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Review

From marine to freshwater environment: A review of the ecotoxicological effects of microplastics

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ABSTRACT

Microplastics (MPs) have been widely detected in the world's water, which may pose a significant threat to the ecosystem as a whole and have been a subject of much attention because their presence impacts seas, lakes, rivers, and even the Polar Regions. There have been numerous studies that report direct adverse effects on marine organisms, but only a few have explored their ecological effects on freshwater organisms. In this field, there is still a lack of a systematic overview of the toxic effects and mechanisms of MPs on aquatic organisms, as well as a consistent understanding of the potential ecological consequences. This review describes the fate and impact on marine and freshwater aquatic organisms. Further, we examine the toxicology of MPs in order to uncover the relationship between aquatic organism responses to MPs and ecological disorders. In addition, an overview of the factors that may affect the toxicity effects of MPs on aquatic organisms was presented along with a brief examination of their identification and characterization. MPs were discussed in terms of their physico-chemical properties in relation to their toxicological concerns regarding their bioavailability and environmental impact. This paper focuses on the progress of the toxicological studies of MPs on aquatic organisms (bacteria, algae, Daphnia, and fish, *etc.*) of different trophic levels, and explores its toxic mechanism, such as behavioral alternations, metabolism disorders, immune response, and poses a threat to the composition and stability of the ecosystem. We also review the main factors affecting the toxicity of MPs to aquatic organisms, including direct factors (polymer types, sizes, shapes, surface chemistry, *etc.*) and indirect factors (persistent organic pollutants, heavy metal ions, additives, and monomer, *etc.*), and the future research trends of MPs ecotoxicology are also pointed out. The findings of this study will be helpful in guiding future marine and freshwater rubbish studies and management strategies.

1. Introduction

Plastics are widely used in all aspects of our daily life because of their light weight, durability, low price, and good ductility (Meng et al., 2020; Kawecki and Nowack, 2019), which makes them the universal materials (Rillig, 2012; Lambert et al., 2014). In the past 70 years, the production of plastic has increased dramatically, and its annual production has risen to 368 million tons in 2019 (Wang et al., 2021a), which means that we are living in a plastic world (Campanale et al., 2020). Due to its large-scale application and improper disposal, an estimated 5–13 million tons of plastic garbage enter the marine environment each year, with the weight of these plastics expected to equal that of fish by 2050 (Wang et al., 2019). More than hundreds of different types of polymers and mixtures of polymers are produced in industry. Among them, polyethylene (both high-density and low-density, HDPE and LDPE) is the dominant product with 29% share. The rest are polypropylene (PP, 17%), polyvinyl chloride (PVC, 9%), polyethylene terephthalate (PET, 8%), polyurethane (PUR, 7%), and polystyrene (PS, 6%). These make up approximately 80% of plastics production and contributing significantly to marine littering (Kershaw et al., 2019).

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Once entering into the aquatic environment, plastics degrade slowly into so-called secondary microplastics (MPs) with sizes smaller than 5 mm (Thompson et al., 2004; Cole et al., 2011) under the action of environmental physical, chemical, and biological process (Anon, 2019). MPs are also produced for industrial abrasives, personal care, and medical products (Hidalgo-Ruz et al., 2012; Mitrano and Wohlleben, 2020; Browne, 2015). Primary MPs may also enter into the water environment through industrial drainage systems, domestic, surface runoff, and the discharge of wastewater treatment plants eventually. In 2012, Eriksen, et al (Eriksen et al., 2013). found that the average abundance of MPs in the Great Lakes of North America is 43,000 particles/km^2, most of which are floating debris. Perhaps due to human activities, the abundance of MPs in Taihu Lake reached 6800,000 particles/km^2, which is two orders of magnitude higher than that of the Great Lakes (Su et al., 2016). Hurley, et al (Hurley et al., 2018). reported that an area of the Tame Riverbed had a maximum concentration of 517, 000 plastic particles/m^2, which were almost double the previously recorded values for any marine or freshwater regions. Recent studies showed that MPs have been detected in deep-sea sediments (Van Cauwenberghe et al., 2013), Tibet plateau (Zhang et al., 2016), or even the North Pole (Obbard et al., 2014). Due to its widely disperse and unique physiochemical properties, known as about 92% of global marine plastic debris (Eriksen et al., 2014), MPs may pose a threat to the freshwater and marine systems (Bucol et al., 2020; Rodrigues et al., 2018). Numerous studies have reported the biological effects of MPs in the aquatic environment. MPs may be easily eaten as food by aquatic organisms of various trophic levels due to their comparable size to plankton (Bessa et al., 2018; Su et al., 2019). Herein, MPs might be accumulated at a higher trophic level, enter the food chain, and endanger human health (Hwang et al., 2019). The potential negative effects of MPs on aquatic organisms contain behavioral, hematological, and biochemical changes, histopathological damage, embryotoxicity, and neurotoxicity (Chen et al., 2017; Yin et al., 2018; Lei et al., 2018a; Hamed et al., 2019; Li et al., 2020a). VOSviewer, a software tool for constructing and visualizing bibliometric networks, was used in this study to verify MPs abundance and research drive content. We collected the keywords, microplastics and aquatic organisms, using the Web of science searching. As shown in Fig. 1, studies on MPs Studies with aquatic organisms have focused on plastic debris, persistent organic pollutants, ingestion, bioaccumulation, degradation, toxicity, and human health.

Although there are many review articles about the toxic effects of MPs, studies about the influence of MPs on the food chain in freshwater and marine system are rare. The factors influencing the toxicity of MPs to aquatic organisms are not incomprehensively explored. To better understand the MPs pollution and potential risks in an aquatic environment, this review focused on the progress of the toxicity of MPs to aquatic biota with different trophic levels and elucidated the potential toxic mechanism, as well as the main direct and indirect effects on the toxicity of MPs to the aquatic organisms. Furthermore, we also briefly overviewed the analytical methods for identifying and charactering microplastics in complex environmental samples.

2. Methodology of literature review

We collected and analyzed the available literature published from 2004 to 2022 based on the database of Web of Science (https://www.webofscience.com), Google Scholar (https://scholar.google.com), and ScienceDirect (https://www.sciencedirect.com) using the keyword included but were not limited to: "microplastics" OR "microplastic" in conjunction with "aquatic", "toxic effects" AND "heavy metal". From the results obtained, we selected a total of 254 publications based on the h-index of citations, mostly from the previous 10 years, and further divided them into several subtopics, *i.e.,* ecotoxicology, toxic mechanisms, influencing factors, and characterization of MPs.

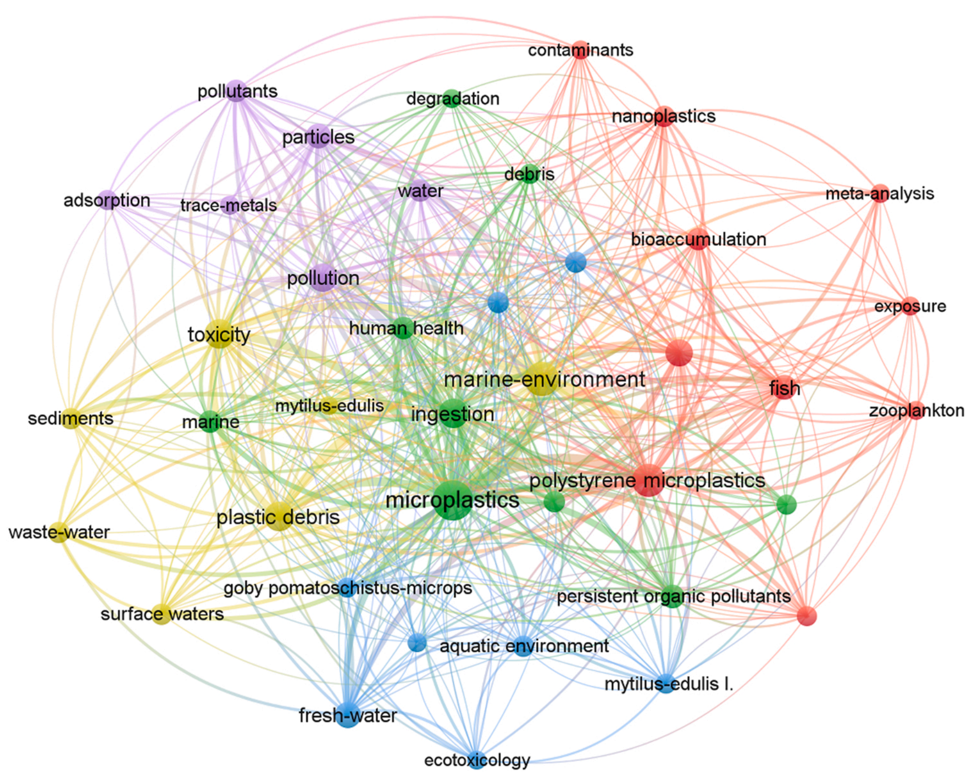

Fig. 1. The network of top 40 keywords about microplastics.The size of the dot means the rate of occurrence of the keyword, the connectingline means the international cooperation between the keywords. The data wasobtained by Web of Science.

