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Review

Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection

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ABSTRACT

The continuous increase in synthetic plastic production and poor management in plastic waste have led to a tremendous increase in the dumping into our aqueous environment. Consequently, microplastics commonly defined as sizes less than 5 mm are produced and stay in both seawater and freshwater environment. The presence of microplastics as a new type of emerging contaminant has become a great issue of concerns from public and government authorities. The sources of microplastics to freshwater systems are many with the largest portion from wastewater treatment plants. The abundance of microplastics varies with the location, from above 1 million pieces per cubic meter to less than 1 piece in 100 cubic meters.

Microplastics can cause several harmful physical effects on humans and living organisms through such mechanisms as entanglement and ingestion. The microplastics can act as carriers of various toxins such as additives from industrial production processes and persistent contaminants by the sorption in waters. Those toxins may cause great health problems to humans. A few studies on the fishes demonstrated that the microplastics and the associated toxins are bio-accumulated and cause such problems as intestinal damage and change in metabolic profiles.

In studies of microplastics, fresh water is first sampled by the nets with typical mesh size of 330 μm for collection of microplastics. After the volume reducing process, the samples will then go through the purification process including density separation by such inorganic salts as sodium chloride and digestion process by oxidizing agents or enzymes. The sequence of these two processes (namely purification and digestion) is dependent on the sample type. The purified samples can be studied by several analytical methods. The commonly used methods for the qualification studies are FTIR spectroscopy, Raman spectroscopy, pyrolysis-GC/MS, and liquid chromatography. A tagging method can be used in the quantification study. Our literature study finds that there is still no universal accepted quantification and qualification tools of microplastics in fresh waters. More work is anticipated so as to obtain accurate information on microplastics in freshwater, which can then be used for the better assessment of the environmental risk.

Contents

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1. Introduction

Plastic products have such outstanding features as light-weight, being durable and versatile, and production with low cost (Hammer et al., 2012; Ivleva et al., 2017). However, plastic debris has raised global concerns over its wide distribution and associated environmental consequences. The annual global production of plastic product in 2016 alone was around 322 million tonnes (Europe, 2016). An estimation of up to 10% of plastic fragments would end up in marine environment as per suggested by Cole et al. (2011), due to extensive usage and increasing production in plastic product, and poor management (Rochman, 2015). While diminishing aesthetic value of water environment, plastic debris is likely to pose threats to public health and cause biodiversity loss (Thompson et al., 2009; Gall and Thompson, 2015).

Microplastics are widely defined as synthetic polymers with an upper size limit of 5 mm and without specified lower limit (Thompson et al., 2009). They can be categorized into primary microplastics and secondary microplastics.

The definition of primary microplastics is the microplastics, which are originally manufactured to have a size less than 5 mm and mainly found in textiles, medicines and such personal care products as facial and body scrubs (Cole et al., 2011; Browne, 2015). These primary microplastics can be transported by rivers, discharge from water treatment plants, wind and surface run-off into either fresh water and seawater environments (Gall and Thompson, 2015).

Secondary microplastics are derived from fragmentation or large plastic debris due to such processes as photo-degradation, physical, chemical and biological interactions (Thompson et al., 2009; Galgani et al., 2013). The origins of secondary microplastics include fishing nets, industrial resin pellets, household items and other discarded plastic debris (Eerkes-Medrano et al., 2015). Notably, it was found that the majority of microplastics are secondary microplastics (Eriksen et al., 2013) and that the abundance in waters would increase along with the increase in input of plastic debris from different origins, leading to continuous transformation of secondary microplastics (Cole et al., 2011). When microplastics are exposed in the environment, there is a higher possibility of break-down of microplastics to nanoplastics that may have higher environmental risks due to the nature of nano-sizes.

Microplastics can originate from both land- and ocean-based sources (Hammer et al., 2012). The ocean-based sources, due to commercial fishing, vessels and other activities in marine environment, only contribute 20% of total plastic debris in marine environment (Andrady, 2011). The microplastics from terrestrial sources contribute the remaining 80%. Terrestrial sources include different origins that mainly are personal care products, air-blasting process, improperly disposed plastics and leachates from landfill (Cole et al., 2011). Once terrestrial microplastics are released into the natural water systems, most of them would be transported to oceans by rivers, while the remaining would reside in fresh water environment, including such isolated water systems as remote mountain lakes (Browne et al., 2010; Free et al., 2014).

Microplastics are of great public concerns for the ubiquitous presence and persistence in the aquatic environment. The global presence of microplastics has been found in recent years. From horizontal perspective, microplastics were reportedly found in tropical areas (Ng and Obbard, 2006; Nor and Obbard, 2014); they were even seen in the polar waters of Antarctica and Arctic (Barnes et al., 2010; Bergmann et al., 2015). When one looks at vertical distribution, microplastics exist in benthic zone of water bodies, water columns, surface waters and beaches. Some reports have shown the concentrations in surface water vary from 10^{-5} to 10^{5} pieces/m^3 (Liebezeit and Dubaish, 2012; Desforges et al., 2014; Frias et al., 2014; Lima et al., 2014; Auta et al., 2017) and 40 to 400 pieces/L in sediments (Zurcher, 2009; Browne et al., 2011; Antunes et al., 2013; Frias et al., 2014; Nor and Obbard, 2014). Most recently, China Central Television website (cctv.com) reported a group of Chinese scientists have discovered the presence of microplastics in Antarctic waters (China.org.cn, 2018). Furthermore, the distribution shows clear geographical variations (Fossi et al., 2012; Collignon et al., 2014; de Lucia et al., 2014; Desforges et al., 2014).

The factors affecting the distribution include such large-scale forces as currents driven by wind and geostrophic circulation (Law et al., 2010), turbulence and oceanographic effects (Ballent et al., 2012; Turra et al., 2014). As key factors, the inherent properties of microplastics such as density, shape and size of microplastics can affect transportation and distribution patterns (Eerkes-Medrano et al., 2015).

The aforementioned factors are more likely to play important roles in a large freshwater environment like riverine systems; however, they become limited on smaller isolated fresh water systems, where natural factors and long water residence time dominantly affect quantity of microplastics (Free et al., 2014). Hence, microplastics in open and dynamic fresh waters would eventually end up in marine environment, while microplastics in isolated and static waters bodies would remain and accumulate in the waters.

