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Review

Assessment of microplastics in freshwater systems: A review

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Abstract

The reliance on plastic for a vast number of consumer products, many of them single-use, results in their continuous entry into aquatic environments. Plastic waste can fragment into smaller debris, some with a diameter <5 mm (microplastics). Microplastics are of growing concern especially since 2014, however to date research on microplastic pollution has mainly focused on marine environments, partly because it has been mistakenly thought that sewage treatment plants could remove all plastic debris. To understand the impact of microplastic pollution in freshwater environments, an assessment of research on the sources, distribution and effects of microplastics, and trends in their analysis and policy has been carried out. Main sources of microplastic found in freshwater environments include synthetic textiles, personal care products, industrial raw materials and the improper disposal of plastic waste. Microplastic pollution is a global issue that presents with a broad range of concentration: for example, 3.5 x 10^3 microplastic units·L^{-1} were reported in sediment of Lake Huron, in the US and as low as 1.2×10^{-4} units·L^{-1} in countries with sparse population such as Mongolia. The main polymer constituents of microplastics found in freshwaters have been identified as polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET), accounting for

70% of the total, each with a very similar frequency of occurrence. Despite microplastics being relatively inert, they are found to cause some effects in aquatic organisms. Future work should focus on monitoring microplastic pollution in regions from where there is currently scarce published data (e.g. South America, Africa and North Asia) and the study of their sources, stability, transport and effects to freshwater ecosystems. The establishment of standardized monitoring methods will allow for the comparison of data from different geographic areas. This information will inform measures to reduce the release and occurrence of microplastics in aquatic environments.

Keywords:

Fibres; microplastics; fate; sampling; characterization; freshwater

1 Introduction

Plastic products are widely used, making the annual output of plastic products worldwide exceeded 3.48×10^8 tonnes and is increasing at a rate of 0.2×10^8 tonne acre^{-1} (Statista, 2017). Based on their mass production and use, plastic products inevitably enter the aquatic environment: for example, more than 2.5×10^5 tonnes of plastic waste were estimated to be floating on the global ocean surface (Eriksen et al., 2014). In the aquatic environment, plastic waste can be fragmented into microplastics (debris < 5 mm in diameter) by physical, photo and bio-degradation (Law and Thompson, 2014). The investigation of microplastic pollution has mainly focused on the marine environment (Cole et al., 2011; Ivar do Sul and Costa, 2014), including Canada (Desforges et al., 2014), Brazil (Santana et al., 2016), the UK and neighbouring countries such as the Netherlands (Barnes et al., 2009), China (Zhang et al., 2017; Zhang et al., 2019), Antarctica (Cincinelli et al., 2017) and in deep-sea Arctic sediments (Kanhai et al. 2019).

Marine microplastic debris can be a possible contributing factor to biodiversity loss and a potential threat to human health. The impacts plastics on aquatic life are influenced by the size of the debris: large plastic debris, such as discarded fishing lines and nets, often cause entanglement among invertebrates, birds, mammals and turtles (Gall and Thompson, 2015; A. Lusher, 2015). Smaller plastic items, such as bottle caps and less dense plastics can cause intestinal obstruction (Law and Thompson, 2014). Plastics and their degradation products are ingested by a variety of aquatic life ranging from invertebrates to fish with varied consequences,

many of which are under current investigation – for example, a trend of fishes, mussels, turtles, seabirds etc. to consume less prey has been observed (Cannon et al., 2016; Foley, et al., 2018; Lusher et al., 2013). Human health could be affected via food chain transmission of microplastics (Hollman et al., 2013). Furthermore, the physical and chemical properties of microplastics have been found to facilitate contaminant sorption to their surfaces, hence microplastics may serve as a vector of contaminants to organisms following ingestion (Carbery et al., 2018; Kontrick, 2018). The presence of plastic debris in the environment is considered among the main environmental issues and an emerging threat that may affect the ability of humans to conserve biodiversity (Sutherland et al., 2010; Auta et al., 2017).

Microplastic pollution is particularly acute in estuaries, indicating that terrestrial river input is an important source of microplastics to coastal and marine environments (Gallagher, et al., 2016; Sadri and Thompson, 2014; Vendel et al., 2017). However, knowledge of the impacts that microplastic pollution has in freshwater environments is still in its infancy when compared to that of marine environments, despite the fact that freshwater is a source for drinking water. Recent reviews of microplastic pollution in freshwater environments have focussed on methodology (Koelmans et al. 2019; Pico and Barcelo, 2019; Mendoza and Balcer, 2019; monitoring occurrence of microplastic in biota (Connor et al., 2019; Triebskorn et al. 2018); toxicity and methodology (Horton, 2017); occurrence, impact and analysis (Li et al. 2018); overarching discussion of microplastic pollution, however not focused on distribution (Wagner and Lambert, 2017) or focused in a specific geographic area (Fu and Wang, 2019; Shahul Hamid et al. 2018). Therefore, the focus of this review is to assess the magnitude of global

85 microplastic pollution in freshwater environments, providing information compiled from
86 recent research associated with the sources, occurrence, fate and effects of microplastics in
87 freshwater environments. In addition, this review provides a discussion of the analytical
88 approaches employed for the study of microplastics and the current state and development of
89 policy related to microplastic pollution.
90
91 **2 Microplastic sources**
92 The rate of fragmentation and degradation of plastics is unknown even for marine environments
93 (Law and Thompson, 2014). Varying degrees of physical forces, such as waves in oceanic
94 systems; environmental conditions, such as sunlight, pH and temperature; and the physical and
95 chemical properties of the plastic itself are thought to play a role in plastic degradation. Plastics
96 in freshwater systems also undergo physical and environmental degradation despite milder
97 physical forces than in marine environments (Andrady, 2011). Some environmental conditions
98 may have a larger impact within freshwater, for example Free et al. (2014) showed that plastic
99 fragments may undergo relatively intense weathering because of high ultraviolet penetration in
100 poorly nourished lakes (Free et al., 2014). However, overall degradation patterns of
101 microplastics in freshwater were found to be similar to those in the marine environment: cracks,
102 pits, and adherent particles (Imhof et al., 2013; Zbyszewski and Corcoran, 2011).
103
104 The degree of weathering to the surface of microplastics can be used to track the history of the
105 particles. Hence, surface features can show whether plastic debris underwent mechanical
106 degradation, for example from the action of waves, sand friction (Zbyszewski et al., 2014),

oxidative weathering such as from the exposure to UV-B (Zbyszewski et al., 2014), or biodegradation such as by the action of hydrocarbon-degrading microorganisms (Zettler et al., 2013). Insights into the effect of organic matter on microplastic degradation in sedimentary environments such as beaches and muddy coastlines were also reported by Zbyszewski et al. (2014). Identifying the degradation patterns of plastics in different environments is important as this can reveal how particles interact with the environment and how various factors affect their stability, transport, fate, and indicate potential effects to organisms (Ballent et al., 2016).