3. Results and discussion

3.1. Toxic effects of MPs on aquatic organisms

Bacteria play a vital role in the natural environment. Once released, the interaction between bacteria and MPs increases, and its effects on the environment would not be ignored (Qiu et al., 2022). The effects of MPs on bacteria are mainly reflected in toxicity.

3.1.1. Effect of MPs on bacteria and algae

In spite of increasing research efforts, there is still little understanding of how MPs might interact with bacteria in the aquatic environment, which depends on the types of MPs and bacteria (Table 1). Due to the composition of bacteria cell walls, this may show a different behavior (toxic or nontoxic) under MPs exposure, such as bacterial susceptibilities, and attachment (Qiu et al., 2022). Microorganisms can also form biofilms on MPs, thus forming a habitat for them (Qiu et al., 2022). Upon entering an aquatic environment, MPs may quickly become adhered to the surface by microorganisms and algae (Ivleva et al., 2017; Lobelle and Cunliffe, 2011; Oberbeckmann et al., 2015). MPs have become a new biological habitat and the relative studies on the aquatic microorganisms attached to MPs can be traced back to the 1970 s (Carpenter and Smith, 1972). Owing to their low surface polarity, MPs tend to be more hydrophobic and make microorganisms colonized easily and the biological communities in their attached biofilm are called "plastisphere". Previous studies have demonstrated that a variety of pathogens are attached to the surface of MPs, which can be transmitted long-distance in water with the help of ocean currents (Goldstein et al., 2014; Feng et al., 2020a; Li et al., 2019; Yousefi et al., 2019a). For instance, MPs act as the carriers for the enrichment of vibrio in the estuarine environment with high salinity, increasing the potential environmental risk of MPs in the aquatic system (Li et al., 2019). Microorganisms attached to the surface of MPs and then enter into a new area, which could cause microbial invasion and pose a threat to the ecological environment. McCormick, et al (McCormick et al., 2014). reported that MPs acted as a bio-sponge for pathogens and parasites, which led to higher concentrations of harmful bacteria, including *Stenotrophomonas maltophilia*, *Bacillus cereus*, and *E. coli*, on the MPs' substrate off the coast of Belgium. The biofilm contains conditional pathogens, which may also cause harmful risks (Gregory, 2009).

Due to their small size, rapid reproduction, and sensitivity to toxins, algae have been used as model organisms to evaluate the potential toxicity of MPs in aquatic systems. Previous studies showed that MPs can inhibit the algal cell growth (Table 1), *e.g.,* both 20 and 50 nm PS nanoplastics (NPs) caused a significant growth inhibition on *C. vulgaris* and it took a long time for the effect to become apparent (Hazeem et al., 2020). The negative effects might be due to the impaired photosynthesis caused by MPs exposure (Qiu et al., 2022; Bhattacharya et al., 2010; Mao et al., 2018; Lagarde et al., 2016; Yousefi et al., 2019b), and such effects could be caused by the down-regulation of photosynthetic gene expression (Lagarde et al., 2016). In addition, PS could also reduce the contents of Chlorophyll A, inhibit the efficiency of the photosystem, reduce organic synthesis, and induce oxidative damage (Hazeem et al., 2020; Lanctôt et al., 2020; Li et al., 2020b; Sendra et al., 2019; Zhang et al., 2017). Most of the literature reported that the adsorption of nano-plastics or MPs on the surface of cells algae is the main reason leading to the reduction of photosynthesis through the shading effect, hindering the material exchange, and reduced CO_2 uptake (Bhattacharya et al., 2010; Saido et al., 2014). Conversely, positive effects on the growth of algae have been also reported. For example, Canniff and Hoang (Canniff and Hoang, 2018) found that *R. subcapitata* grew more in the PE MPs (63–75 μm, 130 mg/L) exposure media than without MPs media. The additive chemicals (UV stabilizers and phthalates) leached from MPs may explain why PE can promote the *Dunaliella salina* growth (Chae et al., 2019). Similarly, PS leachate may also enhance the contents of the photosynthetic pigment of algae (Li et al., 2020b; Feng et al., 2020b). In

Table 1
Toxic effect of MPs on bacteria and algae.

Test organism	Polymer/Size	Effect Criteria	Reference
Streptomyces coelicolor M145	PS 20 nm, 100 nm, 1 μm and 1 mm	Cytotoxicity, reactive oxygen species (ROS) level, permeability, the trends were size and concentration-dependent.	(Liu et al., 2021).
Escherichia coli (E. coli)	PS, 200 nm	Size and functional modification depend, ROS generation, DNA damage.	(Ning et al., 2022).
E. coli	PS, 10, 50 and 500 nm	Size-dependent effect on gene transfer, ROS generation, cell membrane permeability.	(Zha et al., 2022).
E. coli and Bacillus sp.	PS, 60, 220, 430, 700, 1040, 1700, and 2260 nm	ROS generation, Nano-PS accumulated inside bacterial cells, Micro-PS inhibited the growth of *E. coli* and *Bacillus sp.* cells.	(Kim et al., 2022).
Halomonas alkaliphila	PS, 50 and 55 nm， 1 μm	Size-dependent toxic effect，ROS generation, nano-MPs disrupt the ecological function of bacteria.	(Sun et al., 2018).
Vibrio fischeri	PE, 1–500 μm	No acute toxicity	(Gambardella et al., 2019).
E. coli and Bacillus cereus (B. cereus)	PS,	PS inhibited *E. coli* growth but promoted *B. cereus* growth	(Yi et al., 2021).
Scenedesmus obliquus	PS, 5 μm	Algal growth decreased with increasing MPs concentration.	(Chen et al., 2020).
Chlorella vulgaris (C. vulgaris)	PS, 20, 50 and 500 nm	20 and 50 nm PS showed toxicity to algae. Reduction cell viability, chlorophyll a contents and ROS increased, and cell wall damage.	(Hazeem et al., 2020).
Stylophora pistillata	PE, 106–125 μm	High concentration PE affected the photosynthetic efficiency and altered the metabolite profiles of coral.	(Lanctôt et al., 2020).
Chlamydomonas reinhardtii (C. reinhardtii)	PS, 300–600 nm	Exposure dose-dependent, EPS released by microalgae was inhibited, MDA levels increased.	(Li et al., 2020b).
Phaeodactylum tricornutum	PS, 50 and 100 nm	Toxicity, internalization or strong adsorption.	(Sendra et al., 2019).
Skeletonema costatum	PVC, 1 μm, 1 mm	Toxicity of MPs depends on particle size, No-shading effect.	(Zhang et al., 2017).
Karenia mikimotoi	PS, 65 nm, 100 nm and 1 μm	Nano/micro-PS inhibited the algal growth and caused oxidative damage. Size-dependent toxicity, cell structure damage.	(Zhao et al., 2020a).
Raphidocelis subcapitata (R. subcapitata)	PE, 63–75 μm	MPs promoted the algal growth.	(Canniff and Hoang, 2018)
Phaeodactylum tricornutum	PE, 1–500 μm	No acute toxicity	(Gambardella et al., 2019).

addition, no effect on the growth of algae under MPs exposure was also found by Seoane et al. (2019), which may be attributed to the oil body produced by algae.

