

Review

# Microplastic Contamination in Freshwater Environments: A Review, Focusing on Interactions with Sediments and Benthic Organisms

Arianna Bellasi <sup>1</sup>, Gilberto Binda <sup>2</sup>, Andrea Pozzi <sup>1</sup>, Silvia Galafassi <sup>3</sup> , Pietro Volta <sup>3</sup> and Roberta Bettinetti <sup>4,\*</sup> 

<sup>1</sup> Department of Science and High Technology, University of Insubria, Via Valleggio 11, 22100 Como, Italy; Arianna.bellasi@uninsubria.it (A.B.); andrea.pozzi@uninsubria.it (A.P.)

<sup>2</sup> Department of Theoretical and Applied sciences, University of Insubria, J. H. Dunant, 3, 21100 Varese, Italy; gilberto.binda@uninsubria.it

<sup>3</sup> CNR IRSA, Pallanza, 28922 Verbania, Italy; silvia.galafassi@irsa.cnr.it (S.G.); pieter.volta@irsa.cnr.it (P.V.)

<sup>4</sup> Department of Human and Innovation for the Territory, University of Insubria, Via Valleggio 11, 22100 Como, Italy

\* Correspondence: roberta.bettinetti@uninsubria.it

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**Abstract:** Plastic is one of the most commonly produced and used materials in the world due to its outstanding features. However, the worldwide use of plastics and poor waste management have led to negative impacts on ecosystems. Plastic degradation in the environment leads to the generation of plastic particles with a size of <5 mm, which are defined as microplastics (MPs). These represent a global concern due to their wide dispersion in water environments and unclear potential ecotoxicological effects. Different studies have been performed with the aim of evaluating the presence and impacts of MPs in the marine environment. However, the presence of MPs in freshwater systems is still poorly investigated, making data retrieval a difficult task. The purpose of this review is to identify the main aspects concerning MPs pollution sources in lakes and rivers, with a focus on freshwater sediments as a site of accumulation and as the habitat of benthic organisms, which are key components of food webs and play a fundamental role in energy/contaminant transfer processes, but are still poorly considered. Through this review, the sources and fate of MPs in freshwater are analysed, ecotoxicological studies focused on sediments and benthic fauna are exposed, the most frequently used sampling and analysis strategies are reported, and future trends of MPs analysis in this field are proposed.

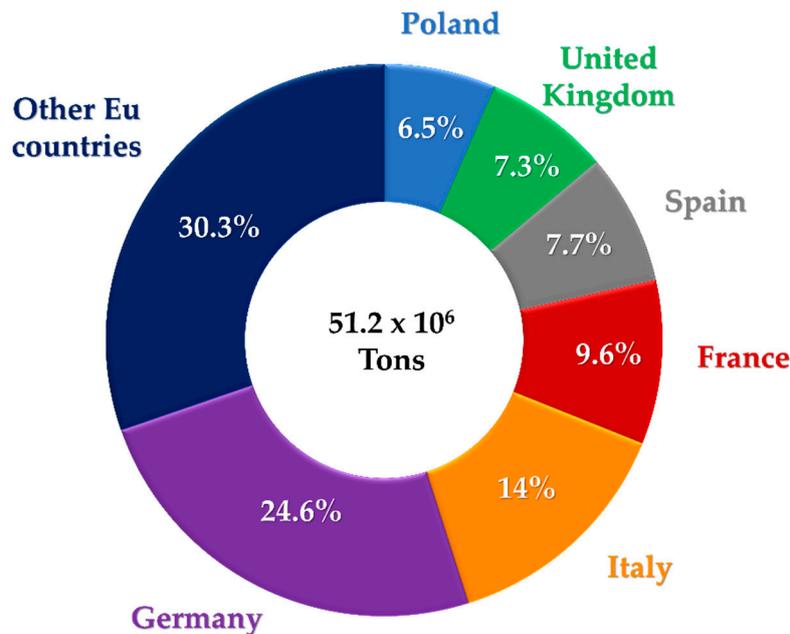
**Keywords:** microplastic; contaminants; freshwater ecosystems; lakes; rivers; benthos; sediments

## 1. Introduction

Plastic (from the Greek “plastikos”, meaning mouldable) is made of synthetic organic polymers, which are usually produced through the polymerization of monomers derived from oil, gas, or coal [1]. Synthetic polymers were first discovered in the 19th century, with the invention of vulcanized rubber and polystyrene [2]. Mass production started in 1950 [3] and nowadays approximately 30,000 polymer materials are registered in the European Union [4].

Despite the availability of many hundreds of polymers, 75% of total plastic demand is limited to a few kinds of plastic: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyvinylchloride (PVC), and polyurethane (PU). In 2013 plastic production exceeded 288 million tons per year, in 2016 the annual global production of plastic was around 322 million tons, and by 2050 it is estimated that the production will increase to a colossal 33 billion

tons [4,5], with 10% ending up in the oceans [6]. From a global perspective, Europe is one of the most important markets for plastics (together with China and North America), with a constant production of synthetic polymers of 64.4 million tons per year and a plastic demand of 51.2 million tons per year [7]. Within Europe, the leading countries in terms of demand are Germany, Italy, France, the United Kingdom, and Spain (Figure 1). Plastic has changed human life, since it is used for a wide range of purposes [3,4] due to its outstanding features: it is light-weight, durable, versatile, and can be produced at low cost [1,8]. However, there are drawbacks to the present “plastic age”, including the long half-life of plastics, excessive use, and inefficient management of waste which causes an unpleasant accumulation of these materials in the environment [9].



**Figure 1.** EU plastic demand in 2017 with a focus on the countries with an annual demand higher than 3 million tons. Data are from PlasticsEurope, 2018 [7].

Plastic debris has become a global concern due to its wide distribution and associated environmental consequences; over the years, plastics have been accumulating in the environment and are present in every environmental compartment and matrix. Moreover, most of the plastic widespread in the environment is finally deposited in aquatic environments [10,11]. Around 4812.7 million tons per year enter the oceans [12] representing the 50%–80% of waste on beaches, floating on the ocean surface, and on the seabed [3]. Plastic and waste can enter aquatic environments through direct discharge or they can be transported from the mainland. For the marine environment it has been estimated that 80% of aquatic litter is delivered into aquatic systems by land-based sources [13]: public littering, improper waste disposal, waste dump run-offs, tourism, industrial activity, and combined sewer systems contribute dramatically to the pollution of the aquatic environment with plastic. It has been predicted that the cumulative amount of plastics available to enter the ocean will increase by one order of magnitude by 2025, assuming no improvement of the waste management infrastructure [10,14].

The residence time of plastic when released in the environment has been estimated in the range of tens to hundreds of years [15]. High resistance leads to extremely low degradation and long half-life of plastics under environmental conditions [16], so their durability is in fact a two-edged sword, causing the widespread persistence of MPs. Plastics litter is of serious concern for economic and ecological reasons: while diminishing the aesthetic value of water environments [17], plastic debris is likely to pose threats to biodiversity due to easy uptake by aquatic organisms. Plastic can transfer chemicals, which can be additives or water pollutants, to living organisms [3]. Indeed, plastic pellets

have the capacity to adsorb hydrophobic pollutants and to discard these into habitats or organisms by desorption [14,18–20].

While increasing studies about MP contamination in freshwater systems exist, knowledge gaps [21] cause trouble in understanding the full extent of the problem, since this environment remains less studied than the sea (Figure 2). The present paper aims to contextualize the problem of MPs in freshwater systems from a global point of view, focusing on the role of MPs in sediment, and, in turn, the interaction with benthic organisms: sediments can be a sink of MPs [22] and uptake of MPs from the surrounding environment occurs by benthic organisms [23]. Furthermore, sediments can act as a retention site for contaminants and toxic elements [24], which may interact with plastics and increase their potential bioaccumulation [25].

This review will be organized as follows:

- Firstly, the main sources, formation mechanisms, and accumulation routes in freshwater systems will be presented;
- The main impacts of MPs in freshwater systems observed in recent studies will be exposed;
- In the central part of the paper, the ecotoxicology of MPs in freshwater systems will be discussed, focusing on the main issues for sediments and the benthic community, which are poorly understood;
- The most used sampling and analytical techniques will be presented, analysing their advantages and drawbacks;
- Finally, the future perspectives for MP studies to understand impacts, especially on freshwater sediments and benthic biota, will be presented.

## 2. From Plastic to Microplastic (MP): Sources and Aquatic Environments

The dispersion of larger plastic items results in well-known risks for marine life and environments [12]; moreover, different hazardous categories of plastic classified by size exist, posing unclear adverse effects. Plastics are usually classified as mega-debris (100 mm), macro-debris (20 mm), meso-debris (20–5 mm), and micro-debris (<5 mm) [3]. Since 2004 the term microplastics (MPs) has been widely used to refer to anthropogenic debris: it is a collective term to describe a heterogeneous mixture of particles ranging in size from a few microns to several millimetres [26]. These particles can present different shapes and composition depending on the source of origin [27].

MPs originate from a variety of sources, but it is possible to point out four main mechanisms of formation: deterioration of larger fragments, direct release into waterways, accidental loss of industrial raw material, and discharge of macerated waste [14]. According to those factors, MPs fall into primary and secondary categories. Primary MPs are specifically manufactured in the micrometre size range and are likely to be washed down from industrial or domestic drainage systems and into wastewater treatment streams [4]. Primary MPs are used in a wide range of industrial activities, from the production of air-blasting media to the production of boat hulls [28]. Despite this, one of the most important sources of primary MPs remains in personal care and cosmetic products such as lotions, soaps, scrubs, and toothpastes [29,30]. Even laundry washing machines discharge a large amount of synthetic fibres into wastewater [31]. Secondary MPs are formed as the result of meso- and macro-plastic litter fragmentation due to prolonged exposure to UV light and physical abrasion [3]. Indeed, plastic is an UV susceptible material and its lifetime outdoors tends to decrease because UV radiation can start oxidative reactions, leading to degradation [32]. Mechanical degradation is another important aspect since the recalcitrant material is shredded into smaller particles by friction forces occurring during movement through the different environmental habitats. MPs widespread in water systems can float on the surface or sink into sediments depending on the density of the polymer. However, there is a correlation between the typology of MPs and the position of these in water systems, with primary MPs being possibly more concentrated in proximity to wastewater effluent sites [28].

Nowadays plastic litter is dispersed throughout the world's oceans: on highly impacted beaches, MP concentrations can reach 3% by weight as compared to natural sediment weight [33].

Another important source of MP pollution is in tire wear particles (TWP): debris generated mechanically by the rolling shear of tread against a surface, or by volatilization, which results in the generation of much smaller particles that are usually nanosized ( $<2.5 \mu\text{m}$ ). Generation of these particles is quantitatively consistent, accounting for 5–10% of total MPs ending up in the oceans per year [34]. The generation of TWPs depends on different factors such as the age and typology of tires, driving speed, and type of road surface. TWPs are also linked to the nature of contact between the tires and road, and to the intensity of road traffic. The frequency of trucks and buses passing by further influences the amount of TWPs released into the environment [35], and for these reasons a specific treatment of road runoff waters is usually foreseen in order to avoid percolation into groundwater and dispersal into surface water systems [36]. An attempt to quantify the impact of TWPs in freshwater systems was made by Wagner et al. [35], who estimated total production at about 1,327,000 tons/year for the European Union and 1,120,000 tons/year for the United States. The mass of TWPs ultimately entering the aquatic environment strongly depends on the extent of collection and treatment of road runoff, which is highly variable, and for this reason Wagner et al. [35] made an estimate for Germany alone, reporting that up to 11,000 tons/year of TWP reach surface waters respect to an estimated produced total of 133,000 tons/year. Rainwater runoff [34] is one of the main factors influencing the dispersal of TWPs in the environment, causing direct discharge into surface waters or sewers.

In addition to the MPs that flow into wastewater treatment plants (WWTPs) through rainfall and then accidental discharge in waters, some types of MPs are directly washed into domestic drainage systems and wastewater treatment streams. For these reasons is crucial to understand the role of WWTPs in the dispersion processes of MPs in water ecosystems. In areas characterized by a high population density, WWTPs are one of the most important sources of microplastics [37]. Cheung and Fok [38] estimated that 80% of the microbead emissions to aquatic environments in mainland China (around 209.7 trillion microbeads, 306.9 tons per year) is due to WWTP effluents. A study from 17 WWTPs in the United States estimated that between 50,000 and 15 million MPs per day are discharged into effluents by WWTPs [39], whereas for the city of Vancouver (Canada) alone, the release has been estimated at around 30 billion annually [40]. Although these numbers may seem incredibly high, WWTPs are doing their job: many studies recently investigated the effectiveness of WWTPs in removal efficiency of MPs, reporting removal rates of 97–99% [30,40]. It is important to emphasize that even low concentrations of MPs in effluents may contribute significantly to MP pollution in the environment due to the large volumes being treated [30]. In the case of strong rainfall events, however, WWTPs represent another serious risk in terms of pollution when there is overflow of untreated wastewater or the capacity of wastewater treatment plants is exceeded. Another source of risk is related to the leachates generated at the temporary storage and transfer stations located at the plants and in the collection network [41]. MPs trapped in sewage sludge can return to the environment if the sewage is reutilized for agriculture, land filling, or green construction, and are thus able to run off again into watercourses. The use of sewage sludge as a fertilizer for agricultural applications is often economically advantageous and is common in many developed regions, since regulations do not usually consider MPs as harmful substances and allow their utilization. In Europe and North America about 50% of sewage sludge is processed for agricultural use [42].

Atmospheric fallout can also transport anthropogenic fibres into the water. In a study carried out by Dris et al. [43] in the area around Paris, an average atmospheric fallout of  $110 \pm 96$  particles/m<sup>2</sup>/day was estimated, with around 29% of fibres containing plastic polymers.