Fresh waters may accumulate numerous microplastic particles and fibers; however, less efforts have been made to monitor the microplastics in fresh waters than those in seawaters. Such fresh waters can be the sources (like waste water plants), transferring media (like rivers) and sink (like isolated lakes) of microplastics, which may differ from those in seawaters because large variations in quantity can be expected (Klein et al., 2018). Meanwhile, the properties of microplastics can be quite heterogeneous. For instance, microplastics in sewage are heavily contaminated by organic contents and exist as relatively large pieces; on the other hand in clean fresh waters are nearly free of organic contents and hardly seen by naked eyes (Orb, 2017). In addition, some lakes or rivers with fresh water are close to the areas with high population, where higher microplastics abundance was detected (Eriksen et al., 2013). Another significant characteristic of microplastics studies in freshwater systems is that the sample sizes are small. However, large sampling areas are necessary to adequately reduce the large variations due to spatial and temporal changes (Ryan et al., 2009). As a result, we found that there was an urgent need to review the current research work and methodologies on microplastics in fresh waters in order that appropriate sampling, quantification and identification approaches can be developed for the study in freshwater samples.

The objective of this review paper is to reveal the current knowledge about microplastics in fresh waters for a better

understanding of microplastics contamination and potential risks. Summaries of sampling methods and comparisons of different quantification and identification approaches are presented. Several key challenges are discussed and suggestions are provided for further research work.

2. Microplastics in freshwater systems

2.1. Occurrence

Most of efforts on the research of microplastics have been placed on seawater environment. Less than 4% of microplastics-related studies are reportedly associated with freshwaters (Lambert and Wagner, 2018). The limited information, however, revealed that the abundance of microplastics in freshwaters is comparable to that of marine environment (Peng et al., 2017) and the distribution is highly heterogeneous (Klein et al., 2018). Table 1 summarizes some of the relevant studies on the microplastics abundance in freshwater matrices. The mean/averaged values of microplastics abundance in fresh water systems varied greatly from almost none to several million pieces per cubic meter. This significant difference results from such key factors as sampling locations, human activities, inherent natural conditions and sampling approaches (Eerkes-Medrano et al., 2015).

Many terrestrial sources contribute the microplastics. Among them, wastewater treatment is one of the dominant sources of microplastics (Magnusson and Norén, 2014; Talvitie, 2014; Estahbanati and Fahrenfeld, 2016; Murphy et al., 2016; Dyachenko et al., 2017; Mintenig et al., 2017). Table 2 lists the key results from the microplastics studies on several wastewater treatment plants (WWTPs). Though WWTPs can remove up to 95% of microplastics (Talvitie, 2014; Talvitie et al., 2017) and tertiary treatment can have a 90% removal efficiency of fine particles of size larger than 10 μm (Wardrop et al., 2016), there is substantial amount of microplastics being discharged into natural waters via WWTPs.

Rochman et al. (2015) used the published data by Magnusson and Norén (2014) and Martin and Eizhvertina (2014) to estimate

Table 1
Studies on microplastics contamination in natural fresh water systems.

Location	Collection	Collection Cut-off size (μm)	Depth	Purification	Identification	Separation substrate	Mean abundance[a]	Maximum abundance[a]	Reference
Austrian Danube, Austria	Stationary conical driftnets	500	0.5 m	–	Visualization	–	0.317p/m^3	141.7 p/m^3	(Lechner et al., 2014)
Rhine river	Manta net	300	–	Enzyme + H_2O_2	FTIR	Sieves	8.93×10^5 p/km^2	3.9×10^6 p/km^2	(Mani et al., 2015)
Dutch river delta and Amsterdam canals	Bulk water 2 L	–	–	–	FTIR	0.7 μm glass filters	1×10^5 p/m^3	1.87×10^5 p/m^3	(Leslie et al., 2017)
Great Paris	Plankton net	80	0.1 −0.35 m	–	Visualization	1.6 μm filter	30 p/m^3	106 p/m^3	(Dris et al., 2015)
Great Paris	Manta Trawl	330	0–0.3 m	–	Visualization	1.6 μm filter	0.35 p/m^3	0.45 p/m^3	(Dris et al., 2015)
Lake Geneva	Manta Trawl	300	–	–	Visualization	–	4.81×10^4 p/km^2	–	(Alencastro, 2012)
Three Gorges Dam, China	Trawl	112	–	–	FTIR	1.6 mm stainless sieve	8.47×10^6p/km^2	1.36×10^7 p/km^2	(Zhang et al., 2015)
Three Gorges Dam, China	Teflon pump and stainless steel sieve	48	1 m	30% H_2O_2	Visualization + Raman	0.45 μm glass microfiber filter	4.70×10^3 p/m^3	1.26×10^4 p/m^3	(Di and Wang, 2017)
Yangtze Estuary	Teflon pump and stainless steel sieve	32	1 m	30% H_2O_2	Visualization	1.2 μm cellulose nitrate filters	4.14×10^3 p/m^3	1.02×10^4 p/m^3	(Zhao et al., 2014)
Lakes, Wuhan, China	Teflon pump and stainless steel sieve	50	0.2 m	30% H_2O_2	FTIR	0.45 μm glass microfiber filter	–	8.93×10^3p/m^3	(Wang et al., 2017)
Taihu lake, China	Plankton net	333	0.3 m	30% H_2O_2	FTIR + SEM-EDS	100 μm polycarbonate filter	–	6.8×10^6 p/km^2	(Su et al., 2016)
Taihu lake, China	Bulk water 5 L	–	–	30% H_2O_2	FTIR + SEM-EDS	5 μm polycarbonate filter	–	2.58×10^4 p/m^3	(Su et al., 2016)
Lake Hovsgol, Mongolia	Manta trawl	333	–	30% H_2O_2	Visualization	Tyler sieves	2.03×10^4 p/km^2	4.44×10^4 p/km^2	(Free et al., 2014)
Lake Winnipeg, Canada	Manta trawl	333	–	30% H_2O_2	SEM-EDS	250 μm sieve	1.93×10^5p/km^2	7.48×10^5p/km^2	(Anderson et al., 2017)
Los Angeles river, San Gabriel river, Coyote Creek	Hand net, Manta trawl	800, 500, 333	–	–	Visualization	Tyler sieves	–	1.29×10^4p/m^3	(Moore et al., 2011)
29 Great Lakes tributaries, USA	Neuston net	333	0.2 −0.35 m	30% H_2O_2+Fe[b]	Visualization	125 μm sieve	4.2 p/m^3	32 p/m^3	(Baldwin et al., 2016)
Laurentian Great Lakes, USA	Manta trawl	333	–	2 M HCl	SEM-EDS	Tyler sieves	4.30×10^4 p/km^2	4.66×10^5 p/km^2	(Eriksen et al., 2013)
Raritan River, USA	Plankton net	153	–	30% H_2O_2+Fe[b]	Visualization	Sieves	–	–	(Estahbanati and Fahrenfeld, 2016)
Goiana Estuary, Brazil	Conical plankton net	300	–	–	Visualization	45 μm mesh	3.1×10^{-4} -2.6×10^{-3} p/m^3	0.19 p/m^3	(Lima et al., 2014)

[a] Data were standardized for consistency.
[b] Wet peroxide oxidation

Table 2
Microplastics detected in waste water treatment plants.

