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Review

Microplastics in Freshwater: What Is the News from the World?

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Abstract: Plastic has become a "hot topic" for aquatic ecosystems' conservation together with other issues such as climate change and biodiversity loss. Indeed, plastics may detrimentally affect habitats and biota. Small plastics, called microplastics, are more easily taken up by freshwater organisms, causing negative effects on growth, reproduction, predatory performance, etc. Since available information on microplastics in freshwater are fragmentary, the aim of this review is twofold: (i) to show, analyse, and discuss data on the microplastics concentration in freshwater and (ii) to provide the main polymers contaminating freshwater for management planning. A bibliographic search collected 158 studies since 2012, providing the scientific community with one of the largest data sets on microplastics in freshwater. Contamination is reported in all continents except Antarctica, but a lack of information is still present. Lentic waters are generally more contaminated than lotic waters, and waters are less contaminated than sediments, suggested to be sinks. The main contaminating polymers are polypropylene and polyethylene for sediment and water, while polyethylene and polyethylene terephthalate are mainly found in biota. Future research is encouraged (1) to achieve a standardised protocol for monitoring, (2) to identify sources and transport routes (including primary or secondary origin), and (3) to investigate trophic transfer, especially from benthic invertebrates.

Keywords: lakes and rivers; aquatic monitoring; plastics contamination; gap analysis; metadata analysis; secondary microplastics; primary microplastic

1. Introduction

Plastics are synthetic organic polymers produced for human use since the 20th century. Plastic items, such as clothes, sponges, bottles, and gloves are commonly used in everyday life. Additives increase specific properties of plastic polymers; for example, those called butyltins stabilise polyvinyl chloride polymers (butyltins are found also in animal and human livers probably due to diet uptake [1]).

Plastics may be dispersed into the environment as mismanaged wastes and indiscriminately contaminate water, soil, and air [2,3]. Environmental contamination by plastics is enhanced by anthropogenic factors, such as the presence of urban centres [4,5], in particular, the low efficiency of urban and industrial wastewater treatments [6,7], use of plastic mulches [8], and application of sewage sludge to fields [9]. Natural factors such as wind, size of the water body and residence time [6], storms, and floods [10–12] contribute to the distribution of plastics.

To date, plastic contamination is widespread globally and is considered one of the main problems of environmental protection and management of aquatic resources. This stimulated researchers and experts in the field of environmental protection to increase and improve monitoring activities to assess the impacts of plastics on environment and biota [13,14]. Diverse studies focused on plastics origin, occurrence in water, and environmental spread revealed intense interactions between plastics and biota (see references throughout the text), highlighting how plastics act as a new colonisable matrix (the "plastisphere") mainly for microorganisms [15]. Plastics also have detrimental effects on aquatic biota. Macroscopically, physical interactions with plastic items can cause death by suffocation, entanglement, and ghost fishing of several fish, aquatic mammals, birds, and reptiles [16–18]. Microscopically, small plastics may cause negative effects on organisms. For example, hydrophobic chemicals may be adsorbed by plastics and desorbed in the gut of organisms, so plastics may act as a vector increasing the bioavailability of pollutants (e.g., persistent organic pollutants) and therefore the probability of being bioaccumulated [19]. The same process is observed for plastic additives, which may be toxic to the organism and could be released into the gut and absorbed by the organism [1]. The effects of plastics should be more studied as they are likely to increase in aquatic environments [20].

1.1. Microplastics in Aquatic Ecosystems

Plastic items, which can be of different polymers or shape, can be classified according to size, and in particular, items ranging within 1 µm and 5 mm are called microplastics (MPs) [21,22]. MPs are a varied contaminant suite which originated from many different product types, composed of various polymers and chemical additives, characterised by a broad range of colours and shapes [23]. They can be divided into primary or secondary, according to their origin. Primary MPs (pMPs) are the final products of industrial activities, e.g., used in cosmetics [24], while secondary MPs (sMPs) are products of plastic litter degradation. In fact, physicochemical factors can accelerate the degradation time of plastics by mechanical stimuli, biological, thermal, and photo-oxidative degradation [25].

Based on laboratory studies, MPs are emerging contaminants of the aquatic biota. MPs have a detrimental effect on the molecular, cellular, systemic, and organismic levels, e.g., neurotoxicity, death, and altered behaviour [19,26,27].

In nature, MPs contaminate inland and marine waters. Assessments of the hazard due to MPs within inland water ecosystems focus on several different topics (such as bioaccumulation and biomagnification, sedimentation, spreading, etc. [28]). Moreover, rivers are proposed as the main source of plastics for seas and oceans [29,30]. However, inland waters are poorly investigated compared to marine waters to date [31]. Although literature on plastics in freshwater is increasing [32], data are globally fragmented regarding the field assessments of concentrations of MPs and impacts on environmental freshwater matrices and biota [31,33].

The absence of a standardised protocol for monitoring activities contributes to the actual incomplete situation [34]. Indeed, scientific literature uses different protocols for monitoring freshwater microplastics (fMPs), enhancing the difficulties for comparing research information [35].

1.2. Aim

The aim of this overview is to discuss the scientific progress and to highlight scientific gaps in fMPs by analysing the current state-of-the-art and by comparing lentic and lotic ecosystems. In particular, the aim is twofold: (i) to show, analyse, and discuss global data on the concentration of microplastics in freshwater by presenting research trends on MPs occurrence in both abiotic and biotic matrices (excluding toxicological experiments on biota) and (ii) provide the main polymers that contaminate freshwater globally, in particular describing the main methodologies adopted by scientific literature to identify fMPs polymers and the origin of the main contaminants (primary or secondary fMPs). Based on the results of scientific literature, future perspectives for contributing to the scientific debate on fMPs are discussed.

2. Materials and Methods

2.1. Bibliographic Collection

Data collection was performed among peer reviewed international scientific articles via Web of Science and Scopus (without a lower time limit, until 25 May 2020). The keywords were "lakes" and "microplastic", and "rivers", and "microplastic". Results were selected for including articles on the detection of fMPs in sediments, waters, and biota and on the transport modelling of fMPs in strictly lentic and lotic freshwater (i.e., transitional areas such as estuaries and brackish lakes are excluded). Some records were selected from the literature of articles found on Web of Science and Scopus. The selection process was represented by the graphical method "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA) [36] (Figure S1).

2.2. Collection of Qualitative Information Data

Qualitative information was collected from all articles on the following topics: the type of ecosystem (i.e., lentic, lotic), year of publication, study areas (i.e., continent, country, and sampling site), affiliation country of the authors, and topics examined (e.g., sediment, water, fish, etc.).

If available, information was collected on the analysis of polymeric type of fMPs collected from the field (i.e., identification technique and type of polymer) and the source of fMPs (i.e., primary or secondary).

