Research on ecotoxicology of microplastics on freshwater aquatic organisms

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ABSTRACT
As a new type of pollutant, microplastics are an emerging scientific and social concern in the environment and are widely distributed in the aquatic environment and organism. Nowadays, researches on microplastic pollution mainly focus on the marine environment. As a bridge for the migration of microplastics from the terrestrial environment to the marine environment, the freshwater environment has been deserved more attention. Published articles on microplastics in freshwater environments were reviewed in this paper, and four typical behaviors of microplastics were summarized: biological ingestion, biological attachment, adsorption of pollutants and release of plasticizers. In addition, the progress in research and results on the ecological toxicity of microplastics to freshwater organisms was also analyzed. Finally, emphasis on future research on the toxicity of microplastics to freshwater aquatics organisms was made throughout this review as a tool in microplastic risk assessment research.

1. Introduction

Plastics have the characteristics of portability, cheapness, versatility, durability and strength [1], which make plastics widely used in today’s life. Since the 1940s, global plastic production has doubled, reaching 322 million tons in 2015 [2]. Because of the large-scale use of plastic products, an average of 5–13 million tons of plastic waste enters the marine environment every year [3]. These plastic products, under the action of physics, ultraviolet radiation and biology [4], degrade into so-called ‘microplastics’, which are usually less than 5 mm in diameter. Microplastics are usually reported as particles, fragments, thin films and fibers [5], and the surface morphology of microplastics are mostly irregular and cracked, and are highly hydrophobic [6].

Microplastics can be divided into primary microplastics and secondary microplastics. Primary microplastics refer to the small micron size plastic particles produced in the industrial production process, which are mainly used in textiles, pharmaceuticals and cosmetics such as facial and body grinding [7]. Secondary microplastics are formed due to the fragmentation or degradation of large plastic fragments [8]. Crushing agricultural plastics and synthetic clothing fibers are important sources of secondary microplastics, and other sources include the plastic fragments produced by fishing industry, automobile industry and household industry [9,10]. The fibers of synthetic clothing are mainly released by washing into sewage treatment plants and eventually into aquatic ecosystems. The heavy use of agricultural plastics allows plastics to enter the soil, degrade into microplastics, and reach the ground or surface water through the action of wind and runoff.

The massive occurrence of microplastics in water can have toxic effects on organisms and cause serious ecological risks [11–13]. Toxicity mainly comes from three pathways: (1) pressure of ingestion, such as physical blockages, energy consumption of ingestion; (2) leakage of plastic additives, such as plasticizers; (3) exposure to microplastic-related contaminants [12,14,15].

In this review, we summarized the environmental behaviors of microplastics in the aquatic environment and the research on the toxicological effects of microplastics on freshwater organisms in recent years. In addition, the future research emphases are put forward.

2. Freshwater systems

2.1. Distribution and sources of microplastic

The United Nations Environment Programme has designated the theme of World Environment Day 2018 as “Beat Plastic Pollution”, showing that plastic pollution has become an important issue of global concern. Micro-scale plastic particles were discovered in the marine environment in the early 1970s [16], and the exploration of marine microplastics has been started since then. In recent years, more and more scholars have begun to realize that there is a lack of research on microplastic pollution in the freshwater environment, which is important as a source of microplastics in the marine environment. The first study on microplastics in

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freshwater systems was in 2011 when Zbyszewski and Corcoran found small plastic particles in Lake Huron, Canada [17]. So far, only 23 out of 195 countries in the world have studied microplastic pollution in freshwater systems, most of which are in North America and Europe, and also include China, India, Mongolia and other countries [18,19]. The research direction is mainly about the distribution and ecological effect of microplastics. In general, the research on microplastics in the freshwater environment is still in the initial stage.

Current limited information shows that the abundance of microplastics in freshwater environments is comparable to the level of the marine environment [20], and exist extensively in rivers and lakes. Microplastics in the great lakes of North America have an average abundance of 43,000 ind/km², mainly in the form of fragments floating on the water [21]. Microplastics have also been detected in Geneva, Costance, Neuchatel, Maggiore, Zurich and Brienzi lakes in Switzerland, with the highest microplastics abundance of Lake Geneva and Lake Maggiore, with as much as 220,000 ind/km² [22]. Taihu lake is located in the developed areas of China, and closely related to human activities, its water and sediment are heavily polluted, and the highest content can reach up to 6,800,000 ind/km², which accounts for an average abundance which is two orders of magnitude higher than in the great lakes [23]. A global model of plastic inputs from rivers into oceans based on waste management, population density and hydrological information shows that the rivers of China accounts for about two-thirds of the plastic flow into the ocean [24]. In the remote areas of Lake Hovsgol in Mongolia, with a sparse population, the average abundance of microplastics is 20,264 ind/km² [25], with the majority of microplastics in fragments and thin films and less fiber. These studies show that microplastics migration is affected by human activities, as well as by wind and run off, resulting in significant differences in the abundance of microplastics in different regions.

