microplastics

Review

Microplastics in Freshwaters: Implications for Aquatic Autotrophic Organisms and Fauna Health

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Abstract: Microplastics (MPs) represent small plastic particles with sizes between 1 μm and 5 mm, are insoluble in water, andclassified as primary (these are originally produced in small sizes) or secondary (the result of the degradation of plastic) types. MPs accumulate in all ecosystems, including freshwater environments, where they are subjected to degradation processes. Due to their ubiquitous nature, freshwater ecosystems, which have a vital importance in human life, are permanently subjected to these small plastic particles. In this context, MPs pollution is considered to be a global issue, and it is associated with toxic effects on all the elements of the freshwater environment. In this review, we present, in detail, the main physical (density, size, color, shape, and crystallinity) and chemical (chemical composition and modification of the MPs' surface) properties of MPs, the mechanism of biodegradation, and the consequences of autotrophic organisms and fauna exposure by focusing on the freshwater environment. The toxicity mechanisms triggered by MPs are related to the critical parameters of the particles: size, concentration, type, and form, but they are also dependent on species exposed to MPs and the exposure route.

Keywords: microplastics; freshwater; toxicity; biodegradation; aquatic autotrophic organisms

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1. Introduction

Plastic production has had an explosive expansion since the end of World War II, which has led to an increased accumulation of plastic particles in the environment. On account of the increased production of plastics (approximately 400 million tonnes per year) and the fact that not all of them can be completely biological and chemical degraded, plastics can remain in nature for extended periods. These topics are studied as ones of the most important and global issues of the future due to their potentially disturbing effects on nature and human homeostasis [1].

From the chemical composition point of view, there can be many types of plastics, but the ones that are most prone to quickly decomposing into small plastic particles are photodegradable bioplastics, bio-based bioplastics, compostable bioplastics, and biodegradable bioplastics [2].

Based on the size of the particles present in nature, plastic can be grouped into five main categories: nanoplastics (<1 μm), microplastics (≥1 μm to <5 mm), mesoplastics (≥5 mm to 5 cm), macroplastics (>5 to 50 cm), and megaplastics (>50 cm) [3]. Among these, significant interest is paid to microplastics (MPs), small solid plastic particles with dimensions that are smaller than 5 mm, which are products of plastic pollution and can be harmful to the environment and humans as well [4]. Considering the origin of MPs, they can be classified into primary and secondary MPs. Primary MPs groups include particles of a small size that are originally produced (remnants of cosmetics, care products, and drugs) [5,6], while secondary MPs represent fragments or particles resulting from the physical, chemical, or biological degradation of plastic products (bags, bottles, and food containers, etc.) [6,7].

In recent years, the use of plastic in important and numerous applications has generated a high number of literature studies focused on the potentially toxic effects induced by MPs, especially in marine ecosystems [8–12]. However, the research regarding freshwater pollution with MPs is still limited compared to that of marine environment. Freshwater has a vital importance in our life and survival: it represents the source of drinking water and has a critical role in agriculture [13], erosion prevention, food supply, and tourism [14]. Moreover, as an essential resource for human life, freshwater has no substitutes, and unfortunately, it is continuously changed by various factors, including: climate, hydrologic factors, land-use, chemical inputs, or invasion of aquatic species [15]. Besides these, the contamination with MPs represents another changing factor of a real concern for autotrophic organisms, fauna, and humans. A first step to prevent the contamination of freshwaters with MPs is to pay attention to the circuit of plastic in nature, to reduce the plastic use, and to introduce plastic collection for the recycling process in daily activities. Through this review, we intend to highlight and update the most important implications of MPs for autotrophic organisms and fauna present in freshwaters. The literature research was performed using specific keywords of the topic and associations with different keywords (e.g., freshwater, microplastics, freshwater organisms, microplastics color, microplastics shape, microplastics degradation, freshwater flora, and microplastics toxicity, etc.).

2. Physico-Chemical Properties of Microplastics

The main properties of MPs can be identified from their definition and general characterization: MPs are solid particles, with dimensions that are smaller than 5 mm, and they are insoluble in water, non-degradable, and composed of synthetic materials with a high content of polymers [16]. However, for a deeper characterization and understanding, the features of MPs are grouped by their physical and chemical properties. The physical properties are represented by the density, color, shape, size, and crystalline structure of the particles [17]. The chemical properties refer mainly to the chemical composition and surface chemistry [18].

2.1. Physical Properties

Density is a key parameter in MPs' spatial distribution over the column of freshwater [19,20], and particles with a lower density than the water float on the surface of the water or are suspended. The density range is a specific characteristic of every plastic type. Thus, the densities of polyvinyl chloride, polyethylene terephthalate, polycarbonate, polystyrene, polyethylene, and polypropylene are in the ranges of 1.38–1.51 g/cm^3, 1.38–1.41 g/cm^3, 1.20–1.22 g/cm^3, 1.04–1.08 g/cm^3, 0.89–0.98 g/cm^3, and 0.85–0.91 g/cm^3, respectively [21].

Color. MPs can have various colors: blue, black, red, orange, yellow, and white [22], and this is an essential characteristic to be considered when one is evaluating the toxicity of MPs.

Regarding the spreading of them in freshwaters, the color of the MPs depends on the region. Transparent, white, black, and colored (e.g., red, blue, green, and gray) MPs were most frequently identified in China [23], while in the Central Pomeranian Region, Poland (Słupia and Łupawa rivers), the dominant color of the MPs was similar with that of the ecosystem elements (food, water, and sediments) [24].

Additionally, those in the Maozhou River (within Guangdong-Hong Kong-Macao Greater Bay Area) were transparent, white, black, yellow, and blue MPs, with transparent MPs representing the dominant type, which was followed by the other ones [25]. Regarding the color preferences for the intake of MPs, freshwater fishes (*Japanese medaka* and zebrafish) have a higher preference for ingesting red, yellow, and green MPs compared to gray and blue ones [26].

Shape. MPs are often classified in three main shape categories: spherical, irregular, and fibrous [27]. However, the MPs can be also found in other variable shapes, including films, foams, fragments [28,29], flakes or pellets [17], line, filament and foil [30].