2.3. fMPs Concentration in Field

Data on fMPs concentration in the environmental matrices and biota were extracted either from articles or supplementary materials. If exact digits were available, they were collected and their unit of measurement was noted. Otherwise, data were extracted by approximating the values of fMPs concentration from the graphical results. The graphic processing of the results was carried out by RStudio software (version 1.1.463, produced by RStudio, Boston, MA 02210, U.S.A.).

3. Results

3.1. Bibliographic Search by Year and Type of Ecosystem

A thorough review of the literature revealed 158 articles on fMPs, of which the oldest was from 2012. Since that year, publications have steadily increased over time. Studies on fMPs are more frequent for lotic (113 articles) rather than lentic waters (55 articles; Figure 1), and only 10 investigated both lotic and lentic ecosystems (Tables S1 and S5).

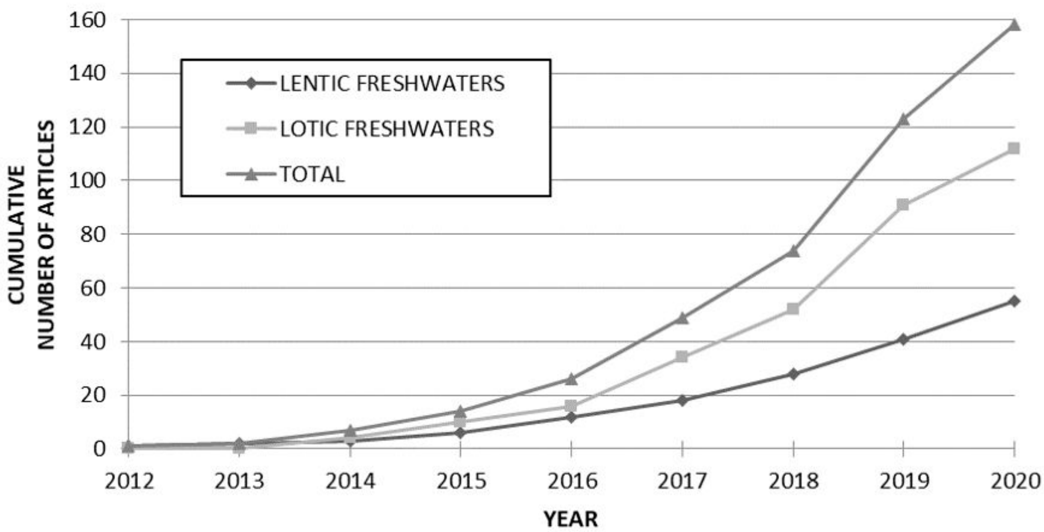

Figure 1. Number of articles published on the freshwater microplastics contamination updated to 25 May 2020.

3.2. Study Areas Overview

For the first time, the presence of fMPs is detected on all continents (Antarctica is excluded because studies on fMPs have not been conducted yet; Figure 2). The number of articles varies among continents: Oceania (5 articles), Africa (7 articles), America (including North and South America)

(37 articles), Europe (50 articles), and Asia (57 articles). North America, Asia, and Europe are the most studied areas; however, the studies mostly focus on particular water bodies. In particular, the most researched lakes are the Laurentian Great Lakes, the lakes of the Chinese regions of Hubei and Hunan (which have the largest number of lakes sampled globally), Lake Taihu, and Lake Garda. The most studied rivers are North Shore Channel, Yangtze, Pearl, Beijiang, Xiangxi, Seine, Marne, and Rhine (Tables S2 and S5).

Based on the distribution of the study areas, global fMP investigations focus on the temperate biome.

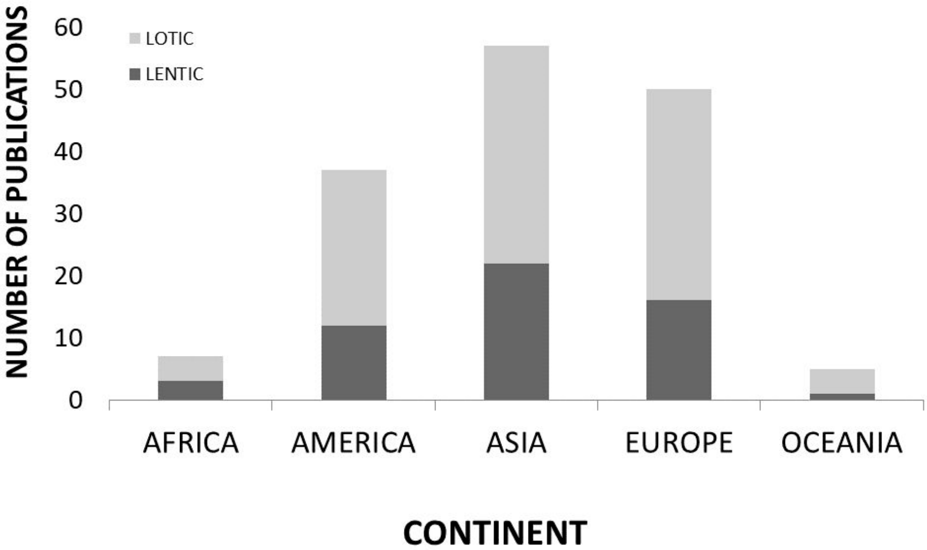

Figure 2. Number of publications on freshwater microplastics per study area.

3.3. *Overview of Authors' Affiliate Countries*

Although the majority of research is conducted in the same country where the research team is affiliated, some sampling sites are investigated by researchers whose affiliations belong to different countries. This means that the country where the freshwater was sampled did not fund studies on fMPs but another country did. For example, a study carried out in Nigeria was performed by researchers belonging to German research institutions (see Akindele et al. [37]). Hence, researchers' affiliations were counted to represent which countries fund more fMP research (Figure 3). Researchers from Chinese, USA, and German research institutions are the most active on fMPs. In fact, the studies conducted until 2020 by research institutions from China are 44, from USA are 30, and from Germany are 21. On the other hand, there are research institutions of many countries that have recently started to research fMPs, as are the cases of India, Kenya, Malaysia, Pakistan, Portugal, Saudi Arabia, South Africa, Tunisia, and Uganda.