A spatial correlation has been found between the types of microplastics found at particular sites and human activities in surrounding areas (Lechner et al., 2014). In addition, the type of polymer and their concentration can be used to link microplastics with their origin. For example, microplastics found in the Great Lakes of North America are similar in size, shape, colour, and elemental composition to those found in facial cleansers (Eriksen et al., 2013). At the same time, microplastic particles in the effluent of a sewage treatment plant were very similar in colour, shape and size to those in toothpaste formulations, revealing that the plastic particles in personal care products may be among the sources of microplastic pollution in freshwater environments (Carr et al., 2016). Industrial sources of microplastics can also be identified even in large rivers such as the Danube River (Lechner et al., 2014). As opposed to rivers, stationary bodies of water such as lakes may accumulate more microplastics (Free et al., 2014; Imhof et al., 2013). Industrial resin particles and microspheres were found to be abundant in Lake Erie near the Huron Lake industrial zone (Eriksen et al., 2013; Zbyszewski and Corcoran, 2011). Large amounts of secondary microplastics (or microplastics derived from fragmentation of

other plastics) were found along the shores of sparsely populated mountain lakes, where there was scarce primary microplastic pollution (Free et al., 2014). Areas near tourist sites are also especially affected by microplastic pollution, and a representative example is the concentration of microplastics (i.e. 5,000-757,500 units Km^{-2}) found in China's Qinghai Lake (Xiong et al., 2018).

Direct sources of microplastic pollution include discharge from sewage treatment plants (Browne, 2015), weathering and degradation of plastic waste in water bodies (Eerkes-Medrano et al., 2015), and terrestrial input from soil erosion or surface runoff (Horton et al., 2017). The contribution of these sources remains controversial. Carr et al. (2016) found that nearly no microplastics were detected in the discharge of a tertiary sewage treatment plant in Southern California, and the abundance of microplastics in the effluent of the secondary sewage treatment plant was also low (with an average of only one microplastic particle per 1.14 litres of effluent). In contrast, most microplastics were found in the primary treatment stage (oil skimming). Also, Murphy et al. (2017) investigated a large secondary sewage treatment plant in Glasgow, Scotland (daily capacity 260,954 m^3) and found that although the final removal rate of microplastics was as high as 98.41%, approximately 6.5×10^7 microplastic particles per day were still discharged into the receiving water, indicating that the sewage treatment plant was an important source of the microplastic pollution (Murphy et al., 2017). Therefore, the different operative conditions applied in each plant could lead to varied efficiencies in the removal of microplastics, and at this stage, more data is needed to understand the magnitude of the problem. Comparable removal rates of fibres were found in the Seine Aval (Paris, France)

151 wastewater treatment plant, which were estimated to be 83-95%. With reference to the treated
152 effluents, the number of fibres in the samplers used for their monitoring was $\times 10^5$ greater than
153 the number of irregular microplastic fragments, which ranged between $6 \cdot \times 10^{-5}$ and $3 \times \cdot 10^{-4}$
154 microplastic units L^{-1} (Dris et al., 2017). Hence, it can be concluded that the contribution of
155 sewage treatment plants to microplastic pollution may be related to their scale, location,
156 residence time and type of influent.
157
158 Microplastics can also enter rivers and lakes through surface runoff and atmospheric deposition
159 (Dris et al., 2017). An example is the large amount (with a maximum abundance of 660 units.
160 kg^{-1}) of large-size (1-4 mm) microplastics in sediments downstream of storm drainage outlets
161 that input into the Thames River, UK. These microplastics were mainly sheet-shaped, which
162 the authors thought might be from painted roads in the surrounding urban area. After being
163 washed away by rainwater, the microplastics were eventually deposited in the sediments of the
164 Thames River (Hortonet al., 2017). In addition, Klein et al. (2015) also found high
165 concentrations of microplastics (228-3,763 units kg^{-1}) in sediments along the banks of the
166 Rhine River in Germany, which further confirms the importance of the terrestrial input to
167 microplastic pollution of freshwater environments.
168
169 Among the origins of microplastics entering wastewater, the cleaning of synthetic fabrics such
170 as clothing (grey water) constitutes a major contribution (Browne, 2015; Peng et al., 2017).
171 When the process of washing clothes in a household washing machine was simulated in the
172 laboratory, the drainage of the washing machine contained a large amount of fibre-like

173 microplastics (Hernandez et al., 2017). When using detergent, the content of microplastics in
174 the drainage of the washing machine was much higher than that of washing without detergent.
175 For example, washing a five-year-old PET fleece jacket released microfibers with a 0.00111
176 weight percentage (wt%) (with no detergent); 0.00123 wt% (with detergent); and 0.00136 wt%
177 (with detergent and softener), having the release of microfibers increased when detergent and
178 detergent plus softener were used (10.8% and 22.5% increases respectively) (Pirc et al, 2016).
179 The various sources contributing to microplastic pollution of freshwater environments have
180 been summarized in the Graphical Abstract.
181