MPs have been classified as “emerging contaminants” by Scotland's centre of Expertise for Waters [27] due to their small dimensions which make it difficult to remove them from the environment and for their potential to be ingested by organisms [3,26]. If ingested they can reduce feeding, decrease ecophysiological functions, and introduce chemicals into the food chain [5,9]. In addition, MPs can

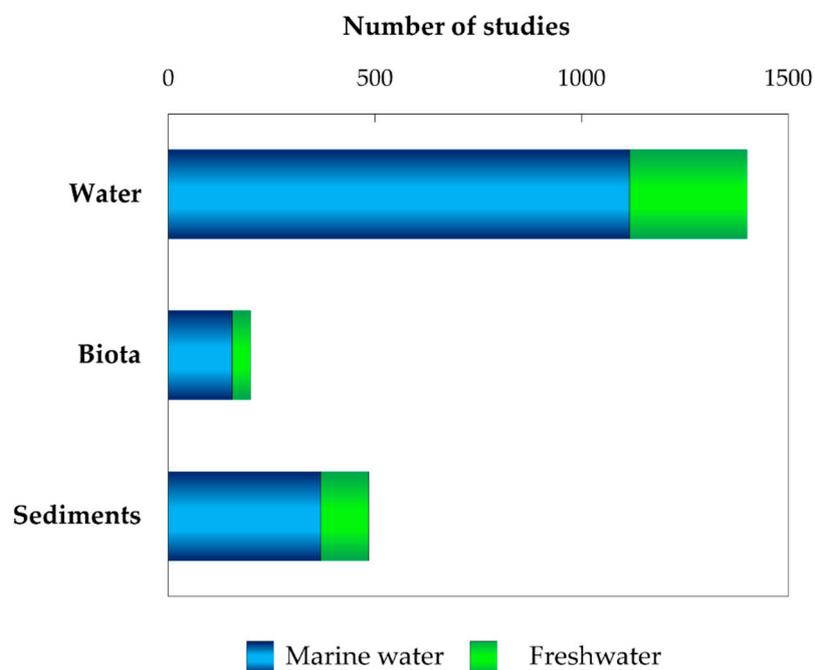
adsorb harmful algal species [44] and persistent organic pollutants which are present in water column or in sediments [45].

Concern about MPs has led to the development of management guidelines by several organizations. The United Nations Expert Panel of the United Nations Environmental Programme (UNEP), the United Nations Environment Programme/Mediterranean Action Plan (UNEP-MAP), the Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR), and the Baltic Marine Environment Protection Commission—Helsinki Commission (HELCOM) have developed guidelines for assessing marine litter including microplastics [46]. However, MPs continue to pose a real threat for the economy, ecosystem conservation, and biodiversity. It has been estimated that the amount of MPs will continue to increase if nothing is done to solve the problem [47].

### 3. Microplastics in Surface Freshwater Systems

It is plausible that MPs are present as a contaminant in surface water worldwide, but their concentration and distribution in each environmental sphere (water column, water surface, sediments) depend on different variables, e.g., geographical position, wind, and currents. An aspect common to all the areas is that around 90% of recovered plastics are of low- or high-density polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), or polyethylene terephthalate (PET) [12].

Whereas studies about plastics and MPs in marine environment are relatively abundant and generally quite recent [6,8,44,46,48–51], limited research exists on plastic pollution in freshwater systems [52]. In Figure 2 we report the records present in the Scopus database (<https://www.scopus.com/home.uri>) regarding microplastics in different water environments and their subdivision into marine water and freshwater systems, highlighting the higher abundance of studies in marine water ecosystems.



**Figure 2.** Studies concerning the contamination of different water compartments by microplastics (MPs), with emphasis on marine and freshwater ecosystems. Data from Scopus database.

With specific reference to the European freshwater ecosystems, scientific studies were performed for different lakes (Table 1) [14,53–55]. A monitoring campaign was also carried out to evaluate MPs presence in the main Italian lakes by the environmental association “Legambiente” [56,57], reflecting the growing concern about this issue.

Plastic particles in lakes and rivers may have different origins: tributaries, on-water activities, tourism, and improper dumping of disused or abandoned plastic wastes of terrestrial origin. Furthermore, stormwater events, rainwater drainage, flooding, and wind can collect and transport MPs that have been dispersed or generated on the land to freshwater ecosystems. Plastic litter released on the land can be efficiently fragmented via processes similar to those on sea beaches, such as photo- and oxidative degradation and physical damage by human activities such as plastic that is broken to fragments by crushing by vehicles [58]. Moreover, rivers and lakes can become active secondary MP producers via the fragmentation of the plastic litter abandoned on riverbanks, floodplains, and beaches that has been rendered brittle by weathering and is easily breakable by the water current and waves, similarly to what happens at sea [13,58].

The quantity of MPs which can be present in lakes depends on the water residence time and size of the water body, type of waste management used, and amount of sewage overflow [59]. Nonetheless, the most important factors influencing the concentration of MPs in water are human population density in the area and proximity to the urban centre. Even though northern Italy and North America are two very different areas, the analogy of MP distribution in lakes could be interesting. In Lake Garda, concentrations around 100 items/m<sup>2</sup> were detected in southern shores and around 1100 items/m<sup>2</sup> in northern sediments [53], while in the Laurentian Great Lakes the concentration of MPs ranged from 0 to 34 plastic fragments/m<sup>2</sup> at the shoreline of Lake Huron and from 0.2 to 8 items/m<sup>2</sup> in Lake Erie [9]. From the evaluation of these data and by considering geo-political characteristics of these lakes, it emerges that higher quantities of MPs are principally related to the magnitude of human activity. Beside this, distribution of MPs depends also on large-scale forces such as currents driven by wind [3,20,60]. In the case of Lake Garda, shores downwind can have greater quantities of MPs than shorelines upwind.

MPs are ubiquitous in freshwater systems and they have also a vertical distribution along the water column, with a top-down distribution gradient, even in benthic areas. Density of plastic affects the partitioning of organic matter and contaminants in surface water, the water column, and sediments [11]. Plastics with higher density than water are expected to sink, but some studies have shown that low-density polymers are also deposited on the substrates of aquatic basins due to biofouling by bacteria, algae, and other organisms [61]. Due to biological processes the density of microplastics in sediments may be several orders of magnitudes higher than that in surroundings water [62].

**Table 1.** MP concentrations in water and sediments of some EU lakes.

LAKES	WATER	SEDIMENTS	REFERENCE
Garda	$2.5 \times 10^4 \pm 14,900$ p/m <sup>2</sup>	1108 ± 983 p/m <sup>2</sup> (north) 108 ± 55 p/m <sup>2</sup> (south)	Imhof et al., 2013 [53]; Sighicelli et al., 2018 [56]
Maggiore	$3.83 \times 10^4 \pm 20,666$ p/m <sup>2</sup>	average: 1100 ± 2300 p/m <sup>2</sup> min-max: 20–6900 p/m <sup>2</sup>	Faure et al., 2015 [54]; Sighicelli et al., 2018 [56]
Iseo	$4.04 \times 10^4 \pm 20,333$ p/m <sup>2</sup>		Sighicelli et al., 2018 [56]
Geneva	$4.81 \times 10^4$ p/km <sup>2</sup>	average: 2100 ± 2000 p/m <sup>2</sup> min-max: 78–5000 p/m <sup>2</sup>	Faure et al., 2012 [14]; Faure et al., 2015 [54]
Constance	61,000 ± 12,000 p/km <sup>2</sup>	average: 320 ± 220 p/m <sup>2</sup> min-max: 140–620 p/m <sup>2</sup>	Faure et al., 2015 [54]
Neuchâtel	61,000 ± 24,000 p/km <sup>2</sup>	average: 700 ± 1100 p/m <sup>2</sup> min-max: 67–2300 p/m <sup>2</sup>	Faure et al., 2015 [54]
Zurich	11,000 ± 2600 p/km <sup>2</sup>	average: 460 ± 350 p/m <sup>2</sup> min-max: 89–800 p/m <sup>2</sup>	Faure et al., 2015 [54]
Brienzi	36,000 ± 23,000 p/km <sup>2</sup>	average: 2500 ± 3000 p/m <sup>2</sup> min-max: 89–7200 p/m <sup>2</sup>	Faure et al., 2015 [54]
Bolsena	-	1922 ± 662 p/m <sup>2</sup>	Fischer et al., 2016 [55]
Chiusi	-	2117 ± 695 p/m <sup>2</sup>	Fischer et al., 2016 [55]

### *MPs in Rivers, A Major Route for MP Transport*

Rivers play an important role in the transport of plastic into lakes, seas, and oceans [31,63,64]. It is nowadays broadly accepted that the dominant input of plastic into oceans is from land-based sources, whereas only a minority is produced directly at sea from vessels, platforms, fisheries, and water breeding [65].

As an example, the mass load of the principal European rivers has been estimated in numerous studies. Lechner et al. [66] reported that the Europe's second largest river, the Danube, can release an average amount of  $316.8 \pm 4664.6$  items per  $1000 \text{ m}^3$  into the Black Sea, which results in a mass load of  $4.8 \pm 24.2 \text{ g}$  per  $1000 \text{ m}^3$ . They estimated an average input of about  $7.5 \text{ g}$  per  $1000 \text{ m}^3$ , resulting in a total entry of 4.2 tons per day at the average flow rate (1533 tons per year). A larger overview is given by the results of a European Commission DG Environment-funded project [67], indicating that the river transports 20–30 tons of plastic litter per year to the North Sea and that the Italian Po river is estimated to transport about 120 tons of plastic litter per year to the Mediterranean Sea.

Nevertheless, although high levels of plastic pollution are found in European rivers, worldwide the major inputs of oceanic MPs come from Asia. A recently published global model, computed considering geospatial information on waste management, population density, and hydrology, estimates that between 1.15 and 2.41 million tons of plastic are currently flowing into the oceans through the riverine system every year [63]. The rivers which pollute the most, as predicted by the model and using information derived from observational studies [68], are located in Asia: the Yangtze, Xi, and Huangpu rivers (China) and the Ganges river (India and Bangladesh) occupy some of the top positions. Asian rivers represent 86% of total global input, whereas European rivers account for only the 0.28%, with a range of 2310–9320 tons per year. In fact, Yangtze river samples in the Wuhan region, the largest city in central China, showed a MP concentration of  $2516.7 \pm 911.7$  particles per  $\text{m}^3$ , an incredibly high number compared to the 0.3168 particles per  $\text{m}^3$  found in the Danube (Austria [66]) and 0.028 particles per  $\text{m}^3$  found in the Tamar Estuary (England [69]).

Research within the European region has been focused on MP concentrations both in water and in sediments of different major rivers: the Seine [31,70]; the Danube [66]; the Rhine [37,67]; the Thames [71]; the Po [67]; the Tamar Estuary [69]; the Solent estuarine complex [72]; and the delta and canals of Amsterdam's rivers [73]. Concentrations of MP found in rivers all over the globe are shown in Table 2. In this table is possible to note that different authors use different units to express MP densities. As discussed in the conclusion of this review paper, this is one of the main problems affecting this research area. Applying different sample protocols and experimental designs leads to the assessment of MP presence using several different units of measurement. In the five considered studies on the evaluation of MPs in waters, only the authors of [37,69] sampled floating plastic particles using only manta nets, whereas the authors of [66] sampled MPs in the Danube using stationary driftnets. The authors of [67,70] assessed the presence of MPs by using combined sample methods. With regard to MPs in sediments there are also discrepancies with respect to measurement units: while the author of [10] expressed the number of MPs as particles/ $\text{m}^2$ , the authors of [71] considered the number of MPs per 100 g of sediment. The lack of a standardized sample method is reflected in the difficulty in understanding the conditions of MP contamination and in the inability to compare different sites.

Beside the difficulties behind the comparison between measurements, rivers present a high level of complexity when evaluating MP concentrations, especially when it is not possible to sample a whole transect, as frequently occurs for logistical problems. Many different phenomena can change the measured concentration: point sources can determine a mosaic situation since complete mixing may not occur until a considerable distance downstream of the confluence is reached; currents, water turbulence, and wind can accumulate floating debris in meanders; and braking of river flow can produce sinking of denser fragments or biofouling if the braking is associated with eutrophication, thus causing variations along the river course. In addition, each set of measurements represents a "snap-shot", which makes it very difficult to estimate the total flux of particles averaged over a representative time period [67,74].

**Table 2.** MP concentrations in water and sediments of some EU rivers.

LOCATION	COMPARTMENT	MPs DENSITIES	REFERENCE
Danube river, Austria, Europe	Surface water	Average: $0.3168 \pm 4.6646 \text{ p/m}^3$	Lechner et al., 2014 [66]
Rhine river, Germany, Europe	Surface water	Average: $892,777 \pm 1,063,042 \text{ p/km}^2$	Mani et al., 2015 [37]
Rhine river, Germany, Europe	Sediment	Min–max: $1784\text{--}30,106 \text{ p/m}^2$	Klein et al., 2015 [10]
Seine river, France, Europe	Surface Water	Min–max: $0.28\text{--}0.47 \text{ p/m}^3$	Dris et al., 2015 [31]
Po river, Italy, Europe	Surface water	Average: $2,043,069.8 \pm 336,637.4 \text{ p/km}^2$	Van der Walt et al., 2015 [67]
Tamar Estuary, United Kingdom, Europe	Surface water	$0.028 \text{ p/m}^3$	Sadri and Thompson, 2014 [69]
Thames river, United Kingdom, Europe	Sediment	Min–max: $18.5 \pm 4.2\text{--}66 \pm 7.7 \text{ p/100 g}$	Horton et al., 2017 [71]

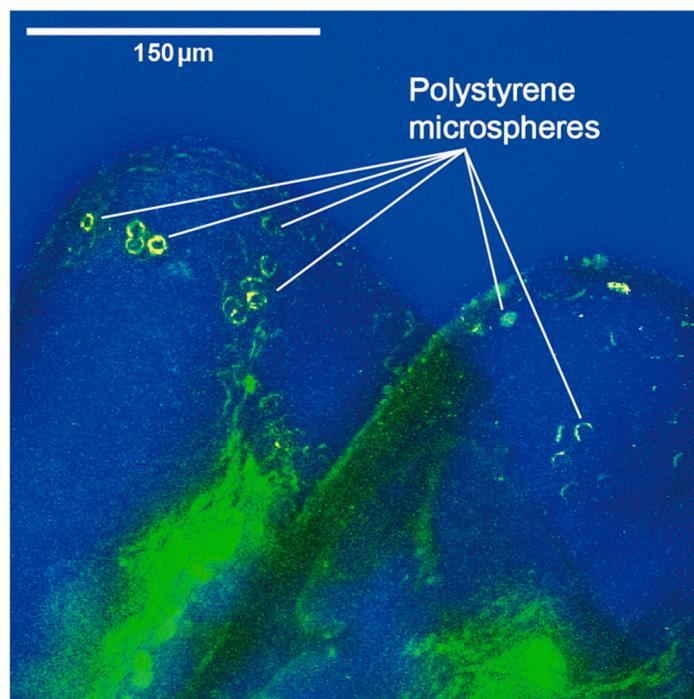
The land-use composition of the territory that composes the watershed has been demonstrated to affect the MP concentration in rivers. For example, the MP concentrations along the Rhine river increase with the river flow towards the sea, except for the tidal zone [37]. A correspondence has been found between population density in the river basin, land use, and MP concentration both in the estuary of Chesapeake Bay, United States [75], and in the Japanese riverine system [58]. Furthermore, various recent publications designed mathematical models of riverine MP transport by using waste management, population density, and hydrological information [63,64], highlighting the relationship between MP concentration and waste management, basin characteristics, and the hydrological regime. Some exceptions have been reported, for example, the Dalälven river (Sweden), a clean river that flows in a scarcely inhabited basin, presents loads of plastic debris higher than expected considering population density and waste management practice. This discrepancy has been linked to intense recreational fishing activity [67].

