwater. Some authors [113] described how the bacterial concentration was 10^5/mL at the surface level, up to a concentration of 10^3/mL at a depth greater than 1000 m. A second significant physical parameter of groundwater microbiology is temperature because it directly regulates metabolic pathways, chemical reactions and metabolite diffusion [114–116].

Moreover, the microbial diversity in the karst aquifer system is defined by pH, Eh (redox) potential and ionic strength. Here, pH forms microbial metabolisms in various paths; pH represents a significant environmental indicator that determines the composition and activity of groundwater microorganisms [117], considering that it controls the nutrient's bioavailability, geochemical reactions [118,119] and activities of extracellular enzymes [120,121]. Bacteria typically live in a range of 3–4 pH units. They can be distinguished into three groups: acidophiles growing in a range of pH < 5, neutrophiles at pH between 5 and 9, and alkaliphiles growing above pH 9 [122,123]. The microbial community mediate oxidation-reduction reactions influencing the redox conditions in groundwater systems [124,125]. Meng et al. [126] highlighted that the reductive dissolution and the processes of Fe^{2+} and Mn^{2+} oxidising precipitation are affected by microbial pathways. In addition, the bacterial communities of groundwater systems, which inhabit the same water depth, play a crucial role in water saturation and oxide-reduction potential.

The microbial community's stability changes with spatial–temporal variations of the chemical–physical parameters, characterising the aquifer status. Water contamination by human activities is a significant factor in transforming groundwater microbiology [127].

This change can lead to three different dynamics of transformation of the bacterial communities: (i) an increase of specific bacterial strains already present in the aquifer [128–130]; (ii) inclusion of new alien bacterial strains [130]; and (iii) development of new bacterial strains. In addition, the contamination type and the class of pollutants impact groundwater microbiology.

Groundwater is reputed to be less vulnerable than surface water to pathogenic bacterial pollution by faecal substances, even though contaminated groundwater still accounts for an excessive proportion of reported outbreaks of water-borne diseases in developing countries and rural regions [131,132]. Several water-borne diseases may be related to the water bodies' contamination following a breakdown of wastewater treatment systems, livestock manure use in agriculture and other agricultural activities in rural and less urbanised areas. Fecal coliforms and streptococci show that groundwater is often polluted by sewage and animal waste. Some authors [133] highlight how small clay particles represent suitable carriers of a high percentage of bacteria. In the United States of America, severe cases of pathogenic contamination bacteria in drinking water systems have been reported following high rainfall, causing 2300 cases of intoxication and seven deaths [134]. Several studies have shown the presence of faecal bacteria in carbonate wells [135,136] and aquifers of Silurian dolomite in Wisconsin.

Ji et al. [137] found a correlation between the high abundance of *Aeromonas veronii* in lakes and groundwater. This microorganism is a pathogen in aquatic environments, causing diseases in humans and freshwater fish [138,139]. In addition, large concentrations of *Comamonas testosteroni* [137] and *Brevundimonas diminuta* were found in adjacent groundwater. Unfortunately, this pathogen can pass through disinfection filters, resulting in partially harmful infections and sometimes even death [140].

4. Influences of the Temporal Dynamic of Karst Systems

Karst aquifers characterised by gullies, gaps and fracture networks within the karst architecture are mainly subjected to rapid transport of pollutants from surface to groundwater, affecting water quality [48]. Therefore, the water cycle is pollutant transfer's primary driver and carrier [141]. It follows that the equilibrium of karst groundwater systems strongly depends on climate. Climate change is expected to deeply affect water availability, influencing the depth of the water table and recharge. Some authors [142] demonstrated with models that the pollution groundwater variability is strongly dependent on average rainfall. In this regard, it is vital to consider the vulnerability of karst aquifers with water recharge linked to seasonal variability, overall environmental conditions and climate changes.

Other authors [143], considering Mediterranean countries' karst aquifers' sensitivity to climatic change, provided a new method to acquire spatiotemporal information on the recharge and groundwater flow dynamics changing hydroclimatic conditions (extremely wet and extremely dry). They concluded that a nonlinear relationship between precipitation and aquifer recharge rate subsists. The Mediterranean study area investigated by the authors is more susceptible to the decrease of precipitation than its enhancement.

Climate simulation studies suggest that in the future, Mediterranean regions will be subjected to growing temperatures and decreasing precipitation combined with improving extreme events in terms of hydrological droughts and floods. The effects of climate change on karst aquifers and water resources take time to evaluate.

Therefore, continuous monitoring of physicochemical features and groundwater levels is essential to provide information about aquifer structure and measure recharge rate over time to avoid over-exploitation of groundwater systems and suggest predictions in response to climate changes [144].