182 **3 Microplastic distribution in freshwater**

183 In marine environments, properties of microplastics such as their small size and low-density
184 result in transport over long distances, particularly via ocean currents (Ballent, et al., 2016;
185 Cole et al., 2011). Their occurrences have been reported along the coasts of continents (Browne,
186 2015; Ivar do Sul and Costa, 2014), in remote areas such as the central Atlantic Islands (Ivar
187 do Sul and Costa, 2014), sub-Antarctic region (Eriksen et al., 2014), the Arctic (Obbard et al.,
188 2014), and even in deep-sea habitats (van Cauwenberghe et al., 2015; Kanhai et al., 2019). The
189 different units of concentration used throughout the research and within review papers hinders
190 comparison between findings (Kang et al., 2018; Li et al., 2018). For example, recent review
191 papers (e.g. van Cauwenberghe et al., 2015) tabulate research findings with different units,
192 which hinder comparison among the concentrations. Table 1 compiles recent studies that report
193 microplastics in freshwater environments, and highlights that it difficult to compare the
194 concentrations found by each study. In Table 1, authors present the average of the

concentrations found by each study. According to the approximate average of plastic of 1 $g \cdot mL^{-1}$ and the size of particles, an estimation – $C_{number\ per\ volume} = C_{mass\ per\ volume} / (d_{plastic} \times V_{plastic})$ (where C corresponds to concentration; "d" corresponds to density and "V" corresponds to volume), – can be made to derive comparable concentration values from different studies using the same unit, i.e. number per volume. Thus, all values can be compared and analysed intuitively. It is noticeable that the concentration of microplastics in sediments is higher than that in water, this may be due to a combination of factors including their hydrophobic nature and density, and as a result, they tend to accumulate in sediments. Figure 1 intends to show where microplastic research is currently focussed and highlights places where microplastic monitoring is currently lacking, e.g. South America, Middle East, Africa, and Russia.

From the data and map, one of the most striking studies is from the Great Lake Basin of North America, where the average abundance of microplastics floating on the surface was as high as 43,000 units km^{-2} (Eriksen et al., 2013). The greatest presence of microplastics in Europe, to the best of our knowledge, has been reported in Lake Geneva, Switzerland, reaching 48,146 units km^{-2} (Florian Faure, 2012). However, microplastic pollution in freshwater environments of Asia may be more serious than those from other parts of the world (Wu et al., 2018). Notably, Free et al. (2014) found microplastic contamination in the surface water of Lake Hovsgol in northern Mongolia, Asia, with an average abundance of 20,264 units km^{-2}. As the geographical location of the region is remote, and the population is sparse, this study suggests that microplastic pollution here may be more influenced by runoff, monsoon rains and atmospheric fallout, among other factors. Concentrations and location of microplastics in recent monitoring

217 studies (period 2011-2019) in the freshwater environment are compiled in Table 1.
218 Microplastics detected in these studies include data from water and sediments, and different
219 compositions (Table 1).

220 Table 1 Concentrations and sizes of microplastics found in samples from freshwater environments.

221

Lat, Lon	Country	Location	Average Concentration from the studies	Estimated MP units· L^{-1}	Sample	Size	Methods	Reference
55.367, -3.96142	UK	Kelvin River	0.26685 g/L	296.5	Sediment	Size classes: 2.8 mm-11μ m	SEM-EDS	Blair et al. (2019)
29.00896, 116.69785	China	Poyang Lake	0.2034 g/L	226	Sediment and Surface water	Size classes:< 0.5 mm	Raman	Yuan et al. (2019)
44.37996, -108.03899	Europe	Carpathian basin	0.4716 g/L	524	Sediment and surface water	Size classes: <0.3mm	FTIR	Bordós et al. (2019)

37.27442, 9.87391	Tunisia	the lagoon of Bizerte	2.106 g/L	2340	Sediment	Size classes: 5 mm – 0.2 mm	FTIR	Toumi et al. (2019)
34.37526, 107.09683	China	Wei river	0.918 g/L	1020	Sediment and surface water	Size classes: <5 mm	Microscope with digital camera	Ding et al. (2019)
4.74974, 6.82766	Belgium	Flemish rivers	0.0153 g/L	17	Water	Size classes: <5 mm	FTIR and Raman	Slootmaekers et al. (2019)
-32.1058579, 115.9381508	Australia	Bloukrans River	0.216 g/L	240	Sediment	Size classes: 500µm	Visual Inspection	Nel et al., (2018)
2.3923759, 112.8471939	Malaysia	Surface water in Malaysia	0.108 g/L	120	Surface water	Size classes: 3 µm - 178 µm	Visual Inspection	Praveena et al., (2018)
-37.718524, 145.234919	Australia	Maribyrnong and Yarra Rivers	2.5803 g/L	2867	Surface water	Size classes: <2 mm	Visual Inspection	Kowalczyk et al. (2017)

52.13191, -97.26176	Canada	Lake Winnipeg	1.7397 g/L	1933	Surface water	Size classes: <5 mm	SEM-EDS	P. J. Anderson et al. (2017)
9.5949193, 76.3942857	India	Vembanad Lake	0.27 g/L	300	Sediment	Size classes: 0.2 mm – 1 mm	Raman	Sruthy and Ramasamy (2017)
52.2379891, 5.5346074	Netherlands	Dutch wastewater treatment plant effluent	0.00297 g/L	3.3	Wastewater treatment plant effluent water	Size classes: <5 mm	Visual Inspection	van Wezel et al., (2016)
61.0666922, -107.9917071	Canada	Canadian lakes and rivers	0.495 g/L	550	Sediment and surface water	Size classes: 2 mm - 5 mm	Visual Inspection	J. C. Anderson et al, (2016)

32.0000002, 89.9999998	China	Remote lakes in Tibet plateau	0.5067 g/L	563	Sediment	Size classes: <5 mm	Raman	Zhang et al. (2016)
42.64326, 11.98514	Italy	Lake Chiusi and Lake Bolsena	2.5 particles / m^3	0.025	Sediment and surface water	Size classes: <5 mm microplastics	Visual inspection	Fischer et al. (2016)
31.23825, 120.1414	China	Taihu Lake	123 particles / L	123	Sediment and surface water	Microplastics with a size of 100–1000 μm	FTIR and SEM/EDS	Su et al. (2016)
-22.9333191, -43.1147684	Brazil	Jurujuba Cove, Niterói, RJ	0.099 g/L	110	Sediment and surface water	Size classes: <5 mm	FTIR	Castro et al., (2016)
-28.816623, 24.991639	South Africa	Five urban estuaries of KwaZulu-Natal	0.288 g/L	320	Sediment and surface water	Size classes: <5 mm	Visual Inspection	Naidoo et al., (2015)