Originally, MPs in the form of beads and pellets were considered to be primary or native, while the other shapes, resulting from the photochemical, mechanical, and biological degradation processes in the marine habitat, were categorized as secondary MPs [31].

MPs with different forms have been distributed in freshwater ecosystems all over the world. Fragment, fiber, and film MPs were identified as the most common shape in the Brisbane River in Australia [32], whilst in the Nakdong River in South Korea, MPs were discovered with fragment, fiber, and sphere shapes [33]. Microbeads MPs were detected in the Rhine River in Germany [34], and pellet MPs (together with foam, fiber, and fragment particles) were identified in the Wen-Rui Tang River, China [35].

Size. MPs have sizes that are between 1 μm and 5 mm, and this is a property of great importance in the interactions between the MPs and elements of freshwater ecosystems. Due to their small sizes, MPs can be easily ingested or incorporated by freshwater organisms. Additionally, the small size influences the transport, accumulation, and retention time of MPs [36]. The MPs clearance in freshwater fishes (*Oncorhynchus mykiss* and *Cyprinus carpio*) is particle size dependent, and the retention time differs between small and large MPs. The retention time increases with the expansion of the MPs' size. Moreover, the retention time is influenced by the gastrointestinal morphology of the fish [37]. Additionally, the size of the particles (e.g., polyethylene) has a primordial role in the MPs' potential to induce toxic effects (oxidative stress, apoptosis, DNA damage, ubiquitination, and autophagy) in freshwater fishes (*Danio rerio* and *Perca fluviatilis*) [38].

Crystalline structure or crystallinity refers to the organization of atoms, ions or molecules in a three-dimensional arrangement. This property decides the location of the MPs in water due to its influence on density, and it controls the degradation process [39], and it is particularly important in determining the MPs sorption capacity of different additives [40]. On the other side, the degree of the MPs' crystallinity can be affected by biofilm formation [41].

2.2. Chemical Properties

Chemical composition. Generally, MPs are organic polymers composed of carbon and hydrogen atoms bonded together in the polymer chains. However, the analysis of their chemical composition revealed that MPs can be associated with individual particles or different chemicals. MPs can associate with two types of chemicals: (i) additives and polymeric raw materials originating from the plastic, which are added during the production for the improvement of its properties, and (ii) chemical compounds present in the MPs' environment [42].

MPs have a high affinity for heavy metals and on the basis of this association, MPs can act as a vector for heavy metals by introducing them into freshwater organisms or the human body [43]. In freshwater, MPs can adsorb on their surface various heavy metals: Cr, Cu, Zn, Pb, Ag, Cd, Hg, Ni, Co, Ti, and As [44,45].

The mechanism by which metals are adsorbed onto the MPs' surface is complex and controlled by the MPs' surface. MPs can interact with heavy metals by the oxygen-containing functional groups formed after the MPs' surface oxidation. Additionally, MPs and heavy metals can be associated through electrostatic interactions and hydrogen bondings [46]. Furthermore, the mechanism of adsorption is controlled by various factors related to the plastic characteristics and environmental parameters. The characteristics of the plastics which influence the association of MPs with heavy metals are: the polymer type, crystallinity, density, size, surface area, and zeta potential, whereas the category of environmental factors includes variables such as: the pH, temperature, salinity, and particulate matter [47]. Moreover, a critical role in the adsorption of heavy metals by the MPs is related to the microbial biofilms developed on the MPs' surface [48].

Besides heavy metals, other hazardous additives from the composition of MPs are considered to be organic synthetic compound bisphenol A, phthalates, or flame-retardants compounds [42].

Surface chemistry. The MPs' surface is modified after the particles enter terrestrial and aquatic environments or are formed from larger particles. Thus, they suffer from biodegradation processes which involve the formation of functional groups on the MPs' surface as a result of oxidative reactions [18]. The functional groups present on the plastic surface can be of the aldehyde, ketones, ester, lactone, or acid type [49]. The surface chemistry of the MPs influences their impact on freshwater biofilm communities [50] and controls the association of the MPs with different additives.

The physical and chemical properties of MPs are summarized in Figure 1.