5. Perspectives of Monitoring and Control Strategies of Karst Systems

The growing awareness of environmental pollution from anthropogenic activities has led to the need to undertake adequate measures to protect groundwater resources. Activities, such as the quarries cultivation, the management of landfills, the treatment of wastewater and the spreading of pesticides and fertilisers [21,127], favour the release on the soil of variable quantities of chemical and bacteriological pollutants, which the action of rainwater then conveys to the groundwater. The problem takes on particular importance when we are in the presence of extensive fractured and karstified carbonate outcrops, which, as is well-known, favour the rapid absorption of rainwater.

The complexity and multiplicity of scientific contents inherent to the intrinsic aquifer vulnerability concept require a multidisciplinary approach. The definition of the intrinsic vulnerability of aquifers to pollution is accompanied, from a cartographic–operational point of view, by the definition of integrated vulnerability. The latter is obtained by superimposing on the intrinsic vulnerability of the flow field of the aquifer and the geo-referenced identification of danger centres (i.e., producers of point pollution), sources of danger (i.e., producers of diffuse pollution, for example, agricultural pollutants) and subjects at risk (i.e., targets of pollution).

For this reason, researchers and experts in the sector are implementing aquifer monitoring with innovative and integrated approaches.

First of all, it is necessary to set up a multi-methodological approach to create: (i) improved indicators for adequate protection and monitoring of karst water sources [145]; (ii) knowledge-based approaches for chemical fingerprint inspections [146]; and (iii) identify the sources of contamination if of natural or anthropic origin with biomolecular methods [21].

Other authors have based the monitoring by examining fingerprinting, using flow cytometry of bacterial cells in groundwater and faecal indicator bacteria to assess whether this technique can provide more rapid and descriptive information on microbial pollution through such karst aquifer systems [147]. Other studies base their approach on integrated transport models to minimise the subjectivity in estimating intrinsic resource vulnerability and provide additional parameters, such as pollutant concentration from solute transport, to enhance the vulnerability analysis [148].

There is also no lack of approaches with statistical methods based on the principal components analysis and multiple linear regression analysis to characterise the dominant sources relating to agricultural and livestock use [149]. Last but not least, there are approaches based on the application of machine learning techniques to predict the spatial distribution of water quality in the world's most ecologically fragile karst watershed [150].

6. Concluding Remarks and Future Perspectives

The significant concerns related to karst systems regard their vulnerability in terms of emerging pollutants due to the pressures concerning the rapid increase of population growth, economic expansion, contamination and over-exploitation of ecosystem services, consumption of energy and waste generation.

However, most emerging contaminants still need to be subjected to regulation in environmental matrices and, therefore, not included in groundwater monitoring programs, generating new challenges.

Although progress in monitoring, methodological approaches and modelling groundwater is increasing, the dynamicity at which new pollutants are entering the environmental framework may outpace current progress.

Emerging contaminants include an extensive group of chemicals with variable physical and chemical properties with different potential toxic compositions, degradation processes and subsequent fate in karst. Their study in karst aquifers is a relatively new area of scientific research. It requires further knowledge to assess their presence in karst systems and their impact on human and environmental health. Some of these compounds are useful for identifying sources of pollution and catchment delimitation of karst aquifers.

Other worries regarding groundwater safety mainly concern the excessive use of fertilisers and new formulations of pesticides. Agricultural practices focused on herbicide input reduction, such as crop rotations, cover crops and low-rate and strong-sorbing herbicides would improve karst aquifers' quality.

It is necessary to act on a heterogeneous set of phenomena influencing the ecological status of the water resource and to bring together the awareness of a fragmented audience of subjects who have skills in using water and the territory. It is honest to recognise that this path is full of difficulties that must also pass through cultural evolution, shifting from the concept of the right to use to its sustainability.

The various legislations of the states provide the protection and management of groundwater resources. These laws regulate preventive measures, the use and management of water in riparian zones and the protection of water-dependent ecosystems. To make these assessments, predominant criteria are usually based on distance and flow, and sensitive areas are delineated in concentric spheres upstream from the source. Many studies have highlighted the inadequacy of traditional strategies for protecting karst water sources and stressed the need to develop an integrated approach [151–153].

Therefore, based on what has already been reported, a comprehensive protection and control approach should be implemented considering these components: (1) design and implementation of a groundwater monitoring system considering the intrinsic and integrated vulnerability, (2) establish protection zones, (3) implement conservation measures for better land use management, (4) eliminate any sources of pollution (which is found with exceeding the regulatory limits), and (5) increase public awareness [154].

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