44.83141, 9.41722	France	River Seine, urban area	3 particles / m^3	0.03	River water	100–5000 µm	Visual inspection	Dris et al. (2015a)
23.1118934, 113.3341061	China	Pearl River Estuary	0.468 g/L	520	Sediment and river water	Size classes: 0.315 mm – 5mm	Visual Inspection	Fok and Cheung (2015)
50.22062, 99.91705	Mongolia	Lake Hovsgol	1.2 x 10^4 particles/ km^3	0.00012	Lake water	Size classes: 0.355–0.999 mm, 1.00–4.749 mm, and >4.75 mm	Visual inspection	Free et al. (2014)
-27.11667, -109.36667	Chile	Easter Island	0.072 g/L	80	Sediment and surface water	Quadrat: 0.25 m^2; Depth: 2 cm; Sieve: 1 mm	Visual Inspection	Hidalgo-Ruz and Thiel (2013)

46.91807, -104.00437	South Korea	Heungnam beach	0.3285 g/L	365	Sediment and surface water	Quadrat: 0.25 m^2; Depth: 5 cm; Sieve: 2 mm	Visual Inspection	Heo et al. (2013)
55.670249, 10.3333283	Denmark	Danish waters	0.0324 g/L	36	Sediment	Size classes: 38 µm – 1 mm, 1 – 5 mm and >5 mm	FTIR	Strand et al., (2013)
45.66132, 10.6851	Italy	Lake Garda	1.7 x 10^3 particles/ m^3	17	Sediment	Size classes: 9–500 µm, 500 µm–1 mm, 1–5 mm, >5 mm	Raman	Imhof et al., (2013)
42.30919, -87.8501	USA	Great Lakes	1.6 x 10^7 particles / km^3	0.016	Surface water	Size classes: 0.355–0.999 mm, 1.00–4.749 mm, >4.75 mm	SEM/EDS	Eriksen et al., (2013)

61.60713, -149.309	Switzerland	Various lakes	2 x10^3 particles / m^3	20	Sediment and surface water	Size classes: <2 mm, <5 mm (sediments) <5 mm, >5 mm (water)	Visual inspection	Faure et al., (2012)
44.65031, -82.2819	USA and Canada	Lake Huron	3.5 x 10^{11} particles / km^3	3499	Sediment	Size classes: <5 mm plastic pellets, >5 mm broken plastic, polystyrene	FTIR	Zbyszewski and Corcoran, (2011)

222

Whilst there are numerous reports of microplastics in freshwater environments such as in the Great Lakes basin of North America; the Thames and Rhine rivers of Europe; and the Taihu basin of China (Table 1), microplastic pollution of freshwater environments has been studied to a lesser extent, when compared with marine environments. However, microplastic contamination of freshwater environments has been found even in remote regions; although studies are limited, this suggests that microplastics are distributed in freshwater systems throughout the world. Therefore, more systems should be studied to fill the gap in our knowledge of the distribution of microplastic pollution in freshwater environments globally.

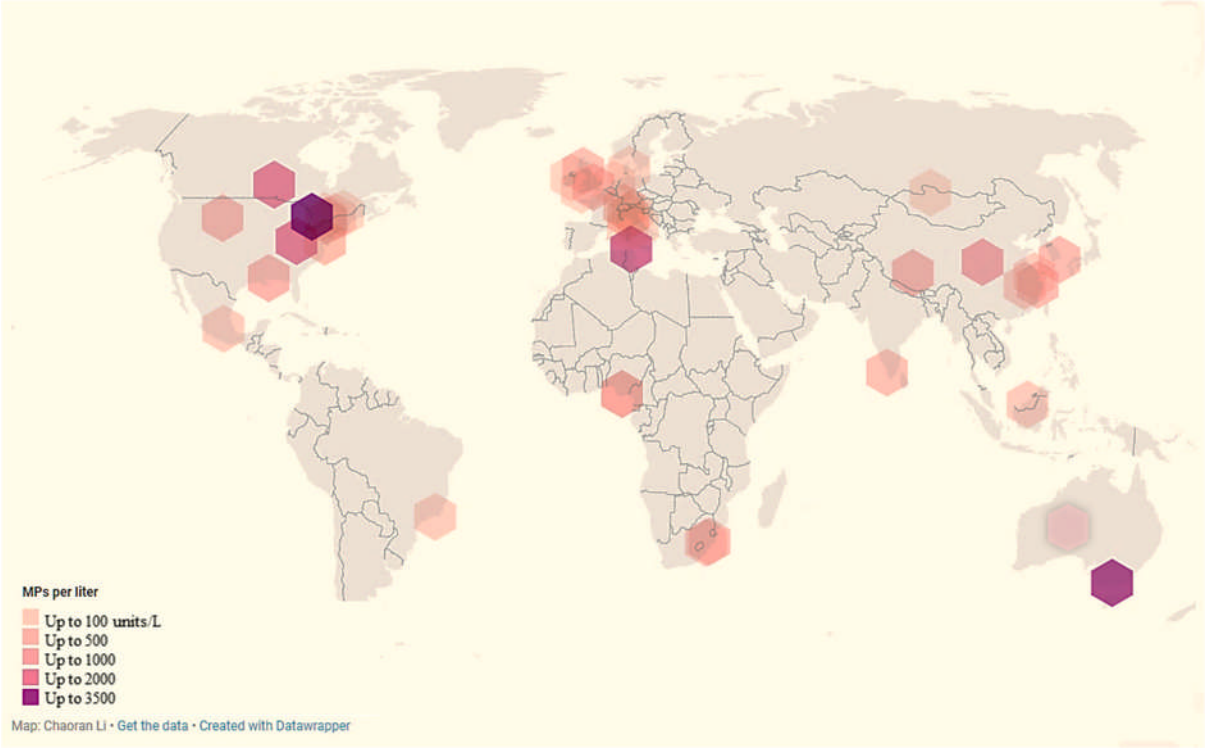


Fig. 1. Map of distribution of microplastics in freshwater systems (based on data in Table 1)