At present, there is no clear conclusion on the source of freshwater environmental microplastics. It has been proposed that the main sources of freshwater environmental microplastics are wastewater discharge from sewage treatment plants, degradation of bulk microplastics in water and use of sludge [21,26]. Waste from sewage treatment plants is an important point source of freshwater environmental microplastics [27,28]. Although studies have shown that wastewater treatment plants can remove plastic particles with an efficiency of 99% [29,30]. However, there is still a large amount of microplastics continuously entering the freshwater environment through the discharge of wastewater and accumulating. Some microplastics also enter into the sludge (often used as agricultural landfills and fertilizers) during sewage treatment, and then are transported to rivers and lakes as a result of surface runoff and even into the marine system [31].

2.2. Environmental behavior

After a large number of microplastics enter the water environment under industrial, agricultural and human actions, some are suspended in the waterbody, and some are deposited in the bottom, composed of sediments. Small sized microplastics are easily consumed by aquatic organisms of differing trophic levels and have been shown to biomagnify along the food chain [14]. Previous studies have confirmed the intake of microplastics by 39 freshwater species, including 4 species of fish and 35 species of invertebrate [32]. The average concentration of microplastics ingested by Tubifex tubifex exposed to sediments in urban UK was 129 ± 65.4 particles g⁻¹, mostly in form of fiber [33]. Microplastics were found in 61 of 63 Asian clam samples (2–4 clams per sample) a field investigation [34]. The content of microplastics in wild clams of Taihu lake in China is 38 ~ 3810 times that of sediment, indicating that benthic animals may have more microplastics. Laboratory experiments have also found the presence of microplastic fibers in the intestines of Daphnids [35]. Fish that feed on plankton are more likely to ingest microplastics [36] and are more present in the intestinal tract. Indigestion and intestinal damage are the most common hazards for organisms exposed to microplastics [35].

When some microplastics enter the aquatic environment, microorganism and algae will quickly adhere to the surface [14,37,38], which also includes the adhesion of organic and inorganic suspensions and eventually forms ‘biofilms’ [39]. Biofilms can change the physical properties of plastics, such as increasing the density of microplastics and reducing its hydrophobicity. As the density of microplastics increases, they will gradually deposit under water to form the composition of sediments [14,40]. Biofilms may carry certain pathogens, for example, members of the genus Vibrio have been found on particles [41]. Another study on biofilms found the presence of multiple microbial communities on microplastics, such as harmful dinoflagellates Ostreopsis sp., Coolia sp., Alexandrium sp. [42]. Adsorption of pollutants is also one of the most common behaviors of microplastics in the water environment and relates to the large surface area and strong hydrophobicity of microplastic particles. In previous studies, the adsorptive binding of microplastics on heavy metals, polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenylethers (PBDEs), polychlorinated biphenyls (PCBs), and dichlorodiphenyltrichloroethane (DDT) has been proved [7,43,44]. In the study of the interaction between metals and microplastics, plastic pellets can associate with metals, which can potentially accumulate at concentrations equivalent to, or greater than, those in the surrounding sediments or water [45]. There is also a lack of information that binds to other important pollutants, such as
pharmaceuticals and endocrine-disrupting compounds (EDCs). The chemical load of freshwater microplastics remains to be studied.

In addition, in order to improve the physical properties of plastics, such as color, fire resistance and hardness, various additives are often added in the production process of plastics [46,47], such as plasticizers, stabilizers, pigments, fillers and flame retardants. Microplastics can constantly release additives in water environments, some of which have been shown to be toxic, carcinogenic or endocrine disruptors [14]. Fries et al. [48] tested various plastic additives in Microplastics and found the presence of phthalates. Wagner and Oehlmann proved that plastic leaching EDC [49,50]. These chemicals may migrate as microplastics through the food chain to higher nutrient levels [11], potentially harming the ecosystem.

3. Ecotoxicological effects on freshwater organisms

At present, studies on the ecotoxicity of microplastics are mainly focused on marine animals, and little is known about the toxicological effects of freshwater aquatic organisms exposed to microplastics. The toxic effects of microplastics mainly include the impact of microplastics on the death rate, growth and development, food intake, reproductive capacity and gene expression. In this review, the toxicity of microplastics to aquatic organisms is summarized as the toxicity of microplastics itself and the combined toxicity of pollutants.