#### 4. Ecotoxicology of MPs in Freshwater

##### 4.1. MPs in Freshwater Food Webs

The fact that MPs can enter various aquatic organisms at different trophic levels has been well-established by different studies [28,45,76–82]. The two main routes of MPs uptake are respiration and ingestion [83]. Most studies, in fact, focus on the potential bioavailability of MPs to organisms in the food web [5,48]. Indeed, within marine and freshwater food webs, MPs have been detected in the gut of a number of taxa of organisms at nearly every trophic level [84]. MPs in freshwater may have domino effects on terrestrial ecosystems through the food web, since many freshwater organisms are preyed upon by terrestrial organisms [59]. A positive correlation between the amount of ingested plastic in birds and PCB tissue concentrations has been reported [85]. Moreover, this effect presents a critical issue for human consumption [86].

In addition, adherence can facilitate MP uptake [87]: some studies have revealed that MPs are present not only in organs such as the liver, stomach, or breathing apparatus, but also on the body of zooplankton and mussels [88,89]. For example, the authors of [88] carried out a study to assess the MP exposure of *Carcinus maenas*, confirming the intake of microplastics by crabs through the gills (Figure 3).



**Figure 3.** Raman microscopy image (imaged at  $3050\text{ cm}^{-1}$ ) of two gill lamellae tips of *Carcinus maenas*, presenting 8- $\mu\text{m}$  microspheres adhering to the outside of the surface. Reprinted with permission from Watts et al. [76]. Copyright 2014 American Chemical Society.

The toxic effect of MPs in freshwater systems is not well understood [12,90], although it has been estimated that between 32% and 100% of freshwater invertebrates organisms ingest MPs [88].

The possibility to ingest MPs by organisms depends on their abundance and particle size, the presence of natural prey, and the physiological and behavioural traits of the organism. Indeed, the size of particles that can be captured depends on organism physiology and morphology [45]. An example is represented by *Daphnia magna*, which usually feed on algae. Although they can consume particles between 1 and 70  $\mu\text{m}$  in size [91], *Daphnia* organisms are unable to distinguish range and quality of particle size [92], implying a lack of selection and likely ingestion of MPs. In general, ingestion does not directly imply fatal effects for organisms, but chronic effects (e.g., oxidative stress, starvation) can be problematic [33].

#### 4.2. Interaction of MPs with Micropollutants

MPs can be considered direct sources for toxic chemicals: synthetic polymers do not directly provide the desired material properties, and therefore, additives are used to improve physical plastic properties [18]. Softeners, stabilizers, blowing agents, and flame retardants added to polymers can be either directly toxic or have endocrine disruptor properties (e.g., phthalates, nonylphenol, bisphenol A, and brominated substances) [49]. These substances are weakly bound to the polymer, so they will leach out of the plastic over time. It is therefore expected that these pollutants can be transferred from plastic particles to the water ecosystems by desorption processes, consequently negatively affecting organisms [18,93].

As well as sources, microplastics can be sinks of waterborne contaminants: because of the nature of the plastic surface, hydrophobic pollutants (PCBs, DDT, PAHs, dioxins, metals and other PBT substances) are adsorbed according to hydrophobic partitioning [94] onto pellets from the surrounding water [95], as occurs for natural particulate organic matter (POM). One research study [95] has reported the presence of low-chlorinated congeners of PCB (CB-11, 28, 44, 52, 66 and 101) in around 51% of total plastic samples analysed. As a result of this mechanism, organic pollutants can become more

concentrated on the surface of the plastic than in surrounding water [26], with a concentration factor up to 106, similar to that of POM. Moreover, pharmaceuticals and personal care products (PPCPs) have also been observed to be affected by adsorption on MPs [96,97].

The sorption rate of these pollutants on plastics debris can vary among polymers: shape, crystallinity, surface functional groups, and ageing of particles affect the sorption capacity of pollutants [98,99]. For example, polyethylene pellets have a higher affinity for PCB than those of polypropylene [95,100]. The higher affinity of PE is the result of the larger volume of the inertial cavities, which allows the diffusion of compounds into the polymer [101]. Moreover, physicochemical properties of water affect adsorption equilibria [98,102].

While this trend should not be applied to all contaminants, there is evidence that these types of pollutants sorb about 100 times better on plastic debris than on POM [49,84]. Due to the high uptake of contaminants by plastics, even a small amount of contaminated plastic may release a considerable amount of the adsorbed compound. Furthermore, it seems that the increase in surface area accompanying the fragmentation of weathered plastic will increase their capacity for uptake and the transport of hydrophobic compounds [84]. Biofouling can also influence the sorption rate: it may decrease the exchange of substances, as plastic surface can be covered by live organisms [49].

Therefore, MPs can act as vectors in the transport of contaminants in water systems and in organisms by ingestion [4,12,45,85,98,103]. Entering aqueous systems, MPs loaded with contaminants can increase the aqueous concentrations of pollutants by desorption processes, and this may be especially significant in continental freshwater, where concentrations of these chemicals are expected to be higher than in marine systems [104]. As a consequence, attention is needed to evaluate the potential synergic effect with respect to toxicity to water-borne organisms, as well as bioaccumulation through the food web [28].

The interaction between POPs and MPs is also less well understood for sediments: only a few studies have attempted to combine the adsorption and desorption of POPs by MPs and sediments. For example, Wang and Wang [99] observed a higher adsorption rate of PAHs on different polymer pellets than in natural sediments. Nonetheless, the equilibria between the adsorbed and dissolved phase need to be further investigated to understand the final sink of POPs and consequent ecotoxicological effects [105,106].

In addition to the interaction with organic pollutants, plastic particles can operate both as sinks and sources of metal contaminants. Additives of plastic can also contain trace metals [107], which can be released into the water environment after plastic degradation [108]. Adsorbed metals have also been reported to be adsorbed on MP surfaces in several studies [109–112]. The sorption of metal by MPs seems to be relatively low [110]. In fact, until recent times interactions between metals and microplastics had not been considered. More recently, different studies have reported non-negligible concentrations of toxic elements adsorbed on MPs [110,111,113,114]. The mechanical degradation of MPs (with increasing porosity and surface area) and biofilm growth on aged plastics seems to enhance the metal adsorption on plastic particles as well as the values of dissolved organic carbon [112,115,116]. This phenomenon can easily increase toxic element bioavailability and alter the uptake route to water organisms, especially for the benthonic community, since sediment is the final sink of anthropogenic metals (e.g., Pb, Cd, Hg) [117]. Moreover, in peculiar geological settings, even naturally occurring trace elements can be present at high concentrations [118,119]. Recent studies investigated the adsorption and desorption kinetics of metals on MPs in order to clarify their possible interactions in the water environment, observing different polymers and tuning the physicochemical properties of water (i.e., pH, salinity, redox potential) [116,120–122], but partitioning with the water–sediment interface is still poorly investigated [123]. Consequently, the dynamics of this unexpected interaction between plastic and metals need to be further investigated, especially for ecotoxicological investigation on freshwater communities.

Nonetheless, the role of MPs as vectors of contaminants still presents contradictory interpretations [124,125]: while some ecotoxicological studies have determined that plastic can

have a synergic effect with respect to pollutants (e.g., [120,126,127]), other studies have reported negligible changes in contaminant uptake in the presence of MPs (e.g., [128–130]).

Moreover, some ecotoxicological studies (e.g., [131,132]) have indicated that the adsorption of pollutants on MP surfaces presents an antagonistic interaction with uptake by the organism since MPs act as scavengers of the dissolved pollutant, which then results less available for the target organism. Nonetheless, these assumptions were made in laboratory conditions. Considering the sedimentation of MPs in natural conditions, MPs loaded with pollutants can sink and accumulate in sediments, causing a higher concentration in this compartment and increasing the toxicological risk for benthic fauna. Therefore, the adsorption–desorption equilibria and the fate of pollutants adsorbed on microplastics in environmental conditions need to be addressed in future studies [124].

#### 4.3. Ecotoxicological Effects of MPs on Benthic Organisms

As observed above, studies examining the ecotoxicological effect of MPs are still mostly focused on pelagic organisms, while knowledge on toxic effects on benthic organisms is still limited [62]. It can be seen by the articles published since 2010 for all the scientific journals indexed in Scopus that in total 44 studies have been published concerning the impacts of plastic particles on benthic organisms, with the majority on benthic marine organisms and only 10 on freshwater benthos.

Table 3 shows the principal studies aiming to observe the effects of uptake of different MP types on freshwater benthic organisms, specifying the effect investigated and eventual evidence. The effects of MP intake in freshwater benthos is an important issue, since benthic invertebrates contribute up to 90% of fish prey biomass [133], and sediment becomes a sink of different organic and inorganic pollutants [134,135]. Therefore, bioaccumulation of MPs in sediment can enhance contaminant biomagnification. Moreover, MP ingestion by benthic freshwater invertebrates could impact sediment bioturbation [12].

As shown in Table 3, most of the research has been carried out using amphipods, which are a key component in aquatic food webs, acting as carriers of nutrients and energy to higher trophic levels [136]. Negative effects have been assessed for polyethylene (PE), polystyrene (PS), polypropylene (PP), polyvinylchloride (PVC), polyamide (PA), and polyethylene terephthalate (PET), which are the most commonly diffused plastics [20,48]. For these laboratory studies both particles and microfibers were considered: a research study carried out by Berglund et al. [137] showed that much of the plastic found inside mussels is synthetic fibre, which can be ascribed to the MPs found in textiles [138].

The risk posed by plastic pollution to benthic fauna is considerably high due to their inability to discriminate between MPs and food particles [139,140]; ingestion has been confirmed by the presence of microplastics in the gut of organisms. Experiments have shown that MP ingestion adversely affects the feeding rate. The presence of MPs in the digestive tract gives a sense of satiety, causing a reduced uptake of food and decreased energy intake, causing starvation [141,142]. As a consequence, growth, survival, fecundity, and reproduction rate are also negatively affected, impacting general fitness [141,143–146]. This lower energy production is also evident in the low emergence rate of sediment-dwelling organisms [145].

Besides accumulation in vital organs such as the gut, the smallest MPs are also able to penetrate the biological tissues in mussels [147]. The effects of MPs in haemolymph still need to be fully explored, but a notable histological change is observable in mussels [87]. This phenomenon makes mussels a useful bioindicator of MP pollution in freshwater [147].

**Table 3.** Studies related to the effects of MPs on benthic species.

ORDER	SPECIES	POLYMER	UPTAKE	EGESTION	PARAMETER	EFFECTS	REFERENCES
Amphipoda	<i>Gammarus fossarum</i>	PMMA <sup>1</sup>	+		1-Feeding rate 2-Assimilation efficiency 3-Weight change	1-No sign. effect 2-Decrease of efficiency 3-Weight loss	Straub et al., 2017 [141]
Diptera	<i>Chironomus tepperi</i>	PE	+		1-Survival 2-Growth 3-Emergence rate	1-MP size-dependent 2-MP size-dependent 3-Decrease from 90% to 17.5%	Ziajahromi et al., 2018 [145]
Myida	<i>Dreissena polymorpha</i>	PS	+		1-Cellular stress 2-Oxidative damage 3-Neurogenotoxicity	1-No sign. effect 2-Increase of CAT <sup>1</sup> and decrease of GPx <sup>2</sup> 3-Increase of DOP <sup>3</sup>	Magni et al., 2018 [147]
Cladocera	<i>Daphnia magna</i>	PS	+		1-Filtration capacity	1-Decrease of filtration capacity	Colomer et al., 2019 [148]
Amphipoda	<i>Gammarus pulex</i>	PS	+		1-Mortality 2-Growth 3-Feeding rate	1-No sign. effect 2-Reduction in size 3-No sign. effect	Redondo-Hasselerharm et al., 2018 [142]
Amphipoda	<i>Hyaella azteca</i>	PS	-	-	1-Mortality 2-Growth 3-Feeding rate	1-No sign. effect 2-No sign. effect 3-No sign. effect	Redondo-Hasselerharm et al., 2018 [142]
Isopoda	<i>Asellus aquaticus</i>	PS			1-Mortality 2-Growth 3-Feeding rate	1-No sign. effect 2-No sign. effect 3-No sign. effect	Redondo-Hasselerharm et al., 2018 [142]
Sferida	<i>Sphaerium corneum</i>	PS			1-Mortality 2-Growth 3-Feeding rate	1-No sign. effect 2-No sign. effect 3-No sign. effect	Redondo-Hasselerharm et al., 2018 [142]
Lumbriculidae	<i>Lumbriculus variegatus</i>	PS		+	1-Mortality 2-Growth 3-Feeding rate	1-No sign. effect 2-No sign. effect 3-No sign. effect	Redondo-Hasselerharm et al., 2018 [142]
Oligochaeta	<i>Tubifex</i> spp.	PS		+	1-Mortality 2-Growth 3-Feeding rate	1-No sign. effect 2-No sign. effect 3-No sign. effect	Redondo-Hasselerharm et al., 2018 [142]

Table 3. Cont.