Location	Collection	Collection Cut-off size (μm)	Purification	Identification	Mean abundance[a]	Maximum abundance[a]	Reference
Effluents of 17 WWTPs, USA	Extraction pump + Tyler sieves	355, 125	30 %H_2O_2	Visualization	50 p/m^3	195 p/m^3	(Mason et al., 2016)
East Bay Municipal Utility District's WWTP, USA	Sieves	355, 125	30 %H_2O_2	FTIR	–	169 p/m^3	(Dyachenko et al., 2017)
Viikinmäki WWTP, Finland	Pumping through filters or metallic beaker	300, 100, 20	–	FTIR	–	Influent: 9×10^5 p/m^3 Effluent: 3.5×10^3 p/m^3	(Talvitie et al., 2017)
WWTP, River Clyde, Glasgow	Steel buckets	65	–	FTIR	Influent: 1.57×10^4 p/m^3 Effluent: 250 p/m^3	–	(Murphy et al., 2016)
Effluents of 12 WWTPs in Lower Saxony, Germany	A custom made pumping device	10	Enzymes + H_2O_2	FTIR	–	9×10^3 p/m^3	(Mintenig et al., 2017)
WWTP, St. Petersburg	A specific filter device	300, 100, 20	–	Visualization	Influent: 1.6×10^5 p/m^3 [b] Effluent:7×10^3 p/m^3 [b]	–	(Talvitie, 2014)
Långeviksverket, Lysekil, Sweden	Ruttner sampler	300	–	FTIR	Influent: 1.5×10^4 p/m^3; Effluent: 8.25 p/m^3	–	(Magnusson and Norén, 2014)
7 WWTPs, Netherland	Glass jars (2 L)	–	–	FTIR	Influent: 7.3×10^4 p/m^3 Effluent: 5.2×10^4 p/m^3	Influent: 5.66×10^5p/m^3 Effluent: 9.1×10^4 p/m^3	(Leslie et al., 2017)
Ljubljana, Slovenia	–		–	–	Effluent: 13.9 mg/m^3 PE (estimated)	–	(Kalčíková et al., 2017)

[a] Data was standardized for consistency.
[b] Data represents the number of synthetic particles.

the amount of microbeads discharged into the waterways in the USA. They came to a conservative conclusion that 8 billion microbeads (pieces) released from the municipal WWTPs per day. Mason et al. (2016) studied the effluent samples of 17 WWTPs and predicted that the average discharge of microbeads from US municipal WWTPs was 13 billion pieces per day, similar to that from Rochman et al. (2015). Given the fact that high volume of treated and untreated wastewater is released globally and only 60% of municipal wastewater is treated (Mateo-Sagasta et al., 2015), a huge amount of microplastics would enter the environment via the discharges from WWTPs. In addition, other sources such as surface run-off, atmospheric fallout (Dris et al., 2016) and direct waste disposal contribute the increase in the microplastics flow into aqueous environment.

In September 2017, Orb Media, a nonprofit journalism organization, published a report that claims the presence of microplastics in drinking water. This cross-border research tested 159 drinking water samples from five continents found out that 83% of them were contaminated with tiny plastic debris (Orb, 2017). Since microplastics can directly enter human bodies, it may be a long-term exposure if people drink microplastics-containing water. These findings would trigger public concerns over the safety of drinking water and food.

2.2. Environmental impacts

The concerns over microplastics are about the potential harms that can impose on organisms and humans. The environmental impacts can be catalogued to physical, chemical and biological impacts, as described below. The findings on the impacts are mainly based on marine environment, but can be used for fresh water environment.

Physical impacts mainly include entanglement and ingestion based on the work on the microplastics in sweater. The study conducted by Laist (1997) has shown over 200 marine species suffered from the entanglement and ingestion of plastic debris. Though to what extent of physical effects would affect organisms remain uncertain, entanglement effect that is often associated with comparatively large animals is visible when we compare it with ingestion. Entanglement could cause severe impacts on aquatic species; they can even be fatal by the means of drowning, suffocating, strangulating or starving (Allsopp et al., 2006). The vulnerable species include sea turtles, mammals, seabirds, and crustaceans (Gilardi et al., 2010). When these animals drown in ghost nets, they may suffer suffocation and starvation; when their predators appear, they are bound to die (Derraik, 2002).

However, there is no report on entanglement incidence in freshwater bodies. Nevertheless, the occurrence of entanglement in marine organisms has provided a clear indication to the situation in freshwater environment.

Ingestion does not directly impose fatal effects on organisms, even though it has well been observed. The chronic effects however become a key issue (Wright et al., 2013a). Ryan (1987) conducted a survey on the potential effects of plastic ingestion on domestic chicks, in order to simulate the biological behavior of ingested plastics on seabirds. It was found that there was a positive relationship between reduced food consumption and feed of plastics. A research team suggested that a negative correlation between the fitness of seabirds and the ingested plastic debris (Spear et al., 1995). The negative correlation was also observed on the fish (Lusher et al., 2014). The pathways of microplastics entering the tract include direct and indirect ingestion. Fish mainly ingests microplastics via predation activities; the accumulation effects can be observed in higher trophic levels, such as seabirds, seals and sea lions (McMahon et al., 1999; Eriksson and Burton, 2003; Romeo et al., 2015). The concentration factor of microplastics from surrounding waters to seals was reported to be as high as 160 times (Eriksson and Burton, 2003; Wright et al., 2013b; Eerkes-Medrano et al., 2015).

It was found that entanglement happened more frequently than ingestion. 55% of marine organisms incidences are associated with entanglement; ingestion contributes to 31% of incidences (Gall and

Thompson, 2015). Other solid matters contribute the rest (14%).

Entanglement of microplastics mainly happens to comparatively large marine organism. On the other hand, ingestion of microplastics can be found throughout almost all the trophic levels, such as zooplankton taxa (Cole et al., 2013), marine lugworm (Wright et al., 2013a), mussel (von Moos et al., 2012), oyster (Sussarellu et al., 2016), fish (Rochman et al., 2013), sea turtles (Bugoni et al., 2001), dolphins (Denuncio et al., 2011), whales (Walker and Coe, 1989) and seabirds (Derraik, 2002).

The chemical and biological impacts play key roles. After ingestion, microplastics cause toxicity effects to humans and living organisms through several pathways and mechanisms. The polymeric compounds used in production of plastics, the additives such as coper ions used during production of plastics are toxic. More importantly, various toxins in waters that are initially sorbed onto microplastics may subsequently be desorbed inside of human and animal bodies.

