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# Biofilm on microplastics in aqueous environment: Physicochemical properties and environmental implications

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ABSTRACT

The excellent properties of plastics make them widely used all over the world. However, when plastics enter the environmental medium, microplastics will inevitably be produced due to physical, chemical and biological factors. Studies have shown that microplastics have been detected in terrestrial, aquatic and atmospheric environments. In addition, the presence of microplastics will provide a new artificial adhesion substrate for biofilms. It has been proved that the formation of biofilms could significantly change some properties of microplastics. Some studies have found that microplastics attached with biofilms have higher environmental risks and eco-toxicity. Therefore, considering the widespread existence of microplastics and the ecological risks of microplastic biofilms, the physical and chemical properties of biofilms on microplastics and their impact on microplastics in aqueous environment are worth reviewing. In this paper, we comprehensively reviewed representative studies in this area. Firstly, this study reviews that the existence of biofilms could change the transport and deposition of microplastics. Subsequently, the presence of biofilms would enhance the ability of microplastics to accumulate pollutant, such as persistent organic pollutants, heavy metals and antibiotics. Moreover, the effect of biofilms on microplastics enrichment of harmful microorganisms is summarized. Finally, some future research needs and strategies are proposed to better understand the problem of biofilms on microplastics.

## 1. Introduction

Plastics are mainly composed of carbon, hydrogen, oxygen, chloride and silicon, while involving some contaminants such as plasticizers (phthalates), additives (organotin compounds and nonylphenols (NP)) and monomers (BPA) (Gonzalez-Pleiter et al., 2019; Teuten et al., 2009). The basic properties of lightweight, durability and resistance to degradation have driven the use of plastics as the material of choice, so plastic waste is widely distributed around the world (Osborn et al., 2014). According to reports, global plastic production reached 360 million metric tons in 2018, and the figure is still increasing every year (Amelia et al., 2021; Oberbeckmann et al., 2016; Shen et al., 2019). Although the life span of plastics is considered to be very long, they may become brittle and shatter after long-term exposure to natural environment conditions, such as solar radiation, temperature changes, physical effects of wind and wave, and biological effects (Barnes et al., 2009; Ye et al., 2020). Over a long period of time, large pieces of plastics break into smaller pieces, and eventually become smaller particles (Auta et al., 2017; Barnes et al., 2009).

Microplastics were first proposed in 2004 and are usually defined as fragments smaller than 5 mm in size (Thompson et al., 2004). They are commonly found in all circles of human life (Cole et al., 2013; Dong et al., 2020; Duncan et al., 2019; Galloway et al., 2017; Gonzalez-Pleiter et al., 2019; Rillig et al., 2017, 2020), and even in the Arctic and Antarctic (Bergami et al., 2020; Peeken et al., 2018). The aqueous environment is one of the main tendencies for microplastics in the environment. Plastics can spread from land or wastewater treatment facilities to the freshwater environment and eventually to the ocean

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(Arias-Andres et al., 2019). One study estimated that between 4 and 12 million tons of plastics entered the ocean in 2010, which caused about 1.5–5.1 billion floating plastic particles circulating in the ocean (Geyer et al., 2017). By 2025, the amount of plastic waste entering the ocean from land will increase by an order of magnitude (Amaral-Zettler et al., 2020; Jambeck et al., 2015).

According to the source, microplastics can be categorized as primary microplastics and secondary microplastics. Primary microplastics are manufactured principally for specific industrial or domestic applications, including facial cleansers, toothpaste and cosmetics, air-jet medium and as drug carriers for medicine (Auta et al., 2017; Cole et al., 2011). Secondary microplastics originate from large plastics which are broken into smaller fragments by complex environmental conditions such as wave action, ultraviolet radiation and physical abrasion (Duncan et al., 2019; Guo et al., 2020). Meanwhile, textile garments, vehicle transport and artificial turf play an important role in secondary microplastics sources (Guo et al., 2020). Due to long-term weathering in the environment, microplastics are characterized by large specific surface area, high porosity and strong hydrophobicity (Dong et al., 2020). These properties make microplastics easily used as carriers of contaminants and harmful microorganisms in aquatic environment.

A large number of studies have pointed out that the surface of microplastics would form biofilms because of the accumulation of a large number of microbial communities in aquatic environment (Di Pippo et al., 2020; Guan et al., 2020; Michels et al., 2018; Verhagen et al., 2011). The process is dynamic and highly regulated. Due to difference between microplastics and the surrounding natural environmental matrix, microplastic biofilms are distinctive and considered as a new ecological niche. The biofilms change some of the properties of microplastics through physical, chemical and biological behaviors, thus changing the behavior and state of microplastics in the environment.

Most of current research focuses on the pollution status, adsorption and toxicology, and few studies summarize the effect of biofilms attachment on microplastics. However, it is common and unavoidable for microplastics to be covered by biofilms in aqueous environment. Therefore, this review will summarize the research status of this field. The transport and deposition behaviors of microplastics in aqueous environment after the attachment of biofilms are explored. The influence of biofilm formation on the pollutant enrichment of microplastic is discussed. Moreover, the effect of biofilms on microplastics enrichment of harmful microorganisms is summarized. Finally, some future research needs and strategies are proposed to better understand the problem of biofilms on microplastics.

## 2. Microplastics

Microplastics, as a widely distributed pollutant, have become the research topic for scholars (Fig. 1). Scholars around the world have showcased research on microplastics from different aspects. These scholars have summarized different viewpoints through the results of many researches. In particular, the pathways of microplastics into the environment and the effects of microplastics on creatures and humans are two indispensable contents.

## 3. Pathways of microplastics into the environment

Microplastics particles enter the aquatic (marine and freshwater), terrestrial (soil) and atmospheric environments in various ways (Fig. 2) (Bradney et al., 2019). Entry from the land through surface runoff, fisheries and aquaculture are the main sources of microplastics in the ocean (Andrady, 2011; Guo et al., 2020; Waldschlger et al., 2020). Due to their floatability and persistence, microplastics may be widely distributed in the ocean by hydrodynamic processes and ocean currents (Ng et al., 2006). Diffusion, transportation and accumulation of microplastics from the ocean surface to the seafloor make microplastic pollution ubiquitous and persistent in the ocean (do Sul et al., 2014). The important pathway for microplastics to enter the freshwater environment is wastewater treatment plants, followed by shipping, fishing and atmospheric deposition which cannot be ignored (Edo et al., 2020; Gonzalez-Pleiter et al., 2019; Martínez-Campos et al., 2021; McCormick et al., 2014; Waldschlger et al., 2020). Sources of microplastics in the soil include agricultural application sludge, degradation of waste agricultural film, landfills and soil amendments (Dong et al., 2020; Guo et al., 2020). Microplastics enter the air through plastic debris on clothing and furniture, materials in buildings, garbage incineration, landfills, particles released by traffic, and synthetic particles used in horticultural soils and so on. In particular, the erosion of synthetic textiles and urban dust are the most important sources of microplastics (Prata, 2017; Waldschlger et al., 2020). There are many ways for microplastics to enter the environment. Hence, in order to control the number of microplastics in the environment, it is necessary to pay attention to the various ways that may produce microplastics.

## 4. The effects of microplastics on the creatures and humans

To curb microplastic pollution, it is important to reach an understanding of not only the pathways of microplastics into the environment, but also their effects on creatures and humans. Microplastics have been shown in many experiments to have an obvious impact on the life activities of animals and plants in the aquatic environment (Fig. 3a) (Ashton et al., 2010; Browne et al., 2008; Thompson et al., 2004). The results of some studies have shown that microplastics may inhibit photosynthesis and reduce chlorophyll concentration in algae (Besseling et al., 2014; Bhattacharya et al., 2010; Nolte et al., 2017). It may also cut

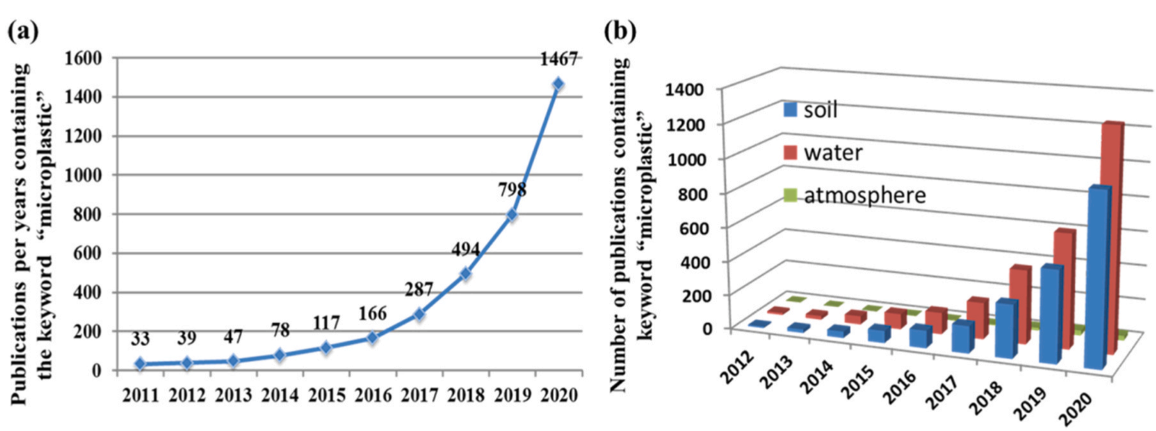


**Fig. 1.** (a) Publications per year containing the keyword "microplastic" on indexed journals between 2011 and 2020 (b) Number of publications containing the keywords "microplastic" related to soil, water and atmosphere. (data from Web of Science).