ORDER	SPECIES	POLYMER	UPTAKE	EGESTION	PARAMETER	EFFECTS	REFERENCES
Rhabditidae	<i>Caenorhabditis elegans</i>	PA, PE, PP, PVC, PS	+		1-Mortality 2-Body length 3-Reproduction 4-Intestinal Ca levels	1-Sign. effect (size-related for PVC and PS) 2-Reduction 3-Inhibition 4-Decrease (concentration-related for PS)	Lei et al., 2018 [149]
Amphipoda	<i>Gammarus fossarum</i>	PA and PS	+	+ (PA)	1-Assimilation efficiency 2-Feeding rate 3-Weight change 4-Mortality	1-Reduced for PA. No effect for PS 2-No sign. effect 3-No sign. effect 4-Increase	Blarer et al., 2016 [139]
Unionida	<i>Anodonta anatina</i>	Microfibers, PA	+				Berglund et al., 2019 [137]
Amphipoda	<i>Hyalella azteca</i>	PE and PP	+	+	1-Mortality 2-Growth 3-Reproduction (PE)	1-Dose-dependent 2-No sign effect (PE). Dose-dependent (PP) 3-decrease	Au et al., 2015 [150]
Littorinimorpha	<i>Potamopyrgus antipodarum</i>				1-Mortality 2-Dimension 3-Reproduction 4-Embryos without shell	1-No sign. Effect 2-Decrease in juveniles 3-No sign. Effect 4- No sign. effect	Imhof and Laforsch, 2016 [151]
Rhabditidae	<i>Caenorhabditis elegans</i>	nanoPS	+	+	1-Intestinal ROS <sup>4</sup> production 2-Locomotion behaviour 3-Brood size 4-Intestinal permeability	1-Increase 2-Decrease 3-Reduction of size 4-Increase	Zhao et al., 2017 [152]
Cladocera	<i>Daphnia magna</i>	PET	+		1-Mortality 2-Growth	1-Higher in non-pre-feeders 2-No sign. effect	Jemec et al., 2016 [143]

Sign.: significant; PMMA: Polymethyl methacrylate; PE: polyethylene; PS: polystyrene; PVC: polyvinylchloride; PA: polyamide; PP: polypropylene. <sup>1</sup> enzyme catalase; <sup>2</sup> glutathione peroxidase; <sup>3</sup> neurotransmitter dopamine; <sup>4</sup> reactive oxygen species.

Benthic organisms even comprise filter feeders. For these organisms, MP uptake increases as particle size decreases, showing size-related effects [143,145,149]. Furthermore, effects are more evident in vulnerable organisms or in those in early life stages [145], because adult healthy organisms prefer to ingest larger particles as food [150]. In addition to problems related with feeding inhibition (including consequent effects on reproduction and growth), some authors also consider physiological effects on cellular stress and oxidative damage [146,147]. This issue needs to be further studied to identify the existence of a trend in cellular response correlated with the presence of MPs.

Another mechanism causing toxic effects of MPs is their interaction with different toxic compounds. As stated in Section 4.2, pollutants can be adsorbed on MP surfaces and then ingested by organisms [6,45]. Therefore, MPs can become vectors of contaminants, enhancing biomagnification [3,104]. The alteration and ageing of MPs over different environmental timescales may affect also affect the vector effect of pollutants. If aged and contaminated, particles can have the potential for greater chemical transfer than virgin particles [18,20,31,33,95]. Leaching and desorption from MPs are mechanisms which can highly enhance MP toxicity. Several studies reported in this review (e.g., [18,143]) assessed the risk for benthic organisms to be endangered by leaching of chemicals from MPs. Laboratory experiments showed no significant effects for PET leachate [143], whereas plasticized PVC and polyurethane caused immobility for *Daphnia magna* [18]. Moreover, studies on TWP leachate highlight the reduction of total reproductive output and growth in *Hyalella azteca*, as well as long-term effects (EC<sub>50</sub>, in the range of 0.01–1.8 g rubber/L) on *Ceriodaphnia dubia* [153]. This evidence implies that leaching phenomena are strictly correlated with polymer physicochemical features, leading to contrasting conclusions about the effects on the biota and highlighting the need for more detailed research.

To shed light on the real potential risk of MPs as vectors of pollutants, the complex adsorption–desorption equilibrium of contaminants on MP surfaces needs to be addressed in future studies, especially in those simulating real environmental conditions.

Therefore, from an ecotoxicological point of view, there are many issues that may need to be further addressed in future. A recent review by [124] critically analysed ecotoxicological analyses performed on freshwater fishes and invertebrates, highlighting the lack of harmonization between measurement units for particle concentrations used (i.e., mg/L; mg/kg; particles/L etc.). Moreover, they reported the need for tests with environmentally comparable particle concentrations, since most of the studies reported thus far analysed extremely high concentrations of MPs. These issues need to be addressed to clearly understand the real impact of MPs on the freshwater biota. Moreover, the role of MPs as vectors of contaminants still needs to be validated in the environmental context for benthic fauna, since the complex interaction between MPs and chemicals in water and at the water–sediment boundary is not well understood [100,125].

## 5. Sampling and Analysis of Environmental MPs

To concisely understand the effects of MPs on the freshwater community, real environmental samples need to be collected and analysed for MP abundance, shape, and composition. MPs are difficult to detect because of their small size and heterogeneous physicochemical features, as well as different particle sizes and shapes [28].

After adequate site selection, which is the first element that needs to be evaluated to obtain a representative sample (according to the hydrodynamic conditions and environmental features of the area), different environmental matrices can be collected to evaluate the impact of microplastics on freshwater systems [154].

Unfortunately, no standard protocols exist for sampling plastic particles, making data comparison unreliable [26,155]. A unified MP analysis in aquatic environments, consequently, is needed to overcome this issue [1]. In this review, therefore, we report the most frequently used techniques in literature, discussing advantages and drawbacks of the different methods in order to understand the most reliable analytical tools to assess the ecotoxicological effect of MPs.

### 5.1. Sampling of Floating MPs and Those Along the Water Column

Low-density plastic particles tend to float on the water surface and these have to be collected using a Manta trawl along a transect selected considering the dominant wind directions [14,27,31,56,156]. The sieved material is dried to determine the solid mass in the sample and then subjected to further treatments.

High-density plastic particles with additives or biofilm on the surface tend to sink in sediments or in the deep part of the water column. Therefore, when the main scope of the study is the quantification of MPs in water, surface water sampling alone will inevitably cause underestimation. Sampling of the column water to detect MPs is not a common practice; nonetheless, a few studies found a decreasing concentration of MPs with increasing water depth [157,158].

Sampling of the water column can be done by direct filtration of water or by the acquisition of batch samples [154]. In one study [157], for example, a rotating drum sampler was used. All samples collected in water (both on the surface and along the column) need to be dried before applying other treatments to the solid phase.

### 5.2. Sampling of Beaches and Sediments

Sampling beaches for microplastics requires only a non-plastic sampling tool, a frame, or a corer to specify the sampling area, and a non-plastic container to store the sample [156]. For beaches, samples have to be collected from the surface layer (from 0 to 5 cm depth) of the substrate [1,51,159,160], while subtidal sediments can be sampled from vessels with grabs [26]. Afterward, the sample is dried in an oven at 60–70 °C to stabilize the weight [51,160,161].

### 5.3. Sampling of Biota

Depending on the research question and the target organisms, freshwater biota can be collected in traps, creels, or grasps (benthic invertebrates), by manta or bongo nets (planktonic invertebrates), or by trawls or gill nets (fish, crustaceans, or bivalves) [162]. After collection, living individuals have to be frozen, desiccated, or preserved in fixatives (e.g., formalin or formaldehyde) [73]. Then, general morphological metrics (i.e., wet weight and dimensions), age, and sex of the sampled organisms are analysed, if possible [154].

### 5.4. Sample Processing

After initial preparation, the environmental samples have to undergo further processing before identification of MP can be performed. The processing depends on the matrix of sample collected as well as the main focus of the study.

#### 5.4.1. Separation of MPs from the Inorganic Matrix

For the separation of plastic particles from the inorganic matrix, which is applied for samples collected in sediment and beaches, density fractionation is the most commonly used technique. In this way the sample is mixed with a liquid of defined density, shaken, and stirred. Afterward, the mixture can settle and the low-density particles (MPs) start to float. Usually density separation can be performed using these suggested separation fluids: NaCl (density: 1.2 kg/L), ZnCl<sub>2</sub> (1.6–1.7 kg/L), or NaI (1.6 kg/L) [31]. Because of the higher density, a zinc chloride solution may be considered the most effective media [104,160] for the separation of high-density polymers such as PVC, but it is important to take into account that ZnCl<sub>2</sub> is corrosive and environmentally hazardous [1]. For this reason, the most commonly used liquid is a saturated sodium chloride (NaCl) solution because it is available, inexpensive, and non-toxic [59].

After the supernatant containing MPs has been filtered on fiberglass filters [159–161], these must be rinsed with distilled water, air-dried, and checked by visual-sorting [159,160]: the first visual inspection

of the whole sample is important to ensure that the separation of MPs from the environmental matrix was successful.

Another method which can be applied for the separation of MPs from the inorganic matrix is the elutriation technique, a process that separates heavy particles from lighter ones using an upward stream of gas or liquid [161]. Following this principle, the Munich Plastic Sediment Separator (MPSS) can be used, as it permits the separation of plastic particles from the environmental matrix [163]. Nonetheless, recoveries for real samples are relatively low and this technique is more expensive than other separation methods [164].

Moreover, for large sediment samples, the use of an electrostatic separator is proposed to separate the nonpolymeric matrix by up to 90% [165]. Particles are transported through an electric field (up to 30 kV) and polymers, which present low electrical conductivity, separate from the conductive matrix. This method is fast and does not require chemicals but does imply another round of separation to purify the sample [154].

#### 5.4.2. Removal of Organic Matter

The digestion of organic material is a necessary step for the analysis of MPs in biological samples. This step is also applied for sediment samples after density separation. The identification of microplastic particles, in fact, could be complicated by organic debris that floats in saturated salt solutions and can adsorb on microplastics during density separation. Thus, the destruction of biological debris is crucial to minimize the possibility of incorrectly quantifying the plastic particles [154].

Chemical digestion uses corrosive reagents to dissolve organic matter, with subsequent separation of the MPs. The most commonly used techniques for organic material removal include acid, alkaline, or oxidative digestion. Moreover, more recently, enzymatic degradation was investigated as a potential alternative treatment.

Acid digestion is generally applied using  $\text{HNO}_3$ , which is most often used since it shows high degradation of organic matter (>98% weight loss of biological tissue) [162]. However, Claessens et al. [161] showed that dissolution of PS and PE occurred, causing underestimation of the results. Hydrochloric acid, in contrast, is not recommended since it is inefficient in organic matter digestion [154].

Another option for digestion is the utilization of alkali (e.g., NaOH or KOH). NaOH has an high efficiency of organic matter digestion, but can also degrade several polymers (e.g., PC, cellulose acetate, PVC, and PET) [166]. KOH, in contrast, is less aggressive. The authors of [167] investigated North Sea fish and added 10 M of KOH solution to the sample. They observed a total destruction of the organic matter after 2–3 weeks. In contrast to NaOH, most polymers are resistant to the usage of KOH (except for cellulose acetate) [154]. To obtain a faster dissolution, avoiding loss of time, digestion at 60 °C overnight was tested with 10 M of KOH [168].

Regarding oxidative digestion,  $\text{H}_2\text{O}_2$  is an efficient oxidizer for removing organic material. Samples are treated with 10% or 30% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) solution [160,169]. The polymers only changed slightly, becoming more transparent, smaller, or thinner when using 30%  $\text{H}_2\text{O}_2$  [51]. However, only 70% of microplastics was extracted with 30%  $\text{H}_2\text{O}_2$ , which was probably due to the formation of foam, causing the loss of material [170].

Another emerging approach to remove organic matter is enzymatic degradation. In this case, microplastics samples are incubated with a mixture of enzymes [169]. This innovative digestion seems very promising for biota samples, since it specifically hydrolyses proteins and breaks down tissues. In contrast to chemical digestion, enzymes avoid any destruction, degradation, or surface change of MPs. This method, nonetheless, is more time-consuming than other types of digestion, making it difficult to apply in large-scale sampling and monitoring [162].

Lusher et al. [162] critically reviewed different methods for biota digestion and reported that KOH and enzymatic digestion protocols are the most widely tested and effective digestive treatments currently available. Nonetheless, since a standard digestion protocol has not yet been presented, to

obtain the most comparable and reliable results the use of multiple digestion protocols is still needed to reach a consensus on the obtained data, comparing the drawbacks and advantages of different digestion processes.

### 5.5. Qualification and Quantification of MPs

The most commonly used techniques for the qualitative identification of plastic particles are spectroscopic methods, in particular FT-IR and Raman spectroscopy [27,56,159]. FT-IR and Raman spectroscopy generally involve a laser light source and return a spectra which can be compared to references or commercially available databases [1]. Both these techniques have the advantages of being non-destructive for the samples, permitting further analyses after spectroscopy. They can also be coupled with optical microscopies, permitting 2D imaging of the samples which can highlight the morphological features of particles [171–173].

In more detail, Fourier-transform infrared spectroscopy (FT-IR) or vibrational spectroscopy is a non-destructive analysis technique by which it is possible to identify materials via the analysis of vibration of chemical bonds. It is based on the absorption of infrared radiation, in the range 0.7–1000  $\mu\text{m}$ , on the materials. From a practical point of view, the spectrometer emits infrared radiation toward the sample with the aim of measuring the intensity of the absorption at different wavelengths. The signal is then automatically processed to obtain spectra which provide qualitative and quantitative information about the chemical groups characterizing the sample (Figure 4). For the analysis of MPs, FT-IR can be conducted in attenuated total reflection (ATR) mode or in transmission mode. It is important to ensure that the filter used in the analysis phase is IR-transparent and the particles are sufficiently thin to avoid the total absorption or scattering of the IR light [173,174].

Raman spectroscopy is (like FT-IR) a vibrational form of spectroscopy, based on inelastic scattering of monochromatic light typically in the near-UV, visible, and near-IR range. The light emitted by the laser source is absorbed by the sample and then re-emitted: part of the re-emitted radiation is not subjected to inelastic scattering, determining the Rayleigh scattering, while part of the radiation loses energy, resulting in a difference in frequency with respect to the Rayleigh emission. This shift provides information about vibrational, rotational, and other low frequency transitions in molecules. Then, turning this signal into a spectrum provides information about the sample composition.

Raman spectroscopy, like FT-IR, is a non-destructive technique. Using Raman spectroscopy, characteristics of the sample such as shape, size, and thickness do not affect the analysis performance.