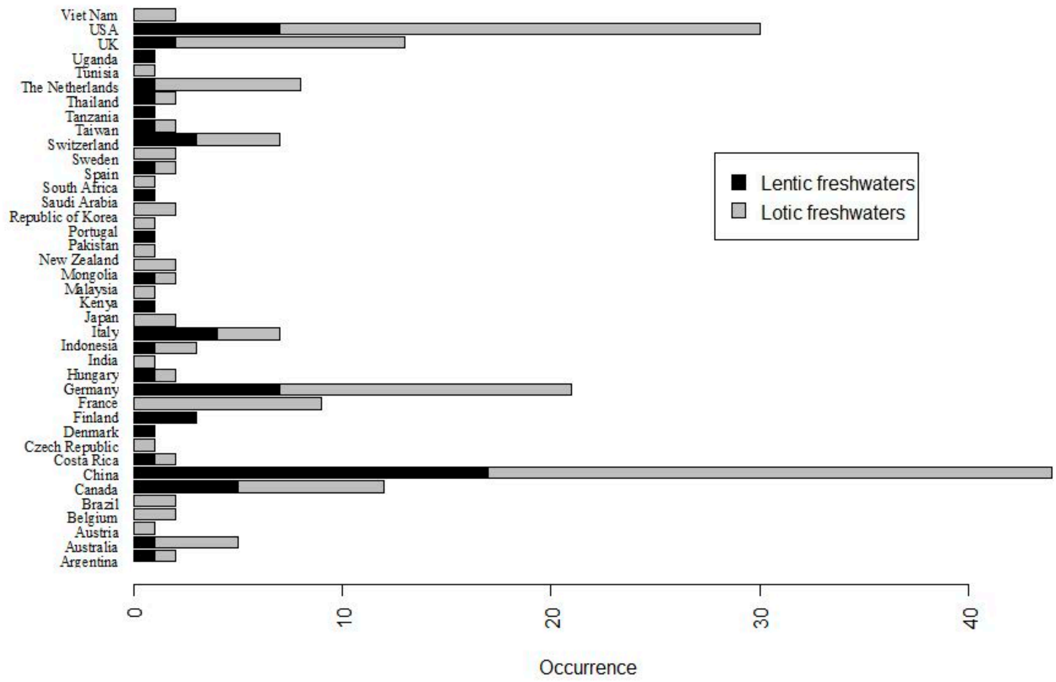

Figure 3. Affiliate countries of researchers studying freshwater microplastics.

3.4. *fMPs Topics of Investigation*

fMP scientific literature focuses on several topics of investigation. The main investigative efforts of the researchers are conducted on the evaluation of the environmental availability of fMPs in waters and sediments by sampling (149 articles) or models (18 articles) (Figure 4 and Table S1). After water and sediment, biota (50 articles) contamination is the third topic of greatest research interest.

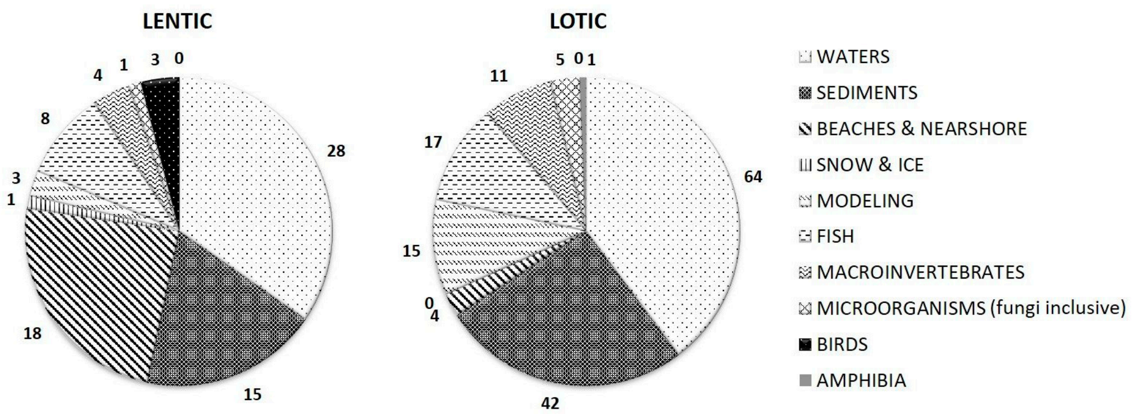

Figure 4. Topics examined on freshwater microplastics (fMPs): the number of studies in freshwater that analyse the different categories is reported.

3.5. *fMPs Contamination of Water and Sediment*

Comparison of the bibliographic search results shows a high variability of the protocols used and therefore in the units of measurement in which the results are expressed in both water studies (i.e., items/kg, items/L, items/m^3, mg/km^2, items/km^2, and g/m^3) and sediment (i.e., items/kg, items/L, items/m^3, items/m^2, g/m^2, and mg/g). Based on the number of studies, the concentration of fMPs in freshwater is expressed mainly in items/m^3, especially with regard to lotic waters and in items/kg for sediments (Table 1; Tables S2 and S5). However, items/m^2 is preferred in studies concerning beach litter. The main results on the concentration of fMPs in water and sediment are represented graphically (Figures 5 and 6). Based on current sampling efforts and unstardardised methods, the most polluted areas are Southeast Asia and Europe with regard to water, while sediments are highly contaminated also in North Africa and North America (Table S2). The lack of data does not allow to determine whether some regions of the world, such as Australia or South America, are more polluted than the Asian and European regions.

The units of measurement items/m^3 and items/L are homologated in items/kg to allow the graphic presentation of a greater number of values, according to the following assumptions. In nature, the weight of 1 L of water sample depends on different sources, for instance, whether it is pure distilled water or collected from marine or inland waters. In the latter cases, factors such as diluted minerals can increase its weight. For the purpose of standardising our dataset, the density of water is approximated at 1 kg/dm^3. Therefore, the following assumptions are made: 1 items/L × 1 dm^3/kg = 1 items/dm^3 × dm^3/kg = 1 items/kg and 1 items/m^3 × 1 dm^3/kg = 1 items/l0^3 dm^3 × dm^3/kg = 10^{-3} items/kg; therefore, 1 items/L = 1 items/kg and 1 items/m^3 = 10^{-3} items/kg.

At the end of this conversion process, a subsample is obtained with the concentration of fMPs expressed in items/kg in water and sediment samples (Figure 7). Data on the beach and nearshore are excluded because the units of measurement cannot be homologated and the comparable values are less than 10.

Table 1. Average concentration of fMPs depending on units of measurement. Af: Africa; Am: America; As: Asia; Eu: Europe; Oc: Oceania. For references, see Tables S2 and S5.

Matrix	Type of Water Body	Number of Sampled Water Bodies	Mean Value	Range across Water Bodies	Region Represented
Water	River	168	11,128 items/m^3	0–510,140	Am, As, Eu, Oc
		12	591.7 items/L	0.1–2083.5	Af, Am, As, Oc
		10	4,087,325 items/km^2	17,127,500–40,873,250	As
		4	0.019 g/m^3	0.005–0.034	As
		2	10.2 items/kg	6.8–13.6	As
		1	0.638 items/m^2	one water body	As
	Lake	62	2561.959 items/m^3	0.0005–8925	As, Eu
		18	92,032 items/km^2	2779–400,500	Am, As, Eu
		10	12.2 items/L	0.8–21.7	Am, As
		1	1200 mg/km^2	one water body	Am
		1	0.407 items/m^2	one water body	Af
Sediment (beaches and nearshore inclusive)	River	96	1161.452 items/kg	0.0000303–32,947	Af, Am, As, Eu, Oc
		5	4835 items/m^2	5–13,759	Am, As
		2	0.18983 items/g	0.16665–0.213	Am, As
		1	87 items	one water body	Am
		1	223 items/L	one water body	Am
		1	0.00077 items/m^3	one water body	As
	Lake	47	525.0905 items/kg	0.2733–13,925	Af, Am, As, Eu
		14	891 items/m^2	17–3508	Am, As, Eu
		3	29.8 g	12.3–42.1	Eu
		1	35 items	one water body	Am
		1	2.9 items/cm^3	one water body	Eu
		1	537.5 items/m^3	one water body	As
		1	4 items/L	one water body	Eu
		1	0.24 mg/g	one water body	Eu
		1	2680.5 g/m^2	one water body	As