4 Detection and analysis of microplastics

The difficulty in separating microplastics from benthic and planktonic habitats has limited the

available knowledge of their spatial and temporal distribution (Galgani, et al., 2013; Hidalgo-Ruz et al., 2012). Most current methods to detect and monitor microplastics are time consuming and inadequate in identifying all particles (Galgani et al., 2013; Mendoza and Balcer, 2019). Challenges in the detection of microplastics primarily comprise three aspects: the ability to capture plastic particles from water or sediment samples; the separation of plastic fragments from other matter (organic and inorganic); and the identification of plastic types (Eriksen et al., 2013; Hidalgo-Ruz et al., 2012). Microplastics are not regularly monitored as there is a lack of understanding of their possible effects on humans (Wright and Kelly, 2017). For this reason, further research on the spectrum of microplastics in freshwater (i.e. size range, type, and effects of microplastics) is required.

4.1 Sampling and separation methods

The sampling methods used for capturing microplastics have consisted of selective sampling (such as sieving, filtration, floatation, density separation and charge separation) and bulk or volume-reduced sampling (Hidalgo-Ruz et al., 2012). Selective sampling (consisting of visual sorting) has been mainly utilised for surface sediments, whereas bulk or volume-reduced sampling, has been used to analyse microplastics from sediments or water samples (Eerkes-Medrano et al., 2015).

Separating microplastics from other particles such as sand can be achieved through different flotation methods because plastics are relatively less dense compared to other particulate matter. Fine filters (generally with a cut-off of 150 μm) and salts (such as NaCl and NaI) are added to

the water samples to increase water density (Hidalgo-Ruz et al., 2012) and facilitate the separation of microplastics. However, separating low-density microplastics, with diameters < 500 μm, is still challenging (Imhof et al, 2012). Some methods may be able to overcome this difficulty however. For example, through the use of a dense fluid, the Munich Plastic Sediment Separator can isolate various sizes (1μm - 1mm), types and density of microplastic particles in water (Imhof et al., 2012), and has been used in the analysis of microplastics in freshwater from Lake Calda (Italy) and made possible the identification of microplastics as small as 9 μm (Imhof et al., 2013). An effective way for separating microplastics from sediment involves washing samples with nitric acid, which led to an extraction efficiency of 93-98% (Claessens et al., 2013). A low-cost approach proposed used castor oil to separate microplastics from sea and river water. This method was found applicable for the extraction of microplastics larger than 300 μm. Methods for improving the separation of microplastics of all sizes and types are emerging and improving our ability to effectively sample and separate microplastics. As new methodology is still emerging, it is too early to reach a unified approach.

4.2 Microplastic morphological characteristics

Morphological characteristics of microplastics are important parameters for the classification of microplastics and determination of their source. Particle size is closely related to the migration behaviour of microplastics in the environment. It also directly determines the ease of entry of microplastics into organisms. On a practical note, it also determines the required mesh size (0.038–5.000 mm) of sampling sieves (Hidalgo-Ruz et al., 2012). Particle size grading is mainly achieved through sieving and filtering during the sample pretreatment stage.

281 According to Hidalgo-Ruz et al. (2012), sediment samples usually pass through 2-4 sieve nets,
282 while water samples pass through 4-9 sieve nets.
283
284 Microplastic morphological features are a good indicator of microplastic degradation and can
285 be important in identifying their source. Microplastic degradation is largely driven by external
286 forces such as biodegradation, photodegradation and chemical weathering. Chemical
287 weathering causes cracks on the surface of the plastic and can break particles into smaller
288 pieces. Different morphologies of microplastics can be found in Fig. 2. The characterization of
289 surface morphology needs to be conducted at a high magnification (50-10,000 times) (Wang et
290 al., 2017a). For this reason, current methods employ scanning electron microscopy techniques
291 (Aytan et al., 2016) such as scanning electron microscopy-energy dispersive X-ray analysis
292 (SEM-EDS), and environmental scanning electron microscopy-energy dispersive X-ray
293 analysis (ESEM-EDS). However, characteristics such as shape and colour still rely heavily on
294 visual inspection, with tools such as fluorescence labelling that can be used to enhance the
295 distinction between microplastics and environmental substrates in cases where they are difficult
296 to distinguish.
297