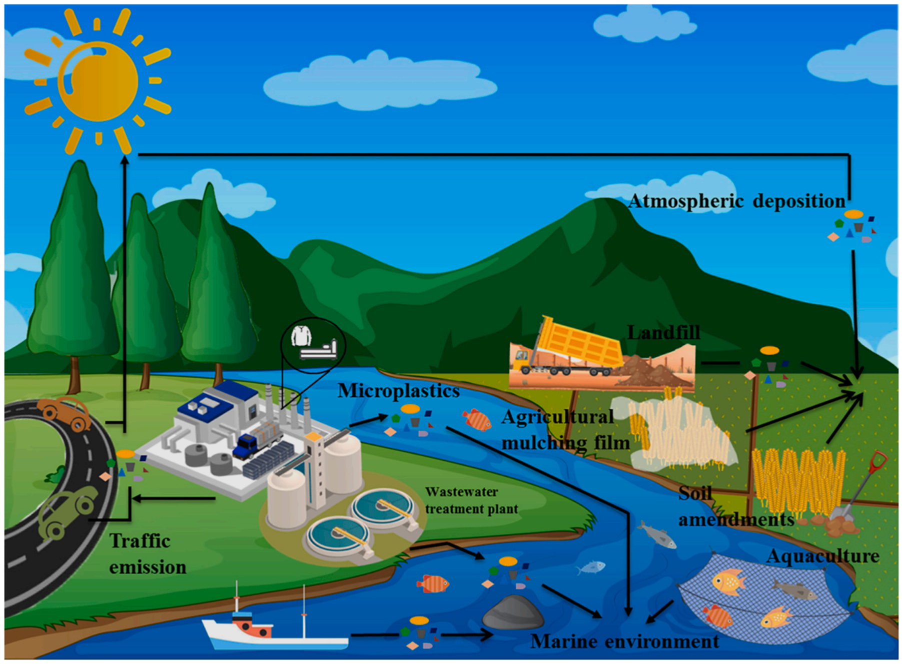


**Fig. 2.** The ways of microplastics enter the environment, including terrestrial (soil), aquatic (marine and freshwater) and atmospheric environment.

down the length of algal roots and decrease the activity of root cells (Kalcikova et al., 2020). It has been found that microplastics may weaken the viability of copepods, such as reducing their feeding activity (Lee et al., 2013; Wright et al., 2013). This will result in the normal growth, maturation and reproduction of the organism to be affected. Studies have also found that microplastics can accumulate in fish, which may disrupt the lipid and energy metabolism structure of fish liver, and cause the imbalance of microflora in the fish intestine and damage the intestinal mucosa, leading to inflammation and metabolic disorders (Lu et al., 2016; Qiao et al., 2019; Yan et al., 2021). Hirt et al. (2020) reported that rodents have been observed to absorb microplastic particles by endocytosis, transcellular and paracellular diffusion (Hirt and Body-Malapel, 2020). These microplastic particles may distribute in various organs in the body, and reduce the activity and phagocytic ability of blood cells (Ashton et al., 2010; Browne et al., 2008; Hirt and Body-Malapel, 2020). It may even induce cell death through cell membrane rupture and oxidative stress, thus affecting animal embryo development (Della Torre et al., 2014). Moreover, the presence of microplastics reduces the heat absorption rate of marine sediments, which may interfere with the sex of turtle eggs (Cole et al., 2011).

Microplastics have become ubiquitous in our living environment, and have long been closely related to our lives. Furthermore, microplastics are being produced every day by human production and living activities. As described previously, many studies have shown that microplastics have toxic effects on organisms. Today's researches are not limited to copepods, fish, algae and so on. More and more researches are in relation to the impact of microplastics on the human body (Fig. 3b). In existing studies, microplastics have two main ways to affect the human body, namely breathing and eating. Firstly, the small and light nature of microplastics makes it possible for them to bypass the human nasal cilia and enter the trachea and lungs, which could cause chronic or acute inflammation (Amelia et al., 2021; Bradney et al., 2019; Wright et al., 2017). Moreover, microplastics have been detected in common foods such as seafood, salt and honey. When people eat foods containing microplastics, some of microplastics will inevitably remain in the digestive system. Microplastics which are resistant to chemical degradation in the body may form stagnant or embedded and then move with blood into the human organ system, thus posing a potential risk to human health (Wright et al., 2017).

The way microplastics entering the environment almost involves all aspects of life, and the toxic effects of microplastics on creatures and humans cannot be underestimated. And microplastics are persistent in aquatic environment, and they may become good substrates for some organisms to colonize. Therefore, a better understanding of microplastics is essential. The study of biofilms on microplastics may provide more new directions for the research of microplastics in the future, and contribute to the prevention and control of microplastics in the future.

## 5. Physicochemical properties of biofilms on microplastics

### 5.1. Biofilm formation on microplastics

Biofilms are composed of one or more biological communities, including bacteria, fungi, algae and protozoa with different development and function (Rummel et al., 2017; Tien et al., 2013). Furthermore, biofilms can form not only on biological surfaces but also on non-biological surfaces (Davey et al., 2000). Compared with hydrophilic materials, microorganisms adhere to hydrophobic non-polar surfaces more quickly, as well as the degree of microbial colonization increases with the increase of surface roughness (Donlan, 2002). As a matter of fact, microplastics are easily become an effective substrate for microbial colonization due to their small size, the rough surface hydrophobic and long half-life, forming a small ecological niche known as the "Plastisphere" (Fig. 4a-b) (Kalcikova et al., 2020; Michels et al., 2018; Wang et al., 2020a; Zettler et al., 2013). As new ecological niches, microplastics are more tolerant of microorganisms than the surrounding environment (Xie et al., 2021; Zhang et al., 2021a). The high hydrophobicity of microplastics will produce a strong interface in aqueous environment, but it is quickly covered by organic matter, which reduces surface hydrophobicity and promotes microbial settlement (Jin et al., 2020; Wright et al., 2020). It has been found that biofilm formation is a dynamic process, which generally involves microbial adhesion, extracellular polymeric substances (EPS) secretion and microbial proliferation (Tu et al., 2020).

From the previous studies, we have summarized the process of biofilm formation on microplastics (Fig. 4c). The processes are as follows:

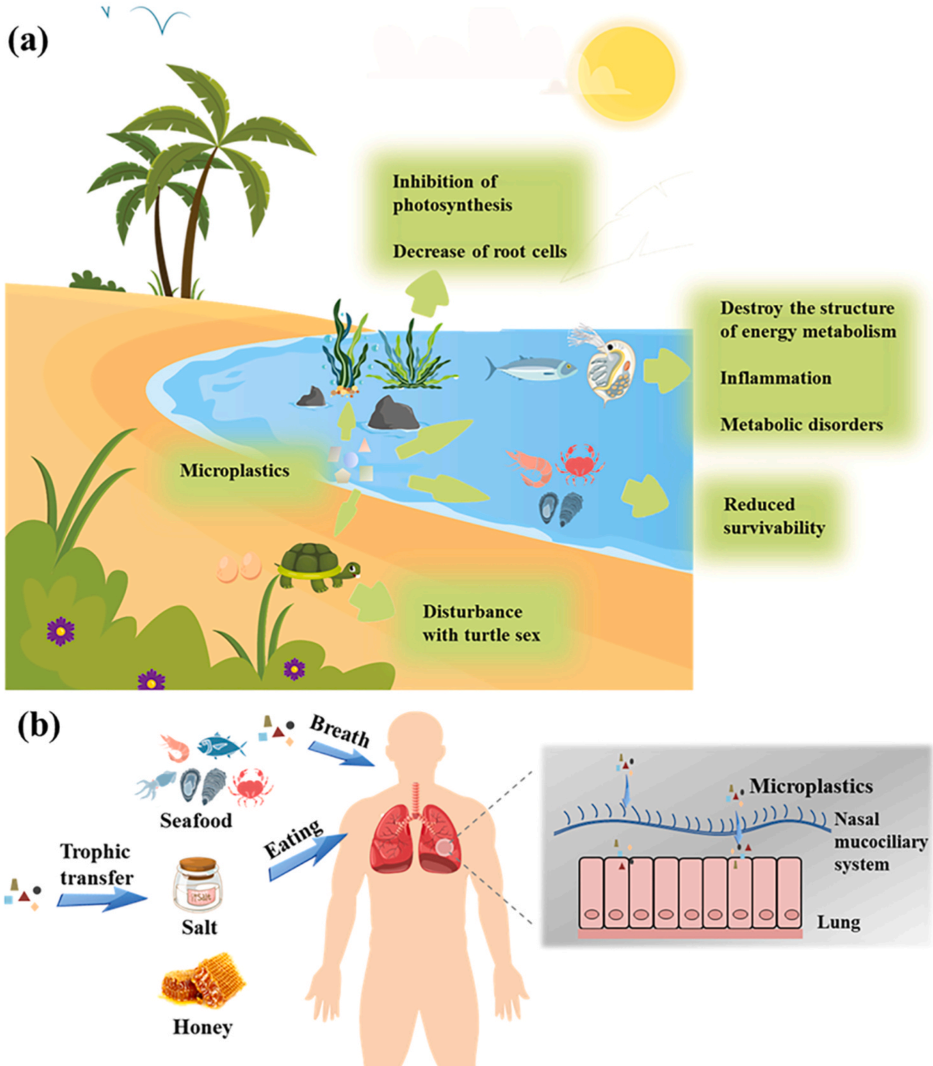


**Fig. 3.** The impact of microplastics on the (a) creatures and (b) humans.

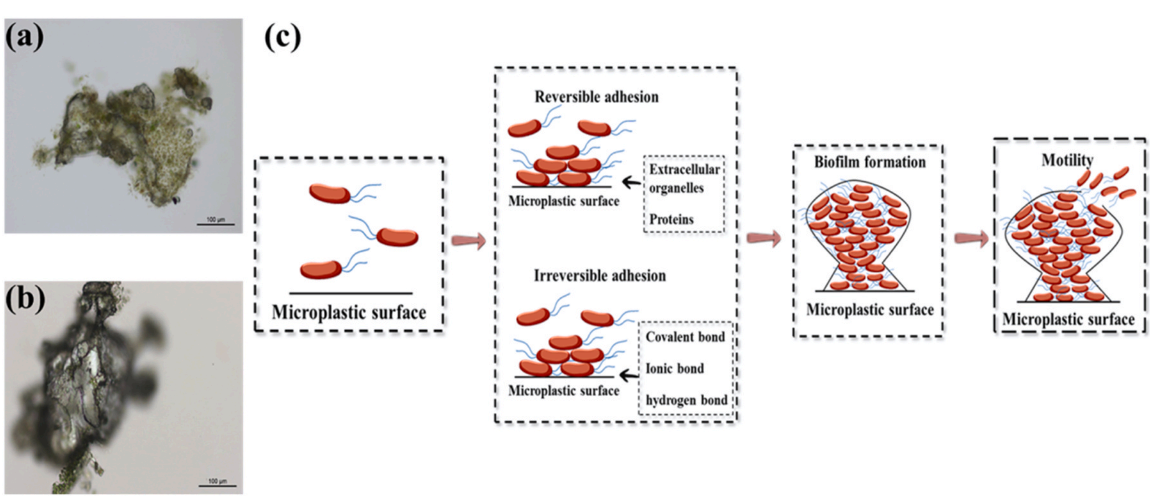


**Fig. 4.** (a, b) Microplastic particles with developed biofilm. (c) The process of biofilm formation on microplastics.
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(1) Reversible adhesion: First microorganisms reach the microplastic surface by motion (Brownian motion, van der Waals forces, hydrophobic interactions, etc.) (Katsikogianni et al., 2004; Renner et al., 2011). Then, they use various extracellular organelles and proteins to form multilayer clusters of cells on the surface of the microplastics (Katsikogianni et al., 2004; Renner et al., 2011). The cells are bridged to the microplastic surface and thus the cells reversibly adhere to the microplastic surface (Hori et al., 2010).
(2) Irreversible adhesion: At high ionic strength, flagella and EPS of bacterial cells may promote cells to penetrate the energy barrier and achieve irreversible adhesion (Hori et al., 2010). The transition from reversible to irreversible adhesion involves a variety of short-range forces, such as covalent, ionic or hydrogen bonds, that keep bacteria and microplastics surface combine firmly (Cazzaniga et al., 2015; Hori et al., 2010; Palmer et al., 2007).
(3) Biofilm formation: The organisms adsorbed on the plastic surfaces grow into small communities, which mature into biofilms with cell replication and EPS accumulation (Renner et al., 2011).
(4) Some microorganisms that are not directly attached to the surface of microplastics turn off mucus production or support the regulatory mechanisms of mucus depletion (Katsikogianni et al., 2004). Thus, the purpose of shedding from the biofilm regions is achieved. Then they move freely to a new environment, and form new biofilms by recolonization processes (Hall-Stoodley et al., 2004; Renner et al., 2011).

The special structure of biofilms reduces the probability of cell leaching, enhances their biochemical resistance and promotes intercellular communication by quorum sensing (Lawniczak et al., 2011). The unique structure of biofilms will affect the physical and chemical properties of microplastics. Changes in the properties of microplastics make microplastics pollution more complex and more potentially harmful. Therefore, it is particularly important to discuss biofilms on microplastics.

### 5.2. The uniqueness of the microplastics biofilms

Microplastics are special microbial habitats, which affect not only the structure of the microbial community, but also the ecological function of the microbial community in the aquatic ecosystem (Miao et al., 2019). Usually in a short time of contact between microplastics and water, the surface of microplastics adsorbs organic and inorganic substances to form a conditioning membrane (Rummel et al., 2017). The layer of membrane is conducive to the colonization of microorganisms on the microplastics, which is a highly controlled and regulated process (Lobelle et al., 2011). The microorganisms attached to the microplastics secrete EPS composed of DNA, proteins, lipids and lipopolysaccharides during the growth process (Flemming et al., 2010; Hori et al., 2010; Renner et al., 2011). The generation of EPS plays a key role in the formation of microplastic biofilms. Firstly, EPS could make microplastics stickier, so that more bacteria, algae, and invertebrates attach to the surface of the microplastics (Flemming et al., 2001; Lobelle et al., 2011; Michels et al., 2018; Rummel et al., 2017). Secondly, EPS can also specifically control the physical and chemical properties of microorganisms, so as to protect internal cells from the external environment (Flemming, 1993; Gilbert et al., 1997). More and more different types of organisms are attached to the surface of microplastics and gradually gather into biofilms.

The unique initial surface properties of microplastics accumulate microorganisms that are different from natural substrates, generating with different EPS, thus forming unique biofilms (Feng et al., 2020; Rosato et al., 2020; Xu et al., 2019). For example, Wu et al., showed scanning electron microscope (SEM) images of biofilms of rocks, microplastics and leaves (Fig. 5a-c) (Wu et al., 2019). It could be seen

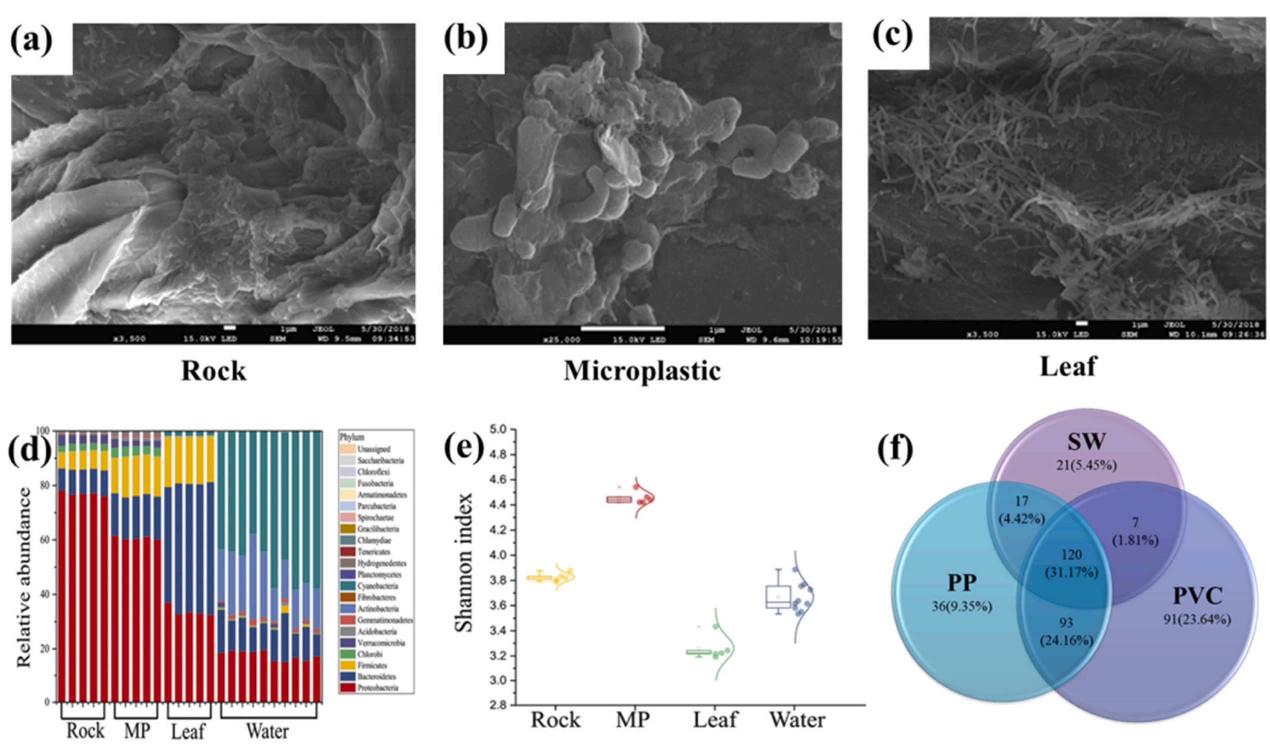


**Fig. 5.** SEM images of biofilms formed on (a) rock, (b) microplastic and (c) leaf after 14 days of cultivation in the river. Histograms of phyla abundances (d) and sample diversity (e) in three types of biofilms and river water. (f) Venn diagram showing overlaps between OTUs found in the surrounding seawater (SW) and microplastics (PP and PVC) in July 2017.
(a, b, c) Reproduced with permission from ref (Wu et al., 2019). Copyright 2019 Elsevier. (d, e) Reproduced with permission from ref (Wu et al., 2019). Copyright 2019 Elsevier. (f) Reproduced with permission from ref (Xu et al., 2019). Copyright 2019 Elsevier.

that there are obvious differences in the biofilms formed on the surface of three different substrates. The microplastic biofilms have clear contour and smooth surface. Actually, the dominant bacteria in microplastic biofilms are different from other biofilms (Fig. 5d). *Pseudomonadaceae*, *Proteobacteria* and *Campylobacteraceae* are the most common species on microplastic biofilms (McCormick et al., 2014; Wang et al., 2020a). And it has found that the abundance of *Burkholderia* in microplastics is high, while a high proportion of actinomycetes and phagocytes in non-plastic matrix biofilms (Ogonowski et al., 2018). Moreover, microplastics, as small particles, have a significantly higher diversity of microorganisms on the surface of biofilm than other large particles (Mughini-Gras et al., 2021). Wu et al., found the Shannon-Wiener index of microplastics biofilm was significantly higher than that of rock biofilm, leaf biofilm and water, indicating that the community diversity in microplastic biofilm was higher (Fig. 5e) (Wu et al., 2019). Xu et al. (2019) obtained similar results that the operational taxonomic units (OTUs) contents on polypropylene (PP) and polyvinyl chloride (PVC) microplastics were 9.35% and 23.64%, respectively, which were both higher than the OTUs in surrounding seawater (Fig. 5f). This suggested that the microbial communities were more abundant on the surface of microplastics than in the surrounding seawater.