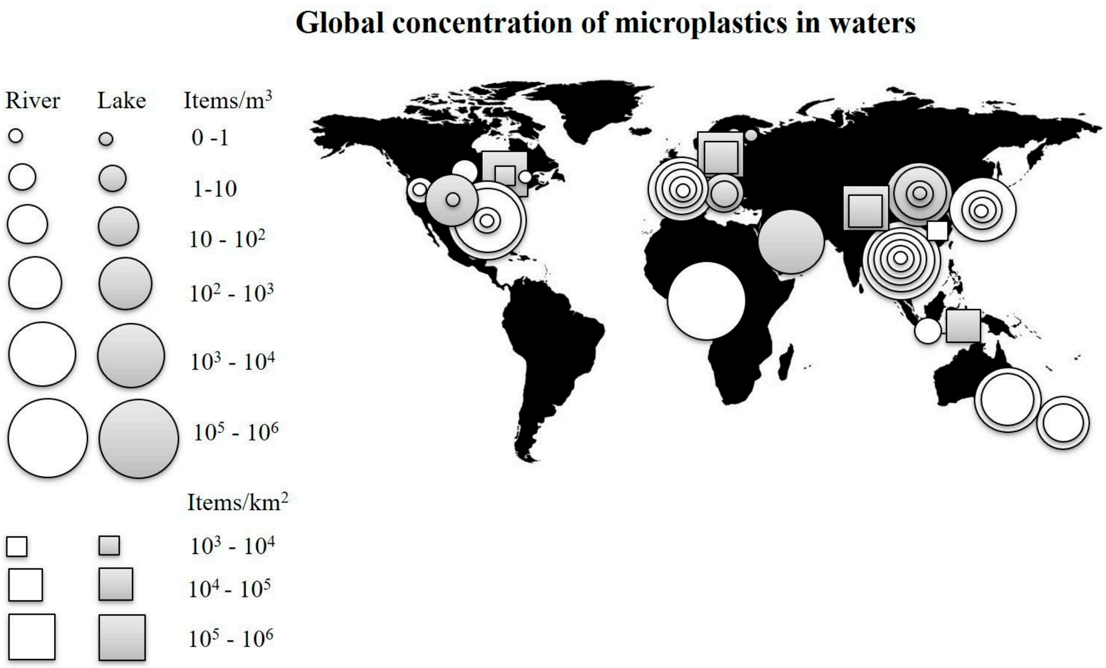

Figure 5. Global concentration of microplastics in lotic and lentic waters expressed in items/m^3 and items/km^2: Stacked concentric circles and rectangles indicate the presence of multiple different concentration values in the same study area.

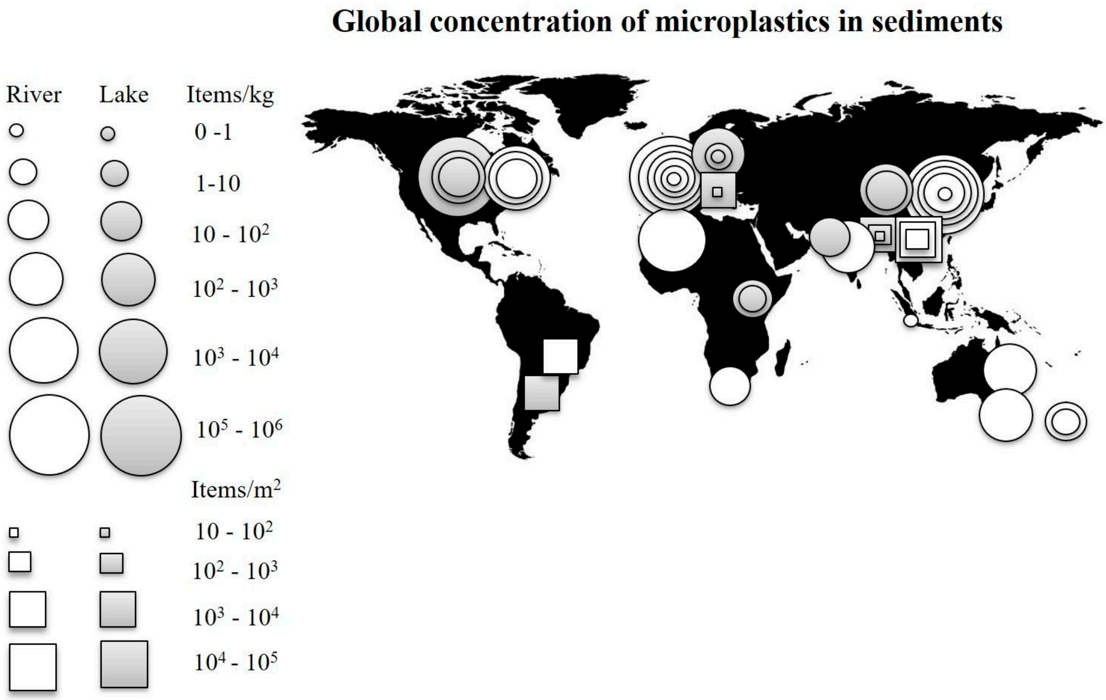

Figure 6. Global concentration of microplastics in lotic and lentic sediments (including beaches and near shore sediments) expressed in items/kg and items/m^2: Stacked concentric circles and rectangles indicate the presence of multiple different concentration values in the same study area.

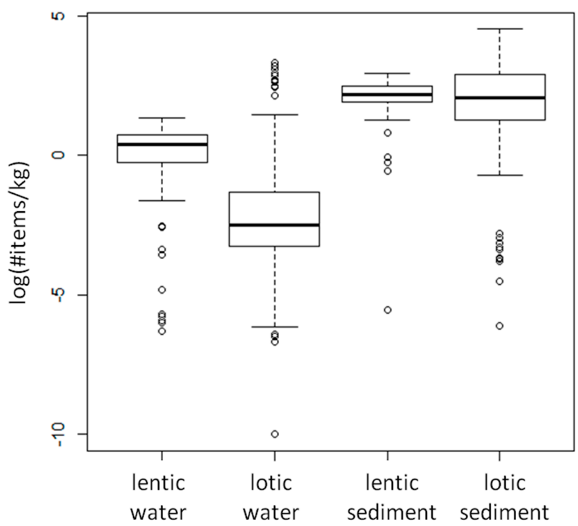

Figure 7. Concentration of microplastics in different sites: water and sediments of lentic and lotic freshwater.