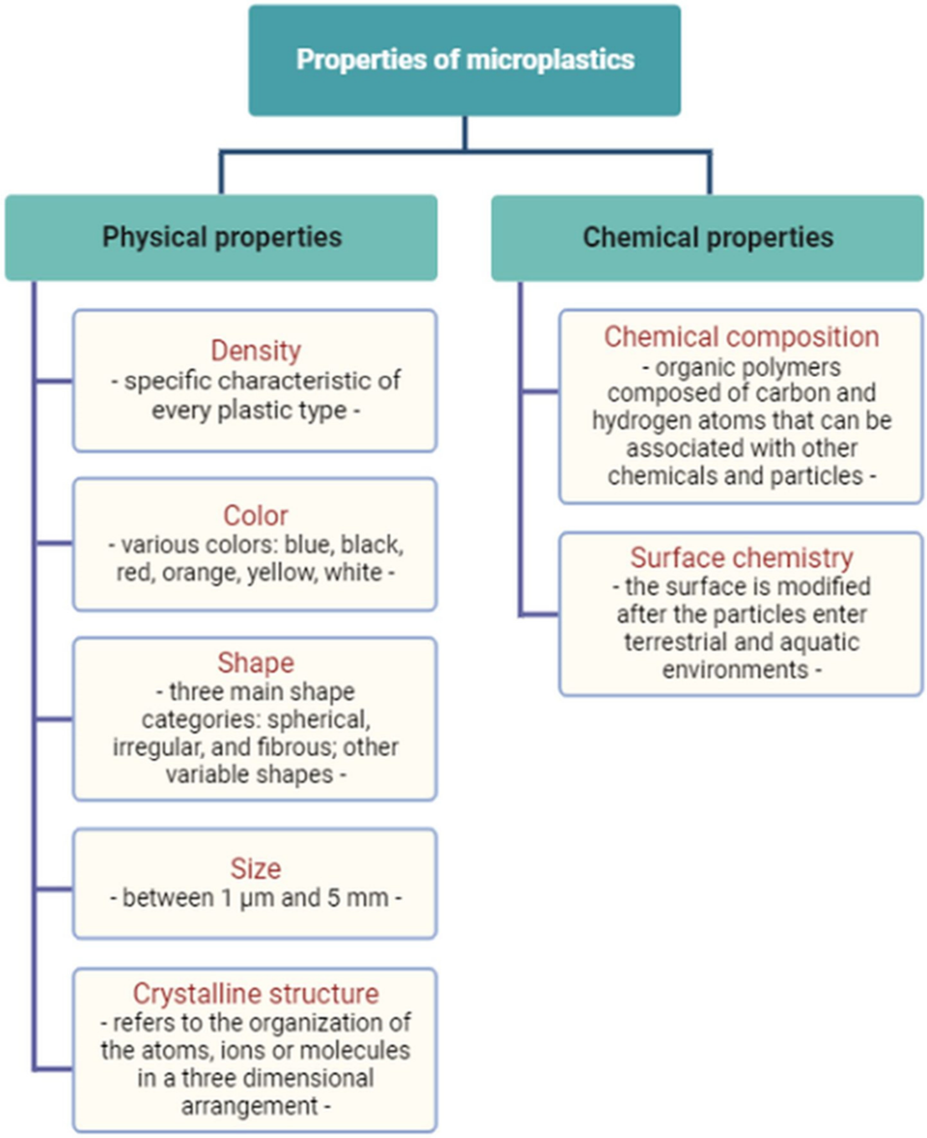

Figure 1. Physical and chemical properties of MPs. Diagram created in https://biorender.com/ (accessed on 11 September 2022).

Furthermore, it was demonstrated that the physical and chemical properties influence the cytotoxic potential of MPs [51], and these are aspects that are discussed in Sections 4 and 5 of this review.

3. Biodegradation of Microplastics in Freshwaters

After the plastic waste enters the environment, they are broken down, leading to the formation of smaller fragments, including MPs. Plastic can be degraded through biotic (biodegradation and biodisintegration) or abiotic (UV irradiation, heat, chemicals, or mechanical stress) processes [52]. After the MPs' formation, the particles are subjected to further mechanical, chemical, or biological degradation [53]. This section of the review focuses on describing the factors and process involved in the biological degradation of MPs, known also as biodegradation or biotic degradation, which is performed mainly by the microorganisms developed in the ecosystem, which use the plastic as a carbon source [54,55]. Depending on the oxygen presence, the biodegradation processes of MPs can be classified into aerobic and anaerobic ones. In aerobic conditions, oxygen acts as an electron acceptor, and the polymers are reduced to carbon dioxide, water, and residuals of carbon. In anaerobic degradation, the organic polymers are broken down into smaller compounds as in aerobic degradation, to which methane is added. In this situation, different compounds, such as nitrate, iron, sulphate, manganese, and CO_2 represent electron acceptors, instead of oxygen (Figure 2) [2,56].

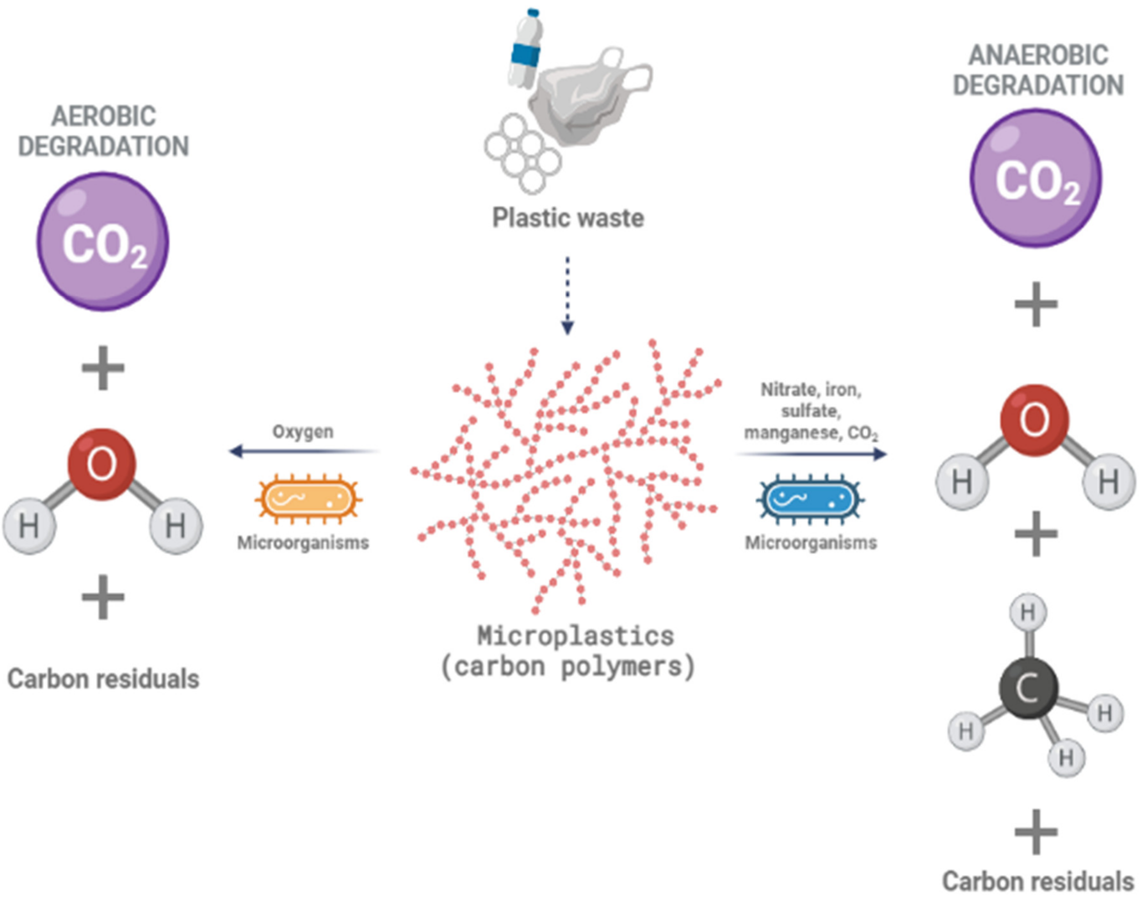

Figure 2. Schematic representation of aerobic and anaerobic decomposition of plastic by microorganisms. The aerobic degradation of MPs by microorganisms leads to their disintegration to CO_2, H_2O, and carbon residuals, while in the anaerobic degradation, CO_2, H_2O, CH_4, and carbon residuals are formed. Diagram created in https://biorender.com/ (accessed on 27 July 2022).