### 5.3. Colonization of microbial community and its influencing factors

The microbial structure in biofilms includes multiple levels of structural and functional complexity, thus constantly adapting to environmental conditions (Caruso, 2020). In order to conduct a deeper study of biofilm colonization on the surface of microplastics, after consulting a large number of literatures, it is concluded that there are three main factors influencing the colonization of biological communities on surfaces, which are environmental factors, the properties of matrix and the properties of attached organisms.

#### 5.3.1. Environmental factors

Biofilm communities are different in different aqueous environments, among which flow state, nutrient status, seasonal changes and pH are all important factors that affect the growth of biofilms (Fig. 6a-b). First of all, the flow state could affect the colonization of biofilms. For example, the colonization rate and steady state of biofilms are faster, but the steady state coverage rate is lower. High flux will reduce the adhesion rate of strains, while high flow rate will result in a denser and thinner biofilm (Davey et al., 2000; Katsikogianni et al., 2004; Korber et al., 1989; Stoodley et al., 1998). Furthermore, the nutrients required for biofilms accumulation include carbon, nitrogen sources and phosphorus sources. Typically, biofilm growth is more uniform and thinner when the carbon source is unstable, and there is no significant cell association (Costerton, 1995). It has been proved that nutrients (TN and TP) are positively correlated with biofilm growth rate, while salinity has negative relationship with the growth rate of biofilms (Li et al., 2019a). Additionally, seasonal changes cause variations in temperature, light and dissolved oxygen. Compared with other seasons, summer has high temperature, long illumination time and low dissolved oxygen, which will lead to high cell metabolism speed and high enzyme activity (Chen et al., 2019; Zeraik et al., 2012; Zhang et al., 2021a). Therefore, the biofilms growing on the surface of microplastics have greater biomass and faster growth rate in summer. Dissolved oxygen plays a major role in the abundance of autotrophs in biofilms, and lower levels of dissolved oxygen lead to higher chlorophyll formation (Artham et al., 2009). Finally, the interaction between the microorganisms and the surface of the substrate is the sum of van der Waals forces and Coulomb forces

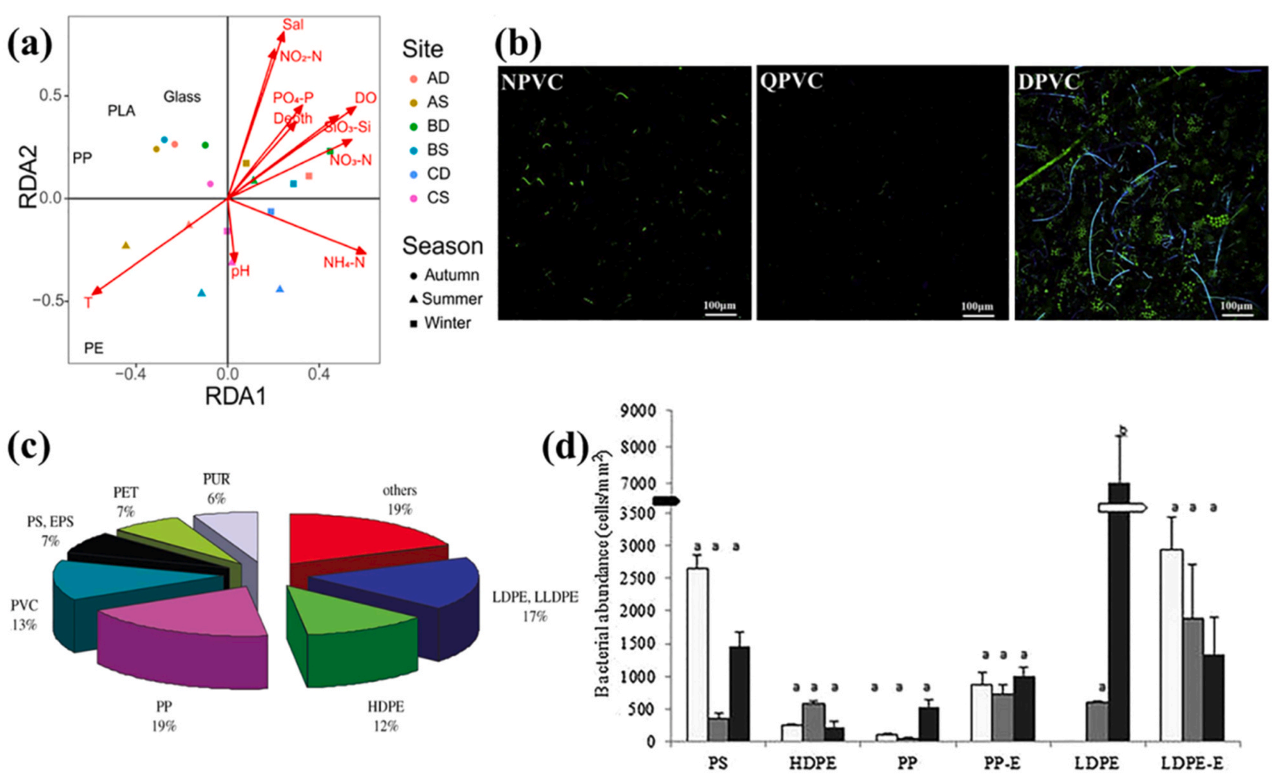


**Fig. 6.** (a) Redundant analysis (RDA) of the correlation between biomass environmental factors such as nutrient status, seasonal changes and pH. (b) 2D CLSM images of biofilms attached to PVC microplastic in three aquatic ecosystems (N, the Niushoushan River; Q, the Qinhuai River; and D, Donghu Lake). (c) World plastic materials demand by resin types 2006. (d) Bacterial abundance at week 8 of DAPI staining under fluorescence microscopy.

(a) Reproduced with permission from ref (Zhang et al., 2021a). Copyright 2021 Elsevier. (b) Reproduced with permission from ref (Miao et al., 2021b). Copyright 2021 Elsevier. Copyright 2009 The Royal Society. (c) Reproduced with permission from ref (Andrady et al., 2009). (d) Reproduced with permission from ref (Hossain et al., 2019). Copyright 2019 Wiley.

(Hori et al., 2010; Katsikogianni et al., 2004). Van der Waals interactions usually show attraction, while Coulomb interactions usually show attraction or repulsion due to the electrical layer overlap between the cell and the substrate surface (Katsikogianni et al., 2004; Morisaki et al., 2009). And pH will affect which interaction is dominant (Morisaki et al., 2009). So attachment situations will be different. Different bacteria have different preferences for pH. Therefore, the species and numbers of bacteria in biofilms are different under acidic, neutral and alkaline conditions. In summary, various factors will affect the growth of biofilms, including their morphology, steady-state coverage, growth rate and so on.

#### *5.3.2. The properties of microplastics*

As a widely used material, plastics naturally have many different types. A survey showed that PP, PVC, PS and polyethylene terephthalate (PET) accounted for nearly half of the total demand (Fig. 6c) (Andrady et al., 2009). Several studies have compared biofilms on different microplastics, and some of the results are as follows: (i) the larger specific surface area and lower bond energy made PVC more available for microbial adhesion than PP (Xu et al., 2019); (ii) biofilms grown on PP and PS microplastics showed tighter aggregation (Frere et al., 2018); (iii) compared with high density polyethylene (HDPE) and PS, higher bacterial abundance were observed on low density polyethylene (LDPE) (Hossain et al., 2019).

The difference of biofilms on different microplastics is roughly related to the following properties of microplastics, including surface energy, surface roughness, hydrophobicity and so on (Artham et al., 2009; Briand et al., 2012; Carson et al., 2013; Lee et al., 2008; Oberbeckmann et al., 2014; Xie et al., 2021). Especially in the early stages of biofilm development, the influence of the types of microplastics is more obvious (Fig. 6d) (Caruso, 2020; Hossain et al., 2019). Different types of microplastics may have different charges on their surfaces, and different charges affect the adhesion of bacteria (Gottenbos et al., 2001; Hossain et al., 2019). For example, studies have found that PE and PS microplastics are negatively charged, which is relatively unfavorable for bacterial adhesion. The positive charge on the surface of some microplastics may damage the cell membrane of gram-negative microorganisms. In addition, the increase of the surface roughness of microplastics not only increases the surface area, but also increases the affinity for microorganisms, providing a more comfortable environment for microorganisms to attach (Fotopoulou et al., 2012; Zhao et al., 2021). The rough and inert surface of microplastics could concentrate the nutrients dispersed in the surrounding environment and provide sufficient nutrients for the formation of biofilms (Hossain et al., 2019; Zhao et al., 2021). The hydrophobicity of microplastics will affect the degree of algae which are often the first organisms to colonize on microplastics growth, and thus affect biofilms growth (Miao et al., 2021a; Wright et al., 2020). Moreover, the difference of nutrient richness on the substrate surface will also affect the adhesion of microorganisms. Previous studies have shown that *Salmonella* spp and *Escherichia coli* have stronger adhesion to nutrient-poor substrates, while *L. monocytogenes* and *Staphylococcus sciuri* have a higher adhesion rate to nutrient-rich substrates (Stepanovic et al., 2004; Zeraik et al., 2012).