3.1. Toxicity of microplastics alone

Microplastics, once ingested, can affect aquatic life in various ways [32,51]. Single toxicity of microplastics is mainly manifested by physical damage, clogging the intestinal tract of organisms, reducing the absorption of nutrients. These affect the growth and development of organisms, and even causing death [47] (Table 1).

Experiments [52] on freshwater animals with *Hyalella azteca* exposure to polypropylene fiber and polyethylene particles showed that microplastics can affect the digestive function of organisms, reducing growth and reproduction. Experiments also found that the toxicity of microplastic fibers is greater than that of microplastic particles, which may be related to the longer duration of fiber in the intestinal tract. In addition, the accumulation of microplastic particles in zebrafish (*Danio rerio*) and nematode (*Caenorhabditis elegans*) [53] can cause intestinal damage, including cracking of villi and splitting of enterocytes. And nematodes exposed to 1 μm microplastic particles had the highest mortality rate, which was higher than that exposed particles size of 5 μm, indicating

| Test organism          | Polymer       | Concentration   | Time     | Effect criteria                                                                 |
|------------------------|---------------|-----------------|----------|--------------------------------------------------------------------------------|
| *Hyalella azteca*      | PP; PE        | 0.2-20,000 ind/mL | 10d      | Mortality                                                                       |
| *Danio rerio*          | PE, PA, PS, PVC | 0.01-0.08 mg/L   | 2d       | Survival; body length; reproduction                                             |
| *Caenorhabditis elegans* | PA, PS, PVC    | 0.01-0.08 mg/L   | 2d       | Survival; body length; reproduction                                             |
| *Gammarus pulex*       | PET           | 0.8-4000 ind/mL  | 24 h; 48 h | Survival; development; metabolism and feeding activity                         |
| *Chlorella* and *Scenedesmus* | PS       | 0.08-0.8 mg/L    | 2 h      | Algae growth inhibition; chlorophyll-a content                                  |

Table 1. The compound toxicity of microplastics on aquatic organisms. PP, Polypropylene; PE, Polyethylene; PA, Polyamide; PVC, Polyvinyl chloride; PS, Polystyrene; PET, Polyethylene terephthalate; d, days; min, minute; h, hour; m, month.

PP, Polypropylene; PE, Polyethylene; PA, Polyamide; PVC, Polyvinyl chloride; PS, Polystyrene; PET, Polyethylene terephthalate; d, days; min, minute; h, hour; m, month.
that the smaller the microplastic particle size, the greater the hazard. *Hydra attenuata* exposure to microplastics can significantly reduce the amount of food intake, and the degree of reduction is negatively significant correlated with the microplastic concentration [54]. In the study of the exposure effect of freshwater invertebrate *Gammarus pulex* [55], we found that the amount of microplastics in juvenile sample (average total length: 6–9 mm) was higher than that in adult sample (average total length: 12–17 mm). After long-term exposure for more than 48 days, the microplastics had no significant influence on the survival, development (molting), metabolism (glycogen, lipid storage) and feeding activity of *G. pulex*. This may be due to the habitat of the *G. pulex*, adapted to feed on indigestible materials. It also indicates that there are differences in the toxic effects of microplastics among different species.

Nano-microplastics adsorbed on *Chlorella* and *Scenedesmus* form physical blockages on light and air, which hinder photosynthesis of algae and promote the production of reactive oxygen species [56]. In the presence of microplastics, the chlorophyll concentration and population growth rate of the *Scenedesmus obliquus* significantly decreased, indicating potential chronic effects [57].

In conclusion, the research on ecotoxicological effects still exists at the individual and tissue level, and more studies on the cellular gene level are needed to provide information for the further understanding of the toxicity principle.

### 3.2. Toxicity of compound

In addition to physical damage, microplastics can also release toxic of substances (additives) or combine with other pollutants in the water environment, causing combined pollution to aquatic organisms and toxic effects (Table 2). The research on composite toxicity, as microplastic itself, is focused in marine animals, and there are few researches on freshwater organisms. Current studies in marine organisms have shown that the short-term exposure of juveniles European seabass (*Dicentrarchus labrax*) to combined microplastics and mercury pollution affects the swimming speed and resistance time of the fish [58]. The conclusion of the common goby (*Pomatoschistus microps*) exposed to microplastics adsorbed pyrene showed that the fish exhibited greater pyrene metabolites accumulation and altered mortality [59]. Another study on goby (*P. microps*) has shown that cefalexin and microplastics both have significant effects on gobies, and when they co-exist, they interact with each other. At 25°C the presence of polyethylene plastic reduces the toxicity of cephalixin [60]. Microplastics that bind to PAHs [61] have a significant ability to transfer PAHs to exposed