The main mechanism by which MPs are biologically degraded is based on the action of the microorganisms (bacteria, actinomycetes, algae, and fungi) present in the environment, and this involves several stages: the synthesis of extracellular enzymes, their attachment on the MPs' surface, the degradation of the MPs into smaller structures, and their mineralization into oxidized metabolites [57,58].

The abundance of MPs in the environment leads to the adaptation of the microorganisms to survival by the synthesis of the enzymes involved in the degradation processes. This process is highly complex. Firstly, the microorganisms produce extracellular enzymes, which increase the hydrophilicity of the MPs through the formation of alcohol or carbonyl groups on their surface, thus allowing further degradation to occur [57–59]. Secondly, the chemical groups generated on the MPs' surface facilitate the anchorage of microorganisms, which cleave the polymers in smaller fragments (oligomers and monomers). Thirdly, the MPs fragments are internalized into the microorganisms' cells [60]. At this stage, other enzymes (MPs-degrading enzymes) are involved in the degradation and mineralization of the MPs' monomers [57,58], which are used as a carbon source for microbial growth [61]. Furthermore, it is postulated that the complete degradation of plastic into CO_2, H_2O, and other metabolic products is mediated by the tricarboxylic acid cycle (TCA) [62]. The schematic representation of the biodegradation process is illustrated in Figure 3. Given that the degradation of MPs by a single species of microorganisms leads to the formation of toxic products, microbial communities have adapted and developed the combined degradation by different microbial species, which limits the disadvantages induced by a single mechanism of degradation [63].

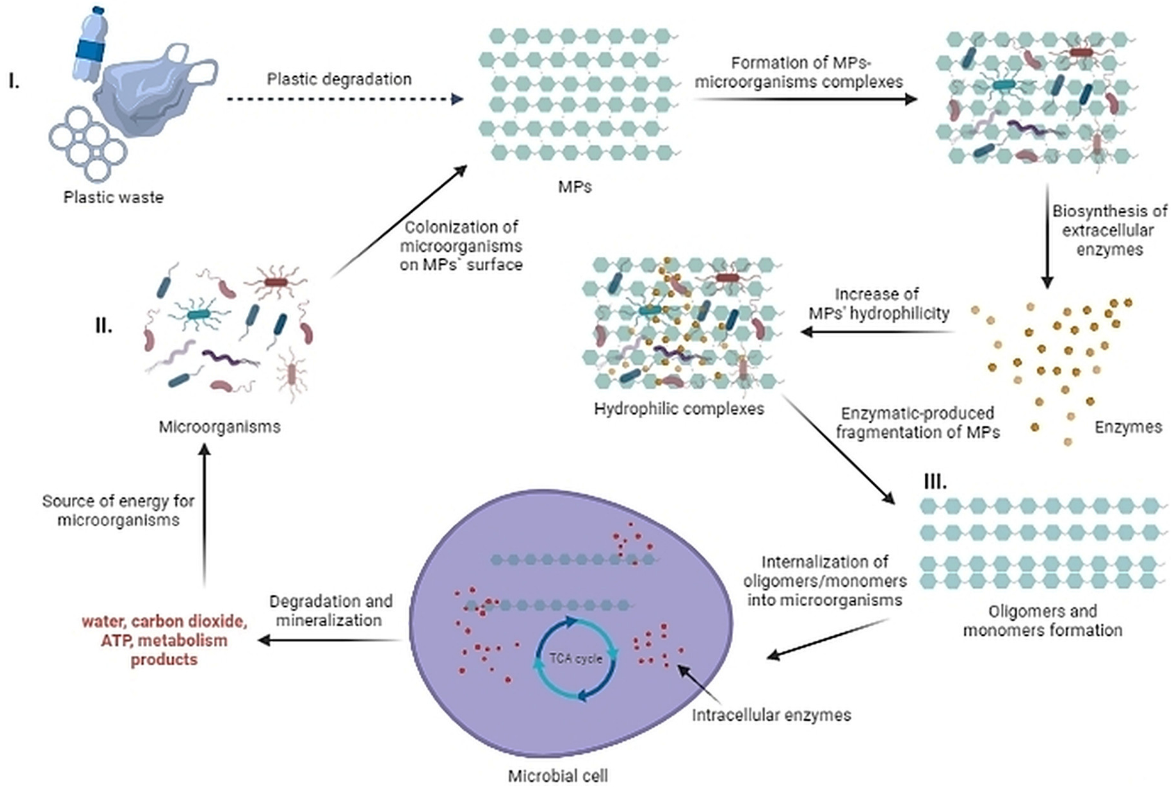

Figure 3. Biodegradation process of MPs by microorganisms. (**I**) Plastic waste is degraded to microplastics. (**II**) Microorganisms colonize the MPs' surface and produce extracellular enzymes which degrade MPs to monomers and oligomers. (**III**) Monomers and oligomers are internalized into microbial cells, and under the action of intracellular enzymes, these are disintegrated to metabolic products, which are used as a carbon source by microorganisms. Image created in https://biorender.com/ (accessed on 4 August 2022).

In freshwater environments, the biofilms community which colonizes the surface of the MPs is characterized by a large diversity. As example, an investigation of biofilm formation on poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) revealed the presence of species from the *Burkholderiales* order (*Acidovorax*, *Undibacterium*, and *Chitinimonas* genera) and their function as degraders of plastic [64]. Besides these, members of the *Planktophila* order and the *Betaproteobacteria* class were found to develop biofilm bacterial communities on high-density polyethylene, polyethylene terephthalate, and polystyrene incubated with freshwater samples [65].

As a consequence of the microorganisms' variability and the intrinsic substrate specificity of the enzymes, the MPs-degrading enzymes are characterized based on the MPs types that can be disintegrated. In this direction, relying on the current research, MPs-degrading enzymes can be grouped in various categories: the polyethylene-group-degrading enzyme, polyethylene terephthalate-degrading enzyme, polystyrene-degrading enzyme, polypropylene-degrading enzyme, and the polyvinyl-chloride-degrading enzyme, etc. [57]. Besides the degradation induced by the microorganisms, in the biodegradation category, enzymatic degradation, which relies on high-specificity enzyme species produced by organisms found in the insect digestive tract and in terrestrial and aquatic environments, is also included [63].