3.1.2. Effect of MPs on Daphnia magna

Due to MPs' small size, they are easily ingested *via* water and food by aquatic organisms (Zhao et al., 2016; Mazurais et al., 2015; Jabeen et al., 2017), including vertebrates, invertebrates, and zooplanktonic organisms (Coverton et al., 2019; Kokalj et al., 2018; Ašmonaitė et al., 2018). MPs can enter the body of Daphnia through water uptake or feeding behavior. The MPs that are adsorbed on the surface of algae can enter the Daphnia as food (Gross et al., 2016). Therefore, MPs are often found concentrated in the intestine, which may further influence the growth, feeding, movement, and reproduction of *D. magna (*Besseling et al., 2014; Ogonowski et al., 2016; Rehse et al., 2016). For instance, Eltemsah and Bøhn (Eltemsah and Bøhn, 2019) tested the toxicity of PS-MPs with a size of 6 μm to the juvenile and adult *D. magna* and found that the EC_{50} values are 34.3 and 52 mg/L in 120 h, suggesting that a juvenile was about 50% more sensitive to the toxicity than that an adult's. Chenxi, et al (Chenxi et al., 2022). selected *Daphnia pulex* (*D. pulex*) as the model animal and found the survival function of *D. pulex* was inhibited by 2 mg/L of 500-nm PS NPs and the body length was reduced by 4 mg/L after 14 and 21 days of exposure, respectively. However, previous study also found that PE MPs filled the gut of *D. magna*, the survival and reproduction did not seem to be affected (Canniff and Hoang, 2018). Scherer et al. (2017) demonstrated that *D. magna* could ingest up to 6180 particles/h, which is higher than any other aquatic organisms they have tested, including *Lumbriculus variegatus* (8 particles/h), *Gammarus pulex* (10 particles/h), *Physella acuta* (118 particles/h) and *Chironomus riparius* (226 particles/h). Besides, Mattsson et al. (2015) suggested that nano-PS (24 and 27 nm) can transfer from algae (*Scenedesmus sp.*) and Zooplankton (*D. magna*) to Crucian Carp (*Carassius carassius*), demonstrating that PS MPs could be transferred to a higher trophic level along the food chain and showed severe effects on both metabolism and behavior in fish.

3.1.3. Effects of MPs on other aquatic animals

Studies of microplastic impacts on marine organisms have mainly been conducted in the field and in laboratories (Rehse et al., 2016), such as zooplankton (Vroom et al., 2017), corals (Hall et al., 2015), fish (Karbalaei et al., 2019), fur seals (Perez-Venegas et al., 2018), and whales (Moore et al., 2020; Besseling et al., 2015) who ingested the MPs with the different trophic levels from the marine environment. In the lab conditions, many studies have also confirmed that the marine organisms ingested the MPs, *e.g.,* mussels (Farrell and Nelson, 2013), freshwater bivalve (Oliveira et al., 2018), krills (Dawson et al., 2018), pelagic species (Lee et al., 2013), amphipods (Wright et al., 2013a), and fish (Jabeen et al., 2017). Studies under lab conditions also found the transfer of MPs from zooplankton to mysid shrimps (Setälä et al., 2014) as well as from mussels to crabs (Farrell and Nelson, 2013). For instance, Lei et al. (2018b) investigated the toxic effects of five types of MPs (polyamides (PA), PE, PP, PVC and PS) on *Danio rerio* (*D. rerio*) and found that all MPs caused gut damage including splitting of enterocytes and cracking of villi. When microplastics (50 m) accumulate in goldfish larvae's digestive tracts, they can undergo oxidative stress, organ damage, and inhibition of growth and movement, and microplastics (70 nm) can penetrate into muscle tissues through the epidermis of larvae, resulting in greater adverse effects (Yang et al., 2020a). It is also believed that the nanoplastics may be able to pass the blood-brain barrier, resulting in brain damage and behavioral abnormalities in the crucian carp fish (Mattsson et al., 2017). The accumulation and tissue distribution of PS-MPs in the red tilapia (*Oreochromis niloticus*) followed the order gut > gills > liver ≈ brain (Ding et al., 2018). They also found that PS inhibited the AchE activity in the brain and disturbed metabolism in the liver of red tilapia (Ding et al., 2018). On the contrary, other studies find no adverse effects on aquatic toxic biomarkers when MPs were used (Kaposi et al., 2014; Van Cauwenberghe et al., 2015). Even though, data on the absorption, bioaccumulation and metabolism fates will help to further understand the toxicological effects of MPs on the aquatic organisms (Table 2), however, several studies conducted in the field have demonstrated that fish retain only a small amount of microplastics, which are unlikely to accumulate in the intestinal tract for very long periods of time and are unlikely to adversely affect wild fish, specifically top predators, for an extended period of time (Chagnon et al., 2018). Therefore, there is still a lot of work need to be done to quantitatively study the complete process on the MPs and organisms at environmentally relevant concentrations.

3.2. Potential toxic mechanisms of MPs to aquatic organisms

The exact toxic mechanisms of MPs are still under study. However, some mechanisms have been proposed to explain the toxicity of MPs to aquatic organisms. This includes the lack of energy supply, intestinal damage, metabolism disturbance, immunotoxicity, neurotoxicity, reproductive toxicity, and oxidative damage, which are listed in Table 3.

Recent studies showed that the potential toxic mechanism of MPs on bacteria and algae mainly includes oxidative stress caused by ROS generation (Ning et al., 2022; Sun et al., 2018), interface effects (Bhattacharya et al., 2010), and cell membrane damage (Déniel et al., 2020). The toxicity of MPs to microalgae may also contain such shading effects, genotoxicity, however, these mechanisms need to be further demonstrated. ROS has been reported to be responsible for the toxicity of MPs toward marine bacterium such as *Halomonas alkaliphila (*Sun et al., 2018*)*, *Chlorella pyrenoidosa (*Mao et al., 2018*)*, *Microcystis aeruginosa (*Zheng et al., 2021*)*, *etc.* Due to interaction of the algal exudates, solution chemistry, plastic surface properties, and electrostatic interaction, NPs could adsorb on *Chlorella* and *Scenedesmus* to form a physical blockage that hinders the entry of light and CO_2 uptake, then inhibit photosynthesis and promote ROS generation (Bhattacharya et al., 2010).

Considering the similar specific gravity of algae and MPs, which makes MPs become a prey analog for plankton and may be treated and ingested similarly as food (Brillant and MacDonald, 2000). The ingestion of MPs items is widely recognized in aquatic organisms. Based on their physical properties, MPs constitute the first-line contact with aquatic organisms by ingesting plastic debris of different sizes. Ingestion of MPs may lead to asphyxia or a decreased sense of feeding when MPs are permanently retained in the digestive tract, which would cause digestive dysfunction, reduce the absorption of nutrients and then inhibit growth and the reproduction of aquatic organisms, and even cause death (Li et al., 2018; Au et al., 2015). For instance, Jemec et al. (2016) found that PE MPs blocked the gut in all dead Daphnis. Compared with PE beads exposure, the survival of *D. magna* under PE fragments exposure was reduced by 50%, which may ascribe to the two-fold greater retention time of PE fragments in the digestive tract (Ogonowski et al., 2016). Similarly, Cappello et al. (2021) reported the number of MPs accumulated in mussel digestive glands and caused osmoregulation, energy, and protein metabolism disorders. These findings suggest that the accumulation and the long retention time of MPs in the gut contribute to the death of *D. magna* and other aquatic organisms.

In the marine and freshwater systems, more than 150 fish species have been reported that can ingest MPs (Jabeen et al., 2017). Once the MPs ingestion, structural and functional deteriorations may happen in the gut, causing nutritional and growth problems for fish. Ingestion of MPs may change the metabolic homeostatic (Mattsson et al., 2015; Lu et al., 2016), disturb the innate immune system (Greven et al., 2016), induce inflammatory responses (Lu et al., 2016), disturb the balance of intestinal flora (Qiao et al., 2019a) and cause oxidative stress (Lu et al., 2016; Qiao et al., 2019a; Yousefi et al., 2017), DNA damage (Sökmen et al., 2020) and neurotoxicity (Bhagat et al., 2020) in fish. For instance, Zhao et al. (2021) reported that PE-MPs have a significant impact on zebrafish phospholipid metabolism, affecting cell membrane stability and the ability of cells to bind essential proteins. The previous study also

Table 2
Toxic effect of MPs on fish species.