As graphical data show, fMPs are generally less abundant in riverine water compared to lacustrine water. On the other hand, the difference in riverine and lacustrine sediments is not evident, although the median is lower in rivers (121 items/kg) than in lakes (150 items/kg). Furthermore, the concentration of fMPs in riverine sediments is higher than in lotic waters. Similarly, the concentration of fMPs is different between sediments and waters in lentic habitats (Figure 7).

3.6. fMP Impacts on Wild Biota

Biota studies examine two types of impact: the colonization process on fMPs and the contamination of organisms. Regarding colonization, microorganisms and fungi are observed on fMPs [38–43]. The colonization of microorganisms on fMPs has an average of 2233 operational taxonomic units (OTUs) (Tables S3 and S5), while fungi colonization is assessed at 16,390 OTUs [41].

Regarding the contamination of organisms, fish are the most studied taxa, being the focus of research in 49% of the studies on biota (Figure 4; Supplementary Material Table S2). Indeed, the presence of fMPs is confirmed in fish digestive tracts of most specimens (Supplementary Material Table S4) and in livers and gills [44,45]. Moreover, ingestion of fMPs is investigated in other vertebrates, i.e., birds [46–48]. Ingestion studies test the presence of fMPs within the animal's gastrointestinal (or stomach) tract by dissecting or digesting it. A study on ingestion which investigated a larger number of freshwater bird specimens (n = 350) of 16 species currently attests that the average contamination of individuals is at 11% [49]. Among anurans, only one study investigates fMPs [50]; however, no contamination was reported. In addition, a few invertebrates were studied prevalently in running freshwaters (Table S1). Invertebrate contamination is mostly expressed in items/g, showing a variability from <1 item/g in the UK [51] to 17 items/g in the USA [52] (Table S3). An exception to the studies on ingestion is the observation of the incorporation of fMPs in the case of case-making caddisflies (Trichoptera) [53].

Biota data are expressed in various measures: above all g/cm^3, frequency of occurrence (%), items/g, and operational taxonomic units (OTUs) (Table S3). The results are expressed in different units of measurement even within the same taxa, highlighting the need for standardisation.

3.7. Types of fMPs

In total, 8% of articles indicate the type of the investigated fMPs (primary or secondary), with sMPs being the most abundant in all reports (Tables S4 and S5).

Identification of suspected fMPs polymers is conducted in 81% of the scientific literature using different techniques. The Fourier-transform Infrared (FTIR) spectroscopic analysis (including the attenuated total reflectance FTIR) is the most commonly used for water and sediment matrices. Instead, FTIR, μ-FTIR, and Raman are equally used for fMPs in biota (Figure 8).

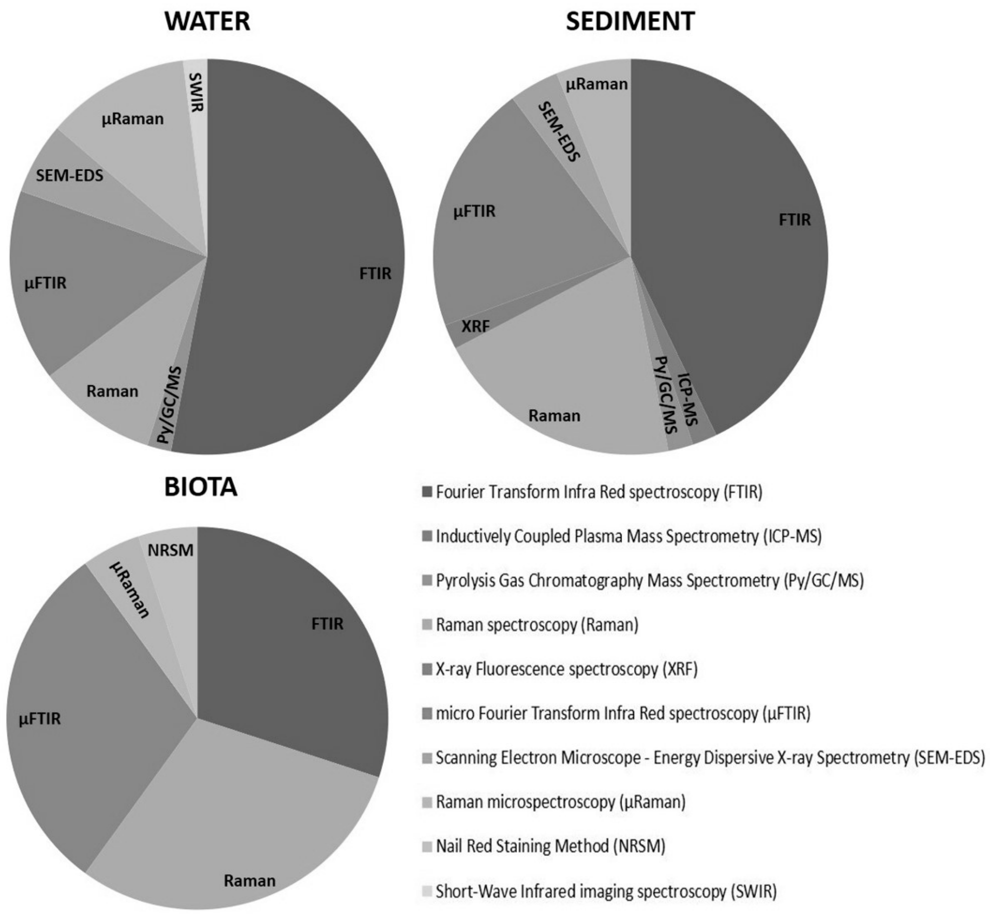

Figure 8. Techniques for identifying the polymers used for microplastics.

The results of the polymeric identification attest that polypropylene (PP) and polyethylene (PE) alone represent more than half of the main fMPs contaminants (52%). They are followed by polyethylene terephthalate (PET) and polystyrene (PS), with occurrence percentages of 10% each (Table S4).

According to available data, PP and PE are the main fMP contaminants in water and sediments, while PE, PP, and PET are the most abundant ones detected in biota (Figure 9; Table S4). According to our results, PP mostly affects Asian countries (35% of total Asian polymers), PE mostly affects American ones (48% of total American polymers), and PET and PE mostly affect European ones (each are 22% of total European polymers); four studies on polymers detected in African countries attest to the presence of contamination mainly from PE and the presence of PP and polyesters (PES); and six reports in Oceania attest to the presence of PES, Poly(hexadecyl) methacrylate (PHM), and PE (Table S4).