Microplastics are made of polymeric compounds that can cause certain health effects. For example, polystyrene (PS), resistant to biodegradation can accumulate in the stomach of fish (Carpenter et al., 1972) and can translocate through blood circulation (Chen et al., 2006). For oysters after being experienced a two-month exposure to PS microplastics, the decreases in oocyte number, diameter and sperm velocity were reported; reproductive disruption for marine filter feeders was expected (Sussarellu et al., 2016).

A variety of additives are added during plastics production, to improve physical properties, such as color, flame resistance, and hardness. They can be low molecular or polymeric, inorganic or organic substances. The most common additive is plasticizer that is for improvement of plasticity or viscosity. For example, polyvinyl chloride (PVC) must have plasticizers like phthalates and bisphenol A in order that thermal and photo-degradation can be minimized (Hammer et al., 2012). Other additives include colorants and flame retardants. It is anticipated that these chemicals would accumulate in human bodies through bioaccumulation process, some of which are well known as endocrine disrupting compounds. Some studies confirmed that such additives as bisphenol A, polybrominated diphenyl ethers, tetrabromobisphenol A and phthalates are present in humans (Talsness et al., 2009).

In addition, some compounds with heavy metals such as chromium, cadmium and lead are often used in production of colorants, stabilizers and plasticizers (Ernst et al., 2000; Murphy, 2001). They can be released from plastic debris into water systems and further enter the food chain to cause bioaccumulation of toxins in organisms.

Microplastics can be a vector for water-borne hydrophobic pollutants (Teuten et al., 2009; Lee et al., 2014; Bakir et al., 2016; O'Connor et al., 2016; Ziccardi et al., 2016). Examples include polychlorinated biphenyls (PCBs) (Velzeboer et al., 2014), and dichlorodiphenyltrichloroethane (DDT) (Rios et al., 2010), which are well known for their high toxicity and persistence in the environment. Due to the large specific surface areas and intrinsic hydrophobicity, the potential of hydrophobic chemical adsorption onto the surface of microplastics has caused great concerns over microplastics (Horton et al., 2017).

PCBs are well known to be carcinogenic, mutagenic, and/or teratogenic (Hammer et al., 2012). DDT can lead to adverse neurological effects and immunodeficiency (Mansouri et al., 2017). The partition studies show the large partitioning coefficients for organic compounds, in the range of 10^4–10^6 (Andrady, 2011). A few studies suggested that microplastics could sorb high amounts of PCBs form the surrounding seawater in coastal areas of USA and Japan (Hammer et al., 2012). Microplastics can sorb lubrication oils and heavy metals (Angiolillo et al., 2015; Hu et al., 2017).

The aforementioned pollutants initially sorbed on the surface of microplastics can be released when microplastics are ingested and stay inside of human body, where pH is rather low, temperature is comparatively high and digestive liquids are present. A few studies confirmed that the desorption rate of sorbed contaminants in organisms was accelerated, substantially faster than that in seawater (Teuten et al., 2007; Bakir et al., 2014).

However, very limited information is available about the real sorption behavior for water-borne pollutants in fresh water on the microplastics. The toxicity of microplastics in fresh waters is not well understood.

A few lab-scale studies have provided some implications on the potential biological hazards from microplastics. A research conducted by Ma et al. (2016) selected *Daphnia magna* as a model fresh water organism to study the toxicity of microplastics together with sorbed phenanthrene. They concluded that nano-sized PS exhibit high toxicity and physical damage to *Daphnia magna* and toxicity was enhanced by sorbed phenanthrene.

A recent study demonstrated that the mixture of antimicrobial florfenicol and microplastics caused higher inhibition level of cholinesterase activity on freshwater bivalve *Corbicula fluninea* than that of florfenicol or microplastics (Guilhermino et al., 2018). It was found that microplastics caused certain levels of biological effects on *Corbicula flumineaand* and their predator *Acipenser transmontanus* (Rochman et al., 2017).

A few studies on larval and adult zebrafish showed that microplastics were first ingested, became accumulated, and consequently caused alternations in locomotion, intestinal damage, and change in metabolic profiles (Lu et al., 2016; Chen et al., 2017; Sleight et al., 2017; Lei et al., 2018). With regards to other fresh water organisms, the toxicity studies on marine organisms may provide suggestive information. Hence, it is of importance to investigate the interaction between microplastics and key compounds in lab-scale experiments, which will be helpful in the risk assessment of microplastics.

The biological effects include the potential to geographically transfer microorganisms (Oberbeckmann et al., 2015). Microorganisms can quickly colonize the surface of microplastics and be transported with the movement of microplastics, as plastics are usually durable and persistent than other media (Thiel and Gutow, 2005). While this interaction is commonly known and possible consequences are raised, such as the introduction of pathogens to a clean environment, limited literatures are available to reveal the diverse biofilms communities, and even less for freshwater environment. (McCormick et al., 2014) conducted high-throughput sequencing analysis on the microplastics collected in an urban river in Chicago, Illinois. They found out some attached taxa were plastic decomposing organisms and pathogens; the findings suggest that microplastics can transport bacterial assemblages in freshwater ecosystems. Their study also emphasized that pathogenic wastewater-associated organisms could be discharged into waterways by means of the microplastics with the attachment of the organisms. A study on microplastics-associated bacteria in Yangtze Estuary also confirmed the presence of potential pathogens on microplastics (Jiang et al., 2018).

Another biological effect is the change in the plastic physical properties because of biofilms that attach onto surface of microplastics. Firstly, the density of microplastics can be increased (Carr et al., 2016), enabling light microplastics to sink in the water column and benthic zones. Furthermore, the biofilms can alter the surface nature of microplastics and make the surface less hydrophobic (Lobelle and Cunliffe, 2011; Zettler et al., 2013). The findings may provide some insights on studies associated with sorption of persistent organic pollutants. Whether the attached biofilms can enhance or weaken the interactions between microplastics and water-borne pollutants remains unknown to us.

3. Microplastics sampling methods

Despite the fact that studies on microplastics have been performed for years, the methodologies on sample collection, sample pre-treatment, quantification and identification are not standardized. The findings from reported studies vary significantly and cannot be easily compared.

The sampling methods can be volume-reduced and bulk sampling (Hidalgo-Ruz et al., 2012). In the volume-reduced sampling, the volume of sample is reduced during the sampling period. In the bulk sampling, no water volume is reduced. In the volume-reduced sampling, neuston plankton net and manta trawl are two commonly adopted approaches, which aim at collecting surface water samples. Only a few studies used the water collected by the bulk sampling approach.

Because of its lower density than water, microplastics tend to float on the surface of water. Therefore, a trawl along a transect can be applied to collect surface water samples (Rocha-Santos and Duarte, 2015). During sampling, a flow meter is used to calculate the entire volume filtered through the mesh, by which the total volume of water can be determined (Free et al., 2014).