Test organism	Polymer/Size	Effect Criteria	Reference
Danio rerio (*D. rerio*)	PE, 38.26 ± 15.64 µm	Toxicity on *D. rerio* embryos and larvae; Negative effect on embryos' hatching rate.	(Malafaia et al., 2020).
D. rerio	PS-MPs, 5 µm and 20 µm	The MPs in fish gills, liver, and gut are 5 µm in diameter, the 20 µm MPs are only found in the liver; inflammation and lipid accumulation in fish liver.	(Lu et al., 2016).
zebrafish larvae	PS, 700 nm	Minimal biodistribution; Internalized MPs caused an immune response; lipid metabolism and oxidative stress.	(Veneman et al., 2017).
D. rerio	PA, PE, PP, PVC with the size of ~ 70 µm and the sizes PS are 0.1 µm, 1.0 µm and 5.0 µm	Intestinal damage, oxidative stress	(Lei et al., 2018b).
Male adult zebrafish	PS, 0.5 and 50 µm	PS MPs induced zebrafish gut microbiota dysbiosis and gut inflammation.	(Jin et al., 2018).
Larval zebrafish	PS, 5 and 50 µm	Gut microbiota dysbiosis, Genes involved in glucose and lipid metabolism are disrupted by MPs; Metabolism disorder.	(Wan et al., 2019).
D. rerio embryos	PS, 0.5 µm	The ingestion, tissue distribution and toxicity on embryos; MPs in the gut and migrated outside the gut epithelium	(Parenti et al., 2019).
Larval zebrafish (*D. rerio*)	PS, 1 µm	MPs enter into the digestive system; MPs decreased the swimming competence of larvae; Inflammation and oxidative stress related genes were significantly upregulated on MPs exposure.	(Qiang and Cheng, 2019)
Pimephales promelas	PS, 5.58 ± 0.06 µm	MPs in gut but not in other organs; short retention time; Higher BCF and BAF values than *D. magna*	(Elizalde-Velázquez et al., 2020).
Juvenile jacopever (*Sebastes schlegelii*)	PS, 15 µm	MPs reduced feeding activity, decreased swimming and exploration ability; PS accumulated in gill and gut which caused significantly gallbladder and liver damaged.	(Yin et al., 2018).
Fathead minnow (*Pimephales promelas*)	PS 41 nm and polycarbonate 158.7 nm	Nano-plastics disturbed the innate immune system of fish	(Greven et al., 2016).
Gilthead seabream (*Sparus aurata L*)	PVC, 40–150 µm	A significant increase was observed in the activity of aspartate aminotransferase and creatine kinase, as well as in the levels of albumin and glucose.	(Espinosa et al., 2017).
Common goby (*Pomatoschistus microps*)	PE, 420–500 µm	The predatory performance and efficiency of fish decreased under MPs exposure.	(de Sá et al., 2015).
European sea bass (*Dicentrarchus labrax*)	PVC, < 0.3 mm	Severe histological changes in distal intestine; Alter intestinal tissues.	(Peda et al., 2016).
African catfish (*Clarias gariepinus*)	LDPE, < 60 µm	Histopathological damaged in the gill and liver; Altered the blood biochemical parameters.	(Karami et al., 2016).
Goldfish (*Carassius auratus*)	ethylene vinyl acetate (EVA) fibers, PS, and PA, < 5 mm	Shape and size-dependent effects; Fibers accumulated in gills, gut and feces; liver damage	(Jabeen et al., 2018).
Silver barb Barbodes gonionotus fry	PVC, 0.1–1000 µm	No histopathological damage to gut tissue or gills; gut mucosal epithelium thickened under PVC exposure.	(Romano et al., 2018).

suggested that PS-MPs can disrupt lipid and energy metabolism in fish liver (Lu et al., 2016), providing new insights into the molecular mechanisms of MPs-induced toxicity. In Addition, exposure to MPs may also induce neurotoxicity and disturb the nerve-related enzymes in aquatic organisms, such as acetylcholinesterase (AchE). As shown in Table 3, AchE may be a sensitive and suitable indicator to evaluate the toxicity caused by MPs.

3.3. Factors influencing the toxicity of MPs to aquatic organisms

Identifying the main influencing factors of the toxicity effects and the potential toxicological mechanism of MPs on aquatic organisms are the important link to improve the ecological risks assessment system of MPs.

3.3.1. Direct factors: physiochemical properties of MPs

In toxicological research, regardless of the research focus, the physicochemical properties of MPs as the basic information must be provided. Physicochemical properties affect and determine their environmental behavior and biological activities. They influence the mobility, transformation, and fate of MPs in the water environment. The physicochemical properties determine the bioavailability and the subsequent biological effects of MPs in aquatic organisms. The main physicochemical parameters of MPs include chemical composition, sizes, shapes, functionalization, and surface charge (Prata et al., 2019) (Table 4).

Firstly, the toxicity of MPs is highly associated with their chemical compositions. For instance, Lagarde et al. (2016) investigated the toxicity effects of PP and HDPE on a model freshwater algae *C. reinhardtii*, the results suggested PP inhibited the growth of *C. reinhardtii*, but HDPE did not. Only PP presence, a hetero-aggregates with a density of 1.2 was constituted with exopolysaccharides, MPs, and algae, which caused the negative effect on the algal growth (Lagarde et al., 2016). Wu et al. (2019b) investigated the effects of MPs on the photosynthesis system of freshwater algae (*Chlorella pyrenoidosa* and *Microcystis flos-aquae*) and found that the toxicity of PVC is more severe than that of PP, which is a non-toxic material used in the manufacture of food production. Similarly, Zimmermann et al. (2020) found that PVC, PUR and polylactic acid (PLA) MPs affect the life history of *D. magna*, and MPs toxicity is dependent on material-specific with PVC showing the strongest toxicity on reproduction.

Size is one of the key factors influencing the bioaccumulation of MPs to enter aquatic organisms and affect toxicity (Gu et al., 2020). In general, the smaller the particle size, the greater the toxicity to microorganisms. A more plausible explanation for MPs' toxic effect on algae is their interaction with microalgae (*i.e.*, adsorption and aggregation, which was described before) than the non-contact shadow effect on small MP particles (Zhang et al., 2017). MPs with a similar range size as plankton, such as algae, could be ingested by various hydrobios, especially for the non-selective foraging organisms (Ma et al., 2020). Many works have studied the size-dependent uptake of MPs by aquatic organisms, and the results showed that the uptake of MPs is highly dependent on the size of particles (Wright et al., 2013b). Amphipods can ingest the sizes of 11–700 µm MPs (Chua et al., 2014), whereas, 1 µm is a normal size of crustaceans' gastric filter interception (Hämer et al., 2014). Hurley et al. (2017) found that 50 nm PS-MPs caused stronger growth inhibition of algae compared with 500 nm and 6 µm PS-MPs at

Table 3
Potential toxic mechanism of MPs to aquatic organisms.