The complexity of MP biological degradation is also influenced by the environmental conditions and plastic characteristics [66]. The plastics characteristics represent the intrinsic factors and include the structure, composition, form of the MPs, and presence or absence of internal additives. The environmental conditions or external environmental factors are considered to be the pH, temperature, oxygen, light, bioturbation, catalyst, and environmental additives [63].

4. Aquatic Autotrophic Organisms Exposure

Plants are fundamental components in the trophic circuit of MPs in a freshwater ecosystem. Accordingly to Bakker et al. (2016), nearly 50% of the produced plant biomass is removed by aquatic herbivores. The freshwater vegetation provides food or nursery habitats for a range of organisms, including periphyton, zooplankton, invertebrates, fish, frogs, and birds. Thus, the aquatic autotrophic organisms' exposure to MPs is a problem that could affect the homeostasis of freshwaters at many levels and further affect human health [67].

The research literature on the impacts of MPs on freshwater autotrophic organisms is not extensive. The first study on the negative effects resulting from the interaction of MPs with freshwater floating plants was reported by Kalčíková et al. (2017), and it showed that 30–600 μm polyethylene microbeads from cosmetic products were adsorbed onto the surface of the roots of duckweed (*Lemna minor)*, and they mechanically blocked their growth reducing the root length. However, leaf growth and the content of photosynthetic pigments in the duckweed leaves were not affected [68]. Another study on duckweed (*Spirodela polyrhiza*) found neither adsorption of them on the plant surface nor negative effects on the plant growth and chlorophyll production when they were exposed to 500 nm MP particles. Only the external attachment of nanoplastics could be observed on duckweed roots [69].

Negative effects of plastic particles on submerged freshwater weeds were also reported. *Myriophyllum spicatum* exposed to 20–500 μm polystyrene microparticles exhibited a reduced main shoot length in a concentration-dependent manner, but no alterations were found for *Elodea* sp. in the same conditions. However, smaller polystyrene particles (50–190 μm) significantly reduced the shoot-to-root ratio for both the macrophytes and side shoot length, relative growth rate, and the root and shoot biomass for *Elodea* sp. [70].

Investigations on the interaction between the plastic particles and freshwater algae were initiated in 2010. Bhattacharya et al. (2010) exposed the green algae *Chlorella* sp. and *Scenedesmus* sp. to positively and negatively charged polystyrene beads of 20 nm. They concluded that nanosized plastic beads were adsorbed onto the surface of algae, which inhibited the algal photosynthetic activities, but only the exposure to positively charged particles led to the amplification of reactive oxygen species (ROS) production [71]. Later, in a similar study, Nolte et al. (2017) proved that any growth inhibition is not the cause of the external adsorption of polystyrene particles onto the algal cell wall [72]. When the microalga *Chorella vulgaris* was exposed to polystyrene particles of different sizes (0.05, 0.5, and 6.0 μm) under laboratory conditions, no effects on microalgal photosynthesis were noticed, except for a high concentration of the smallest particles tested [73]. The contact of polypropylene and high-density polyethylene (>400 μm) with *Chlamydomonas reinhardtii* revealed no alteration in the expression of the genes involved in the stress response and apoptosis up to 60 days of exposure, but a significant overexpression of the genes involved in sugar biosynthesis was noticed [74]. When the same algae were exposed to polystyrene microplastic, physical damage was detected on their surface, which caused changes in the osmotic pressure inside and outside the cells, the alteration of algal photosynthesis, and an increase in the number of soluble proteins, and malondialdehyde (MDA) content [75,76].

Other species from freshwater phytoplankton such as *Scenedesmus armatus* and *Microcystis aeruginosa* might also be injured by polyethylene MPs, which can inhibit their cell growth and gross photosynthesis activity in a time- and concentration-dependent manner, mainly affecting cell respiration [77].

The toxicity of MPs to freshwater autotrophic organisms depends on many factors. Most of the studies reported time- and concentration-dependent effects, but in a similar way, the size, shape, charge, and composition of the MPs are determinant factors of the plastic exposure effects on aquatic plants. It is also shown that the water temperature may influence the interaction of freshwater species with MPs, as it can make plastic float in the cold season and sink more rapidly in the warm season [78]. As well as this, the particle color influences the toxicity. Studies on algal growth revealed that the inhibition effects were dependent on the MPs' color; white MPs inhibited the growth rate significantly, while green MPs induced the smallest cytotoxic effects [79]. MPs can be adsorbed onto plant surfaces and alter the cell growth and photosynthesis of many aquatic species of freshwater autotrophic organisms, and these effects could be transmitted to the food web, which includes humans. This aspect urgently needs more research to be conducted to fill the gaps and acquire a better comprehension of the effects of MPs on the freshwater full ecosystem.

5. Aquatic Fauna Exposure

MPs contaminants present a serious hazardous risk to freshwater fauna, and significant biochemical and molecular modifications in homeostasis have been described: genotoxicity, ROS production, liver changes, and the inhibition of the growth rate, etc. Because of their small sizes, MPs particles can be easily mixed with the food and ingested or incorporated by the freshwater organisms, including: fishes [80–82], bivalves [83], and caddisflies [84], etc. Besides ingestion, another major route of exposure to MPs, which has been described for animals, is inhalation [85].

In fauna studies, great attention is paid to fish exposure to MPs, on account of their importance in human feed as a source of protein [86]. The impact of the MPs' pollution on various freshwater fishes has been examined all over the world (Table 1).

Table 1. Overview of distribution over the world of freshwater fishes subjected to MPs' pollution.