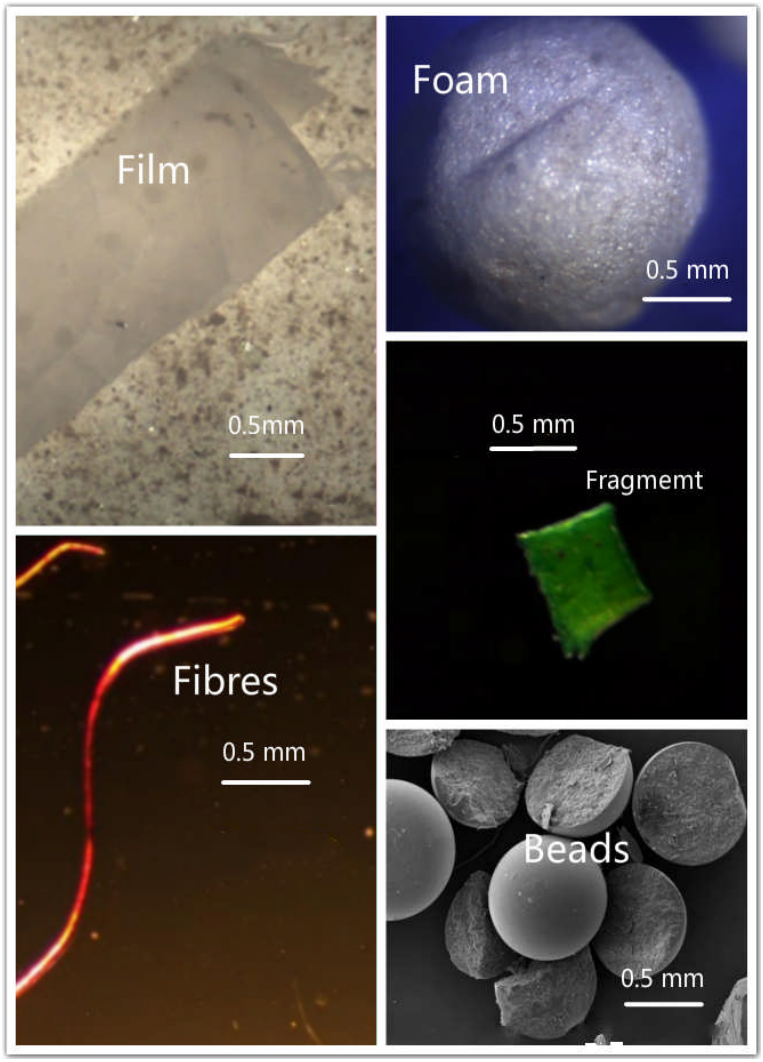


Fig. 2 Examples of types of morphologies in microplastics (Katsnelson, 2015; Wuhan, 2017; Wageningen, 2014)

4.3 Characterization methods of microplastics

The most common approaches used for the characterization of microplastics often utilise complementary techniques. For example, Fourier Transform Infrared Spectroscopy (FT-IR) or Raman, which are primarily stand-alone techniques, are often employed coupled with optical microscopy (micro-spectrometer) (Song et al. 2015). Microplastics of >20µm from drinking water were characterized with µFTIR imaging (Mintenig et al. 2019). Despite their high

selectivity, differentiating microplastics with smaller particle size (i.e. in the low micrometre-range) from natural matter becomes difficult with µFTIR and µRaman imaging and can cause overestimation of the number of identified microplastics (Mendoza and Balcer, 2019). In addition, as a result of the reduction of light transmittance through microplastics, the use of an attenuated total reflectance crystal attached to the microscope (ATR-µFTIR) is preferred. This modality is affected by limited sensitivity however (Pico and Barcelo, 2019), and although it does not require sample treatment, the characterization of microplastics with this technique is still time consuming.

SEM-EDS (or ESEM-EDS) (Zhao et al., 2017) provide greater spatial resolution than µFTIR and µRaman imaging. Compared to the visualization of specimens (from ~10 µm in the case of optical microscopy), SEM modalities makes possible resolutions > 1 nm (Busquets, 2017) at the same time as their inorganic compositional analysis is carried out by EDS. The qualitative analysis that they offer is very localised; hence the lack of homogeneity of the microplastic sample can become an issue if the goal is quantitative analysis. This is also problematic in the analysis of nanoparticles, and it can be overcome by characterizing a very high number of sites within every sample (Dudkiewicz et al., 2015).

In addition to FTIR and Raman based techniques, Pyr-GC-MS has been used to identify the composition of microplastics (Dierkes et al., 2019). Unlike the spectroscopic approach, this technique is destructive; the characterization is based on the pyrolysis of the polymer (0.1-0.5 mg polymer i.e. at 700 °C for 60 s (Nuelle et al. 2014) which leads to cleavage of chemical

bonds and generation of low molecular weight volatile moieties from the non-volatile polymer. These thermal degradation products can be cryo-trapped, separated and identified by their mass spectrum. The identification is carried out by matching the retention time and mass spectrum with that of standards of polymers or the use of spectral libraries. The advantage of this approach is greater sensitivity and selectivity in the identification than when using spectroscopic techniques, but it has drawbacks: Pyr-GC-MS requires high maintenance of the equipment because the relatively heavy moieties arising from the degradation of the polymer can condensate in the capillary between the pyrolysis chamber and the GC and cause blockages and cross contamination. Nuelle et al. (2014) used these techniques to identify the polymer in microplastics from sediments collected from Norderney Island beach after a two-step (fluidization-flotation) sample treatment method that separates microplastics based on their density in saturated solutions of NaCl and NaI. The microplastics in the samples were probably made of polypropylene (PP), polyethylene terephthalate (PET), and polyvinyl chloride (PVC).

Pre-treating the sample before the chromatographic analysis can allow increasing the sample size (up to 100 mg) and overcoming the obturation problems when using Pyr-GC-MS for the analysis of microplastics. This is achieved with TED-GC-MS (Dumichen et al., 2014), which consists of a combination of thermogravimetric analysis (at temperatures about 600 °C) where the volatile products generated are pre-concentrated onto fibres by adsorption. These volatile degradation products will be subsequently desorbed and introduced into the GC-MS (Dumichen et al., 2017).

4.4 Quantitative analysis of microplastics

Traditional quantitative analysis of microplastics is carried out by visual inspection, which implies manual counting of the debris and the counts are then converted into the concentration in the sample (Shan et al., 2018). For mass concentration, all microplastic particles are usually selected by tweezers and weighed. The visual inspection method is not only time-consuming and laborious but also prone to error (Shan et al., 2018).

During recent years, quantitative analysis methods have been complemented by the qualitative characterization of the microplastic with microscopy-Fourier transform infrared spectroscopy (μFTIR); Raman spectroscopy combined with microscopy (μRaman); and pyrolysis–gas chromatography–mass spectrometry (Pyr-GC-MS) (Lares et al., 2018) which greatly improves the analysis accuracy (Shan et al., 2018). Pyr-GC-MS can be used to quantify microplastics. This method can effectively distinguish different components of plastics and is particularly suitable for quantitative analysis of a single type of microplastics (Dumichen et al., 2017). Dumichen et al. (2017) A pre-treatment step based on solid phase extraction (SPE) which consisted of trapping and pre-concentrating the polymer degradation products previous to the GC-MS analysis, allowed increasing the sample size by ~40 times. This has a potential positive impact on increasing the representativity of the analysed sample and sensitivity of the method. This method made possible identifying unique thermal degradation products related to the precursor polymer of the microplastics, which also enhanced the capacity to characterise microplastics, even in a complex substrate environment.

5 Characteristics of microplastic pollution

Microplastic pollution in freshwater environments is global and generalised. This can be observed from a sample of published data (Fig. 3). Data in Fig. 3 were collected from the Web of Science database and included information from every research article that was retrieved with keywords microplastics and freshwater from 2016 to 2019. From the results, microplastic pollution has been mainly reported in North America and Western Europe (Horton, et al., 2017) and parts of China (Peng et al., 2017; K. Zhang et al., 2018) (Fig. 1 and Fig. 3). In addition, microplastics have been reported in Brazil (Castro et al., 2016), Mongolia (Wu et al., 2018), and India (Sruthy and Ramasamy, 2017).