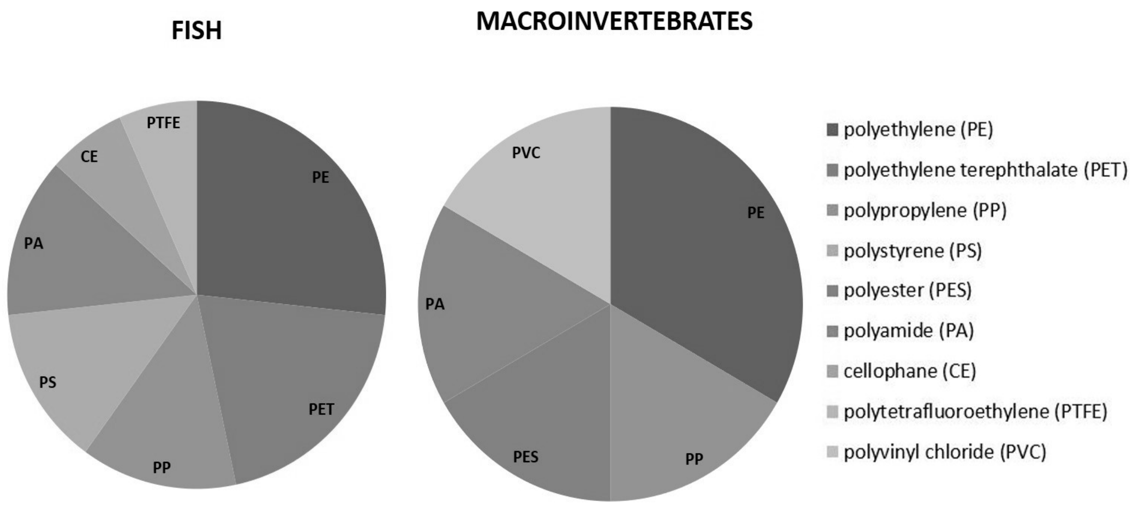

Figure 9. Types of microplastic polymers in freshwater biota (data were available only for fish and macroinvertebrates).

4. Discussion

The scientific literature collected in this work is one of the most complete collections by number of articles on fMPs based exclusively on field data from freshwater ecosystems. In particular, the comparison between lentic and lotic ecosystems is highlighted in our results. Lentic ecosystems are less studied than lotic ones; therefore, increasing data collection on lentic ecosystems could contribute significantly to scientific debate. In addition, this review has brought out that an actual comparison of plastic concentrations worldwide would only be possible by standardising the sampling effort. This collection of scientific literature and datasets is proposed as a reference for established and emerging researchers studying fMPs in lentic or lotic ecosystems (Tables S1 and S5).

4.1. Geographical Distribution of Contaminated Sites

According to scientific literature, lotic ecosystems have been more studied in North America and Europe while Asia and South America have been less investigated [54]. However, Asia has recently increased their contributions to the research on fMPs. To date, Asia is the main area investigated in the lotic and lentic ecosystems, followed by Europe and America (North and South). Based on our results and Li et al. [55], the leading countries on fMPs publications are China, the USA, and Germany for Asia, America, and Europe, respectively. In particular, to date, China is the country with the highest number of reports on fMPs, as opposed to what, recently, Li et al. [55] stated about the USA, probably owing to a difference in bibliographic search methods and the new scientific literature available. Plastic inputs into the sea due to mismanaged waste are different between the USA, Germany, and China: the latter contaminates more than any other country [20]. Therefore, the increasing interest in research towards Chinese and, more generally, Asian countries is of particular relevance for remediation actions as they are considered the main sources of fMPs in seas and oceans [20].

In a recent review, North America is proposed as one of the most concentrated areas of fMP occurrence [55] in contrast to the results of Wu et al. [56], which indicated Asia as the most polluted. The latter agrees that Asia is one of the main fMP sources for marine ecosystems [20], as confirmed by our observations. However, the absence of a standardised method and uneven sampling effort do not allow a significant comparison of different concentrations around the globe.

A relevant knowledge gap is that the worldwide description of fMP contamination is uneven and lacking. For instance, many countries in Africa and Oceania are not sampled. Furthermore, the Philippines and Sri Lanka, belonging to the top five countries for plastic inputs in marine waters [20], are an example of this knowledge gap. In this regard, we stressed that researchers mainly work on fMPs in their own (affiliation) country and that the affiliation groups that work on fMPs are absent especially in the most polluting countries, according to other studies [17,20]. The absence of research in potentially critical countries represents a serious issue for the understanding and contribution to the resolution of the problem of contamination by fMPs. In this regard, the presence of international collaborations can positively contribute to overcoming the obstacles to research in this field with the aim of having a global increased knowledge of fMPs.

Moreover, there is regional variability of the investigation efforts in freshwater sampled within each country. Some specific study areas are at the centre of the research (e.g., the Laurentian Great Lakes, Hunan area). Concentrating resources on a model study area can allow good characterisation of the specific processes of the MPs that are occurring specifically on the site, for instance, by modelling the transport of fMPs in Lake Huron [57]. However, from a regional perspective, there is no territorial coverage to provide a complete assessment of the exposure to fMPs and their sources, transport routes, and sinks.

Usually, areas with anthropogenic impacts are more studied than remote low-density areas. That is probably due to many reasons, such as the accessibility of sampling sites and the fact that cities are considered the main source of fMPs.

4.2. Factors Influencing fMP Contamination

Greater contamination of fMPs is explained by a higher density of the human population that produces plastic waste and by the possibility that fMPs reach freshwater, for example, due to poor waste management systems or surface runoff [6,20,33]. The proximity of lakes to urban areas has been shown to be positively correlated with an increased amount of fMPs in both lakes [5] and rivers [58]. However, even remote lakes with low population density are contaminated by higher than expected fMPs, probably due to the lack of waste management facilities and the absence of emissaries [7]. The lack of environmental protection measures may explain why rural areas were more polluted than urban areas in Lake Dongting (China) [59]. Similarly, microplastic pollution in Australia is widespread in rural and urban areas, with no differences in concentrations between samples from different sites [60]. Therefore, the absence of effective measures to prevent entry of fMPs into freshwater systems is a relevant factor to be considered when modelling plastic inputs. In addition, natural factors such as wind transport and long residence time of water can concentrate fMPs in lakes, especially if they have a small surface of water [6].

4.3. Microplastics in Water and Sediment

Microplastics in environmental matrices can be detected by transport modelling or sampling. Few studies evaluate the distribution of fMPs in environmental matrices through modelling; however, this technique could become very useful for addressing land management planning, for instance, for fMPs remediation efforts. Transport modelling is more studied in rivers than in lakes, probably because of the interest in understanding (micro)plastic loads introduced into the seas and oceans. However, lakes do provide interesting information, such as the fact that fMPs do not accumulate in the middle of lakes in gyres (like the oceans do) but rather on the coast, closer to urban centres [57].

However, most of the scientific literature determines fMPs by sampling in the natural field and by analysing the sample through different protocols. Three major types of information are collected from monitoring of the fMPs and from the impact assessment: the concentration of fMPs on the surface, in a volume, or in a weight. The comparison of research results is rather complicated in this variable context. In fact, methodological standardisations are strongly suggested [29].