As shown in Table 1, the researchers typically use net with 330 μm mesh to concentrate top water samples (Ryan et al., 2009; Hidalgo-Ruz et al., 2012; Eriksen et al., 2013; Zhao et al., 2014; Mani et al., 2015; Baldwin et al., 2016; Su et al., 2016; Anderson et al., 2017). Such a selection is mainly due to the lower size boundary of microplastics of 333 μm suggested by National Oceanic and Atmospheric Administration of USA (Arthur et al., 2009). The advantages of using the trawl are that it can cover large sampling areas and reduce sample volume.

Only one study adopts 500-μm mesh in Austrian, leading to a much smaller content of microplastics (Lechner et al., 2014). However, as the mesh size is comparatively large, it may neglect small particles, which may be more important in fresh waters due to the environmental impacts (Eerkes-Medrano et al., 2015).

Additionally, when the mesh of different sizes is selected, greater difference in abundance of microplastics can always be observed (Lozano and Mouat, 2009; Dris et al., 2015; Zhang et al., 2015; Wang et al., 2017). Lozano and Mouat (2009) found out that when an 80-μm mesh was used, the results were 100,000 times higher than that by a 450-μm mesh. The similar observation was reported in a study of the waters in Great Paris (Dris et al., 2015). Therefore, the concern over comparability and data credibility from studies is being raised.

In addition to common trawl methods, other approaches are used. They include surface microlayer method, hand-net collection and bulk water sampling.

Surface microlayer method enables researchers to collect upper-layer water. This process is performed manually by using a 2-mm sieve to get top 1-mm seawater (Song et al., 2014), while rotating glass drum can automatically collect surface water at upper level of 50–60 μm (Ng and Obbard, 2006).

Hand-net sampling is another common method to collect microplastics samples. When one uses the method, hand bucket is required to collect certain volume of surface water at top 20-cm level followed by the hand-net filtration (Moore et al., 2011; Song et al., 2014).

For bulk water samples, the volume varies among studies. Song et al. (2014) reportedly collected as high as 100-L water samples, while the volume of 100 mL and 2 L was only collected for the studies by Dubaish and Liebezeit (2013) and Leslie et al. (2017), respectively. The results by different sampling approaches were compared by Song et al. (2014). It was concluded that the mean abundance of microplastics present in surface seawater was in the order of surface microlayer, hand net, bulk water and manta trawl. The size distribution and particle compositions were also dependent on sampling methods. For instance, while the PS particles showed 96% of total particles in manta trawl samples, they seldom appeared in surface microlayer samples and only accounted for 0.2% of total particles.

The size of microplastics can be as small as 1.6 μm to the upper boundary of 5 mm (Cole et al., 2011). A study has shown plastic fragments of size less than 50 μm were underestimated (Song et al., 2015). Additionally, for dynamic freshwater bodies, like rivers, the microplastics can quickly transfer to the aquatic environment. However, in isolated and remote freshwater bodies, due to the low human activity, microplastics are trapped in the water and would gradually fragment into tiny microplastics, which have the sizes of sub-micro meters. Hence, underestimation can be expected in the results, when a 330-μm mesh is used to collect samples. It is inappropriate to apply the marine microplastics sampling methods for the work in fresh water environment. It is therefore an urgent need to develop optimized and standardized methods for water sampling.

4. Sample extraction and purification

The microplastics analysis mainly consists of two steps: (1) extraction/purification, and (2) quantification and/or identification. The microplastics must first be separated from the initial matrix in order to improve and simplify the subsequent process for the quantification and/or identification.

For the initial separation, density separation is the most often used approach, which involves the mixing of the sample with a liquid that has the defined density (normally a saturated salt solution). The suspension was then shaken and stirred for a defined time, followed by the settling of the mixture. This process can enable the low-density particles such as the microplastics to float to the upper layer of water and the high-density particles such as inorganic clay to sink to the bottom. Thus, microplastics could be recovered from the supernatant.

The saturated NaCl solution with a density of 1.2 kg/L is the most commonly used to achieve this separating process due to its low cost and no toxicity to humans (Browne et al., 2011; Claessens et al., 2011). Filtered seawater and distilled water are also used as separating solutions (Hanvey et al., 2017). However, for those denser microplastics that contain polyvinyl chloride or polyoxymethylene with densities of 1.16–1.58 kg/L and 1.41–1.61 kg/L, they cannot be recovered by this approach.

Other salt solutions were also reportedly used. They are sodium polytungstate (SPT) solution with a density of 1.4–1.5 kg/L (Corcoran et al., 2009; Corcoran, 2015), calcium chloride ($CaCl_2$) with a density of 1.3 kg/L (Stolte et al., 2015) and sodium iodide (NaI) solutions with a density of 1.8 kg/L (Nuelle et al., 2014). The selection is based on the separation efficiency and the cost of materials.

As a non-toxic separating salt, the SPT was applied for liquid separation for years (Munsterman and Kerstholt, 1996). However, it is rather expensive when it is compared with other salts. A study by Stolte (2015) demonstrated that $CaCl_2$ was not suitable as it caused interference in the measurement.

NaI was used for the microplastics separation in several studies. Although the density of NaI solution is as high as 1.8 kg/L, it is expensive and must be handled with care (Nuelle et al., 2014).

Zinc chloride ($ZnCl_2$) solution with a dentistry of 1.6 kg/L was also used for the microplastics separation (Imhof et al., 2012, 2016); most types of microplastics can be recovered. However, it is hazardous compared to other substances reported. Thus, the subsequent recycling and reuse of $ZnCl_2$ with care are necessary in order to minimize environmental pollution.

The extraction/separation methods are based on the density separation through the combination of fluidization with floatation (Ivleva et al., 2017). 80%–100% of microplastics (particles) could be obtained by saturated NaCl solution following the plain stirring extraction (Fries et al., 2013). However, the drawback is that smaller plastic particles of size <1 mm can only be separated to a small degree of 40% (mass).

Several strategies were proposed and adapted. It was reported that the combination of fluidization in a NaCl solution and floatation in NaI solution gave a very good recovery rate of 99% (Nuelle et al., 2014). The newly developed instrument, Munich Plastic Sediment Separator, in combination with the use of $ZnCl_2$ solution, can yield a particle number recovery of up to 96–100% for larger microplastics and 96% for smaller microplastics, which was mainly used for the microplastics separation for the sediment samples (Imhof et al., 2012).

Another alternative is through the pressurized fluid extraction (PFE). By optimizing the PFE conditions, plastics can physically be separated from waste and soil samples (Fuller and Gautam, 2016). Although the plastic particles as small as 30 μm can efficiently be extracted, the challenges of using the method are that: (1) size distribution cannot well be determined; (2) morphology of microplastics after extraction is changed.