Test organism	Polymer	Toxic Mechanisms	Reference
Halomonas alkaliphila	PS	Oxidative stress	(Sun et al., 2018).
Streptomyces coelicolor M145	PS	Oxidative stress and Cell permeability; Nano-MPs inhibited the transport capacity, primary metabolism and oxidative phosphorylation of bacteria.	(Liu et al., 2021).
Bacillus subtilis, Pseudomonas aeruginosa, E. coli, S. aureus	PE	Oxidative stress, Protein metabolism disorders	(Ustabasi and Baysal, 2020)
E. coli	PS	Oxidative stress, DNA damage	(Ning et al., 2022).
E. coli and Bacillus sp. cells	PS	Oxidative stress, MPs internalization	(Kim et al., 2022).
Skeletonema costatum	PVC	Direct physical interaction: adsorption and aggregation	(Zhang et al., 2017).
C. reinhardtii	PVC	Oxidative stress, Direct interaction between MPs and algae.	(Wang et al., 2020).
Chlorella, Scenedesmus	PS	Oxidative stress, Electrostatics interaction between MPs and algae, physical blockage of light and air.	(Bhattacharya et al., 2010).
Chlorella pyrenoidosa and Microcystis flos-aquae	PVC, PP	Inhibition of photosynthesis, adsorption of nutrients and release the toxic substances of MPs to algae	(Wu et al., 2019a).
C. vulgaris	PE, PA, PLA, and Polybutylene succinate (PBS)	Shading effect, Roughness of surface of MPs, Oxidative stress	(Su et al., 2022).
R. subcapitata	PE	MPs serve as a substrate to promote algae growth	(Canniff and Hoang, 2018)
Daphnia pulex (D. pulex)	PS	Oxidative stress, heat shock protein, energy system	(Liu et al., 2018).
D. pulex	PS	Oxidative stress, MAPK-HIF-1/NF kB-mediated antioxidant system	(Liu et al., 2020).
D. magna	PS	MPs in mechanical interference with *D. magna* on the feeding (clogging filtering function), digestion (gut filled with MPs).	(Eltemsah and Bøhn, 2019)
D. magna	PS	The abundance decreased of DNA-directed RNA polymerase and several digestive enzymes.	(Trotter et al., 2021).
D. rerio	PS	Oxidative stress	(Lu et al., 2016).
Goby	PE	Oxidative stress and neurofunction disorder	(Luís et al., 2015).
Sparus aurata Linnaeus	LDPE	Oxidative stress and inflammatory response	(Solomando et al., 2020).
red tilapia (Oreochromis niloticus)	PS	Oxidative stress	(Ding et al., 2018).
juvenile guppy (Poecilia reticulata)	PS	Oxidative stress	(Huang et al., 2020).
Goldfish (Carassius auratus, C. auratus)	PVC	Oxidative stress	(Romano et al., 2020).
C. auratus Larvae	PS	Oxidative stress	(Yang et al., 2020a).
D. rerio	PE, PS	Immune responses	(Liu et al., 2019).
Dicentrarchus labrax	PE, PVC	Immune responses, Oxidative stress	(Espinosa et al., 2019).
Cyprinus carpio	PE	Immune responses	(Banaee et al., 2019).
Symphysodon aequifasciatus	PS	Immune responses, Oxidative defense	(Wen et al., 2018).
Oreochromis niloticus	PS	Neurotransmitters of AchE decreased in fish brain	(Ding et al., 2018).
Pomatoschistus microps larvae	PE	Neurotoxicity, EROD activity and DNA damage	(Pannetier and Morin, 2020).
Clarias gariepinus	PVC	AchE and antioxidant enzyme activity decreased	(Iheanacho et al., 2020).
D. rerio	PS	AchE activity declined , and neurotoxicity	(Chen et al., 2017).

250 mg/L. When compared with micro-plastics, nano-MPs may be accumulated in tissues, causing higher bioaccumulation and toxicity (Hurley et al., 2017). A previous study found that *D. magna* did not ingest 90 μm PS whereas the other two MPs (1 and 10 μm PS) can be ingested (Scherer et al., 2017). Similarly, 1 μm PE MPs could be ingested by *D. magna* but 100 μm PE did not, and the latter did not show any toxic effects (Rehse et al., 2016). Small-sized NPs may cross physiological and cellular barriers more easily than MPs. NPs entered into the goldfish muscle tissue, displayed neurotoxicity, and showed more hazards than MPs (Yang et al., 2020b). However, Ding et al. (2020) found 5 and 70–90 μm PS-MPs induced serious oxidative damage in red tilapia than 300 nm PS, suggesting that the toxicity of MPs may not be simply determined by the size.

The shapes of MPs such as whether they are spheres, fibers, and fragments can also affect their behavior and toxicity (Albanese et al., 2012). In general, irregular MPs are likely to be more toxic than its spherical counterpart, such as for acute (Frydkjær et al., 2017) and chronic effects in daphnids (Ogonowski et al., 2016). Accumulation of MPs fragments with 63.5 μm was higher than its bead shape with a size of 80.4 μm in the grass shrimp (*Palaemonetes pugio*) (Gray and Weinstein, 2017). Similarly, Ziajahromi et al. (2017) found that PL fiber exposure caused deformities in the carapaces and antennas of freshwater zooplankton *Ceriodaphnia dubia*, whereas PE beads did not produce significant adverse effects. Compare with PS fragments and beads, the fibers could stay longer in the zebrafish gut, which caused a more severe intestinal toxicity (Qiao et al., 2019b). Lei et al. (2018b) also found that MPs fibers have a higher toxic effect on the zebrafish and nematode than its particles, which may be ascribed to the MPs fibers containing a longer duration in the gut. It has been suggested that depleted energy reserves are caused by inflammation, reduction in feeding activity, and longer residence times in the gut (Wright et al., 2013a). Long fibers may risk physical damage to the digestive tract, hampering assimilation efficiency, resulting in decreased food intake, reduced growth rates, as well as longer developmental times, *i.e.*, there could be greater mechanical damage to the gut epithelium that results from fibers rather than the round and smooth-surfaced beads (Blarer and Burkhardt-Holm, 2016). Moreover, the color and density of MPs also influence their fate and toxicity. White, blue, and clear MPs were the most commonly ingested colors of MPs by planktivorous fishes in the North Pacific central gyre, a result of their resemblance to their food sources (Boerger et al., 2010), indicating that color influences visual predators' appetite for MPs (Ory et al., 2017; Cheung et al., 2018). Similarly, as a result of density, MPs

Table 4
Main factors influencing the toxicity of MPs to aquatic organisms.

Test organisms	Polymer/Physical properties	Effects Criteria	Reference
Pseudomonas putida (P. putida), Scenedesmus sp	PS, PE, PP, PVC, PET, 200, 400, and 600 μm	These values follow further sequences: PE > PVC > PS > PP > PET for *Scenedesmus sp.*; PS > PE > PVC > PP > PET for *P. putida*	(Miloloža et al., 2022).
Sea trout Salmo trutta	PS, PET, PE, 3 mm	Genotoxicity: PS > PET > PE	(Jakubowska et al., 2020).
Green mussel Perna viridis	PP, PS, PBS (small MPs; <30 μm, medium MPs; 30–300 μm, and large MPs; 300–1000 μm)	Toxicity of small MPs: PP > PS > PBS ; Toxicity of medium and large-sized: PS > PP > PBS	(Phothakwanpracha et al., 2021).
Rainbow trout (Oncorhynchus mykiss)	PS and PET with 3 mm size, PE (150–180 μm)	PE displayed genotoxicity while PS and PET did not show any negative effects.	(Jakubowska et al., 2022).
D. magna	PP, PE, PVC, PVC and PE range size of 10–100 μm	The toxicity orders: PVC + surfactant > PE+ surfactant > PP > PE	(Renzi et al., 2019).
Thalassiosira seudonana, Dunaliella tertiolecta	PS, 0.05, 0.5 and 6.0 μm	The toxicity orders: 50 nm > 500 nm > 6.0 μm	(Hurley et al., 2017).
Streptomyces coelicolor M145	The differ size of MPs: 20 nm, 100 nm, 1 μm and 1 mm	The size-dependent toxicity effect: nano-MPs > MPs	(Liu et al., 2021).
D. magna	PS, 2 μm and 100 nm	The feeding rates reduced 21% of 100 nm compared with 2 μm PS	(Rist et al., 2017).
Goldfish Carassius auratus Larvae	PS, 70 nm and 50 μm MPs	Nano-MPs are potentially more hazardous than MPs	(Yang et al., 2020a).
D. magna	PS, 1, 10 and 90 μm	Ingested 1 and 10 μm but not 90 μm; The irregular MPs fragments have a longer duration time in gut than the regular one.	(Frydkjær et al., 2017)
D. magna	PE, 4.1 ± 1.0 μm, 2.6 ± 1.8 μm , 4.4 ± 1.1 μm	The irregular secondary MPs showed a higher toxic than the primary MPs.	(Ogonowski et al., 2016).
Marine bacterium Halomonas alkaliphila	NH_2-PS, 50 nm; non-modified nano-MPs, 55 nm;	The toxicity order of PS: NH_2-PS > non-modified.	(Sun et al., 2018).
Dunaliella tertiolecta and Artemia franciscana	40 nm PS-COOH and 50 nm PS-NH_2	PS-NH_2 inhibited the growth of algae while PS-COOH did not.	(Bergami et al., 2017).
Ascidian Ciona robusta (Phylum Chordata)	60 nm PS-COOH and 50 nm PS-NH_2	PS-NH_2 caused the toxicity on the embryonic development, but PS-COOH did not affect.	(Eliso et al., 2020).
Sea urchin embryos (Paracentrotus lividus)	40 nm PS-COOH and 50 nm PS-NH_2	No embryo toxicity was observed for PS-COOH up to 50 mg/L whereas PS-NH_2 caused severe developmental defects	(Della Torre et al., 2014).
nematode C. elegans	PS and PS-PEG, PS-COOH, PS-SOOOH and PS-NH_2, 50 nm	The rank order in toxicity of PS-NH_2 > PS-SOOOH > PS-COOH > PS > PS-PEG (15 μg/L) in reducing locomotion behavior and causing gut barrier deficit	(Qu et al., 2022).

are distributed and disposed of in various habitats and biota as they affect their trajectory, sinking velocity and spatial distribution (Ma et al., 2020; Courtene-Jones et al., 2017; Cole et al., 2016).