Figs. 4 and 5 illustrate the percentage of composition and type of microplastics found in freshwater. These figures were constructed based on the papers listed in Table 1 that included percentage value of composition (Ballent et al., 2016; Bordós et al., 2019; Burns and Boxall, 2018; Horton et al., 2017; Imhof and Laforsch, 2016; Martin et al., 2017; Naji et al., 2017; Peng et al., 2018; Sruthy and Ramasamy, 2017; K. Zhang et al., 2016; W. Zhang et al., 2017) and type (P. J. Anderson et al., 2017; Aytan et al., 2016; Baldwin, et al., 2016; Ballent et al., 2016; Burns and Boxall, 2018; Cincinelli et al., 2017; Gewert et al., 2017; Leslie et al., 2017; Peng et al., 2018; Lei Su et al., 2018; L. Su et al., 2016; Sutton et al., 2016; Wang, et al., 2018; Wang, et al., 2017b; K. Zhang et al., 2018; W. Zhang et al., 2017) of microplastic. The percentages here were then calculated as the average of the percentages given by those papers. It can be seen that, polypropylene (PP), polyethylene (PE), polystyrene (PS), and polyethylene terephthalate (PET), account for nearly ¾ of the pollution in fresh water systems (Fig. 4). PP

and PE have the highest detection rate, possibly because of the high production and utilization of these two types of plastic products, so it is urgent to improve the current sewage treatment methods and reduce the pollution of PP and PE microplastics (Lechner and Ramler, 2015).

According to the morphological characteristics of microplastics, fibres and fragments account for the overwhelming majority (Fig. 5). Fibres account for 59%, probably because of a large amount of laundry wastewater discharge (Kole et al., 2017), and it is a concern because it is not removed by the current wastewater treatment process (Browne, 2015). Fragments account for 20%, and this can be because of the impact of runoff on the crushing of large pieces of plastic (Auta et al., 2017). In addition, beads, films, and foams have also been found in freshwater in proportions <10%, of the total pollutants.

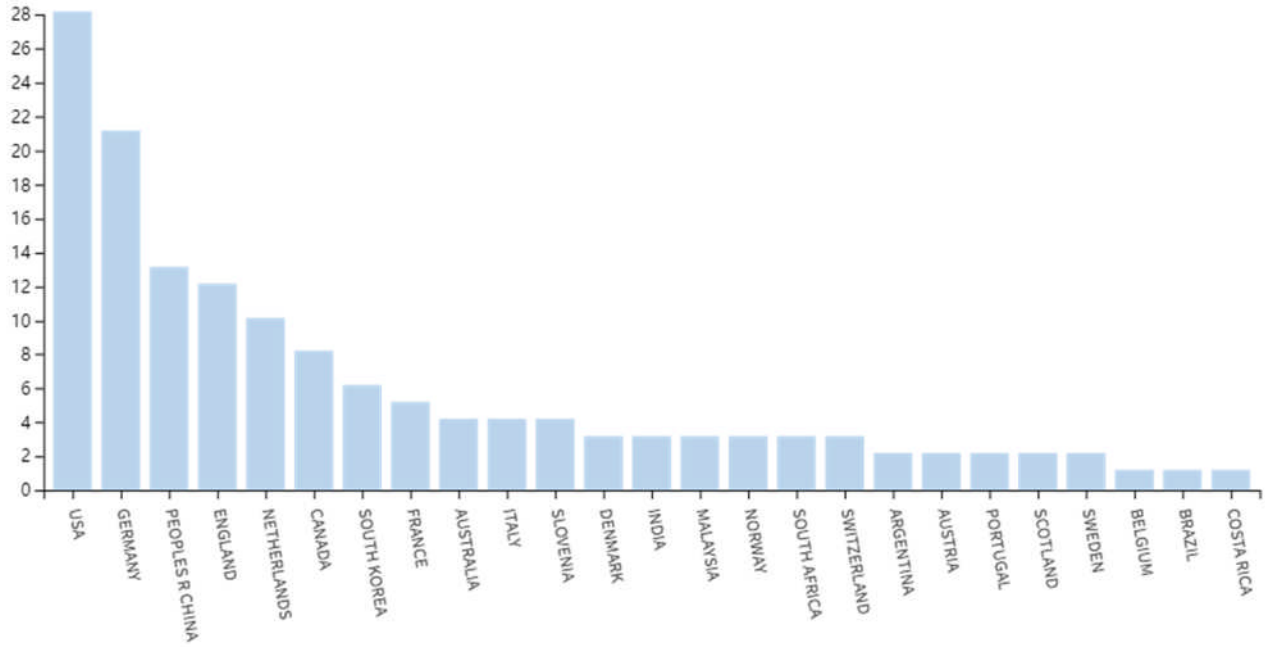
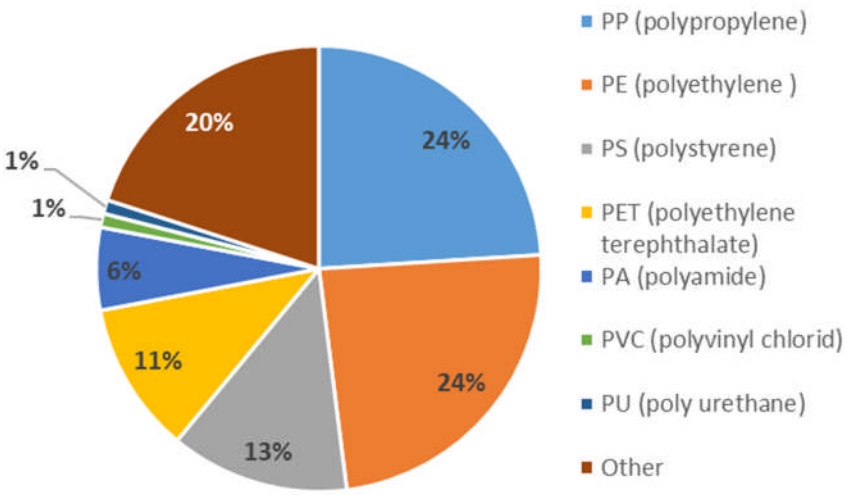