In addition to the density separation, alternative separation techniques are reportedly used. For example, elutriation can effectively isolate microplastics from marine sediments (Claessens et al., 2013). The light microplastics can be retained through the upward water flow and aeration in a self-designed elutriation and floatation apparatus, and then be collected on a 35-μm sieve. An extraction efficiency of 94–98% can be obtained. Its limitation is that it is less applicable for wastewater, which contains a high concentration of organic matter. This is due to the negligible density difference between the microplastics and the naturally occurring particles within these matrices, making the elutriation method difficult to achieve.

To improve the efficiency of the extraction for samples taken from sediment and sewage, density separation to separate the lighter microplastics is always the first step, followed by the purification. This becomes especially important for bulk samples. However, the sequence in the operations is less important for water samples.

The purification process can be divided into two main different categories of chemical degradation and enzymatic degradation, in order that the interfering matters such as organic tissues and inorganic dusts can be removed. In the first approach, the microplastics samples are treated with different chemicals, mainly 10%–30% hydrogen peroxide (H_2O_2) solution (Zettler et al., 2013; Nuelle et al., 2014; Zhao et al., 2017) or peroxide mixed with mineral acids such as sulfuric acid (Imhof et al., 2013).

Cole et al. (2014) studied the efficiency of different acid, alkaline and enzymatic digestion methods for digestion of biological materials, including organisms/microorganisms and attached biofilms from marine surface trawls. The damage of microplastics may be less. The non-oxidizing acids such as hydrochloric acid at low concentrations at room temperature are insufficient, yielding large amount of organic residues after the digestion, whereas the strong oxidizing acids such as sulfuric acid and nitric acid would destroy or damage the microplastics made by polymers with less tolerance at low pH.

A study on digestion of the microplastics-containing samples taken from marine environment by 1-M sodium hydroxide showed a higher effectiveness of 90% (Cole et al., 2014). The digestion efficiency would increase with the increase in the molarity and the temperature. The plankton were digested by 10-M NaOH at 60 °C; however, such a harsh condition would damage the microplastics. As the damage to the microplastics is negligible, the H_2O_2 based method is still the most popular for the sample digestion.

Other chemical approaches such as ultrasonication can be combined with the deionized water or sodium dodecyl sulfate (SDS) solutions for the digestion (Cooper and Corcoran, 2010; Enders et al., 2015). However, some concerns had been raised as tiny microplastics could be generated from the brittle plastic samples (Löder and Gerdts, 2015).

Another emerging approach to remove the organic matters is the enzymatic degradation. During the degradation process, microplastics samples are incubated with a mixture of technical enzymes such as lipase, amylase, proteinase, chitinase, and cellulose (Cole et al., 2014; Löder et al., 2015). Such organic matters as proteins, lipids and carbohydrates can be specifically removed. Cole et al. (2014) reported the application of the proteolytic enzyme (proteinase-K) in the treatment of the marine samples. More than 97% (by weight) of the materials present in plankton-rich seawater samples were removed within a few hours, whereas the microplastic debris present was unaffected. This method should further be optimized as the molecular biological enzyme is costly.

The basic enzymatic purification protocol (BEPP) was used to purify samples for subsequent spectroscopic analyses (Löder et al., 2017). Combined with the detergent sodium dodecyl sulfate (SDS), technical-grade enzymes (e.g., protease, cellulase and chitinase) in phosphate buffer saline solutions and H_2O_2, with the density separation by $ZnCl_2$, the purification efficiency can reach 98.3% for plankton samples.

A universal enzymatic purification protocol (UEPP) different from the aforementioned BEPP was further developed (Löder et al., 2017). In the UEPP, another two more enzymes (lipase and amylase) were added and digestion was processed in tris(hydroxymethyl)-aminomethane (Tris) and sodium acetate buffer. The modified protocol can be implemented on samples from different environmental matrices. Although these two protocols can reduce operation time of microscopy and have more reliable spectroscopic results, it requires long digestion time up to 15 days. Therefore, the new enzymatic purification approach should still be optimized and the flexibility in the large-scale routine field sample purification still needs to be further investigation.

5. Microplastics identification and quantification

After the microplastics-containing water is treated, visual sorting is conducted. This is commonly used by the majority of the research groups, to select the suspected microplastics for further analysis and identification (Derraik, 2002; Hidalgo-Ruz et al., 2012; Rochman, 2015; Qiu et al., 2016). However, visual sorting may not provide accurate information on microplastics abundance due to presence of such particles as clay and algae. If the aftermentioned treatment is not conducted, it is very difficult to visually differentiate the microplastics from other extracted organic and inorganic particles of similar size and shape, especially for the samples that are not pre-treated as aforementioned. Additional approaches such as the spectroscopic approaches are required. They can serve as a more reliable technical tool for the identification of the plastic particles, instead of relying on visual observation that would introduce large variations in the results.

The current techniques for quantitative and qualitative research of microplastics include pyrolysis gas chromatography coupled to mass spectrometry (Fabbri et al., 2000; Fabbri, 2001; Fries et al., 2013; Nuelle et al., 2014), Raman spectroscopy (Cole et al., 2013; Collard et al., 2015; Imhof et al., 2016; Qiu et al., 2016; Wiesheu et al., 2016; Zhao et al., 2017), Fourier-transform infrared (FT-IR) spectroscopy (Song et al., 2014; Besseling et al., 2015; Qiu et al., 2016), liquid chromatography (Hintersteiner et al., 2015; Elert

Table 3
Summary of commonly used analytical techniques for analysis of microplastics.