In this work, the conversion of L and m^3 to kg has been proven useful for showing the concentration of fMPs in sediment and water. However, we cannot exclude that our result may be biased due to a difference in scientific article protocols. Concentrations of fMPs reported for lakes are generally higher than those that have been reported for rivers. Our observation is supported by the results of Koelmans et al. [61], i.e., lakes are more polluted than rivers by fMPs. Several factors may cause the observed outcome, either due to sampling design or processes. Rivers are more sampled than lakes; thus, more data are available on them. Therefore, the lack of sampling may conceal local variations in the concentrations. Furthermore, spatial aggregation and autocorrelation within the selected articles are currently unknown and may represent a bias in the comparison of the results. However, anthropic or natural processes may also cause the differences, as described in Section 4.2.

Regarding the environmental availability of fMPs in sediments, which is usually higher than fMPs in water (both for rivers and lakes), the sedimentation process of fMPs could explain the greater presence [62]. In fact, it has been suggested that sedimentation of fMPs occurs in river sediments [63]. Coastal sediments are also accumulation sites of fMPs [64]. Based on our results, freshwater sediments have reasonably been shown to be fMP sinks. This agrees with the fact that seafloor sediments are suggested as sinks of oceanic microplastic [65].

4.4. Microplastics in Biota

fMP risk assessments in wildlife are a priority [14]. Both lentic and lotic ecosystems are potentially at risk, based on the hazard of fMPs demonstrated in laboratory studies [26,28]. Nevertheless, the environmental risk caused by fMPs is mostly unknown.

A substantial increase in sampled taxa (both vertebrate and invertebrate) and on the study of colonization would provide more information on the ecological impacts of fMPs on the communities. Some taxa (e.g., birds) are more difficult to sample in freshwater if compared to fish [47]. In fact, fish are the focus of biota freshwater studies. Concerning the method of extracting fMPs from fish, it is suggested to collect the whole gastrointestinal tract for the analysis of fMP ingestion to obtain more accurate data than the ones obtained by stomach only [66]. However, the actual results of fMP ingestion are likely to be underestimated by current methods [67]. In addition, livers, gills, and muscles were investigated, finding microplastics in livers and gills but not in muscles [44,45]. Further research is required to detect smaller fMPs than those studied to ascertain whether a smaller microplastic is present in fish muscles.

This review points out that plants are completely absent in wild biota studies, even if they interact with fMPs. For instance, the adherence of MPs to *Lemna minor* (L.) and the consequent ingestion by an amphipod that feeds on *L. minor* is demonstrated in laboratory [68].

In addition to the research on biota regarding risk assessment, some species could become economic and time-saving indicators of the presence of fMPs in environmental matrices. In this regard, molluscs are proposed as fMP bioindicators [37]. In particular, the Bivalvia *Corbicula fluminea* (Müller, 1774) correlates with the environmental availability of fMPs in lacustrine and riverine water and, even more, in sediments; therefore, it is proposed as a biological monitoring tool for fMPs [69].

4.5. Methodological Considerations towards a Standardised Protocol

The actual literature relies on the number of items to describe the concentration of fMPs in freshwater. Changing this trend is not recommended to allow comparisons with previous studies. However, weighing the whole sample and the fMPs is a procedure that we suggest adding to the sampling protocol of fMPs as additional information, i.e., g/m^2, g/m^3, and g/kg. In fact, the use of grams is useful for distinguishing one heavier fMP from another, both counting as one item. Both the number of items and the mass are included in the parameters of the Guidance on Monitoring of Marine Litter in European Seas [70].

To date, few authors prefer to refer to grams of fMPs/km^2 [6,71]. However, we do not encourage the use of g/m^2 when sampling surface waters. In fact, fMPs in the waters are sampled by a three-dimensional liquid, which is more properly expressed in volume. On the other hand, plastic litter found on beaches could be expressed in g/m^2 when only the surface is analysed, according to the protocol.

Furthermore, it is suggested to express the results of the sediment by weight (in particular dry weight) instead of by volume, since the same volume can have different weights depending on the composition of the substrate and the water content (Figure 10).

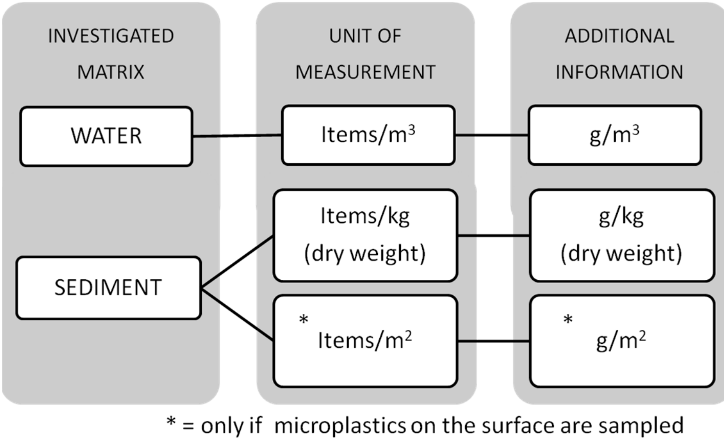

Figure 10. Suggested units of measurement to express the results in the articles on microplastics detected in environment matrices.

In addition, the indication of biogeographical region and habitat in which the sampling is carried out can facilitate the identification of similar study areas. For lotic freshwater, sampled zone (from eucrenon to hypopotamon) and current (riffle and pool) could be indicated, while for lentic freshwater, type of mixing (monomictic, dimictic, and polymictic), extent (holomictic and meromictic), and eventually layer (epilimnion, metalimnion, and hypolimnion) could be indicated.

4.6. The Issue of Identifying Primary and Secondary Microplastics

Knowing whether primary or secondary microplastics are the main fMPs could help develop plans for reducing waste dispersal. However, not many authors specify it. A problem could be how to distinguish primary from secondary fMPs. The observation of the shape of fMPs is considered a parameter for the definition of primary or secondary fMPs [72]. For instance, microbeads can be considered primary while the fragments are secondary [73,74]. Fibres, on the other hand, are considered primary by some authors [75] and secondary by others [76]. Although cracks on the surface of the fMPs can help recognise the altered plastics, it is not sure whether fMPs themselves were part of a larger plastic. In fact, if enough time is allowed (under degrading agents, such as radiation, mechanical factor, etc.), primary fMPs can appear damaged. Therefore, distinguishing them is difficult [77].

In addition, a new third category of fMPs is suggested: weathered plastics, which are fMPs produced by environmental weathering of plastics, such as the abrasion of tires and fibres from clothes [54].

The absence of a clear method of distinguishing primary, secondary, and weathered fMPs when collected in the field is a highly limiting factor for environmental management policies. In fact, the production of primary fMPs can be limited by regulations in some sectors of the industry, for example personal care products (e.g., Italy's law n. 205/2017 Art. 1, 546). However, if the majority of fMPs is of secondary origin, larger plastics are the main source of contamination and therefore remediation plans for environmental conservation are more effective if focused on them.