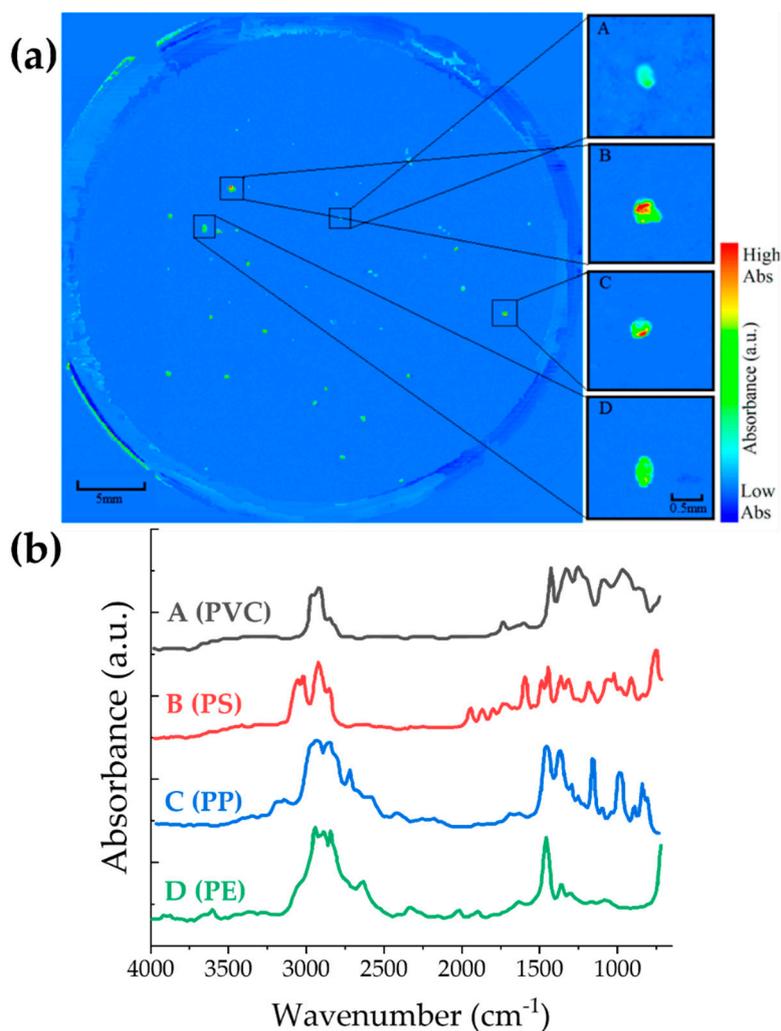
In both the techniques it is possible to analyse only selected filter areas. This implies a need for an extrapolation of the detected amount of microplastics in the analysed area, and this can be very problematic [174].

After the observation of MPs in the sample, both FT-IR and Raman spectroscopy allow the matching of the spectra obtained in the sample with libraries and standards in order to recognize the different polymers and possibly quantify them [171]. With the help of chemometric tools this process could be automatized. As an example, the authors of [175] proposed a method based on FT-IR and partial least squares regression (PLSR) to identify LDPE and PET particles directly in sediments, leading to semi-quantification. These results are encouraging for the use of this technique for a direct analysis even in complex environmental matrices, but so far valuable results can be obtained only in samples with very high microplastic concentrations (>1% in weight).

Comparing these two spectrometric approaches, Raman spectroscopy permits the analysis of particles down to 1  $\mu\text{m}$ , while FT-IR is suitable only for analysing particles in the size range of 10–20  $\mu\text{m}$  [158,173]. The principal characteristics of both techniques are summarized in Table 4.

Raman spectroscopy is less-often used as compared to FT-IR due to its drawbacks: long measurement time caused by the weak intensity of Raman scattering, and proneness to spectral distortion induced by fluorescence which is more marked with the presence of impurities (e.g., colouring agents and degradation products) [176]. On the other hand, a drawback of FT-IR is the

high interference of water. Moreover, FT-IR presents broader bands and lower sensitivity to non-polar functional groups compared to Raman spectroscopy [171,176].



**Figure 4.** Example of MP particle recognition through micro-IR mapping (adapted from Tagg et al. [172], content under creative common license). (a) False-colour images of the total absorbance in the spectra window (4000–750  $\text{cm}^{-1}$ ) showing 4 different microplastic types. Fragments of different polymer types have been selected and magnified. (b) FT-IR spectra of selected and magnified microplastic fragments, permitting the recognition of materials (A: PVC; B: PS; C: PP; D: PE).

A direct comparison between the two techniques was presented for real samples [174], whereby the pros and cons of both techniques were observed. It was concluded that FT-IR is preferable for routine analysis, especially for the fast observation of coarser particles (50–500  $\mu\text{m}$ ). The best results can be obtained only by combining both methods, especially for smaller fractions (<50  $\mu\text{m}$ ), since Raman spectroscopy results more time-consuming but more reliable for small particles.

**Table 4.** Principal characteristics of FT-IR and Raman spectroscopy.

Characteristics	FT-IR	RAMAN SPECTROSCOPY
Typology	Spectroscopic technique	Spectroscopic technique
Operation mode	Absorption of IR radiation	Inelastic scattering of monochromatic light
Source of light	Laser	Laser
Range of light	Infrared	UV, visible, NIR
Detection limit	10–20 $\mu\text{m}$	1 $\mu\text{m}$
Visual response	Spectra	Spectra

After the qualification of polymers in samples, it is possible to investigate the morphological structure of plastic particles by using EDS-SEM microscopy [27,99,159]. This technique can provide extremely clear and high-magnification images of plastic-like particles, permitting the observation of the surface texture of the particles in order to discriminate microplastics from organic particles. Moreover, elemental analysis with energy-dispersive X-ray spectroscopy (EDS) permits the discrimination of plastics from inorganic particles [171,177]. Nonetheless, this technique is more expensive and time-consuming than the others presented, and is only applied for the detailed analysis of small particles [171].

Other methods which can be applied are thermo-analytical approaches such as gas-chromatography coupled to mass spectrometry (GC-MS) or pyrolysis GC-MS [1,59]. The drawback of thermo-analytical techniques is that the sample is destroyed by the analysis and thus will not be available for further investigations. Moreover, these techniques are more time-consuming and data interpretation is limited since only bulk analysis can be performed [171].

Finally, quantification of MPs can be performed by microscopic visual sorting, and results can be expressed as items/kg of (dry) sediment. In this phase it is also interesting to categorize MPs according to shape: fragments, pellets, films, foam, and fibres [169].

## 6. Future Perspectives in Microplastic Research for Freshwaters

MPs represent a global concern because of their distribution and the impact they could have on freshwater ecosystems. From an ecotoxicological point of view, MPs have been detected in different aquatic organisms since they can be ingested, and accumulation along the food web has been observed in different settings [86,90,144]. Moreover, plastic particles can act as vectors of toxic chemicals to the biota [106,178]. Nonetheless, the toxic effects of MPs, especially regarding adsorption–desorption equilibria in environmental conditions, need to be investigated further, since the vector effect of MPs for pollutants still presents contradictory results [124,125].

Through this review, the poor understanding of the effects of MPs on the sediment and benthic fauna was highlighted. Extensive studies are needed in this field: sediment is the final sink of different plastic particles and benthic fauna represent an important link in the whole trophic web [150,179]. Therefore, an understanding of the interaction of MPs and biota in this compartment will also shed light on the other trophic levels [143,180].

Nonetheless, the first issue which needs to be addressed in order to investigate the general impact of MPs in the freshwater system is the harmonization of sampling and pre-treatment protocols for MP analysis, especially for complex environmental matrices [155]. Currently, authors are applying different analysis protocols, making data comparison complicated. Moreover, a harmonization of the different measurement units of microplastic concentrations is necessary to allow clear data comparisons [154]. With the setting of a standard method, data will be comparable, permitting a comparison of the status of different freshwater systems in order to manage the impacts of MPs on ecosystems. Therefore, studies

aiming to compare different treatments and analysis techniques are strongly encouraged (e.g., [166,174]), in order to reach a clear understanding of the best techniques for different environmental matrices.

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## References

- Ivleva, N.P.; Wiesheu, A.C.; Niessner, R. Microplastic in Aquatic Ecosystems. *Angew. Chemie-Int. Ed.* **2017**, *56*, 1720–1739. [CrossRef]
- Andrady, A.L.; Neal, M.A. Applications and societal benefits of plastics. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 1977–1984. [CrossRef]
- Barnes, D.K.A.; Galgani, F.; Thompson, R.C.; Barlaz, M. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 1985–1998. [CrossRef]
- Horton, A.A.; Walton, A.; Spurgeon, D.J.; Lahive, E.; Svendsen, C. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci. Total Environ.* **2017**, *586*, 127–141. [CrossRef]
- Free, C.M.; Jensen, O.P.; Mason, S.A.; Eriksen, M.; Williamson, N.J. High-levels of microplastic pollution in a large, remote, mountain lake. *Mar. Pollut. Bull.* **2014**, *85*, 156–163. [CrossRef] [PubMed]
- Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. Microplastics as contaminants in the marine environment: A review. *Mar. Pollut. Bull.* **2011**, *62*, 2588–2597. [CrossRef] [PubMed]
- PlasticsEurope. Plastics—The Facts 2018. Available online: <https://www.plasticseurope.org/it/resources/publications/619-plastics-facts-2018> (accessed on 28 February 2020).
- Hammer, J.; Kraak, M.H.S.; Parsons, J.R. Plastics in the Marine Environment: The Dark Side of a Modern Gift. In *Reviews of Environmental Contamination and Toxicology*; Whitacre, D.M., Ed.; Springer: New York, NY, USA, 2012; ISBN 9781461434146.
- Wagner, M.; Scherer, C.; Alvarez-Muñoz, D.; Brennholt, N.; Bourrain, X.; Buchinger, S.; Fries, E.; Grosbois, C.; Klasmeyer, J.; Marti, T.; et al. Microplastics in freshwater ecosystems: What we know and what we need to know. *Environ. Sci. Eur.* **2014**, *26*, 1–9. [CrossRef] [PubMed]
- Klein, S. Microplastics in Freshwater Systems: Analysis, Occurrence, and Sorption of Organic Contaminants. Ph.D. Dissertation, Technische Universität Dresden, Dresden, Germany, 2015.
- Li, J.; Liu, H.; Paul Chen, J. Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water Res.* **2018**, *137*, 362–374. [CrossRef]
- Aljaibachi, R.; Callaghan, A. Impact of polystyrene microplastics on *Daphnia magna* mortality and reproduction in relation to food availability. *PeerJ* **2018**, *6*, e4601. [CrossRef]
- Andrady, A.L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62*, 1596–1605. [CrossRef]
- Faure, F.; Corbaz, M.; Baecher, H.; De Alencastro, L.F. Pollution due to plastics and microplastics in lake Geneva and in the Mediterranean sea. *Arch. des Sci.* **2012**, *65*, 157–164.
- Zbyszewski, M.; Corcoran, P.L. Distribution and degradation of fresh water plastic particles along the beaches of Lake Huron, Canada. *Water. Air. Soil Pollut.* **2011**, *220*, 365–372. [CrossRef]
- Eubeler, J.P.; Zok, S.; Bernhard, M.; Knepper, T.P. Environmental biodegradation of synthetic polymers I. Test methodologies and procedures. *TrAC—Trends Anal. Chem.* **2009**, *28*, 1057–1072. [CrossRef]
- Jang, Y.C.; Hong, S.; Lee, J.; Lee, M.J.; Shim, W.J. Estimation of lost tourism revenue in Geoje Island from the 2011 marine debris pollution event in South Korea. *Mar. Pollut. Bull.* **2014**, *81*, 49–54. [CrossRef] [PubMed]
- Lithner, D.; Damberg, J.; Dave, G.; Arsson, Å. Leachates from plastic consumer products—Screening for toxicity with *Daphnia magna*. *Chemosphere* **2009**, *74*, 1195–1200. [CrossRef]

19. Ogata, Y.; Takada, H.; Mizukawa, K.; Hirai, H.; Iwasa, S.; Endo, S.; Mato, Y.; Saha, M.; Okuda, K.; Nakashima, A.; et al. International Pellet Watch: Global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. *Mar. Pollut. Bull.* **2009**, *58*, 1437–1446. [[CrossRef](#)]
20. Browne, M.A.; Crump, P.; Niven, S.J.; Teuten, E.; Tonkin, A.; Galloway, T.; Thompson, R. Accumulation of microplastic on shorelines worldwide: Sources and sinks. *Environ. Sci. Technol.* **2011**, *45*, 9175–9179. [[CrossRef](#)]
21. Blettler, M.C.M.; Abrial, E.; Khan, F.R.; Sivri, N.; Espinola, L.A. Freshwater plastic pollution: Recognizing research biases and identifying knowledge gaps. *Water Res.* **2018**, *143*, 416–424. [[CrossRef](#)]
22. Yao, P.; Zhou, B.; Lu, Y.H.; Yin, Y.; Zong, Y.Q.; Chen, M.T.; O'Donnell, Z. A review of microplastics in sediments: Spatial and temporal occurrences, biological effects, and analytic methods. *Quat. Int.* **2019**, *519*, 274–281. [[CrossRef](#)]
23. Wang, J.; Wang, M.; Ru, S.; Liu, X. High levels of microplastic pollution in the sediments and benthic organisms of the South Yellow Sea, China. *Sci. Total Environ.* **2019**, *651*, 1661–1669. [[CrossRef](#)]
24. Xie, M.; Alsina, M.A.; Yuen, J.; Packman, A.I.; Gaillard, J.F. Effects of resuspension on the mobility and chemical speciation of zinc in contaminated sediments. *J. Hazard. Mater.* **2019**, *364*, 300–308. [[CrossRef](#)] [[PubMed](#)]
25. Schlekot, C.E.; Decho, A.W.; Chandler, G.T. Bioavailability of particle-associated silver, cadmium, and zinc to the estuarine amphipod *Leptocheirus plumulosus* through dietary ingestion. *Limnol. Oceanogr.* **2000**, *45*, 11–21. [[CrossRef](#)]
26. Bergmann, M.; Gutow, L.; Klages, M. *Marine Anthropogenic Litter*; Springer: Berlin, Germany, 2015; ISBN 9783319165103.
27. Blair, R.M. Micro- and Nanoplastic Pollution of Freshwater and Wastewater Treatment Systems. *Springer Sci. Rev.* **2017**, *5*, 19–30. [[CrossRef](#)]
28. Syberg, K.; Khan, F.R.; Selck, H.; Palmqvist, A.; Banta, G.T.; Daley, J.; Sano, L.; Duhaime, M.B. Microplastics: Addressing ecological risk through lessons learned. *Environ. Toxicol. Chem.* **2015**, *34*, 945–953. [[CrossRef](#)] [[PubMed](#)]
29. Carr, S.A.; Liu, J.; Tesoro, A.G. Transport and fate of microplastic particles in wastewater treatment plants. *Water Res.* **2016**, *91*, 174–182. [[CrossRef](#)] [[PubMed](#)]
30. Murphy, F.; Ewins, C.; Carbonnier, F.; Quinn, B. Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. *Environ. Sci. Technol.* **2016**, *50*, 5800–5808. [[CrossRef](#)]
31. Dris, R.; Imhof, H.; Sanchez, W.; Gasperi, J.; Galgani, F.; Tassin, B.; Laforsch, C. Beyond the ocean: Contamination of freshwater ecosystems with (micro-)plastic particles. *Environ. Chem.* **2015**, *12*, 539–550. [[CrossRef](#)]
32. Zepp, R.G.; Erickson, D.J.; Paul, N.D.; Sulzberger, B. Effects of solar UV radiation and climate change on biogeochemical cycling: Interactions and feedbacks. *Photochem. Photobiol. Sci.* **2011**, *10*, 261–279. [[CrossRef](#)]
33. Wright, S.L.; Rowe, D.; Thompson, R.C.; Galloway, T.S. Microplastic ingestion decreases energy reserves in marine worms. *Curr. Biol.* **2013**, *23*, R1031–R1033. [[CrossRef](#)]
34. Jan Kole, P.; Löhr, A.J.; Van Belleghem, F.G.A.J.; Ragas, A.M.J. Wear and tear of tyres: A stealthy source of microplastics in the environment. *Int. J. Environ. Res. Public Health* **2017**, *14*, 1265. [[CrossRef](#)]
35. Wagner, S.; Hüffer, T.; Klöckner, P.; Wehrhahn, M.; Hofmann, T.; Reemtsma, T. Tire wear particles in the aquatic environment—A review on generation, analysis, occurrence, fate and effects. *Water Res.* **2018**, *139*, 83–100. [[CrossRef](#)] [[PubMed](#)]
36. Barbosa, A.E.; Fernandes, J.N.; David, L.M. Key issues for sustainable urban stormwater management. *Water Res.* **2012**, *46*, 6787–6798. [[CrossRef](#)] [[PubMed](#)]
37. Mani, T.; Hauk, A.; Walter, U.; Burkhardt-Holm, P. Microplastics profile along the Rhine River. *Sci. Rep.* **2015**, *5*, 1–7. [[CrossRef](#)] [[PubMed](#)]
38. Cheung, P.K.; Fok, L. Characterisation of plastic microbeads in facial scrubs and their estimated emissions in Mainland China. *Water Res.* **2017**, *122*, 53–61. [[CrossRef](#)] [[PubMed](#)]
39. Mason, S.A.; Garneau, D.; Sutton, R.; Chu, Y.; Ehmann, K.; Barnes, J.; Fink, P.; Papazissimos, D.; Rogers, D.L. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. *Environ. Pollut.* **2016**, *218*, 1045–1054. [[CrossRef](#)] [[PubMed](#)]