Country	Sublocation	Freshwater Fish Species	MPs Type	References
Japan	Tama River Tokyo	*Plecoglossus altivelis*	Not described	[87]
Japan	Komaoi River, Hokkaido	*Tribolodon hakonensis*	Not described	[87]
Poland	Widawa River	*Gobio gobio, Rutilus rutilus*	Not described	[88]
Thailand	Chi River	*Labiobarbus siamensis*	Not described	[89]
		Puntioplites proctozyson		
		Cyclochelichthy repasson		
		Henicorhynchus siamensis		
		Labeo chrysophekadion, Mystus bocourti		
		Hemibagrus spilopterus, Laides longibarbis		
Bangladesh	Jamuna River	*Wallago attu*	Not described	[90]
		Anguilla bengalensis		
		Labeo calbasu, Ailia coila		

Table 1. *Cont.*

Country	Sublocation	Freshwater Fish Species	MPs Type	References
		Cirrhinus reba, Ompok pabda		
		Clupisoma garua		
Brazil	Uruguay River	*Iheringhthys labrosus*	Not described	[91]
		Astyanax lacustris		
Brazil	Goiana Estuary	*Centropomus undecimalis*	Not described	[92]
		Centropomus mexicanus		
Brazil	Pajeú River	*Hoplosternum littorale*	Not described	[93]
United Kingdom	River Thames	*Rutilus rutilus*	Polyethylene	[94]
			Polypropylene	
			Polyester	
Argentina and Uruguay	Río de la Plata Estuary	*Luciopimelodus pati*	Not described	[95]
		Pseudoplatystoma corruscans		
		Oligosarcus oligolepis, Parapimelodus valenciennis		
		Odontesthes bonariensis		
		Astyanax rutilus		
		Cyprinus carpio, Pimelodus maculatus		
		Prochilodus lineatus		
		Hypostomus commersoni		
		Cyphocharax voga		
Belgium	Flemish rivers	*Gobio gobio*	Ethylene vinyl acetate copolymer	[96]
			Polypropylene	
			Polyethylene terephthalate	
			Polyvinylchloride	
			Cellophane	
			Polyvinyl acetate	
			Polyamide (nylon)	
Tanzania	Lake Victoria	*Lates niloticus*	Polyethylene/polypropylene co-polymer	[97]
		Oreochromis niloticus		
			Polyethylene	
			Polyester	
			Polyurethane	
Australia	Greater MelbourneArea	*Gambusia holbrooki*	Polyester	[98]
			Polypropylene	
			Rayon	

Table 1. *Cont.*

Country	Sublocation	Freshwater Fish Species	MPs Type	References
			Polyamid	
			Polyethylene	
			Acrylic	
			Polystyrene	
			Ethylene vinyl acetate	
			Poly (ester amid)	
			Polyurethane	
			Polyvinyl chloride	
USA	Brazos River Basin	*Lepomis macrochirus*	Not described	[99]
		Lepomis megalotis		
USA	Evergreen Lake	*Dorosoma cepedianum*	Not described	[100]
	Lake Bloomington	*Micropterus salmoides*		
China	Qinghai Lake	*Gymnocypris przewalskii*	Polyethylene	[101]
			Polypropylene	
			Polystyrene	
			Nylon	
			Polyethylene terephthalate	
			Ethylene vinyl acetate copolymer	
			Polyvinyl chloride	
			Polycarbonate	
China	Poyang Lake	*Carassius auratus*	Polypropylene	[102]
			Polyethylene	
			Polyvinyl chloride	
			Nylon	
China	Taihu Lake	*Cyprinus carpio*	Cellophane	[103]
		Carassius auratus	Polyethylene terephthalate	
		Hypophthalmichthys molitrix	Polyester, etc.	
		Pseudorasbora parva		
		Megalobrama amblycephala		
		Hemiculter bleekeri		
Canada	East coast of Vancouver Island	*Chinook salmon*	Not described	[104]
Turkey	Karasu River	*Squalius cephalus*	Polyethylene	[105]
		Cyprinus carpio	Polyester	
		Alburnus mossulensis	Poly (vinyl stearate)	
			Polypropylene	
			Cellulose	

The influence of MPs on freshwater fishes can have impacts at all biological levels (the cellular level, organ/tissue, individual, population, community, and the ecosystem) by inducing cell death, the alteration to the metabolic activity, mortality, and the change in population number, diversity, or ecosystem structure [106]. For fish, two possible uptake routes of MPs have been postulated: active and passive. MPs particles can be actively ingested if they are confused with food or passively when accidentally are ingested or transferred from the food chain [107].

Cyprinus carpio is one of the most investigated fish species for the recognition of the consequences induced by MPs exposure in freshwater [108]. Xia and collaborators (2020) reported that polyvinyl chloride induced oxidative stress in the liver, intestine, and gills of *Cyprinus carpio* var. larvae after 30 and 60 days of exposure to different concentrations of polyvinyl chloride MPs (10%, 20%, and 30% MPs by weight). Additionally, a cytoplasmic vacuolation in the liver, altered gene expressions, and an inhibition of weight gain and body length growth were detected [109]. In another study, Chen et al. (2022) found that polyethylene MPs perturbs the intestinal homeostasis of *Cyprinus carpio* by alterations to the gut histology and changes in the intestinal microbiota composition [110]. In agreement with these studies, it was reported that MPs, represented mainly by polyethylene, induced biochemical changes (e.g., elevation of alkaline phosphatase and creatine kinase) in *Cyprinus carpio* after 21 days of exposure [111].

Biochemical alterations have been observed also for other freshwater fish species. *Oreochromis niloticus* individuals presented an inhibition of acetylcholinesterase activity in the brain after 14 days of exposure to polystyrene MPs (dimensions of 0.1 μm and concentrations of 1, 10, and 100 μg/L), which can indicate the potential of these MPs to trigger neurotoxicity [112]. The neurotoxic potential of naturally aged polystyrene MPs was demonstrated also by Guimarães and collaborators (2021), who exposed *Danio rerio* fishes (zebrafishes) to MPs for five days and observed a high acetylcholinesterase activity and numerical changes in the neuroblasts distributed on the fishes' body surface. Besides the neurotoxic potential, polystyrene MPs presented also a cytotoxic potential, which was revealed by the presence of apoptotic and necrotic erythrocytes and the induction of oxidative stress [113]. For the same species, Xue et al. (2021) reported that polyethylene MPs can affect the intestinal microbiota of fishes by changing the composition and proportions of certain phyla and genera [114]. Microbiota dysbiosis was noticed also when zebrafish individuals were exposed to polystyrene MPs for two weeks. Moreover, it was observed that these MPs can induce pathological damages in the gills and intestines, oxidative stress in the liver, and an innate immune disruption [115].