Recently, several countries have started the process of banning primary microplastics, especially microbeads. In particular, in Europe, some countries have regulated or are preparing drafts, such as Belgium, France, Italy, Sweden, and the United Kingdom [78]. In fact, the European Union is working intensively for the sustainable use of resources according to "A European Strategy for Plastics in a Circular Economy" (COM/2018/028). Outside Europe, other countries have banned primary microplastics, such as Canada, New Zealand, South Korea, Taiwan, and the USA [78]. In addition, the United Nations produced a draft resolution on marine litter and microplastics (UNEP/EA.3/L.20) which highlights the problem of plastic pollution. Although the resolution does not suggest banning microplastics, it recognises the need for their reduction.

If the ban process is further developed on a larger scale, the distinction of primary fMPs and secondary fMPs will be obsolete because no new primary microplastics would enter freshwater. Therefore, research could focus on secondary fMPs. This fact could help management plans and activities as it simplifies the problem to secondary sources. To date, there is no global action on banning primary microplastics; however, countries that have already regulated the ban could be monitored to assess changes in contamination over time.

4.7. Polymer Identification Techniques and Contamination of Fresh and Marine Waters

It is confirmed that the methods for the identification of fMP polymers are based on spectroscopic analysis, in particular, FT-IR and Raman for fMPs in water and sediment [34,55]. The preference for µ-FTIR in biota studies may be due to the size of the fMPs studied (e.g., macroinvertebrates can ingest smaller MPs [79]).

The results of the comparisons of the polymer analysis underline that Asia has a prevalence of PP and that Europe and America have PE and PET. The difference could be related to the fact that Asia is the main plastic producer (51%) [80], while Europe and America are consumers. However, the reasons need to be further investigated, in particular, for elaborating plans on reducing waste dispersal. Indeed, these results could be due to the application of different sampling protocols, since different polymers have different densities and can be found floating at different depths or in sediments.

By studying biota in detail, macroinvertebrates are mainly affected by PET and polyester compared to fish, which mainly ingest PE. However, only 8 reports concern macroinvertebrates and 16 fish. Therefore, we encourage research activities in this direction to confirm this observation. In fact, the results from these field observations may be relevant for direct laboratory toxicological experiments.

Some studies describe the polymeric types without clearly indicating which group the item belongs to (i.e., water, sediment, and biota). In this regard, we invite the authors to always specify the original group to which the fMPs were identified by spectroscopic analysis belong. Furthermore, the analysis of subsamples is not encouraged, since the choice to identify only a part of sampled items provides partial results on detected contamination.

A comparison between the main polymeric types of fMPs and marine ones revealed a partial discrepancy. The chemical composition of microplastics in oceans shows a prevalence of PE in surface waters while polyester, polyamide, and acrylic (PP&A) are more abundant in the water column and sediments [81]. Since freshwater is considered one of the main sources of microplastics for marine ecosystems, the abundance of PE is probably linked to river emissions because PE is indicated as the primary fMP contaminant. However, the presence of PP&A in rivers (and generally in freshwater) is detected only by PET concentrations, which are higher in biota but mostly lower than PP and PE in sediments and water of freshwaters. Therefore, the development of global models on the export of fMPs in marine environment is strongly recommended and, in addition to identifying MPs sources and transport, can predict future scenarios and can suggest different analysis approaches, as highlighted by the Global Riverine Export of Microplastics into Seas (GREMis) model [82].

5. Conclusions

There is a general agreement on considering freshwater microplastics as a great concern even if the hazard and probability of an impact are not well described or quantified and many research gaps are highlighted. In fact, data from the scientific literature reveal that the assessment of fMP availability in environmental matrices fails to identify the main sources of dispersion; they are rarely indicated in studies. Therefore, researchers are encouraged to add fMP sources to their studies whenever possible. In this regard, chemical analysis of the suspected fMP items can contribute to the identification of the sources: mapping the occurrences of fMPs and comparing the results with land use maps can highlight where the fMP input is located. This is a suitable method especially in rivers, where the current flow allows to state that the source of fMPs is upstream from the contaminated sampled site. However, this methodology is based on chemical analysis which requires expensive equipment and generally long samples pretreatment procedures. In this regard, the data sets provided here may help researchers conduct further research using metadata analysis in addition to (or by replacing) sampling and chemical analysis. Moreover, the creation of interdisciplinary research collaborations could be useful for overlapping methodological requirements. In addition, methods used in marine-coastal environment for plastic pollution monitoring, such as the use of diversity metrics applied to litter (including plastics) management [83], can also be applied in freshwater and can contribute to the standardization process.

Regarding methodology, the lack of uniform methodological protocols and units of measurement is a further gap because it adds some difficulties in comparing data from literature to today. This represents a limitation for a clear understanding of the global phenomenon of microplastic pollution and for the sharing of knowledge acquired between different research groups. A technical report on riverine litter monitoring (including plastics) is a step forward in the harmonisation process [84].

In conclusion, further investigations should be directed to the following:

1. drafting a standardised freshwater protocol for sampling, analysis, and expression of results, which could facilitate and enhance research on monitoring and risk assessment of fMPs;
2. determining global sources of contamination and transport routes based on spatial data, increasing monitoring activities for remote, low-impact or non-sampled areas and temporal changes by improving diachronic studies and, furthermore, specifying if the detected fMPs are primary or secondary would be a valid addition to the choices made by political planners for environmental protection management actions; and
3. studying the biota contamination in more detail and the interactions with fMPs (and fMPs-mediated pollutants), in particular regarding benthic organisms, which are generally exposed to higher concentrations (as they are strongly connected to sediments) and trophic web transfer, to clarify the hazard to human health.

According to our metadata analysis, sediments are considered sinks for fMPs. In a broader consideration, rivers are considered the main source of microplastic input into oceans, which are another fMP sink. However, as far as fMP outputs from rivers are concerned, we highlight that contamination information is unknown for major global rivers, such as the Nile, Amazon, Yellow, Paraná, Congo, and Mekong rivers. Therefore, it is strongly suggested to extend the research areas to include them since the lack of information can generate a different perception of the phenomena. In fact, Asia is currently considered the main source of microplastics; however, sampling uninvestigated areas could provide new information on the subject. In particular, countries having poor waste management may have a considerable impact, but the situation is currently insufficiently known. Joint political and administrative solutions may obtain the funding needed to expand fMP research in geographical areas, so far, little or not at all researched and on topics with high economic requirements.

Supplementary Materials: The following are available online at http://www.mdpi.com/1424-2818/12/7/276/s1, Figure S1: The Preferred Reporting Items for Systematic Reviews (PRISMA) flow diagram describing the selection process of the articles: Numbers in parentheses stay for the total number of references remaining after the selection process, Table S1: Categories of topics investigated by the articles, Table S2: Concentrations of fMPs in environmental matrices for each site of investigation, Table S3: Value of fMPs in biota investigated, Table S4: Polymers data: origin, identification technique, type, and occurrence, Table S5: References for Supplementary Materials.

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