Method		Methodology	Particle Size by different analytical methods	Advantages	Limitations
Visual Method	Microscopic Counting	Pretreated samples are identified by microscopy. The particles are counted directly.	The particles with size down to micro-meter (μm) range can be identified by the stereomicroscope.	Samples with a relative high amount of large microplastics can be identified by this methods quickly, providing an overall picture of microplastics abundance in short time with low cost.	The nature of the samples cannot be determined and it is necessary to couple with identification methods listed below.
Spectroscopic Method	FTIR	Samples are subjected to infrared radiation with defined range and the excitable vibrations depend on the composition and molecular structure of a substance. Plastic polymers have highly specific IR spectra with distinct band patterns.	Larger particle size of > 500 μm can be analyzed by ATR-FTIR, Smaller particle down to 20 μm can be analyzed by microscopy coupled FTIR.	FTIR based techniques are non-destructive methods. They are well established, fast and quite reliable. The newly emerging automatic FTIR imaging such as FPA makes it possible for the fast acquisition of several thousand spectra within an area through one single measurements and the analysis time is greatly shortened.	Samples must be IR −active; the samples below 20 μm might not yield not enough absorbance interpretable spectra. Non-transparent particles are difficult to be analyzed by this method. The highly specific instruments are expensive and require experienced personals for operation and data processing. The detection is affected by the environmental matrix (e.g., biofilm formation on polymer), which creates difficulty in the data interpretation. The sample must be pretreated to eliminate IR-active water.
	Raman Spectroscopy	The interaction of the irradiated laser light with the molecules and atoms of the sample results in differences in the frequency of the back-scattered light when compared to the irradiating laser. The Raman shift can be detected, leading to substance-specific Raman spectra.	Microscopy coupled Raman Spectroscopy (RS) method is suitable for particles size > 1 μm. It is the only method available for particles falling in the range of 1 to 20 μm.	Microscopy coupled RS allows the analysis for small particles between 1 to 20 μm with high spatial resolution and relatively low sensitivity towards water. It is possible to analyse non-transparent and dark particles using RS method; the fast chemical mapping can be performed by RS method, enabling fast and automatic data collection /processing.	There are great interferences of fluorescence from biological, organic and inorganic impurities, hampering the identification of microplastics. Sample requires purification before analysis; appropriate Raman acquisition parameters (e.g., wavelength, laser power, and photo bleaching) are important. The automatic mapping of micro-RS is still under development. The analysis by RS is time-consuming.
	Scanning Electron Spectroscopy	The sample images are produced by the interaction of an electron beam with the sample to measure the secondary ions.	Particle of sizes down to micro scale can be analyzed.	The high-resolution image of the samples can be produced by this method.	Samples need to be coated at high vacuum; no detailed identification information is available.
Chromatographic Method	Thermo-analytical methods such as pyrolysis GC/MS	Samples are thermally treated under ambient conditions and the released gaseous compounds are trapped, and subsequently transferred to a GC column, coupled to a quadrupole - MS. The spectra of pyrolysis products are then compared to a database of common plastic types.	The method is suitable for samples with sizes > 500 μm, which can be manually handled by tweezers.	The sample can be analyzed together with the organic plastic additives in one run without the use of solvents and thus avoid background contamination. The method is sensitive and reliable. Some common polymers spectra data library is available.	Only one particle with certain weight can be assessed per run. The pyrolysis database is only available for selected polymers only such as PE and polypropylene.
	Liquid Chromatography	Samples are dissolved by selected solvents. Different molar mass distribution is measured by size exclusion chromatography and quantification is based on HPLC analysis.	Sufficient sample size of several milligrams is required for the chemical extraction to conduct this testing.	The recoveries of selected polymers were high.	Inability to determine the physical characteristics, such as size information, and restrictions on polymer types limit its applications to environmental samples. Only small amount of samples can be assessed per run. Only specific polymers such as PS and PET can be analyzed by this method.
Other Methods	Tagging Method	Hydrophobic dye adsorbs onto surfaces of microplastics and renders them fluorescent when they irradiated with blue light.	Microplastics with sample size down to microscale range can be visualized and counted.	This method is straightforward and allows fast screening of microplastics with low cost. The fluorescent particles can be counted and may be identified.	Other particles such as organic debris might be stained by the dye. This would lead to over-estimation of microplastics abundance.

et al., 2017) and the most recently reported tagging method (Shim et al., 2016). Table 3 summarizes most of the analytical techniques, and provides their advantages and limitations.

Among them, manual counting under a stereomicroscope is the most straightforward and thus widely used. Noren (2007) proposed several standardized criteria on a strict and conservative examination of microplastics with the key points as follows:

(1) The particles or fibers that have structure of biological organisms should not be included as microplastics;
(2) The fibers can be counted as microplastics if they have three-dimensional structure;
(3) The particles can be counted as microplastics if they can be homogeneously colored;
(4) The microplastics must be transparent or whitish in color and be studied under high magnification with the help of fluorescence microscopy.

There are a few drawbacks to the aforementioned criteria. The results from the visual sorting are strongly affected by several factors including: (1) personal factors (e.g. carelessness), (2) microscopy quality, and (3) sample matrix. Furthermore, visual counting suffers the drawback of size limitation due to the resolution of the microscopy. Up to 70% error rates can be observed; the number of error increases with a decrease in particle size (Hidalgo-Ruz et al., 2012).

A study was conducted on the microplastics abundance in the sediment samples collected from the North Sea. The results by the visual counting approach seemed overestimated based on the additional study by the focal plane array (FPA)-based micro-FTIR spectroscopy. It was found that, only 1.4% of the particles visually observed were the synthetic polymer by the micro-FTIR spectroscopy. The other particles visually found were of other origins such as organic matters and dusts (Löder et al., 2015).

Among the commonly reported techniques, Fourier transform infrared spectroscopy and Raman spectroscopy are most commonly used to identify microplastics (Besseling et al., 2015; Ivleva et al., 2017; Zhao et al., 2017). In the testing, the microplastic samples are excited, by which the structure specific vibrations can be detected. The produced characteristic spectra with the fingerprint range allow the identification of nature of materials (namely plastics and non-plastics). The polymer identification was achieved by comparing the obtained spectra with the known reference spectra.

For FTIR spectroscopy, the sample is irradiated with IR light with a defined wavelength range and the IR radiation absorbance collected by the equipment is structure specific. The prerequisite for the IR absorption is the change of the permanent dipole moment of a chemical bond, thus this technique is mainly used to analyze the molecules with the polar functional groups such as carbonyl groups.

Raman spectroscopy is a scattering method. The laser with the single wavelength is applied to excite the molecule; the radiation interaction with the sample is detected. The prerequisite of the Raman spectroscopy is the change in the polarizability of a chemical bond and thus this technique is mainly for compounds with aromatic bonds, C—H and C=C double bonds.

FTIR and Raman spectroscopy are complementary vibrational techniques to each other. Both would provide complementary information on microplastic samples.

FTIR spectroscopy is a non-destructive technique with well-established polymer database. There are three different operating modes available for FTIR, namely, transmission, reflection and attenuated total-reflectance (ATR) mode. Larger plastic samples >500 μm can be analyzed using ATR-FTIR.

For smaller particles, the micro-FT-IR spectroscopy is a good tool for simultaneous visualization, mapping and collection of spectra. The micro-FTIR is particularly useful as the membrane filter can be directly used for the visualization with little sample preparation. The micro-FTIR analyses of plastics can be performed in either transmission or reflectance mode. The transmission mode gives high-quality spectra, but requires substrates to be infrared-transparent. The analyses in reflectance mode can be done for thick samples. Meanwhile, irregularly-shaped microplastics would produce non-interpretable spectra due to refractive error (Harrison et al., 2012). Thus only transparent microplastics with certain thickness with regular shape can be analyzed; otherwise the signal will be disturbed/distorted by reflection error caused by light scattering. These drawbacks can hardly be avoided.