#### *5.3.3. The properties of attached organisms*

The structure of microbial cells plays an indispensable role in the initial colonization of microorganisms (Pompilio et al., 2008). Studies have shown that different organelles of bacteria have important roles in the adhesion process (Korber et al., 1989; Mafu et al., 2011). For example, flagella play a key role in the initial reversible adhesion stage, so the strains without flagella are weaker in both surface adhesion and biofilm formation. Biofilms include a variety of microorganisms and the species diversity and close association of microbial communities lead to complex relationships of synergy and competition. The formation of certain biofilms is more stable and productive than the original colonized community, where synergistic interactions between microbial communities play an important role (Rickard et al., 2010; Simoes et al., 2007). Biofilms serve as substrates that allow the exchange of various metabolites, signaling molecules, and genetic material, providing a basis for cooperation between different microorganisms (Flemming et al., 2016). In addition, competitive effects often exist. Pre-colonizing microorganisms could inhibit the colonization of other microorganisms by inhibiting intercellular communication or degrading polysaccharides and protein (Flemming et al., 2016). Some members of the microorganisms that dominate on microplastics have a quorum sensing system, signaling systems between bacteria (Xu et al., 2019). The bacteria control the formation and succession of biofilms by producing and responding to signaling molecules (Xu et al., 2019). Therefore, the microorganisms that colonize first will affect the diversity of later organisms in the biofilms. For example, *Acinetobacter calcium* could act as a bridge in the process of biofilms formation and automatically gather some bacteria to form copolymers. Therefore, microbial properties are one of the factors influencing factors of biofilms formation.

## 6. Effects of biofilms on the behavior of microplastics in aquatic environment

### *6.1. Transport and sedimentation*

Firstly, the long life of microplastics allows their long-distance transport in the aquatic environment (Mughini-Gras et al., 2021). Then, if the density of microplastic particles is less than water, they will float on the surface for a long time and gradually form biofilms (Chubarenko et al., 2016). Over time, the increase of biofilms thickness will cause the size and density of microplastics to increase, and the surface hydrophobicity will decrease, so the buoyancy of microplastics will change (Andrady, 2011; Chen et al., 2019; Ding et al., 2015; Miao et al., 2021a). The microplastic particles floating on the water surface are gradually distributed throughout the water, and some particles even sink deeper into the sediments (Kalcikova et al., 2020). Therefore, the formation of biofilms will adjust the buoyancy of microplastics and change their transport paths, including vertical and horizontal transportation (Chen et al., 2019; Syberg et al., 2015).

Many scholars have conducted researches on the relationship between biofilms and microplastic transport (He et al., 2020; Kvale et al., 2020; Lobelle et al., 2011). The study of Lobelle et al., found that the changes in the hydrophilicity of the microplastics surface were consistent with the formation of biofilms (Fig. 7a-b) (Lobelle et al., 2011). Microplastics initially floated on the surface because of their hydrophobicity, when they were incubated in water. Biofilms were observed in the second week, and the hydrophilicity of microplastic surface was greatly improved. By the third week, microplastics began to sink into the water. What's more, research by Chen et al., showed that during the growth process of biofilms on microplastics, suspended minerals would be embedded into the biofilms (Chen et al., 2019). As biofilms developed, the volume and density of microplastic particles would become larger (Fig. 7c). According to Stokes' law, steady settling velocity of particles is proportional to the square of diameter. Therefore, the increase of particle size accelerates the sinking process of microplastics (Shapiro et al., 2014). Finally, the main components of biofilms, EPS, polysaccharides and humus, can narrow the flow path and roughen the surface in the porous medium, which helps the deposition of plastic particles (Fig. 7d). Therefore, the penetration curve of the microplastic particles covered with biofilms is significantly lower than that of the microplastic particles without biofilms (Fig. 7e). Several other studies have similar conclusions that biofilms attachment leads to higher retention rates for microplastics (Tripathi et al., 2012; Xiao et al., 2013).

### *6.2. Contaminant enrichment*

Some contaminants are concentrated at the water-air interface, and microplastic particles mostly float on the water surface because of their

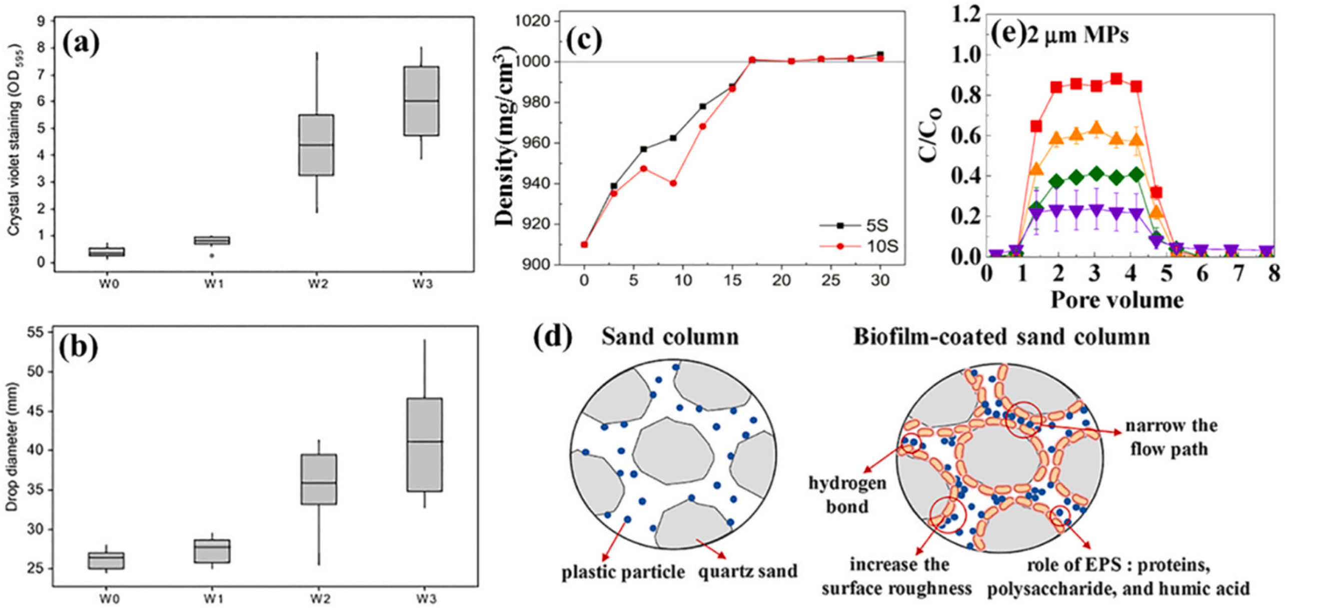


**Fig. 7.** (a) Biofilm formation and (b) surface hydrophilicity of plastics immersed in 2 m depth of the aqueous environment. Copyright 2011 Elsevier. (c) Density changes of 5S (5 ×5 mm) and 10S (10 × 10 mm) microplastics in summer. (d) Changes of sand column before and after biofilms attachment. (e) Breakthrough curve for microplastics in bare sand and biofilm-coated sand, both in 10 mM and 50 mM NaCl solutions.
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strong hydrophobicity, so they are easily adsorb these contaminants (Mato et al., 2001). Then, the strong transport ability of microplastics expands the pollution rage of the contaminants (Jose et al., 2020). Microplastics have received more and more attention as a carrier of contaminants. After being immersed in water for a long time, microplastics gradually attract the colonization of microorganisms and form biofilms (Turner et al., 2015). The complex process inevitably changes the properties of microplastics and adds new variables to the existence of microplastics in the environment (Jose et al., 2020). For example, the specific surface area, group content, roughness and other properties of microplastics have changed, and EPS secreted by microorganisms in biofilms are considered to have good adsorption characteristics (Decho, 2000; Holmes et al., 2012; Lang et al., 2020). All of these changes in nature provide more possibilities for the accumulation of contaminants, such as persistent organic pollutants (POPs), heavy metals and antibiotics (Kirstein et al., 2016; Mato et al., 2001; Xiong et al., 2020).

### 6.2.1. *Persistent organic pollutants*

POPs have long half-life, strong transportation ability, and are easy to accumulate in organisms (Ashraf, 2017; Lohmann et al., 2007; Nadal et al., 2015). They are important pollutants that can't be ignored in the environment. However, the formation of biofilms allows POPs not only to adsorb on the surface of microplastics, but also to enter the interior of microplastics (Turner et al., 2015). On the one hand, the adsorption of POPs on microplastic particles is affected by the adsorption characteristics of microplastic particles. On the other hand, it is also affected by the ability of microorganisms on biofilms to absorb and metabolize POPs (Rummel et al., 2017). For example, some studies found that the level of plastics adsorption of POPs is much higher than the environmental concentration (McCormick et al., 2014; Rios et al., 2010; Teuten et al., 2009). When biofilms are formed on the surface of microplastics, the ability to adsorb POPs may be enhanced. POPs desorption often occurs in organisms. Therefore, the strong POPs adsorption capacity of microplastics attached with biofilms will intensify the bioaccumulation of POPs (McCormick et al., 2014).