In addition to the physical-based toxicity of MPs, the chemical-based toxicity induced by the release of monomers and additives, such as vinyl chloride, styrene, and bisphenol A, has been considered a key factor contributing to the toxicity of MPs. For example, Lithner et al. (2012) evaluated the acute toxicity of *D. magna* after exposure to the leachates from different types of MPs (epoxy resin plastic, acrylonitrile-butadiene-styrene, PVC, PE, and PP) and found that the observed toxicity is MPs-types dependents. The most toxic one is PVC leachate. As additives during the manufacture of plastic products (Rios et al., 2007; Rochman et al., 2014; Yousefi et al., 2021a), once ingestion, these toxic monomers and additives would be transferred and might release from MPs to higher trophic levels (Setälä et al., 2014; Fossi and Depledge, 2014), and then accumulate and being biomagnified, and thus increasing the risk to the aquatic organism and humans eventually (Rochman et al., 2013; Browne et al., 2013; Andrady, 2017; Yousefi et al., 2021b).

Furthermore, surface functionalization and surface charge are also important factors determining the toxic effects of MPs. Surface functionalization and surface charge play a vital role in the “nano-bio” interface for the interfacial dynamics and thus they are responsible for the interactions with living systems (Corsi et al., 2020). The most common studies are amino functionalized (-NH_2) and carboxyl functionalized (-COOH) PS MPs, which display positive and negative surface charges, respectively (Murano et al., 2021). MPs toxicity may also be affected by the functional groups on MPs. Interestingly, PS-NH_2 often exhibited significant toxicity in many aquatic organisms, causing physiological alternations (Bergami et al., 2016, 2017; Yousefi et al., 2016), developmental disorders (Tallec et al., 2019; Mal et al., 2017), and oxidative stress (Feng et al., 2019), while PS-COOH (the negatively charged counterpart) is usually less harmful. For instance, González-Fernández et al. (2021) used 50 nm PS MPs with or without surface functionalization to investigate their environmental risk to marine life, and found that only PS-NH_2 were toxic to brain-derived cell line (SaB-1) from fish cell lines while PS-COOH and PS pristine did not. A recent study employed PS and its modified derivatives (PS, PS-PEG, PS-COOH, PS-SOOOH and PS-NH_2) to investigate the toxic effects on nematode *Caenorhabditis elegans,* the authors also found that the toxic orders is PS-NH_2 > PS-SOOOH > PS-COOH > PS > PS-PEG (Qu et al., 2022). Cationic MPs interact strongly with the phosphate groups of the cell membrane, contributing to the observed toxic effect (Foroozandeh and Aziz, 2018). Taken together, these findings suggested that the toxicity of MPs is strongly correlation with surface charge-dependent to aquatic organisms.

3.3.2. Indirect factors: combined effects of MPs and other contaminants

Beyond the direct toxicity factors caused by MPs that were discussed above, the toxicity of MPs might be also affected by other factors such as the presence of other chemical pollutants, which may cause synergistic or reduced effects. MPs can strongly adsorb and enrich other pollutants, *e.g.,* the adsorption coefficient of PP for DDE is 10 [6, (Mato et al., 2001)]. This indicates that MPs could act as a carrier of contaminants, such as pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and heavy metals which can be adsorbed by electrostatic attraction or hydrophobic interactions.

Adsorption of these pollutants on MPs can enhance their long-distance transport in aquatic environments (Qu et al., 2020). The adsorption also allows MPs to bring these pollutants into aquatic organisms and release them inside the organisms, causing "Trojan effects” that might enhance the toxic effects. Additionally, it will increase the risk of food web transfer and bioaccumulation of toxic substances and further increase their ecological risks of this contaminants (Naqash et al., 2020). For instance, zebrafish gills, guts and livers accumulated cadmium (Cd) 46%, 25%, and 184% after co-exposure to 200 μg/L of

PS-MPs and Cd, which enhanced the levels of the inflammation and oxidative stress in zebrafish tissues when compared with Cd treatment alone (Lu et al., 2018). Lee et al. (2019) found that PS NPs only showed a slight effect on the survival and hatching rate, developmental, and cell death of zebrafish embryos, while Au ions synergized these effects significantly. In addition, Yan et al. (2020) found that PS MPs (2.5 μm, 100 μg/L) promoted the bioaccumulation of Zn, Pb, and Cd in marine medaka *Oryzias melastigma* tissues (*e.g.,* gonads, livers, brains, and guts). Meanwhile, PS MPs mixed with heavy metals increased combined-pollution load, which adversely affected particular bacteria and gut function in the male medaka (Yan et al., 2020). Nevertheless, previous studies have found that MPs (1–5 μm, 0.26 and 0.69 mg/L) adsorbed Hg (0.010 and 0.016 mg/L) and decreased Hg accumulation within tissues (*e.g.,* muscle, brain) of juvenile European seabass *Dicentrarchus labrax*, and that both mixtures altered enzyme activity, caused lipid damage and oxidative stress, and resulted in neurotoxicity (Barboza et al., 2018a, 2018a, 2018b). Moreover, recent studies have also shown that MPs can highly adsorb and concentrate hydrophobic organic contaminants, such as PAHs, on their surface (Zhang et al., 2015). The combination of MPs and organic contaminants can improve its bioavailability along the food chain (Zhao et al., 2020b; Wardrop et al., 2016), and may enter the human being's body eventually (Wang et al., 2021b).

The association between MPs and other contaminants is still under debate. Many studies showed that the existence of MPs may have serious consequences for the ecological risk of environmental contaminants (Xia et al., 2020; Mahdi et al., 2022; Yousefi et al., 2021c), however, some studies considered that the negative effects caused by MPs could be ignored (Bakir et al., 2016; Koelmans et al., 2016). For instance, Trevisan, et al (Trevisan et al., 2019). reported that MPs decreased the toxicity of PAHs in zebrafish. In the presence of Nano-PS, PAHs sorb to its surface, which increased the agglomeration rate of Nano-PS and thus decreased their concentration, uptake, and toxicity (Trevisan et al., 2019). There is no doubt that the different doses and kinds of chemical contaminants may change the associated impacts of co-exposure. However, no consensus has been made on the effects co-existing pollutants on of the toxicity of MPs. Microplastics can therefore act as sources as well as sinks of associated chemical contaminants in a variety of media environments (Alimi et al., 2018),the effects of which need further studies (Wang et al., 2021b).

3.4. Identification and characterization of MPs in complex aquatic environmental samples

Acquiring information on MPs isolation, purification, concentration, fragmentation dynamics, polymer and size distribution, and temporal and spatial changes is a prerequisite to knowing the fate and influence of MPs. Following the detection of MPs throughout the world, microplastics became recognized as a global issue (Yu et al., 2018). Generally, visual inspection is conducted first before determining the type of polymer. To avoid the occurrence of false positives or negatives (da Costa et al., 2017), chemical composition analysis combined with optical and spectroscopic tools were used together.