The micro-FTIR requires tremendous time and efforts to find the suitable microplastics particles for the analytical work. The contact of the crystal with the inorganic particles could impose great damage to the expensive instrumentation.

Levin and Bhargava (2005) used the approach of micro-FTIR mapping, with the sequential measurement of IR spectra at spatially separated and manually-selected points on the sample surface. However, only small areas of the filter paper could be analyzed and the process is very time-consuming.

A FPA-based FTIR imaging with several detectors placed in a grid pattern was applied for microplastics analysis (Tagg et al., 2015). This method allows for detailed and unbiased high-throughput screening of total microplastics on the whole filter paper. It can enable the simultaneous recording of several thousand spectra in a targeted area within a single measurement run, and thus generate chemical images for the whole filter paper. The screening and analysis for the whole sample filter paper may become possible this technique.

This method has several limitations as follows. The lateral resolution of micro-FTIR spectroscopy is always limited to certain diffraction range (e.g. 10 μm at 1000 cm^{-1}). The smaller particles with irregular shape are less applicable. The samples down to 20 μm cannot be analyzed (Löder and Gerdts, 2015). As water is strongly IR active, which produces broad peaks over 3000 cm^{-1}, the sample preparation is required prior to measurement.

Raman spectroscopy as a surface analytical technique allows for the study of large and visually sorted particles. The micro-Raman microscopy combined with Raman spectra imaging technique theoretically allows for the spectra analysis of whole filters at a spatial resolution below 1 μm (Imhof et al., 2016; Zhao et al., 2017). However, as Raman spectroscopy is based on the methodology that the fluorescent samples are excited by the laser, the contaminants by biological residual and others would interfere with the spectra, leading to the difficulty in producing the interpretable spectra.

The thermoanalytical methods such as pyrolysis-GC/MS and TGA-MS have been used for the microplastic analysis. Samples are firstly thermally degraded and the resultant products are subsequently sent to the mass spectrometer for analysis. The collected data are compared with reference to obtain such sample information as identity and concentration.

However, the techniques have a limitation on size of plastic matters. It is difficult to handle samples <500 μm as the tiny small samples cannot be put into the tube. In addition, the method is less applicable for mixtures with high concentration of impurities (Ivleva et al., 2017).

Another thermoanalytical method was used by Duemichen et al. (2014). It allows the direct microplastics assessment from the field environmental samples with ease in sample preparation. The thermogravimetric analysis is combined with the solid-phase extraction (TGA-SPE) and thermal desorption gas chromatography mass spectrometer (TDS-GC/MS). The environmental field sample with certain impurities such as the organics and

microplastics particles (up to 20 mg) was placed in a TGA crucible, followed by a heating process under inert conditions such as N_2 up to 800 °C. The resultant products that are sorbed on the extraction substrate can be tested by GC/MS for the determination of the composition. Only the results of polyethylene (PE) were published.

In addition to gas chromatography, liquid chromatography was applied in quantification of microplastics (Hintersteiner et al., 2015; Elert et al., 2017). The method takes advantage of the different solubility of plastics. Appropriate solvents are used to dissolve different polymers. For instance, tetrahydrofurane and hexafluoroisopropanol could dissolve PS and polyethylene terephthalate (PET), respectively (Elert et al., 2017).

After the preparation of polymer extracts, the samples can be analyzed by High Performance Liquid Chromatography (HPLC) coupled with size exclusion systems. Though unable to determine the sizes of plastic particles, this method reportedly has high recoveries and can quantify the microplastics. However, this method has yet to be applied to real environmental water samples. More studies are required to verify this method for the microplastics samples in fresh waters.

In addition, a simple staining method was reported developed (Shim et al., 2016). Commercially available Nile Red (NR) was applied to stain highly hydrophobic microplastics. The NR molecule is only fluorescent in the presence of a hydrophobic environment. The molecule specifically binds to the microplastics. As a result, the NR stained microplastic particles are visible under a fluorescent microscope, which can easily be counted.

The NR staining could be useful to determine the microplastics abundance. The main drawback is that the NR also can stain the natural organic matters. Hence, pre-purification is required. The staining method cannot be used alone unless the interfering organic matters are fully removed.

6. Challenges and recommendations

Microplastics (polymers in nature) collected from freshwater environment have undergone long-time exposed to variation degradation processes, including UV-induced photo-degradation, thermal degradation, mechanical action, and biological interactions (e.g biodegradation). The surface morphology and properties such as hydrophobicity would be greatly altered from original polymer compositions (Hidalgo-Ruz et al., 2012).

Organisms and microorganisms can colonize microplastics, forming successional biofilms. Such processes in combination with polymer additives would further complicate spectroscopic analysis, because spectral changes result from biofilm/additives and reference spectra are less sufficient for degradation products. It becomes more challenging for smaller particles, where the higher surface to volume ratio makes the signals from the surface material more significant. Thus it is rather difficult to differentiate the microplastics from natural polymers such as chitosan, cellulose, and chitin.

It is difficult to quantify and qualify microplastics from the complex environmental samples using a single analytical method. Thus, combination of multiple methods is preferred, which strongly depends on the sizes of microplastics.

When the sizes of microplastics fall in the range of <1 mm and the minimal cut-off size is tens of microns, the combination of the microscopic analysis with the spectroscopic analysis is highly recommended. If the minimal cut-off size is in a range of a few microns, Raman spectroscopy is preferred as it is the only technique for such sizes. If the samples after digestion/pretreatment have less impurity, thermal methods and liquid chromatography are recommended.

Standardized and robust methods for quantification and identification of microplastics should be first developed and verified so that data from different researches can be more comparable and reliable. Key issues such as environmental risk assessment can then be conducted scientifically. Subsequently, rules and regulations can be formulated and enforced.

7. Conclusions

Microplastics have become one of emerging contaminants in the aquatic environment. The presence has been reported in many places around the world, and has caused great public concerns. However, there is still a lack of sufficient knowledge about microplastics in freshwaters such as their health effects and fast monitoring.

This review article summarized the current status of microplastics contamination in freshwaters, including rivers, lakes, water treatment plants and drinking water. The potential environmental consequences including entanglement, ingestion, vector of waterborn pollutants and possible toxicity were addressed.

The sampling methods of volume-reduced method and bulk sample method were described to provide researchers with suitable one(s) to use. Detailed illustration on sample purification and separation was given. FTIR spectroscopy, Raman spectroscopy, pyrolysis-GC/MS, liquid chromatography and tagging method (for counting) were described and their advantages and disadvantages were discussed.

Through the literature research, we found that it was difficult to use a single method for quantitation and quantification of microplastics from freshwater samples. The combination of different techniques can greatly improve our understanding of this new environmental problem and more reliable data for environmental risk assessment and preparation/enforcement of rules and regulations in the future.

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