40. Gies, E.A.; LeNoble, J.L.; Noël, M.; Etemadifar, A.; Bishay, F.; Hall, E.R.; Ross, P.S. Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada. *Mar. Pollut. Bull.* **2018**, *133*, 553–561. [[CrossRef](#)]
41. Mei, X.; Sui, Q.; Lyu, S.; Wang, D.; Zhao, W. Pharmaceuticals and personal care products in the urban river across the megacity Shanghai: Occurrence, source apportionment and a snapshot of influence of rainfall. *J. Hazard. Mater.* **2018**, *359*, 429–436. [[CrossRef](#)]
42. Nizzetto, L.; Futter, M.; Langaas, S. Are Agricultural Soils Dumps for Microplastics of Urban Origin? *Environ. Sci. Technol.* **2016**, *50*, 10777–10779. [[CrossRef](#)]
43. Dris, R.; Gasperi, J.; Saad, M.; Mirande, C.; Tassin, B. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? *Mar. Pollut. Bull.* **2016**, *104*, 290–293. [[CrossRef](#)]
44. Zettler, E.R.; Mincer, T.J.; Amaral-Zettler, L.A. Life in the “plastisphere”: Microbial communities on plastic marine debris. *Environ. Sci. Technol.* **2013**, *47*, 7137–7146. [[CrossRef](#)]
45. Setälä, O.; Fleming-lehtinen, V.; Lehtiniemi, M. Ingestion and transfer of microplastics in the planktonic food web. *Environ. Pollut.* **2014**, *185*, 77–83. [[CrossRef](#)] [[PubMed](#)]
46. Auta, H.S.; Emenike, C.U.; Fauziah, S.H. Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. *Environ. Int.* **2017**, *102*, 165–176. [[CrossRef](#)] [[PubMed](#)]
47. Forum, W.E. The Fourth Industrial Revolution. In Proceedings of the World Economic Forum Annual Meeting 2016, Davos-Klosters, Switzerland, 20–23 January 2016; pp. 1–47.
48. Thompson, R.C.; Olson, Y.; Mitchell, R.P.; Davis, A.; Rowland, S.J.; John, A.W.G.; McGonigle, D.; Russell, A.E. Lost at Sea: Where Is All the Plastic? *Science* **2004**, *304*, 838. [[CrossRef](#)] [[PubMed](#)]
49. Engler, R.E. The Complex Interaction between Marine Debris and Toxic Chemicals in the Ocean. *Environ. Sci. Technol.* **2012**, *46*, 12302–12315. [[CrossRef](#)] [[PubMed](#)]
50. Cózar, A.; Echevarría, F.; González-Gordillo, J.I.; Irigoien, X.; Úbeda, B.; Hernández-León, S.; Palma, Á.T.; Navarro, S.; García-de-Lomas, J.; Ruiz, A.; et al. Plastic debris in the open ocean. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 10239–10244. [[CrossRef](#)] [[PubMed](#)]
51. Nuelle, M.; Dekiff, J.H.; Remy, D.; Fries, E. A new analytical approach for monitoring microplastics in marine sediments. *Environ. Pollut.* **2014**, *184*, 161–169. [[CrossRef](#)] [[PubMed](#)]
52. Wagner, M.; Lambert, S. *Freshwater Microplastics*; Springer International Publishing: Basel, Switzerland, 2018; ISBN 9783319616148.
53. Imhof, H.K.; Ivleva, N.P.; Schmid, J.; Niessner, R.; Laforsch, C. Contamination of beach sediments of a subalpine lake with microplastic particles. *Curr. Biol.* **2013**, *23*, R867–R868. [[CrossRef](#)]
54. Faure, F.; Demars, C.; Wieser, O.; Kunz, M.; De Alencastro, L.F. Plastic pollution in Swiss surface waters: Nature and concentrations, interaction with pollutants. *Environ. Chem.* **2015**, *12*, 582–591. [[CrossRef](#)]
55. Fischer, E.K.; Paglialonga, L.; Czech, E.; Tamminga, M. Microplastic pollution in lakes and lake shoreline sediments—A case study on Lake Bolsena and Lake Chiusi (central Italy). *Environ. Pollut.* **2016**, *213*, 648–657. [[CrossRef](#)]
56. Sighicelli, M.; Pietrelli, L.; Lecce, F.; Iannilli, V.; Falconieri, M.; Coscia, L.; Di, S.; Nuglio, S.; Zampetti, G. Microplastic pollution in the surface waters of Italian Subalpine Lakes. *Environ. Pollut.* **2018**, *236*, 645–651. [[CrossRef](#)]
57. Legambiente Microplastiche nelle acque interne italiane: Le indagini della Goletta dei Laghi di Legambiente ed Enea. 2018. Available online: [https://www.legambiente.it/sites/default/files/docs/microplastiche\\_nei\\_laghi\\_2016-2017\\_legambiente-enea.pdf](https://www.legambiente.it/sites/default/files/docs/microplastiche_nei_laghi_2016-2017_legambiente-enea.pdf) (accessed on 28 March 2020).
58. Kataoka, T.; Nihei, Y.; Kudou, K.; Hinata, H. Assessment of the sources and inflow processes of microplastics in the river environments of Japan. *Environ. Pollut.* **2019**, *244*, 958–965. [[CrossRef](#)] [[PubMed](#)]
59. Eerkes-Medrano, D.; Thompson, R.C.; Aldridge, D.C. Microplastics in freshwater systems: A review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res.* **2015**, *75*, 63–82. [[CrossRef](#)] [[PubMed](#)]
60. Law, K.L.; Morét-Ferguson, S.; Maximenko, N.A.; Proskurowski, G.; Peacock, E.E.; Hafner, J.; Reddy, C.M. Plastic accumulation in the North Atlantic subtropical gyre. *Science* **2010**, *329*, 1185–1188. [[CrossRef](#)] [[PubMed](#)]
61. Corcoran, P.L. Benthic plastic debris in marine and fresh water environments. *Environ. Sci. Process. Impacts* **2015**, *17*, 1363–1369. [[CrossRef](#)] [[PubMed](#)]

62. Haegerbaeumer, A.; Mueller, M.-T.T.; Fueser, H.; Traunspurger, W. Impacts of Micro- and Nano-Sized Plastic Particles on Benthic Invertebrates: A Literature Review and Gap Analysis. *Front. Environ. Sci.* **2019**, *7*, 7. [[CrossRef](#)]
63. Lebreton, L.C.M.; Van Der Zwet, J.; Damsteeg, J.W.; Slat, B.; Andrady, A.; Reisser, J. River plastic emissions to the world's oceans. *Nat. Commun.* **2017**, *8*, 1–10. [[CrossRef](#)]
64. Schmidt, C.; Krauth, T.; Wagner, S. Export of Plastic Debris by Rivers into the Sea. *Environ. Sci. Technol.* **2017**, *51*, 12246–12253. [[CrossRef](#)]
65. UNEP. *Marine Plastic Debris and Microplastics: Global Lessons and Research to Inspire Action and Guide Policy Change*; United Nations Environment Programme: Nairobi, Kenya, 2016; ISBN 9210601602.
66. Lechner, A.; Keckeis, H.; Lumesberger-Loisl, F.; Zens, B.; Krusch, R.; Tritthart, M.; Glas, M.; Schludermann, E. The Danube so colourful: A potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environ. Pollut.* **2014**, *188*, 177–181. [[CrossRef](#)]
67. Van Der Wal, M.; Van Der Meulen, M.; Tweehuijsen, G.; Peterlin, M.; Palatinus, A.; Kovač Viršek, M.; Coscia, L.; Kržan, A. *Identification and Assessment of Riverine Input of (Marine) Litter*; European Commission: Bruxelles, Belgium, 2015.
68. Zhao, S.; Zhu, L.; Wang, T.; Li, D. Suspended microplastics in the surface water of the Yangtze Estuary System, China: First observations on occurrence, distribution. *Mar. Pollut. Bull.* **2014**, *86*, 562–568. [[CrossRef](#)]
69. Sadri, S.S.; Thompson, R.C. On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England. *Mar. Pollut. Bull.* **2014**, *81*, 55–60. [[CrossRef](#)]
70. Gasperi, J.; Dris, R.; Bonin, T.; Rocher, V.; Tassin, B. Assessment of floating plastic debris in surface water along the Seine River. *Environ. Pollut.* **2014**, *195*, 163–166. [[CrossRef](#)] [[PubMed](#)]
71. Horton, A.A.; Svendsen, C.; Williams, R.; Spurgeon, D.; Lahive, E. Large microplastic particles in sediments of tributaries of the River Thames, UK—Abundance, sources and methods for effective quantification. *Mar. Pollut. Bull.* **2017**, *114*, 218–226. [[CrossRef](#)] [[PubMed](#)]
72. Gallagher, A.; Rees, A.; Rowe, R.; Stevens, J.; Wright, P. Microplastics in the Solent estuarine complex, UK: An initial assessment. *Mar. Pollut. Bull.* **2016**, *102*, 243–249. [[CrossRef](#)] [[PubMed](#)]
73. Leslie, H.A.A.; Brandsma, S.H.H.; van Velzen, M.J.M.J.M.; Vethaak, A.D.D. Microplastics en route: Field measurements in the Dutch river delta and Amsterdam canals, wastewater treatment plants, North Sea sediments and biota. *Environ. Int.* **2017**, *101*, 133–142. [[CrossRef](#)] [[PubMed](#)]
74. GESAMP. Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection Sources. In *Fate and Effects of Microplastics in the Marine Environment: A Global Assessment*; Reports Stud.; International Maritime Organization: London, UK, 2015; Volume 90, p. 96.
75. Yonkos, L.T.; Friedel, E.A.; Perez-Reyes, A.C.; Ghosal, S.; Arthur, C.D. Microplastics in Four Estuarine Rivers in the Chesapeake Bay, U.S.A. *Environ. Sci. Technol.* **2014**, *48*, 14195–14202. [[CrossRef](#)]
76. Watts, A.J.R.; Lewis, C.; Goodhead, R.M.; Beckett, S.J.; Moger, J.; Tyler, C.R.; Galloway, T.S. Uptake and retention of microplastics by the shore crab *Carcinus maenas*. *Environ. Sci. Technol.* **2014**, *48*, 8823–8830. [[CrossRef](#)]
77. Rummel, C.D.; Löder, M.G.J.; Fricke, N.F.; Lang, T.; Griebeler, E.M.; Janke, M.; Gerdt, G. Plastic ingestion by pelagic and demersal fish from the North Sea and Baltic Sea. *Mar. Pollut. Bull.* **2016**, *102*, 134–141. [[CrossRef](#)]
78. Collard, F.; Gilbert, B.; Compère, P.; Eppe, G.; Das, K.; Jauniaux, T.; Parmentier, E. Microplastics in livers of European anchovies (*Engraulis encrasicolus*, L.). *Environ. Pollut.* **2017**, *229*, 1000–1005. [[CrossRef](#)]
79. Pazos, R.S.; Maiztegui, T.; Colautti, D.C.; Paracampo, A.H.; Gómez, N. Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary. *Mar. Pollut. Bull.* **2017**, *122*, 85–90. [[CrossRef](#)]
80. Ory, N.C.; Gallardo, C.; Lenz, M.; Thiel, M. Capture, swallowing, and egestion of microplastics by a planktivorous juvenile fish. *Environ. Pollut.* **2018**, *240*, 566–573. [[CrossRef](#)]
81. Bordós, G.; Urbányi, B.; Micsinai, A.; Kriszt, B.; Palotai, Z.; Szabó, I.; Hantosi, Z.; Szoboszlai, S. Identification of microplastics in fish ponds and natural freshwater environments of the Carpathian basin, Europe. *Chemosphere* **2019**, *216*, 110–116. [[CrossRef](#)] [[PubMed](#)]
82. Sloommaekers, B.; Catarci Carteny, C.; Belpaire, C.; Saverwyns, S.; Fremout, W.; Blust, R.; Bervoets, L. Microplastic contamination in gudgeons (*Gobio gobio*) from Flemish rivers (Belgium). *Environ. Pollut.* **2019**, *244*, 675–684. [[CrossRef](#)] [[PubMed](#)]
83. Weis, J.S. Improving microplastic research. *AIMS Environ. Sci.* **2019**, *6*, 326–340. [[CrossRef](#)]