A series of studies clarified the impact of biofilms on the relationship between different types of POPs and microplastics. Rosato et al., found that there was almost no dechlorination of PCBs in the sterile microplastics, while PCBs in microplastics containing microorganisms were significantly dechlorinated from the second week (Fig. 8a), indicating that biofilms would accelerate the reduction and dechlornation process of PBCs on microplastics and lead to changes in toxicity and bioavailability of microplastics (Rosato et al., 2020). Besides, studies have shown that biofilms on microplastics not only enrich high concentrations of polycyclic aromatic hydrocarbon (PAHs), but also increase the desorption rate of PAHs (Jin et al., 2020; Wu et al., 2017). Under biological/non-biological conditions, the concentration ratio of the three PAHs adsorbed on the microplastics were all lower than 1.0 (Fig. 8b) (Wu et al., 2017). The bacteria abundance is higher in summer, and at the same time the concentration of PAHs is higher (Fig. 8c). In summer, the bacterial abundance was high, and the concentration of PAHs was also high. And the maximum decrease of PAHs concentration occurred after the bacteria abundance reached its maximum. This may be because biofilms can stimulate the succession of PAH-degrading bacteria after enriching high concentrations of PAHs, thereby increasing the conversion rate of PAHs into PAHs homologs (Jin et al., 2020). In addition, toxicological experiments found that microplastics not only enrich phenanthrene, but also have a synergistic effect with phenanthrene to enhance the toxicity of phenanthrene (Xu et al., 2021; Zhang et al., 2021c). The diffusion coefficient of phenanthrene in biofilms is higher than that of microplastics, so microplastics with biofilms will absorb more phenanthrene than microplastics themselves (Fotopoulou et al., 2014). This will cause more oxidative damage to the organism, stimulate immune function and affect the health of the organism (Xu et al., 2021).

### 6.2.2. *Heavy metal*

Global industrial production has led to the discharge of large quantities of wastewater containing heavy metals into rivers and oceans. Heavy metals are easy to accumulate in organisms and can cause high toxicity at low levels (Fu et al., 2011; Tchounwou et al., 2012; Wang et al., 2020c; Yaseen, 2021). However, many studies have demonstrated that microplastics could accumulate heavy metals (Table 1). Although in most cases, plastics don't have a fixed charge, the modification of the

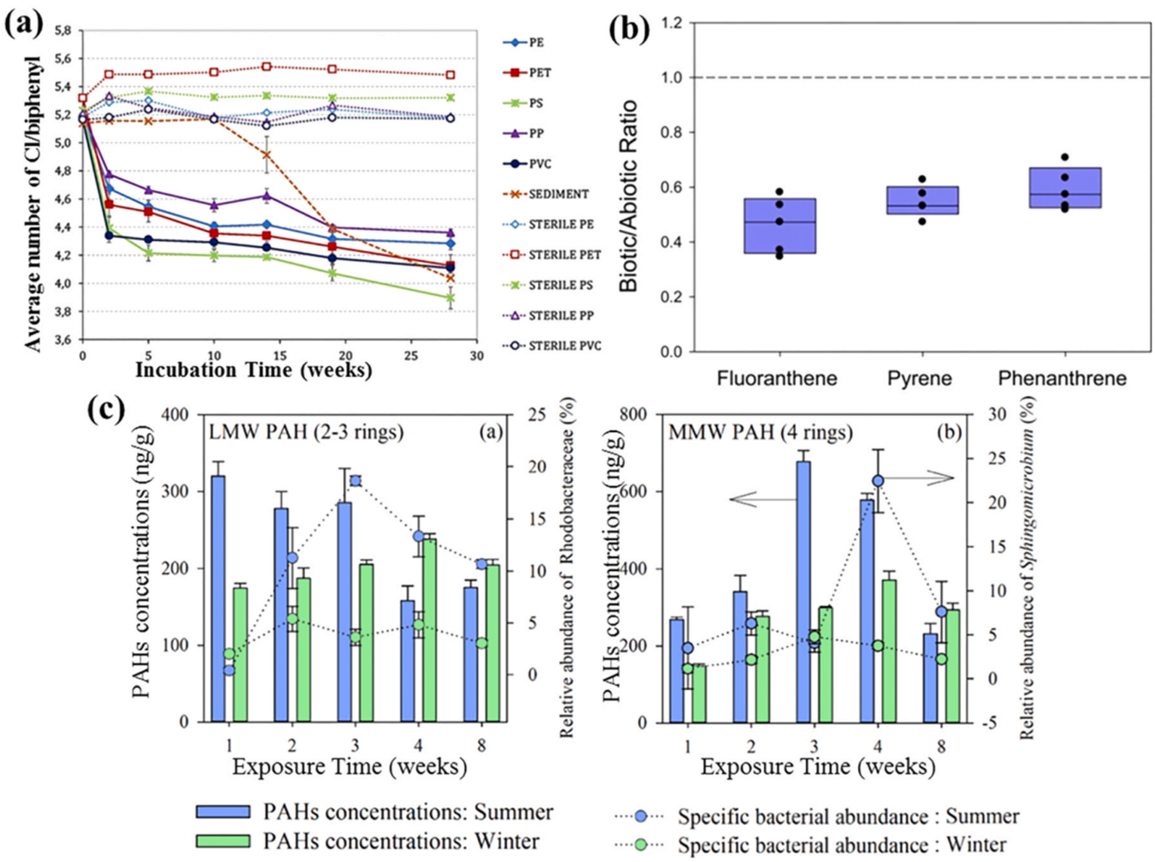


**Fig. 8.** (a) Reductive dechlorination of PCBs adsorbed on sediments and different types of microplastics. (b) The concentration ratios of three PAHs adsorbed by microplastics under biological/non-biological conditions. (c) Time dynamic analysis of PAHs and representative PAH-degrading bacteria in summer and winter. (a) Reproduced with permission from ref (Rosato et al., 2020). Copyright 2020 Elsevier. (b) Reproduced with permission from ref (Wu et al., 2017). Copyright 2017 ACS Publications. (c) Reproduced with permission from ref (Jin et al., 2020). Copyright 2020 Elsevier.

**Table 1**
Studies on adsorption between microplastics and metals.

| Microplastic type | Diameter | Metal type | Adsorption | | References |
|---|---|---|---|---|---|
| | | | Isotherm model | Kinetic model | |
| PS | 2 mm | Pb | Langmuir and Freundlich | Intraparticle diffusion | (Qi et al., 2021) |
| PA/PE/PP/PS<br>PVC | < 5 mm | Pb/Cd/Zn/Cu/Co/Ni | Langmuir<br>Freundlich | NA[a] | (Li et al., 2019b) |
| HDPE/PVC/LDPE/PP | 3 mm | Al/Cr/Mn/Fe/Co/Ni/Zn/Cd/Pb | NA | NA | (Rochman et al., 2014) |
| PET | 2 mm | | NA | NA | |
| Virgin PS<br>Biofilm covered PS | 2 mm | Co/Ni/Cu/Zn/Cd/Ag | Langmuir | Intraparticle diffusion<br>Film diffusion | (Guan et al., 2020) |
| Virgin PE<br>Biofilm covered PE | 60–150 μm | Cu | Freundlich | Intraparticle diffusion<br>Film diffusion | (Wang et al., 2020d) |
| PS<br>PVC | 0.7–0.9 mm<br>0.8–1.6 mm | Cu/Zn | NA | NA | (Brennecke et al., 2016) |
| PE | 4 mm | Al/Fe/Mn | NA | NA | (Ashton et al., 2010) |
| PE/PVC | < 5 mm | Ni/Pb | NA | NA | (Hossain et al., 2019) |
| PS | < 5 mm | Cd | Henry | The second-order kinetic | (Lang et al., 2020) |
| PE | 4–12 μm | Ag | NA | NA | (Kalcikova et al., 2020) |
| PMMA<br>PP<br>PE | 6.3 μm<br>85.4 μm<br>286.7 μm | Pb | Langmuir | The second-order kinetic | (Shen et al., 2021) |
| HDPE | < 5 mm | Cd/Cr/Co/Cu/Ni/Pb/Zn | Langmuir and Freundilich | NA | (Holmes et al., 2014) |

[a] NA: Not available.

plastic surface by microorganisms provides a suitable charging condition for the adsorption of metal ions (Holmes et al., 2014). EPS secreted by microorganisms can provide many functional binding sites, such as carboxyl, hydroxyl, acetyl, phosphate and so on (Ancion et al., 2013; Tien et al., 2013). Meanwhile, the microbial cells provide a huge surface area and total anionic charge (Ancion et al., 2013). All these provide ideal conditions for the adsorption of metal ions. Moreover, the complexation between metals and biofilm components is stronger than the electrostatic affinity of the original microplastics, and the coordination ion stability ($K_f$) value of microplastics attached with biofilms is higher (Guan et al., 2020; Wang et al., 2020d). It not only improves the adsorption capacity of microplastics to metals but also improves the stability of metals on the microplastics (Guan et al., 2020).