The biochemical and molecular responses of fishes to the presence of MPs was species and tissue dependent. So, *Perca fluviatilis* was more liable to polystyrene MPs action compared to *Danio rerio*, with a high DNA damage index in the liver, opposed to that registered in the gills in both of the species. However, the gills presented more a significant alteration of the metabolites than the liver tissue did [116]. In Figure 4 is a summary of the main alterations induced in freshwater fishes as a consequence of MPs pollution.

MPs ingestion by freshwater fishes is influenced by various factors classified in two main categories: biotic factors and plastic properties [107]. In the literature, several reviews outline the factors that control the ingestion of plastic microparticles by freshwater fishes [117,118]. MPs ingestion is a complex and unclear mechanism, but it can be influenced by the concentration of MPs in the environment, the length, color, and density of the particles [107,118], and the living habitat [117].

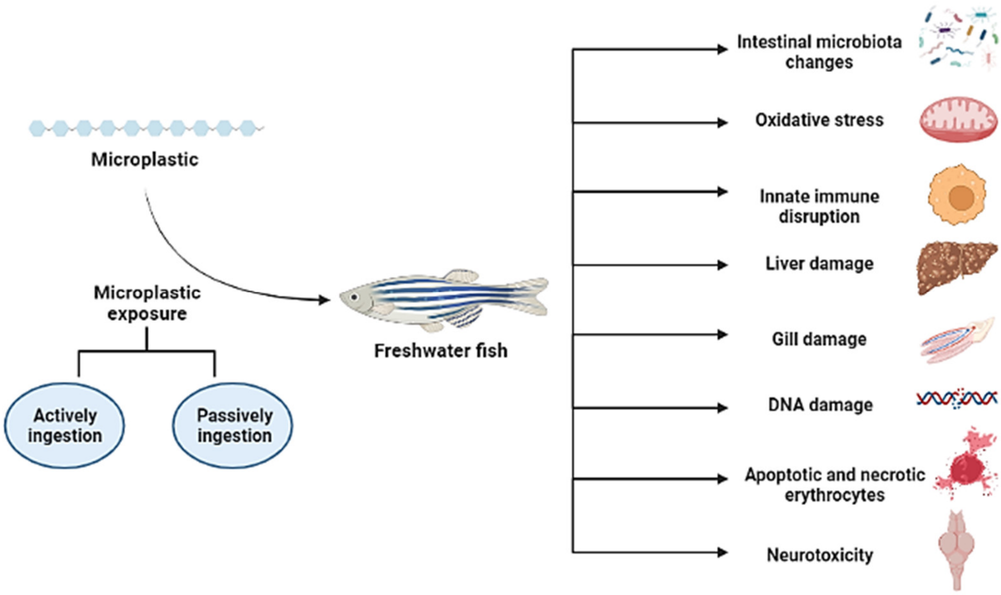

Figure 4. The main histological, biochemical, and molecular modifications induced by microplastic in freshwater fishes. Image created in https://biorender.com/ (accessed on 9 August 2022).

Besides fish, other organisms from the *Animalia* regnum are affected by the exposure to different MPs types. Polystyrene MPs beads can induce acute toxicity in rotifer *Brachionus calyciflorus*, crustacean *Ceriodaphnia dubia*, and benthic ostracod *Heterocypris incongruens*. Moreover, DNA strand breaks and an increase in ROS production were described in *Ceriodaphnia dubia* as a response to the inflammatory processes [119]. Additionally, polystyrene fragments affect the clearance rate of *Dreissena polymorpha*, without influencing the energy reserves or generating oxidative stress [120]. Other toxic effects of polystyrene were observed on freshwater benthic clams *Corbicula fluminea* when various modifications were reported: the activation of an innate immune response, the triggering of the complement and coagulation cascades, and epithelial damage in the intestines [121]. For the same species, it was proved that MPs can induce neurotoxicity through cholinesterase inhibition [122]. Adverse effects of MPs were also reported on crustacean *Daphnia magna*. The long-term exposure (21 days) of *Daphnia magna* to MPs microspheres (1–5 µm diameter; concentrations between 0.04 and 0.19 mg/L) in different conditions of temperature and light intensity reduced their somatic growth and population growth rate, affected reproduction, and induced mortality [123]. The exposure of adult *Daphnia magna* to 2 µm polystyrene MPs for the same time interval was associated with mortality after seven days of treatment, and this effect was associated with food presence rather than MPs concentration. Thus, a relation was observed between MPs ingestion and food availability, which indicates that when food was present, the uptake of MPs by *Daphnia magna* did not increase with the rise in the MPs concentration [124]. Similarly, the importance of feeding in correlation with MPs toxicity was reported. Jemec and collaborators (2016) found that polyethylene terephthalate textile microfibers can induce mortality in *Daphnia magna* only when the individuals were not pre-fed before the experiment [125]. Polyethylene and polystyrene MPs can affect the swimming behavior of *Daphnia magna* by increasing the "spinning" swimming patterns, without affecting the speed or mortality rate [126]. The behavior of *Daphnia magna* exposed to polystyrene MPs (0.125, 1.25, and 12.5 µg/mL, with sizes of 1 and 10 µm) for 21 days was investigated also by De Felice and collaborators (2019) who found that the highest concentrations of both sizes of MPs induced a change in the phototactic behavior and an increase in the swimming activity, body size, and reproductive effort [127]. In addition, it was found that polyethlyene MPs (1 µm) can induce the immobilization of *Daphnia magna* after short-term exposure to them (96 h) [128].