84. Rowland, S.J.; Galloway, T.S.; Thompson, R.C. Potential for Plastics to Transport Hydrophobic Contaminants. *Environ. Sci. Technol.* **2007**, *41*, 7759–7764.
85. Anderson, P.J.; Warrack, S.; Langen, V.; Challis, J.K.; Hanson, M.L.; Rennie, M.D. Microplastic contamination in Lake Winnipeg, Canada. *Environ. Pollut.* **2017**, *225*, 223–231. [[CrossRef](#)]
86. Akhbarizadeh, R.; Moore, F.; Keshavarzi, B. Investigating microplastics bioaccumulation and biomagnification in seafood from the Persian Gulf: A threat to human health? *Food Addit. Contam. Part A* **2019**, *36*, 1696–1708. [[CrossRef](#)]
87. Kolandhasamy, P.; Su, L.; Li, J.; Qu, X.; Jabeen, K.; Shi, H. Adherence of microplastics to soft tissue of mussels: A novel way to uptake microplastics beyond ingestion. *Sci. Total Environ.* **2018**, *610–611*, 635–640. [[CrossRef](#)]
88. Watts, A.J.R.; Urbina, M.A.; Goodhead, R.; Moger, J.; Lewis, C.; Galloway, T.S. Effect of Microplastic on the Gills of the Shore Crab *Carcinus maenas*. *Environ. Sci. Technol.* **2016**, *50*, 5364–5369. [[CrossRef](#)]
89. Wegner, A.; Besseling, E.; Foekema, E.M.; Kamermans, P.; Koelmans, A.A. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). *Environ. Toxicol. Chem.* **2012**, *31*, 2490–2497. [[CrossRef](#)]
90. Li, J.; Lusher, A.L.; Rotchell, J.M.; Deudero, S.; Turra, A.; Bråte, I.L.N.; Sun, C.; Shahadat Hossain, M.; Li, Q.; Kolandhasamy, P.; et al. Using mussel as a global bioindicator of coastal microplastic pollution. *Environ. Pollut.* **2019**, *244*, 522–533. [[CrossRef](#)]
91. Ebert, D. *Ecology, Epidemiology, and Evolution of Parasitism in Daphnia*; National Center for Biotechnology Information: Bethesda, MD, USA, 2005; ISBN 1932811060.
92. DeMott, W.R. The role of taste in food selection by freshwater zooplankton. *Oecologia* **1986**, *69*, 334–340. [[CrossRef](#)] [[PubMed](#)]
93. Endo, S.; Yuyama, M.; Takada, H. Desorption kinetics of hydrophobic organic contaminants from marine plastic pellets. *Mar. Pollut. Bull.* **2013**, *74*, 125–131. [[CrossRef](#)] [[PubMed](#)]
94. Velzeboer, I.; Kwadijk, C.J.A.F.; Koelmans, A.A. Strong sorption of PCBs to nanoplastics, microplastics, carbon nanotubes, and fullerenes. *Environ. Sci. Technol.* **2014**, *48*, 4869–4876. [[CrossRef](#)] [[PubMed](#)]
95. Rios, L.M.; Jones, P.R.; Moore, C.; Narayan, U.V. Quantitation of persistent organic pollutants adsorbed on plastic debris from the Northern Pacific Gyre’s “eastern garbage patch”. *J. Environ. Monit.* **2010**, *12*, 2226–2236. [[CrossRef](#)]
96. Wu, C.; Zhang, K.; Huang, X.; Liu, J. Sorption of pharmaceuticals and personal care products to polyethylene debris. *Environ. Sci. Pollut. Res.* **2016**, *23*, 8819–8826. [[CrossRef](#)]
97. Li, J.; Zhang, K.; Zhang, H. Adsorption of antibiotics on microplastics. *Environ. Pollut.* **2018**, *237*, 460–467. [[CrossRef](#)]
98. Rodrigues, J.P.; Duarte, A.C.; Santos-Echeandía, J.; Rocha-Santos, T. Significance of interactions between microplastics and POPs in the marine environment: A critical overview. *TrAC—Trends Anal. Chem.* **2019**, *111*, 252–260. [[CrossRef](#)]
99. Wang, W.; Wang, J. Different partition of polycyclic aromatic hydrocarbon on environmental particulates in freshwater: Microplastics in comparison to natural sediment. *Ecotoxicol. Environ. Saf.* **2018**, *147*, 648–655. [[CrossRef](#)]
100. Velez, J.F.M.; Shashoua, Y.; Syberg, K.; Khan, F.R. Considerations on the use of equilibrium models for the characterisation of HOC-microplastic interactions in vector studies. *Chemosphere* **2018**, *210*, 359–365. [[CrossRef](#)]
101. Pascall, M.A.; Zabik, M.E.; Zabik, M.J.; Hernandez, R.J. Uptake of polychlorinated biphenyls (PCBs) from an aqueous medium by polyethylene, polyvinyl chloride, and polystyrene films. *J. Agric. Food Chem.* **2005**, *53*, 164–169. [[CrossRef](#)]
102. Bakir, A.; Rowland, S.J.; Thompson, R.C. Transport of persistent organic pollutants by microplastics in estuarine conditions. *Estuar. Coast. Shelf Sci.* **2014**, *140*, 14–21. [[CrossRef](#)]
103. Koelmans, A.A.; Besseling, E.; Wegner, A.; Foekema, E.M. Plastic as a carrier of POPs to aquatic organisms: A model analysis. *Environ. Sci. Technol.* **2013**, *47*, 7812–7820. [[CrossRef](#)] [[PubMed](#)]
104. Horton, A.A.; Jürgens, M.D.; Lahive, E.; van Bodegom, P.M.; Vijver, M.G. The influence of exposure and physiology on microplastic ingestion by the freshwater fish *Rutilus rutilus* (roach) in the River Thames, UK. *Environ. Pollut.* **2018**, *236*, 188–194. [[CrossRef](#)] [[PubMed](#)]

105. Sørensen, L.; Rogers, E.; Altin, D.; Salaberria, I.; Booth, A.M. Sorption of PAHs to microplastic and their bioavailability and toxicity to marine copepods under co-exposure conditions. *Environ. Pollut.* **2020**, *258*, 113844. [[CrossRef](#)] [[PubMed](#)]
106. Verla, A.W.; Enyoh, C.E.; Verla, E.N.; Nwarnorh, K.O. Microplastic–toxic chemical interaction: A review study on quantified levels, mechanism and implication. *SN Appl. Sci.* **2019**, *1*, 1400. [[CrossRef](#)]
107. Hahladakis, J.N.; Velis, C.A.; Weber, R.; Iacovidou, E.; Purnell, P. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. *J. Hazard. Mater.* **2018**, *344*, 179–199. [[CrossRef](#)] [[PubMed](#)]
108. Munier, B.; Bendell, L.I. Macro and micro plastics sorb and desorb metals and act as a point source of trace metals to coastal ecosystems. *PLoS ONE* **2018**, *13*, 1–13. [[CrossRef](#)]
109. Khan, F.R.; Syberg, K.; Shashoua, Y.; Bury, N.R. Influence of polyethylene microplastic beads on the uptake and localization of silver in zebrafish (*Danio rerio*). *Environ. Pollut.* **2015**, *206*, 73–79. [[CrossRef](#)]
110. Ashton, K.; Holmes, L.; Turner, A. Association of metals with plastic production pellets in the marine environment. *Mar. Pollut. Bull.* **2010**, *60*, 2050–2055. [[CrossRef](#)]
111. Vedolin, M.C.; Teophilo, C.Y.S.; Turra, A.; Figueira, R.C.L. Spatial variability in the concentrations of metals in beached microplastics. *Mar. Pollut. Bull.* **2018**, *129*, 487–493. [[CrossRef](#)]
112. Bradney, L.; Wijesekara, H.; Palansooriya, K.N.; Obadamudalige, N.; Bolan, N.S.; Ok, Y.S.; Rinklebe, J.; Kim, K.-H.; Kirkham, M.B. Particulate plastics as a vector for toxic trace-element uptake by aquatic and terrestrial organisms and human health risk. *Environ. Int.* **2019**, *131*, 104937. [[CrossRef](#)] [[PubMed](#)]
113. Li, W.; Lo, H.S.; Wong, H.M.; Zhou, M.; Wong, C.Y.; Tam, N.F.Y.; Cheung, S.G. Heavy metals contamination of sedimentary microplastics in Hong Kong. *Mar. Pollut. Bull.* **2020**, *153*, 110977. [[CrossRef](#)] [[PubMed](#)]
114. Brennecke, D.; Duarte, B.; Paiva, F.; Caçador, I.; Canning-Clode, J. Microplastics as vector for heavy metal contamination from the marine environment. *Estuar. Coast. Shelf Sci.* **2016**, *178*, 189–195. [[CrossRef](#)]
115. Mao, R.; Lang, M.; Yu, X.; Wu, R.; Yang, X.; Guo, X. Aging mechanism of microplastics with UV irradiation and its effects on the adsorption of heavy metals. *J. Hazard. Mater.* **2020**, *393*, 122515. [[CrossRef](#)]
116. Godoy, V.; Blázquez, G.; Calero, M.; Quesada, L.; Martín-Lara, M.A. The potential of microplastics as carriers of metals. *Environ. Pollut.* **2019**, *255*, 113363. [[CrossRef](#)]
117. Zhang, G.; Bai, J.; Xiao, R.; Zhao, Q.; Jia, J.; Cui, B.; Liu, X. Heavy metal fractions and ecological risk assessment in sediments from urban, rural and reclamation-affected rivers of the Pearl River Estuary, China. *Chemosphere* **2017**, *184*, 278–288. [[CrossRef](#)]
118. Binda, G.; Pozzi, A.; Livio, F.; Piasini, P.; Zhang, C. Anomalously high concentration of Ni as sulphide phase in sediment and in water of a mountain catchment with serpentinite bedrock. *J. Geochemical Explor.* **2018**, *190*, 58–68. [[CrossRef](#)]
119. Binda, G.; Pozzi, A.; Livio, F. An integrated interdisciplinary approach to evaluate potentially toxic element sources in a mountainous watershed. *Environ. Geochem. Health* **2019**, 1–18. [[CrossRef](#)]
120. Turner, A. Mobilisation kinetics of hazardous elements in marine plastics subject to an avian physiologically-based extraction test. *Environ. Pollut.* **2018**, *236*, 1020–1026. [[CrossRef](#)]
121. Turner, A.; Holmes, L.A. Adsorption of trace metals by microplastic pellets in fresh water. *Environ. Chem.* **2015**, *12*, 600–610. [[CrossRef](#)]
122. Zou, J.; Liu, X.; Zhang, D.; Yuan, X. Adsorption of three bivalent metals by four chemical distinct microplastics. *Chemosphere* **2020**, *248*, 126064. [[CrossRef](#)] [[PubMed](#)]
123. Boucher, C.; Morin, M.; Bendell, L.I. The influence of cosmetic microbeads on the sorptive behavior of cadmium and lead within intertidal sediments: A laboratory study. *Reg. Stud. Mar. Sci.* **2016**, *3*, 1–7. [[CrossRef](#)]
124. Triebkorn, R.; Braunbeck, T.; Grummt, T.; Hanslik, L.; Huppertsberg, S.; Jekel, M.; Knepper, T.P.; Kraus, S.; Müller, Y.K.; Pittroff, M.; et al. Relevance of nano- and microplastics for freshwater ecosystems: A critical review. *TrAC - Trends Anal. Chem.* **2019**, *110*, 375–392. [[CrossRef](#)]
125. Koelmans, A.A.; Bakir, A.; Burton, G.A.; Janssen, C.R. Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies. *Environ. Sci. Technol.* **2016**, *50*, 3315–3326. [[CrossRef](#)] [[PubMed](#)]
126. Lu, K.; Qiao, R.; An, H.; Zhang, Y. Influence of microplastics on the accumulation and chronic toxic effects of cadmium in zebrafish (*Danio rerio*). *Chemosphere* **2018**, *202*, 514–520. [[CrossRef](#)] [[PubMed](#)]