One study found that the concentration of metals on PS and PVC microplastics was hundreds of times higher than that in the surrounding seawater (Brennecke et al., 2016). Plastics themselves carry a lot of metals, so microplastics that are broken from plastics also contain metals on their surface (Wang et al., 2017). But the metals carried by microplastics not only come from themselves, but from enrichment in the surrounding environment. In addition, Rochman et al., reported that the type of plastic fragments had no significant influence on the accumulation of metals, so the accumulation of metals on plastic fragments might be mediated by biofilms (Rochman et al., 2014). As we can see from the FT-IR spectrum in Fig.9a, the microplastics with biofilms have more peaks than the original microplastics, indicating that the formation of biofilms leads to the appearance of more functional groups. These functional groups may include carboxyl, hydroxyl, amino groups, etc., and their strong complexation makes the adsorption of microplastics on metals change from intra-particle diffusion to membrane diffusion (Guan et al., 2020; Wang et al., 2020d). As shown in Fig. 9b, the intercepts of the fitting lines for Cu (II) adsorption by PE microplastics with biofilms were larger, indicating a large contribution of surface adsorption in the adsorption process of metals by microplastics with biofilms. Besides, the adsorption capacity of microplastics with biofilms was significantly greater than that of original microplastics (Qi et al., 2021; Wang et al., 2020d). At the same time, the newly generated functional groups may generate more adsorption active sites, thus enhancing the ability of microplastics to adsorb metal ions (Guan et al., 2020; Li et al., 2019b; Rochman et al., 2014). Some studies have found that the concentration of metals (such as Zn, Cu, Pb and Cd) was higher on the biofilms, which was obviously a good and rapid accumulation of metals by biofilms (Ancion et al., 2013; Dranguet et al., 2017; Rochman et al., 2014). The data in Table 2 shows that there was an interaction between microplastics and Hg. As the concentration of microplastics increased in water, the attenuation rate of Hg increased greatly, from 33% to 73% (Barboza et al., 2018). The suspension experiments showed that microplastic particles tended to accumulate metals from the water column or the surface, and the possible mechanism was to adsorb metals directly to the charged parts of the surface, as well as in the form of

**Table 2**
The attenuation of Hg (II) concentration in water within 24 h (Barboza et al., 2018).

| Treatments | 0 h Actual Hg conc. (mg/L) | 24 h Actual Hg conc. (mg/L) | Decay (%) |
|---|---|---|---|
| Hg low | 0.007 | 0.006 | 17 |
| Hg high | 0.013 | 0.010 | 21 |
| MPs low+Hg low | 0.007 | 0.005 | 33 |
| MPs low+Hg high | 0.013 | 0.009 | 30 |
| MPs high+Hg low | 0.007 | 0.002 | 73 |
| MPs high+Hg high | 0.013 | 0.004 | 69 |

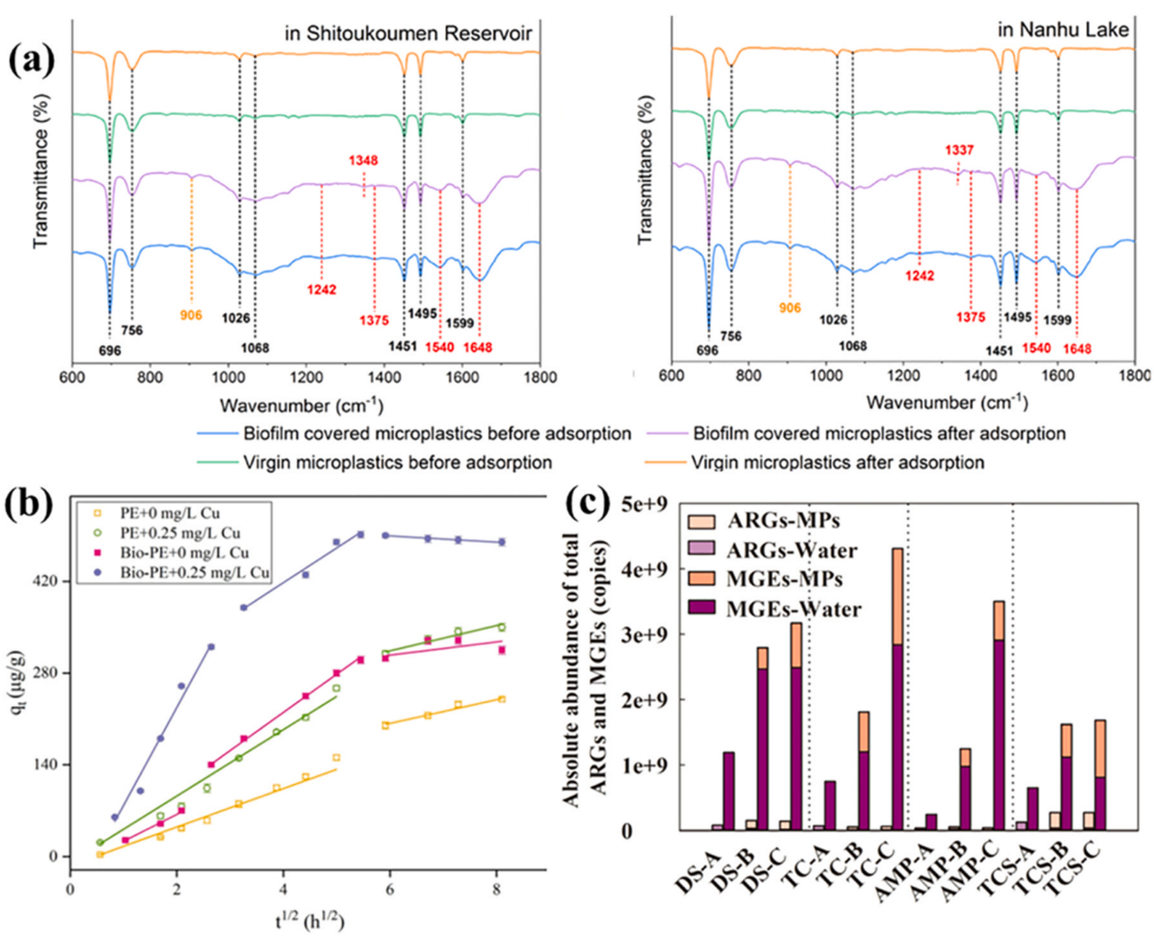


**Fig. 9.** (a) FT-IR of virgin microplastics and biofilms covered microplastics before and after adsorption. (b) Intracellular diffusion patterns of Cu (II) adsorbed by original PE microplastics and biofilms-covered PE microplastics. (c) The absolute abundance of ARGs and MGEs of microplastics samples in different systems, and DW-A, TC-A, AMP-A and TCS-A were not added with microplastics.
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hydrated oxides (Ashton et al., 2010; Wang et al., 2017). In fact, biofilms not only enhance the carrier effect of microplastics on heavy metals, but also enhance their combined toxicity, which undoubtedly poses a potential ecological risk to ecosystems in the aquatic environment (Barboza et al., 2018; Qi et al., 2021).

#### 6.2.3. Antibiotic

Antibiotics are widely used as antibacterial drugs and growth promoters, which can be harmful to the ecosystem when they enter the environment (Guo et al., 2018). In aqueous environment, antibiotics are adsorbed on the surface of microplastics through hydrogen bonding, van der Waals forces, electrostatic interactions and hydrophobic interactions (Guo et al., 2018; Li et al., 2018). All the antibiotics tested in the studies were found to have a tendency to be adsorbed by microplastics (Table 3). And the affinity between antibiotics and microplastics is greater than that between antibiotics and dissolved organic matter (Xu et al., 2018). The adsorption behavior of the aged and virgin microplastics is very different, including adsorption capacity and adsorption mechanism (Liu et al., 2019). The formation of biofilms will increase the number of oxygen-containing functional groups and enhance the bond energy of hydrogen and π-π bonds, which will enhance the ability of microplastics to accumulate antibiotics (Fan et al., 2021; Li et al., 2018; Wu et al., 2016). By comparing the adsorption of the sulfamethoxazole (SMX) to the original and aging microplastics, Guo et al., found that the formation of carbonyl groups during the aging of microplastics increases its polarity and increases the surface volume ratio of microplastics, which may enhance the adsorption capacity of microplastics to SMX (Guo et al., 2019b). However, the existence of biofilms can not only enhance the enrichment of antibiotics, but also promote the accumulation of antibiotic resistance genes (ARGs).

A number of studies have demonstrated that microplastics have a similar enrichment capacity for ARGs and for antibiotics. And it has also been demonstrated that the aging process enhances the possibility of microplastics as an ARGs hotspot. The experimental results showed that the total amount and relative abundance of ARGs stored on microplastics are higher than those in aqueous environment, and the distribution of ARGs in microplastics was largely determined by the bacterial community (Su et al., 2021; Wang et al., 2021; Yang et al., 2019; Zhang et al., 2020). As shown in Fig. 9c, the abundance of total ARGs and mobile genetic elements (MGEs) treated with microplastics is higher than that of untreated samples, which indicates that microplastics may promote the flow of ARGs (Wang et al., 2021). Moreover, the interaction between microplastics and ARGs has been shown to enhance the toxicological effects and promote the occurrence of fish diseases (Dong et al., 2021; Su et al., 2021). Su et al., concluded that the combination of biofilms on microplastics showed stronger enrichment capacity, and the ARG-bacterial symbiosis network is tighter and more stable on the surface of the microplastics (Sun et al., 2021; Zhou et al., 2020). In summary, the water polluted by microplastics may have more ARGs pollution, leading to a stronger pathogenic potential.

### 6.3. Harmful microorganisms enrichment

For survival and long-distance transport, microplastics are good carriers for microorganisms, so microplastics with biofilms attached could act as act as reservoirs for pathogenic microorganisms (Keswani et al., 2016). Several studies have found potential pathogenic bacteria and harmful algae enriched on microplastics, including *Bacilli*, *Cocci* and *Vibrio* (Foulon et al., 2016; Harrison et al., 2014; Keswani et al., 2016; Lehtola et al., 2004; Maso et al., 2003; Tavanolu et al., 2020). In aqueous environment, microplastics not only provide new substrates for pathogenic microorganisms, but also enhance the transport ability of pathogenic microorganisms (Kirstein et al., 2016; Martínez-Campos et al., 2021; Tavanolu et al., 2020; Wingender et al., 2011; Wu et al., 2019). In fact, harmful microorganisms in microplastic biofilms are more resistant and tolerant to antibiotics than planktonic organisms, which naturally increases the toxicity potential of aquatic ecosystems (Kumar et al., 2017).