Other organisms, such as amphipods, nematodes, chironomids, oligochaetes, and amphibians, were negatively influenced by MPs presence in the freshwater environment. The chronic exposure of *Hyalella azteca* amphipod to polyethylene MPs reduced their growth and reproduction rates. Moreover, it was observed that polypropylene particles were more toxic than polyethylene fibers in acute exposure conditions [129]. Another effect that was observed for amphipods (*Gammarus fossarum*) after MPs exposure (polyamide fibers) was the reduction of the assimilation efficiency [130]. When the effects of low-density polyethylene MPs were compared between *Gammarus fasciatus* and *Gmelinoides lacustris* amphipods, it was observed that the two species differed in their response to MP exposure for 14 days: *Gmelinoides lacustris* was more sensitive to oxidative stress, whereas the swimming behavior changed only for *Gammarus fasciatus* [131]. For benthic nematode *Caenorhabditis elegans*, the reproductive dysfunction and decrease in the body length and survival rates were observed after exposure to 5 mg/m^2 MPs. In the same study, it was found that MPs can cause damage and oxidative stress in nematode intestines, dependent on the plastic's size [132]. Similar negative effects (oxidative stress, an intestinal injury through the hyperpermeabilization of the intestinal barrier, and an alteration of the expression of genes related to intestinal development, and a decrease in lifespan) after the exposure of *Caenorhabditis elegans* to MPs were also reported [133,134]. However, the effects of MPs on nematodes are species specific. The exposure of different nematode species to polystyrene beads revealed that *Caenorhabditis elegans* was more susceptible to MPs than *Plectus acuminatus*. Additionally, the particles reduced the carrying capacity of *Caenorhabditis elegans* and accelerated the growth of *Acrobeloides nanus* populations [135]. As well as this, it was reported that polyvinyl chloride MPs can affect the emergence, development, and weight of freshwater *Chironomus riparius* in a concentration-dependent manner [136]. Similarly, polyethylene induced mutagenicity and the cytotoxicity of erythrocytes and morphological changes in *Physalaemus cuvieri* tadpoles [137]. MPs can also be toxic to freshwater oligochaete *Lumbriculus variegatus*. Polyethylene exposure induced important sub-cellular responses in *Lumbriculus variegatus* (the reduction of energy reserves and aerobic energy production and the activation of antioxidant and detoxification mechanisms) after 48 h, while after 26 days, the rates of reproduction and biomass were not affected [138]. Additionally, it has been proved that MPs mixed with sediment are more harmful to lumbriculids than MPs that are layered on the sediment surface [139].

However, excepting the negative effects demonstrated on various species from freshwater fauna, some MPs can also not be harmful to different organisms. It was demonstrated that polyethylene MPs did not have detrimental effects on the survival and reproduction of freshwater oligochaeta *Allonais inaequalis* [140]. Additionally, it was revealed that amphipod *Gammarus duebeni* can ingest 10–45 μm polyethylene MPs by feeding on contaminated *Lemna minor,* but no impact on their mortality or mobility was observed after 48 h of exposure [141]. Similarly, *Daphnia magna* ingested plastic microbeads with a size of 63–75 μm, which filled the guts of the crustaceans, but no significant effects on their reproduction and survival were found [142]. Consistent with these studies, no negative impact of polyethylene terephthalate fragments (10–150 μm) on the survival, development (molting), energy reserves (glycogen and lipid storage), and feeding activity of *Gammarus pulex* after 24 h of exposure was reported [143]. Neither of the polystyrene fragments MPs (<63 μm) induced modifications to the oxidative balance, survival, reproduction, and energy reserves of gastropod *Lymnaea stagnalis*, suggesting that these particles are not relevant stressors for these populations [144]. Similar results were obtained also when freshwater oligochaete *Tubifex tubifex* was exposed to polyethylene microspheres (up to 10 μm, in size) and no significant differences between the exposed and control groups were observed in terms of survival and oxidative balance [145].

6. Conclusions and Outlooks

Despite the large amount of research focused on the effects induced by MPs in marine environments, the studies centered on the outcomes produced by micro-sized plastic particles on freshwater ecosystems are still limited. In this paper, we reviewed the most important implications of MPs in freshwater, considering their properties, the processes to which they are subjected after their entrance into freshwaters, and we examined their potential toxic effects on autotrophic organisms and fauna.

MPs are carbon polymers with dimensions between 1 μm and 5 mm, and they have various shapes (e.g., spherical, irregular, fibrous, films, foams, fragments, flakes or pellets, line, filament, and foil) and colors (e.g., blue, black, red, orange, yellow, and white). MPs can be associated with other particles or chemicals, including heavy metals, for which MPs have a high affinity. Based on this association, MPs are able to act as vectors for heavy metals. After they enter the environment or are generated from the plastic debris found in nature, the MPs are subjected to biodegradation processes that are based, mainly, on the action of microorganisms which use them as a carbon source.

The presence of MPs in the environment is linked with a considerable impact on the fauna and autotrophic organisms. The studies showed that MPs could have no harmful consequences, or they could induce cytotoxic effects, the repercussions being dependent on the particle characteristics, species, and method of exposure. Freshwater autotrophic organisms can be affected by micro-sized particles by the production of reactive oxygen species, inhibition of cell growth, physical damage, and the alteration of the photosynthesis process. Among the biochemical and molecular modifications induced in freshwater fauna, oxidative stress, DNA damage, neurotoxicity, changes to the intestinal microbiota, and inflammation were reported.

Freshwater represents a source of drinking water and food for humans. As a consequence of this essential need of humans, the high impact of presence of MPs in global freshwater systems is linked to the entry of MPs into the human food chain through the ingestion of food or water contaminated with these micro-sized plastic particles, which can induce considerable systemic dysregulations. In terms of the first perspective of this study, the investigation of the MPs' effects on human health could be really interesting and useful in this field. Until now, little research has been conducted in this area. Studies on various human cell types such as colon, intestinal, endothelial, lymphocytes, lung, dermal fibroblasts, and kidneys exposed to different categories of MPs have revealed important morphological and metabolic changes: modifications to the oxidative balance, genomic instability, inflammation, the loss of proliferative capacity, cytotoxicity, the triggering of autophagy, and necrosis, etc. However, the effects are dependent on the size, concentration, properties, and chemical composition of the MPs [146–157]. Along with the knowledge brought by these studies, other ones that are focused on the human systemic effects induced by MPs will be needed in the future.

In terms of a second perspective, the production of MPs will continually increase, affecting freshwater ecosystems, and also, humans life, and this aspect urgently needs to establish the criteria for plastic recycling, and also, the technological, engineering, and social tools used to solve the MPs pollution issue.

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