127. Liu, J.; Ma, Y.; Zhu, D.; Xia, T.; Qi, Y.; Yao, Y.; Guo, X.; Ji, R.; Chen, W. Polystyrene Nanoplastics-Enhanced Contaminant Transport: Role of Irreversible Adsorption in Glassy Polymeric Domain. *Environ. Sci. Technol.* **2018**, *52*, 2677–2685. [[CrossRef](#)]
128. Khan, F.R.; Boyle, D.; Chang, E.; Bury, N.R. Do polyethylene microplastic beads alter the intestinal uptake of Ag in rainbow trout (*Oncorhynchus mykiss*)? Analysis of the MP vector effect using in vitro gut sacs. *Environ. Pollut.* **2017**, *231*, 200–206. [[CrossRef](#)]
129. Grigorakis, S.; Drouillard, K.G. Effect of Microplastic Amendment to Food on Diet Assimilation Efficiencies of PCBs by Fish. *Environ. Sci. Technol.* **2018**, *52*, 10796–10802. [[CrossRef](#)]
130. Schmieg, H.; Huppertsberg, S.; Knepper, T.P.; Kraiss, S.; Reitter, K.; Rezbach, F.; Ruhl, A.S.; Köhler, H.-R.; Triebkorn, R. Polystyrene microplastics do not affect juvenile brown trout (*Salmo trutta f. fario*) or modulate effects of the pesticide methiocarb. *Environ. Sci. Eur.* **2020**, *32*, 49. [[CrossRef](#)]
131. Sleight, V.A.; Bakir, A.; Thompson, R.C.; Henry, T.B. Assessment of microplastic-sorbed contaminant bioavailability through analysis of biomarker gene expression in larval zebrafish. *Mar. Pollut. Bull.* **2017**, *116*, 291–297. [[CrossRef](#)]
132. Wen, B.; Jin, S.-R.; Chen, Z.-Z.; Gao, J.-Z.; Liu, Y.-N.; Liu, J.-H.; Feng, X.-S. Single and combined effects of microplastics and cadmium on the cadmium accumulation, antioxidant defence and innate immunity of the discus fish (*Symphysodon aequifasciatus*). *Environ. Pollut.* **2018**, *243*, 462–471. [[CrossRef](#)] [[PubMed](#)]
133. Weber, S.; Traunspurger, W. The effects of predation by juvenile fish on the meiobenthic community structure in a natural pond. *Freshw. Biol.* **2015**, *60*, 2392–2409. [[CrossRef](#)]
134. Bettinetti, R.; Quadroni, S.; Boggio, E.; Galassi, S. Recent DDT and PCB contamination in the sediment and biota of the Como Bay (Lake Como, Italy). *Sci. Total Environ.* **2016**, *542*, 404–410. [[CrossRef](#)] [[PubMed](#)]
135. Guzzella, L.M.; Novati, S.; Casatta, N.; Roscioli, C.; Valsecchi, L.; Binelli, A.; Parolini, M.; Solcà, N.; Bettinetti, R.; Manca, M.; et al. Spatial and temporal trends of target organic and inorganic micropollutants in Lake Maggiore and Lake Lugano (Italian-Swiss water bodies): Contamination in sediments and biota. *Hydrobiologia* **2018**, *824*, 271–290. [[CrossRef](#)]
136. Väinölä, R.; Witt, J.D.S.; Grabowski, M.; Bradbury, J.H.; Jazdzewski, K.; Sket, B. Global diversity of amphipods (Amphipoda; Crustacea) in freshwater. In *Freshwater Animal Diversity Assessment*; Springer: Dordrecht, The Netherlands, 2008; pp. 241–255.
137. Berglund, E.; Fogelberg, V.; Nilsson, P.A.; Hollander, J. Microplastics in a freshwater mussel (*Anodonta anatina*) in Northern Europe. *Sci. Total Environ.* **2019**, *697*, 134192. [[CrossRef](#)]
138. Napper, I.E.; Thompson, R.C. Release of synthetic microplastic plastic fibres from domestic washing machines: Effects of fabric type and washing conditions. *Mar. Pollut. Bull.* **2016**, *112*, 39–45. [[CrossRef](#)] [[PubMed](#)]
139. Blarer, P.; Burkhardt-Holm, P. Microplastics affect assimilation efficiency in the freshwater amphipod *Gammarus fossarum*. *Environ. Sci. Pollut. Res.* **2016**, *23*, 23522–23532. [[CrossRef](#)]
140. Iannilli, V.; Pasquali, V.; Setini, A.; Corami, F. First evidence of microplastics ingestion in benthic amphipods from Svalbard. *Environ. Res.* **2019**, *179*, 108811. [[CrossRef](#)]
141. Straub, S.; Hirsch, P.E.; Burkhardt-Holm, P. Biodegradable and petroleum-based microplastics do not differ in their ingestion and excretion but in their biological effects in a freshwater invertebrate *Gammarus fossarum*. *Int. J. Environ. Res. Public Health* **2017**, *14*, 774. [[CrossRef](#)]
142. Redondo-Hasselerharm, P.E.; Falahudin, D.; Peeters, E.T.H.M.; Koelmans, A.A. Microplastic Effect Thresholds for Freshwater Benthic Macroinvertebrates. *Environ. Sci. Technol.* **2018**, *52*, 2278–2286. [[CrossRef](#)]
143. Jemec, A.; Horvat, P.; Kunej, U.; Bele, M.; Kržan, A. Uptake and effects of microplastic textile fibers on freshwater crustacean *Daphnia magna*. *Environ. Pollut.* **2016**, *219*, 201–209. [[CrossRef](#)] [[PubMed](#)]
144. Besseling, E.; Wegner, A.; Foekema, E.M.; van den Heuvel-Greve, M.J.; Koelmans, A.A. Effects of Microplastic on Fitness and PCB Bioaccumulation by the Lugworm *Arenicola marina* (L.). *Environ. Sci. Technol.* **2013**, *47*, 593–600. [[CrossRef](#)] [[PubMed](#)]
145. Ziajahromi, S.; Kumar, A.; Neale, P.A.; Leusch, F.D.L. Environmentally relevant concentrations of polyethylene microplastics negatively impact the survival, growth and emergence of sediment-dwelling invertebrates. *Environ. Pollut.* **2018**, *236*, 425–431. [[CrossRef](#)] [[PubMed](#)]
146. Jeong, C.B.; Won, E.J.; Kang, H.M.; Lee, M.C.; Hwang, D.S.; Hwang, U.K.; Zhou, B.; Souissi, S.; Lee, S.J.; Lee, J.S. Microplastic Size-Dependent Toxicity, Oxidative Stress Induction, and p-JNK and p-p38 Activation in the Monogonont Rotifer (*Brachionus koreanus*). *Environ. Sci. Technol.* **2016**, *50*, 8849–8857. [[CrossRef](#)]

147. Magni, S.; Gagné, F.; André, C.; Della Torre, C.; Auclair, J.; Hanana, H.; Parenti, C.C.; Bonasoro, F.; Binelli, A. Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel *Dreissena polymorpha* (Mollusca: Bivalvia). *Sci. Total Environ.* **2018**, *631–632*, 778–788. [[CrossRef](#)]
148. Colomer, J.; Müller, M.F.; Barcelona, A.; Serra, T. Mediated food and hydrodynamics on the ingestion of microplastics by *Daphnia magna*. *Environ. Pollut.* **2019**, *251*, 434–441. [[CrossRef](#)]
149. Lei, L.; Wu, S.; Lu, S.; Liu, M.; Song, Y.; Fu, Z.; Shi, H.; Raley-Susman, K.M.; He, D. Microplastic particles cause intestinal damage and other adverse effects in zebrafish *Danio rerio* and nematode *Caenorhabditis elegans*. *Sci. Total Environ.* **2018**, *619–620*, 1–8. [[CrossRef](#)]
150. Au, S.Y.; Bruce, T.F.; Bridges, W.C.; Klaine, S.J. Responses of *Hyalella azteca* to acute and chronic microplastic exposures. *Environ. Toxicol. Chem.* **2015**, *34*, 2564–2572. [[CrossRef](#)]
151. Imhof, H.K.; Laforsch, C. Hazardous or not—Are adult and juvenile individuals of *Potamopyrgus antipodarum* affected by non-buoyant microplastic particles? *Environ. Pollut.* **2016**, *218*, 383–391. [[CrossRef](#)]
152. Zhao, L.; Qu, M.; Wong, G.; Wang, D. Transgenerational toxicity of nanopolystyrene particles in the range of  $\mu\text{g L}^{-1}$  in the nematode: *Caenorhabditis elegans*. *Environ. Sci. Nano* **2017**, *4*, 2356–2366. [[CrossRef](#)]
153. Halle, L.L.; Palmqvist, A.; Kampmann, K.; Khan, F.R. Ecotoxicology of micronized tire rubber: Past, present and future considerations. *Sci. Total Environ.* **2020**, *706*, 135694. [[CrossRef](#)] [[PubMed](#)]
154. Stock, F.; Kochleus, C.; Bänisch-Baltrusch, B.; Brennholt, N.; Reifferscheid, G. Sampling techniques and preparation methods for microplastic analyses in the aquatic environment—A review. *TrAC—Trends Anal. Chem.* **2019**, *113*, 84–92. [[CrossRef](#)]
155. Hermsen, E.; Mintenig, S.M.; Besseling, E.; Koelmans, A.A. Quality Criteria for the Analysis of Microplastic in Biota Samples: A Critical Review. *Environ. Sci. Technol.* **2018**, *52*, 10230–10240. [[CrossRef](#)] [[PubMed](#)]
156. Masura, J.; Baker, J.; Foster, G.; Arthur, C. *Laboratory Methods for the Analysis of Microplastics in the Marine Environment*; NOAA: Silver Spring, MD, USA, 2015.
157. Ng, K.L.; Obbard, J.P. Prevalence of microplastics in Singapore’s coastal marine environment. *Mar. Pollut. Bull.* **2006**, *52*, 761–767. [[CrossRef](#)]
158. Mai, L.; Bao, L.J.; Shi, L.; Wong, C.S.; Zeng, E.Y. A review of methods for measuring microplastics in aquatic environments. *Environ. Sci. Pollut. Res.* **2018**, *25*, 11319–11332. [[CrossRef](#)]
159. Vianello, A.; Boldrin, A.; Guerriero, P.; Moschino, V.; Rella, R.; Sturaro, A.; Da Ros, L. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. *Estuar. Coast. Shelf Sci.* **2013**, *130*, 54–61. [[CrossRef](#)]
160. Liebezeit, G.; Dubaish, F. Microplastics in beaches of the East Frisian Islands Spiekeroog and Kachelotplate. *Bull. Environ. Contam. Toxicol.* **2012**, *89*, 213–217. [[CrossRef](#)]
161. Claessens, M.; Cauwenberghe, L.V.; Vandegehuchte, M.B.; Janssen, C.R. New techniques for the detection of microplastics in sediments and field collected organisms. *Mar. Pollut. Bull.* **2013**, *70*, 227–233. [[CrossRef](#)]
162. Lusher, A.L.; Welden, N.A.; Sobral, P.; Cole, M. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Anal. Methods* **2017**, *9*, 1346–1360. [[CrossRef](#)]
163. Imhof, H.K.; Schmid, J.; Niessner, R.; Ivleva, N.P.; Laforsch, C. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. *Limnol. Oceanogr. Methods* **2012**, 524–537. [[CrossRef](#)]
164. Pinto da Costa, J.; Reis, V.; Paço, A.; Costa, M.; Duarte, A.C.; Rocha-Santos, T. Micro(nano)plastics—Analytical challenges towards risk evaluation. *TrAC—Trends Anal. Chem.* **2019**, *111*, 173–184. [[CrossRef](#)]
165. Felsing, S.; Kochleus, C.; Buchinger, S.; Brennholt, N.; Stock, F.; Reifferscheid, G. A new approach in separating microplastics from environmental samples based on their electrostatic behavior. *Environ. Pollut.* **2018**, *234*, 20–28. [[CrossRef](#)] [[PubMed](#)]
166. Hurley, R.R.; Lusher, A.L.; Olsen, M.; Nizzetto, L. Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices. *Environ. Sci. Technol.* **2018**, *52*, 7409–7417. [[CrossRef](#)] [[PubMed](#)]
167. Foekema, E.M.; De Gruijter, C.; Mergia, M.T.; van Franeker, J.A.; Murk, A.J.; Koelmans, A.A. Plastic in North Sea Fish. *Environ. Sci. Technol.* **2013**, *47*, 8818–8824. [[CrossRef](#)] [[PubMed](#)]
168. Rochman, C.M.; Tahir, A.; Williams, S.L.; Baxa, D.V.; Lam, R.; Miller, J.T.; Teh, F.C.; Werorilangi, S.; Teh, S.J. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Sci. Rep.* **2015**, *5*, 1–10. [[CrossRef](#)] [[PubMed](#)]

169. Rodrigues, M.O.; Abrantes, N.; Gonçalves, F.J.M.; Nogueira, H.; Marques, J.C.; Gonçalves, A.M.M. Spatial and temporal distribution of microplastics in water and sediments of a freshwater system (Antuã River, Portugal). *Sci. Total Environ.* **2018**, *633*, 1549–1559. [[CrossRef](#)]
170. Avio, C.G.; Gorbi, S.; Regoli, F. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. *Mar. Environ. Res.* **2015**, *111*, 18–26. [[CrossRef](#)]
171. Shim, W.J.; Hong, S.H.; Eo, S.E. Identification methods in microplastic analysis: A review. *Anal. Methods* **2017**, *9*, 1384–1391. [[CrossRef](#)]
172. Tagg, A.S.; Sapp, M.; Harrison, J.P.; Ojeda, J.J. Identification and Quantification of Microplastics in Wastewater Using Focal Plane Array-Based Reflectance Micro-FT-IR Imaging. *Anal. Chem.* **2015**, *87*, 6032–6040. [[CrossRef](#)]
173. Xu, J.L.; Thomas, K.V.; Luo, Z.; Gowen, A.A. FTIR and Raman imaging for microplastics analysis: State of the art, challenges and prospects. *TrAC—Trends Anal. Chem.* **2019**, *119*, 115629. [[CrossRef](#)]
174. Käßler, A.; Fischer, D.; Oberbeckmann, S.; Schernewski, G.; Labrenz, M.; Eichhorn, K.-J.; Voit, B. Analysis of environmental microplastics by vibrational microspectroscopy: FTIR, Raman or both? *Anal. Bioanal. Chem.* **2016**, *408*, 8377–8391. [[CrossRef](#)] [[PubMed](#)]
175. Hahn, A.; Gerdt, G.; Völker, C.; Niebühr, V. Using FTIRS as pre-screening method for detection of microplastic in bulk sediment samples. *Sci. Total Environ.* **2019**, *689*, 341–346. [[CrossRef](#)] [[PubMed](#)]
176. Araujo, C.F.; Nolasco, M.M.; Ribeiro, A.M.P.; Ribeiro-Claro, P.J.A. Identification of microplastics using Raman spectroscopy: Latest developments and future prospects. *Water Res.* **2018**, *142*, 426–440. [[CrossRef](#)] [[PubMed](#)]
177. Wang, Z.-M.; Wagner, J.; Ghosal, S.; Bedi, G.; Wall, S. SEM/EDS and optical microscopy analyses of microplastics in ocean trawl and fish guts. *Sci. Total Environ.* **2017**, *603–604*, 616–626. [[CrossRef](#)]
178. Hodson, M.E.; Duffus-Hodson, C.A.; Clark, A.; Prendergast-Miller, M.T.; Thorpe, K.L. Plastic Bag Derived-Microplastics as a Vector for Metal Exposure in Terrestrial Invertebrates. *Environ. Sci. Technol.* **2017**, *51*, 4714–4721. [[CrossRef](#)]
179. Zhang, D.; Liu, X.; Huang, W.; Li, J.; Wang, C.; Zhang, D.; Zhang, C. Microplastic pollution in deep-sea sediments and organisms of the Western Pacific Ocean. *Environ. Pollut.* **2020**, *259*, 113948. [[CrossRef](#)]
180. Renzi, M.; Guerranti, C.; Blašković, A. Microplastic contents from maricultured and natural mussels. *Mar. Pollut. Bull.* **2018**, *131*, 248–251. [[CrossRef](#)]



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