Through the comparison of absolute abundance of 16S rRNA genes, Zhao et al., concluded that PVC microplastics enriched *Mycobacterium* that has been identified as an attractive pathogen in the surrounding sewage, and the enrichment degree increased with the increase of time (Zhao et al., 2021). Virsek et al., reported for the first time the detection of *Aeromonas salmonella* on microplastics, which is not only one of the main pathogens of fish infections, but also one of the most harmful invasive bacteria in the marine environment (Virsek et al., 2017). It has been found that microplastics can selectively enrich pathogenic *Vibrio*, including *Vibrio coralis*, *Vibrio splenicus*, *V. vulnificus*, etc. (Foulon et al., 2016; Kirstein et al., 2016). Kirstein et al., using matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for species classification analysis, proved that 12 microplastic particles were rich in *Vibrio* spp., and V. parahaemolyticus accounted for up to 59% (Erler et al., 2015; Kirstein et al., 2016). Other studies have also found the presence of *Vibrio* spp, such as Xie et al. (2021) found that two food-borne pathogens, Vibrio parahaemolyticus and Escherichia-Shigella, were detected on microplastics. Furthermore,

**Table 3**
Studies on adsorption between microplastics and antibiotics.

| Microplastic type | Diameter | Antibiotic type | Adsorption | | References |
|---|---|---|---|---|---|
| | | | Isotherm model | Kinetic model | |
| PE/PS/PP/PA/PVC | < 5 mm | SDZ/AMX | Linear | NA[a] | (Li et al., 2018) |
| | | CIP/TMP/TC | Langmuir and Freundilich | | |
| PE | < 5 mm | SDI/TC/CHL/TYL | NA | NA | (Wang et al., 2020b) |
| PVC | 120 μm | TC/AMP | NA | NA | (Zhao et al., 2021) |
| PE | 150–250 μm | TC | NA | NA | (Shen et al., 2018) |
| PE/TWP[b] | 74 μm | CTC/AMX | Freundilich | The pseudo-second-order | (Fan et al., 2021) |
| PA/PE | 100–150 μm | SMT | Freundlich | EMTR | (Guo et al., 2019a) |
| PET/PP/PS/PVC | | | Linear | | |
| PE/PP/PS/PVC | < 75 μm | TYL | Freundlich | The pseudo-second-order | (Guo et al., 2018) |
| PE | 150 μm | SMZ[c] | Linear | The pseudo-second-order | (Xu et al., 2018) |
| PS/PE | < 4 mm | SMX/SMT/CPE-C[d] | Linear | MO | (Guo et al., 2019b) |
| PP/LDPE/HDPE/PVC | < 5 mm | ENR[e]/CIP/NOR[f] | NA | NA | (Xu et al., 2018) |
| PS | 3 μm | TYL | Henry | NA | (Huang et al., 2021) |

[a] NA: Not available;
[b] TWP:Tire wear particles.
[c] SMZ:sulfamethoxazole;
[d] CPE-C:cephalosporin C;
[e] ENR:enrofloxacin;
[f] NOR:norfloxacin;

McCormick et al. (2014) found that the *Campylobacter* family was more than four times more abundant on microplastics in wastewater from sewage treatment plants than in the downstream water column, and more than 13 times more abundant than suspended organic matter. Compared with the control sand substrate, *Flectobacillus* sp. was found to be significantly enriched in biofilms of microplastics up to 11 times (Dung Ngoc Pham, 2021). In addition, Wu et al. (2019) discovered that when biofilms were cultured on microplastics and two natural substrates (rocks and leaves), two human pathogens and one plant pathogen were detected on the microplastic biofilms, while these pathogens were not detected on the natural substrate.

Except for bacteria, eukaryotes are also often found in microplastic surface communities. Kettner et al., detected differences between fungal communities on microplastics and in water, and fungi as pathogens, symbionts or saprophytes can greatly influence the community composition within microplastic biofilms (Kettner et al., 2017). Experiments have found that the fungal colonizers on PET microplastics include *Ascomycota*, *Basidiomycota* and *Synsporophyta* (Oberbeckmann et al., 2016). At the same time, harmful algae were found in floating plastic debris. Maso et al., found that benthic diatoms, micro-flagellates and harmful flagellate algae were observed on microplastic particles collected from the northern coast of the Mediterranean (Maso et al., 2003). Casabianca et al., found that the most abundant algae colonizing the microplastics contained *Pseudo-Nitzschia* spp. and *Ostreopsis* cf. (Casabianca et al., 2019). *Pseudo-Nitzschia* spp. produces a neurotoxin that can cause shellfish poisoning, which is one of the causes of death of many marine life (Casabianca et al., 2019; Trainer et al., 2012).

Microplastics are ubiquitous in the water environment and have become an enrichment center for various pathogenic microorganisms. Microplastics carrying pathogenic microorganisms may not only be ingested by invertebrates and enter the food chain, thereby transferring to different nutrient levels, but may also be mixed into larger sinking aggregates, greatly increasing the risk of benthic organisms (Jiang et al., 2018; Shapiro et al., 2014). Microplastic biofilms not only provide new micro-habitat for the colonization of pathogenic microorganisms, but also increase the possibility of their reproduction (Wu et al., 2019). Moreover, phylogenetic diversity on microplastic biofilms has a higher rate of plasmid DNA transfer than that of free microorganisms, and thus microplastics are also a hot spot for gene transfer of pathogenic microorganisms and the spread of antibiotic resistance (Hirt and Body-Malapel, 2020; Imran et al., 2019).

## 7. Conclusion and outlook

With the development of microplastics research, it is necessary to study the biofilms on the surface of microplastics. This review outlines the main ways of microplastics enter the terrestrial, aquatic and atmospheric environment, and outlines the impact of microplastics on creatures and humans. Whether it is directly ingesting microplastics or entering the ecosystem through the food chain or other means, they will have different effects on the growth, development and reproduction of organisms. In addition, this paper reviews the physicochemical properties of biofilms on microplastics. It includes the formation process of biofilms on the surface of microplastics, the uniqueness of the microplastics biofilms and three factors that affect the formation of microplastic biofilms (environmental factors, matrix properties and the properties of attached organisms). Finally, the effects of biofilms attached on microplastics in aqueous environment are concluded. Biofilms can change the transport and deposition behavior of microplastics in aqueous environment. The distribution of biofilms adjusts the buoyancy of microplastics, thus changing their transport path. It is also possible to promote the rate of sinking of microplastics, eventually becoming permanently buried in the underlying sediments. Microplastics themselves have a high adsorption capacity for a large number of pollutants. The formation of biofilms introduces new variables for microplastics in the environment. The ability of microplastics to absorb persistent organic pollutants, heavy metals and antibiotics changes with the formation of biofilms. Microplastic biofilms may also be reservoirs for pathogenic microorganisms and harmful algae, serving as vehicles for the spread of these harmful microorganisms.

According to the summary and collation of the current research contents, the following aspects need to be considered in future research:

- Current researches on microplastics in the aquatic environment focus on the marine environment. However, there are still few studies on microplastics in the freshwater environment. In fact, wastewater treatment plants are one of the main sources of microplastics. Microplastics enter the freshwater environment first, and then enter the marine environment. Most freshwater environments are eutrophic, which means that microplastics may form biofilms at a faster rate and have a richer microbial community in freshwater environments. Therefore, studies about biofilms on microplastics in urban lakes and reservoirs need to be strengthened.
- The content of microplastics is an important index for evaluating the environmental pollution and biological effects of microplastics (Zhang et al., 2021b). Therefore, establishing a unified and reliable method for quantitative detection and analysis of microplastics in aqueous environment is one of the crucial tasks for future research. More in-depth studies would be better carried out by quantifying the microplastics. At the same time, qualitative analysis of microplastic biofilm communities is also important to better predict the ecological consequences of microplastics.
- Most of the relevant experimental periods are short. But not only do the properties of microplastics in the environment change over time, but also change in the concentration. Meanwhile, the attachment of biofilms to microplastics usually lasts for a long time and deserves more research (Oberbeckmann et al., 2016). In the following studies, the experimental period could be appropriately extended to better investigate the mechanism of biofilm effects.
- Laboratory studies use mostly pure microplastic particles of uniform size. But in the environment, microplastic particles are not uniform in size and dimensions, and may mix with natural suspensions to form polymers. When microplastics exist in aqueous environment, it is not a single solution environment in the laboratory, but an environment of physical, chemical and biological interactions. The in-situ experimental methods can more comprehensively explore the effects of different environments on microplastic biofilms and explore the community composition of microplastic biofilms in different environments. Therefore, it is best to use in-situ methods in future studies to better evaluate the current status of microplastic pollution.
- Currently, most studies on the toxicity of microplastics and the combined effects of microplastics and contaminants focus on virgin microplastics. However, microplastics are easily covered by biofilms, and their surface properties in aquatic environment will affect their toxic effects. For example, biofilms have been shown to enhance the combined toxicity of Pb(II) and microplastics (Qi et al., 2021). In the future, it is possible to strengthen the exploration of the impact of microplastics attached to biofilms on individuals with higher nutritional levels, and to improve the impact of microplastics at the population level (Syberg et al., 2015). Previous studies have found that the presence of biofilms enhances the ability of microplastics to accumulate contaminants. Future research on the adsorption of pollutants by microplastics covered with biofilms will be given priority. The combined effects of microplastics with biofilms and contaminants may show greater toxicity, and further assessment of the potential environmental risks from combined pollution is needed.
- More deep investigation of the microbial colonization and biofilm formation processes on microplastics surface is needed. To further expand the understanding of the relationship between microplastics and biofilms. In conclusion, the future exploration of microplastics

biofilms has great potential, which requires the joint efforts of researchers from all over the world.

## 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

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