3.4.1. Isolation and purification of MPs

Despite years of research on MPs, methods for sample isolation and purification are yet standardized. Studies have reported varying results and cannot easily be compared. Samples can be collected in bulk or by volume reduction (Hidalgo-Ruz et al., 2012). In fact, the bulk sampling approach has been used in only a few studies. For the volume-reduced sampling, manta trawl and neuston plankton net are two commonly used methods to collect surface water samples (Li et al., 2018). Water volume is calculated using a flow meter during sampling by calculating the volume of water that has been filtered through the mesh (Free et al., 2014). A typical mesh size of 330 μm was used for sampling MPs in freshwater and marine environments as suggested by the National Oceanic and Atmospheric Administration of the United States, and this choice was primarily driven by the lower size boundary for microplastics of 333 μm (Arthur et al., 2009).

Plastic purification requires the use of its properties that are different from the surroundings in order to isolate plastic from complex aquatic environmental samples. Hydrophobicity and less density are two characteristics of plastics. In addition, when the samples have low solids content, plastics can be purificated by size-based filtration (Hernandez et al., 2017; Mintenig et al., 2018), density-based (Claessens et al., 2013; Herrera et al., 2018), and hydrophobicity-based approaches (Imhof et al., 2012; Crichton et al., 2017; Grbic et al., 2019). The purification of nanoplastics has been less reported, but adapting methods developed for engineered nanoparticles may prove to be beneficial, such as magnetic field flow fractionation (Gigault et al., 2017), gel electrophoresis (Nguyen et al., 2019), and size-exclusion chromatography (Llorca et al., 2020).

3.4.2. Visual characterization

Visual recognition is a cheap, simple, and fast technique, which can observe MPs *in situ*. Large plastics (1–5 mm) can be seen with the naked eye, while small plastics ($<$ 1 mm) must be identified using microscopy. However, visual analysis of samples is prone to produce false results. For instance, the "blue" fibers identified by visual microscopic were confirmed as a cotton-indigo by μFT-IR (Dyachenko et al., 2017) (Table 5).

Scanning electron microscopy (SEM) can provide high magnification and extremely clear micrographs of plastics, which is helpful to distinguish between MPs and organic matters (Cooper and Corcoran, 2010). Combined with Energy-dispersive X-ray spectroscopy (EDS), SEM-EDS can obtain the elemental composition of MPs, carbon-based MPs would be identified from inorganic particles (Shim et al., 2017; Dehghani et al., 2017). SEM can be used to detect the different shapes and sizes accurately, such as irregular spherule and fiber, and EDS can detect the trace amount of elements of Si, Mg, Ca, Na and Al (Dehghani et al., 2017). When the SEM-EDS is conjunct with optical microscopy, the latter can find the size of plastics range from 70 to 600 μm, and the former can help to identify the characteristic element, such as chlorine, and thus confirm the polymer are chlorinated plastics, such as PVC (Wang et al., 2017). However, the equipment of SEM-EDX is very expensive, the sample preparation steps are cumbersome, and it is also time-consuming to fully inspect all samples, thus it may limit the number of plastic particles that can be detected in a given timeframe.

3.4.3. Confirmation of MPs' composition

The spectroscopic methods can distinguish between natural and plastic particles. The spectra information collected from scattering and absorption phenomena may contain the characteristic frequency caused by the excited state in the sample. As is known, the energy difference between the ground and excited state is a key characteristic of a specific molecular structure and which is shown in the spectral signal. Fourier Transform Infrared Spectroscopy (FT-IR) is a common and powerful used technique for distinguishing the physicochemical properties of MPs (Nguyen et al., 2019). FT-IR and its optimized devices, such as micro FT-IR (μFT-IR) (Vianello et al., 2013), focal plane array (FPA) detector-based μFT-IR imaging (Löder et al., 2015), and attenuated total reflectance (ATR) FT-IR (Cheung et al., 2016), are also used for MPs characterization. The functional groups in the MPs can be determined by the FT-IR spectrum. For instance, FPA μFT-IR imaging has successfully imaged and identified the different types of MPs, such as PE, PP, PVC and PS, etc (Tagg et al., 2015). In addition, ATR FT-IR is superior to μFT-IR in obtaining the spectrum of irregularly shaped MPs, but it's only suitable for the analysis of particles bigger than 500 μm (Löder and Gerdts, 2015). The spatial resolutions of μFT-IR can go down to 5 μm (Elert et al., 2017). To prepare a FT-IR sample, a minimum sample thickness is about 150 nm (Mallikarjunachari and Ghosh, 2016) and the sample needs to be deposited on a special substrate, which makes FT-IR

Table 5
Detection method of MPs in the water environment.

Methods	The types of MPs	References
SEM-EDX	The different sizes, colors, and shapes (polyhedrons, hexagonal, spherule, and fiber) and elements analysis (Na, Mg, Ca, Al and Si)	(Dehghani et al., 2017).
SEM-EDX (FEI XL30 Environmental SEM, Thermo Fisher Scientific Noran System 7 EDS System) and optical microscopy (Leica DFC 420)	PE, PET, PP, and PVC, Degradation fragments from large plastic pieces and manufactured MPs can be found in the fish gut.	(Wang et al., 2017).
A stereoscopic microscope (M165 FC, Leica, Germany) ; μ-Raman spectrometer (ThermoFisher Scientific DXR2, USA)	PP, PE, PVC and nylon	(Yuan et al., 2019).
Leica MX75 stereo microscope, ATR FT-IR, Shimadzu IRAffinity-1S FTIR	PE and PP fibers, PVS and PP Fragments, PE and PVS films, other copolymer films, *etc.*	(Blair et al., 2019).
magnifying glass; Vertical optical microscope (SAIKEDIGITAL SK 2500 H, China); μFT-IR spectrometer (Nicolet iN 10, Thermo Fisher, USA)	PE, PP, PET, EVA, PS, PVA, PAN	(Zhang et al., 2020).
Optical microscope; Optical micro-Raman tweezers spectroscopy	LDPE, PS	(Ripken et al., 2021).
μFT-IR (Thermo Nicolet iN10 MX), SEM (Hitachi S-4800, Japan)/Energy Dispersive Spectrometer (EDS, EMAX).	PET, PS and diatoms	(Li et al., 2016).

not used to detect individual particles smaller than 20 μm (Löder and Gerdts, 2015) (Table 5).

Raman spectroscopy elucidates the vibrational information of the sample's molecular structure, the modern device can achieve less than 1 μm spatial resolutions (Lenz et al., 2015). As complementary to FT-IR detection, Raman spectroscopy provides a better non-polar symmetric bond response, while the former can discriminate the polar groups more distinctly. In addition, one of the advantages of Raman spectroscopy is its identification which is based on the whole wavelength spectrum and it can also detect amorphous carbon (Lenz et al., 2015). For example, even though MPs degraded under UV exposure, the Raman spectrum of MPs did not change significantly (Lenz et al., 2015). As discussed above, Raman microscopy may be a more sensitive tool for identifying MPs when compared with FT-IR (Cabernard et al., 2018) (Table 5). However, some materials show fluorescence, which masks the vibrational information. The Raman signal is heavily influenced by microorganisms (Käppler et al., 2016), dyes (Lenz et al., 2015), inorganic substances (Elert et al., 2017), and organic (Elert et al., 2017). When concurrently used with electron microscopies, such as SEM combined with Raman and FT-IR, it will provide more complete information for the MPs study. For example, Li et al. (2016) took a transparent sphere as $Al_2O_3{\cdot}SiO_2$ by μFT-IR, while SEM analysis confirmed that it was diatoms. This finding emphasized that the different complimentary tools need to be applied to accurately classify the suspected MPs (Li et al., 2016).

MPs are also analyzed using mass spectrometry (MS). For the MS analysis, MPs need to be burned or digested into gas or liquid samples for the next analysis. This method cannot provide the structure information of MPs, such as size and shape, but the type and the number of MPs can be provided.