| Test organism | Polymer | Exposure method | Time | Concentration | Effect criteria |
|---------------|---------|----------------|-----|--------------|----------------|
| *Pomatoschistus microps* | PE | Combined exposure (chromium) | 96 h | 1-5 um | Mortality, post-exposure predatory performance (PEPP), acetylcholinesterase activity, lipid peroxidation levels |
| *Mussels* | PE | Combined exposure (chromium) | 96 h | 1-5 um | Mortality, post-exposure predatory performance (PEPP), actinidylcholine esterase activity, lipid peroxidation levels |
| *Oryzias latipes* | PS | Combined exposure (phenanthrene) | 96 h | 0.184 mg/L | Immunochemical parameters, lysosomal membrane stability, peroxisomal proliferation, antioxidant defences, oxidative stress biomarkers, neurotoxic effects and onset of genotoxicity |
| *Daphnia magna* | PS | Combined exposure (phenanthrene) | 96 h | 0.184 mg/L | Gene expression, histopathological changes, effects on dissociation and degradation of phenanthrene |
| *Scenedesmus obliquus* | LDPE | Combined exposure (phenanthrene) | 96 h | 0.184 mg/L | Gene transcription, blood biochemistry, histopathology |

Table 2. The compound toxicity of microplastics on aquatic organisms.
mussels, and significant accumulation of pyrene has also been observed in digestive tissues. The study on the marine microalgae found that the microplastics did not affect the copper-induced toxicity on *Tetraselmis chuii* within the detection concentration range (from 0.042 to 1.472 mg/l), possibly because the microplastics themselves had no significant influence on *T. chuii* population growth [62].

Researches on freshwater animals show the combined pollution of microplastics and organic pollutants can not only damage the liver cells of Japanese medaka (*Oryzias latipes*) [63], but also affect the gene expression of medaka fish from the genetic level [64]. The combined contamination of microplastics and phenanthrene can even affect the synthesis of proteins in *Clarias gariepinus* [65]. For *Daphnia magna*, the toxicity of microplastics adsorbing phenanthrene is higher than that of single microplastics [66]. In the presence of microplastics, Cr(VI) has a toxicological interaction with microplastics, resulting in oxidative damage in the early 0° age group *P. microps* juveniles [67]. However, the impact on the goby in the two estuaries is significantly different, which may be due to the large difference in the ecosystem environment between the two estuaries.

Based on the above background, it is clear that few research on the toxicity of microplastics to freshwater organisms has been conducted. Therefore, attention must be given on this specific topic in the future in order to master the mechanism of toxicity of microplastics and protect the safety of water environment.

4. Conclusions and recommendations

Over the past 100 years, the extensive use of plastics has brought great facilities to people as well as serious environmental pollution problems. Due to the difficult degradation of plastic products, plastic waste accumulates constantly in the environment, as microplastic particles after degradation, and forms plastic microparticles in the freshwater water environment. Freshwater environments are rich in aquatic species, and the ecological toxicity of microplastic pollution to organisms is not to be underestimated.

In this review paper, the main sources and environmental behaviors of microplastics in the freshwater environment were summarized, and the research progress on the ecotoxicological effects of microplastics on freshwater aquatic organisms is listed. Based on the evidence presented in this paper, it is clear that there is a fundamental gap in our knowledge of the ecotoxicology of microplastics in freshwater ecosystems. All in all, the next most important tasks are:

- **(1) Reinforcement of microplastic ingestion research is needed by environmental toxicologists on freshwater aquatic animals in order to determine the microplastic intake of key freshwater species and transfer in organisms.** Current research confirms the feeding of freshwater invertebrates and fish on microplastics, and there is a lack of research on microplastics in other species, such as zooplankton, freshwater mammals, reptiles and so on. Studies on other groups and properties of plastic on feeding capacity are also urgently needed.

- **(2) According to the different properties of different plastic materials, the mechanism of their toxic effects on aquatic organisms in the water environment is studied, not only at the level of individual organisms, but also at the level of cells and genes. Also, consider the relationship between environmental factors and microplastics toxicity, such as temperature, pH, light intensity, salt, etc.**

- **(3) Strengthen the studies on the interaction of microplastics with other pollutants in the water environment.** Microplastics can absorb persistent organic pollutants, heavy metals, etc., or release additives in the water environment, resulting in the combined toxic effect on aquatic organisms. The adsorption and release mechanism of microplastics should be studied to provide help for the study of the combined toxic effect of microplastics.

- **(4) Study technologies and methods for controlling microplastics pollution.** Source analysis of freshwater microplastics is carried out to reduce the production and use of primary microplastics from the fundamental source. For example, relevant policies and regulations are formulated to strictly control the abuse of plastic products. But more research is needed on how to deal with the ecological pollution of microplastics.

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