Pyrolysis gas chromatography-mass spectrometry (Py-GC-MS) is destructive that identifies polymer types by analyzing the thermal degradation products of MPs (Qiu et al., 2016). The tool can directly detect solid polymer samples without pretreatment, and only a little quantity of sample (5–200 μg) is needed to analyze in one measurement (Silva et al., 2018). Py-GC-MS has been used to distinguish the types of polymers and related plastic additives. For instance, Fries et al. (2013) applied Py-GC-MS to identify the types of polymer in the marine sediment samples, *e.g.,* PS, PE, PP, and chlorinated PE, and found many kinds of organic substances such as diethyl phthalate, diethylhexyl phthalate, diisobutyl phthalate, dimethyl phthalate, 2,4-di-tert-butylphenol, *etc* in the polymer. Similarly, Py-GC-MS also is used to examine the types of MPs collected from the wastewater treatment plant, the results showed that PS, low-density PP, and PE were detected in the effluent samples (McCormick et al., 2016). Herein, Py-GC-MS is reliable for identifying the types of polymer, even if the difference of polymer subtypes cannot be determined, *e.g.,* HDPE *vs.* LDPE (Dehaut et al., 2016).

As a double-edged sword, while the small quantity of samples is an advantage, this limited number may affect the representativeness of sample composition during analyzing complex environmental samples, due to the samples may be uneven in a small range (Dümichen et al., 2017, 2015). To solve these shortcomings, a variant of this technology was used to exploit new methods, such as thermal extraction and desorption coupled with gas chromatography-mass spectrometry (TED-GC-MS) (Dümichen et al., 2017, 2015). This device can quickly and quantitatively analyze a large number of MPs that contains five common polymers, such as PET, polyamide 6, PS, PP, and PE, in environmental samples to ensure the representativeness of their composition, and there is no need to pre-select the MPs in the samples (Dümichen et al., 2017, 2015). Using an untargeted thermogravimetric analysis-Fourier transform infrared-gas chromatography-mass spectrometry (TGA-FTIR-GC-MS) technique, 11 polymers were identified from the South Africa and U.K. beaches, including PE, PP, PS, PET, and PLA, etc (Nel et al., 2021). This is a three-way combination technology that can characterize a wider chemical space by adding physical property analysis without requiring samples to be split or additional processing time (Nel et al., 2021). The standardized protocols for sample identification and quantification analysis are still scarce, which makes it difficult to compare the results between different works (Fig. 2). Therefore, new research methods should be carefully sorted out to formulate standard methods to evaluate the physicochemical properties, behaviors and aquatic environmental fates of MPs.

4. Conclusion and perspectives for future studies

As a new pollutant, MPs are widely distributed in the water environment and aquatic organisms, which becomes a new scientific and social environmental problem. Due to its unique physicochemical properties, plastic products are difficult to degrade and may be accumulated constantly in the aquatic environment. Even after degradation, the plastic debris could form the MPs which causes further concerns and their toxicity and ecological risks. There are abundant aquatic organisms in the marine and freshwater system, thus the ecological toxicity of MPs contamination to these organisms should not be underestimated. In this review, the main environmental behaviors and the ecotoxicological effects of MPs in the freshwater and marine environment were summarized, and our paper progressed to the potential toxic mechanism, the main factor influencing the toxicity, and the identification and characterization of MPs in aquatic organisms are listed. According to the evidence provided in this research, there is a fundamental gap in our understanding of the ecotoxicology of MPs in aquatic ecosystems. In short, the next most important tasks are:

a) Most experiments used an unrealistically high concentration. Further studies of MPs should focus on environmentally relevant concentrations.

b) Currently, MPs have mostly been studied in relation to fish and Daphnia, but knowledge of their effects on other groups of organisms, particularly invertebrates, still need to be studied. Also urgently needed are studies on other groups and the properties of plastic on feeding capacity. Other species such as Planarians *D. japonica* are a suitable model to study the biological responses to environmental contaminant

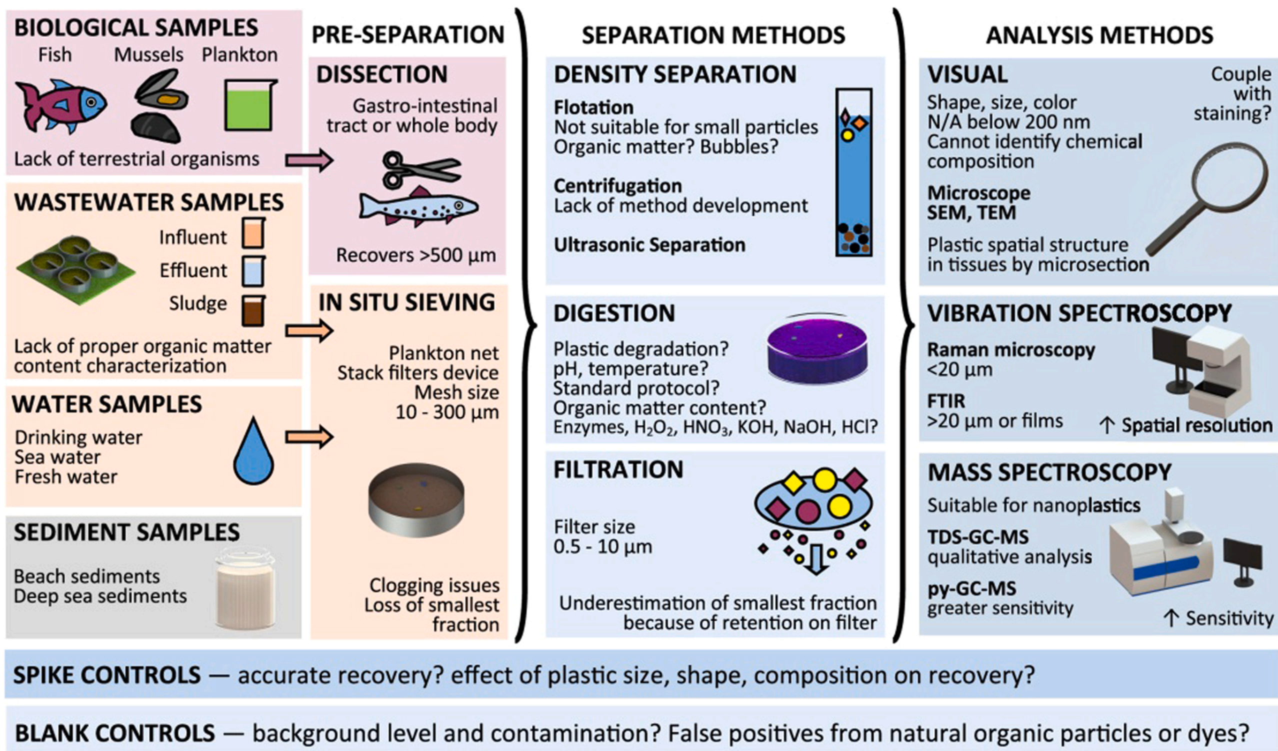

Fig. 2. Overview of MPs and nano-MPs separation and analysis methods in simple and complex matrices (Nguyen et al., 2019). Reprinted (adapted) with permission from Accounts of Chemical Research. Copyright 2019 American Chemical Society.

exposure, especially for MPs, planarians have clear intestinal tissue. The intermediate complex brain system also allows the planarian a suitable model for neurotoxicity studies.

c) The physicochemical properties of MPs play key roles in the toxic effects and toxicological research in aquatic organisms. However, this link is far from established. Further studies are needed to elucidate the quantitative structure relationships. The relationship between MPs toxicity and environmental factors is also needed to consider, such as temperature, pH, light, *etc.*

d) Ecotoxicological research is still being conducted at the individual and tissue levels, while cellular and genetic research is also needed to deepen our understanding of the mechanisms by which MPs are toxic.

e) Compared the field research or the most commonly found in the aquatic environment reports with the types of MPs used in the lab, a significant mismatch existed. In this regard, controlled laboratory studies may not always reflect real-life environmental conditions.

f) Many studies have investigated the combined toxic effects caused by MPs and other contaminants (POPs, heavy metal ions, and additives), however, little is known about the adsorption and desorption mechanism of MPs. The research on these items will help to study the joint toxicity of MPs.

CRediT authorship contribution statement

Xiaowei Li: Writing – original draft, Methodology, Resources. **Yiqing Chen:** Writing – original draft, Resources, Writing – review & editing. **Shujing Zhang:** Resources. **Yuling Dong:** Resources. **Qiuxiang Pang:** Investigation. **Iseult Lynch:** Writing – review & editing. **Changjian Xie:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing, Funding acquisition. **Zhiling Guo:** Writing – review & editing. **Peng Zhang:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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