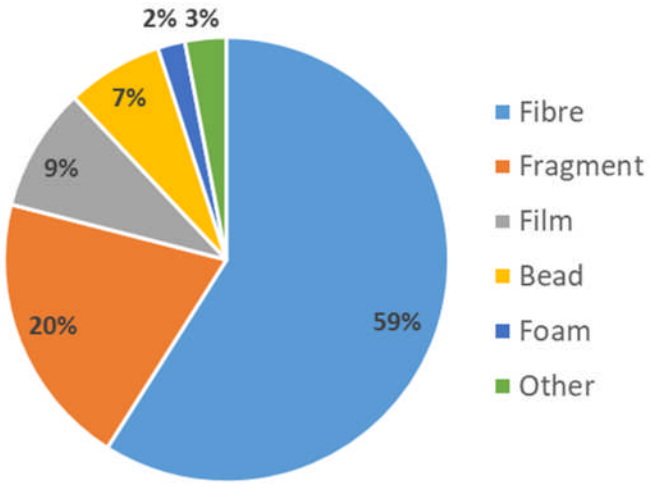
Fig. 3. Reports on microplastics in freshwater worldwide (Y axis indicates the number of published relevant papers)



411

412 Fig. 4. Composition of microplastics found in freshwater samples

413



414

415 Fig. 5. Proportion of microplastics in freshwater samples according to their type

416

417 **6 Fate and effects of microplastics on organisms**

418 To date, studies of the ecotoxicological effects of microplastics have mainly focused on marine
419 organisms. The potential threat of pollution in the freshwater environment can be higher than
420 those in the marine environment because of the nearer proximity of human activities. The
421 effects of microplastics have been reported to take place at various levels: genes, cells, tissues,
422 plants and animals (Zhou et al., 2015). The effects of microplastics on humans and the toxic

mechanism remain scarce, and humans have been exposed to microplastics given that they have been found in edible salts in supermarkets (Iñiguez et al., 2017; A. Karami et al., 2017). The Food and Agriculture Organization of the United Nations (FAO) survey in 2017 concluded that for rodents and dogs, microplastics over 150 µm in diameter would not be absorbed and would be discharged (Wright and Kelly, 2017). Therefore, it is estimated that >90% of the microplastics ingested will not be absorbed by the human (Wright and Kelly, 2017). However, there is a research gap about the effects of the microplastics over 150 µm, when they stay in the body. Moreover, microplastics can enter the circulatory system and harm the human body when they are < 20 µm diameter (Rothen-Rutishauser et al., 2006).

The presence of microplastic in different species indicates their fate within the trophic chain (Besseling et al., 2017). Wild freshwater mussels and benthic invertebrates accumulate microplastics mainly from sediments, while microplastics in non-benthic fish stomach are mainly from microplastics suspended in water. Laboratory studies have further confirmed that microplastics can accumulate in large amounts in the zooplankton *Daphnia magna* (Besseling et al., 2017; Nasser and Lynch, 2016; Rehse et al., 2016; Rosenkranz et al., 2009). Fibres were found to affect the assimilation efficiency of *Gammarus fossarum* (Blarer and Burkhardt-Holm, 2016), an amphipod, but microbeads did not affect *Gammarus duebeni* (Mateos Cardenas, et al. 2019). Microplastics accumulate in digestive and reproductive systems of different trophic freshwater organisms such as *Alella azteca* (Au et al., 2015), *Lumbricus variegates* (Imhof et al., 2013) and *Oryzias latipes* (Rochman et al., 2013). However, recent reports have also revealed that goldfish (*Carassius auratu*s) rapidly excrete microplastics such that they do not

445 accumulate in their gut (Grigorakis et al., 2017), suggesting that microplastics may accumulate
446 in freshwater organisms of different species, and that microfibers may potentially have more
447 impact than microbeads, because microplastics can be enriched via food chain and humans
448 may inadvertently consume aquatic organisms which have accumulated microplastics and may
449 accumulate them in the human body depending on their size.
450
451 Microplastics were found to block the digestive tracts of zooplankton (Au et al., 2015;
452 Besseling et al., 2017; Nasser and Lynch, 2016; Rehse et al., 2016; Rosenkranz et al., 2009),
453 reduce their feeding rate (Nasser and Lynch, 2016), or directly interfere with their feeding
454 process (Au et al., 2015; Blarer and Burkhardt-Holm, 2016) resulting in an energy deficiency
455 and decreased growth, activity, and reproductive capacity and even death (Besseling et al.,
456 2017). In fish, microplastic accumulation can cause liver glycogen depletion and fat
457 vacuolation (Rochman et al., 2013).
458
459 Plastics could cause alterations to aquatic plants and animals: and the nanoplastics were found
460 to adsorb onto the surface of *Pseudokirchneriella subcapitata* (Nolte et al., 2017), *Chlorella*
461 *spp.*, and *Scenedesmus spp.* by electrostatic interaction, and hinder the absorption and
462 utilization of photons and CO_2 by algal cells, thereby reducing algal growth (Bhattacharya,
463 2016), but microbeads (10-45µm PE) where not found to affect plant growth (*Lemna minor*)
464 (Mateos Cardenas, A., et al. 2019)
465
466 In addition to physical damage, microplastics may leach plasticizers, resulting in toxic effects

on freshwater organisms, but due to the limited concentration of the chemicals leaching, effects are assumed to be low. Lithner et al. (2009) studied the effects of various plastic extracts on *Daphnia magna*. It was found that microplastics made from polymers like PVC and PU could produce acute toxicity to *Daphnia magna* when studying concentrations of microplastics in the samples. Overall, the current research on the toxicological effects of microplastics on freshwater organisms is mainly limited to the individual and tissue level. Toxic mechanisms of microplastics at the cellular and genetic levels should be the object of future investigations. Microplastics can also act as carriers of micropollutants given that there are many types of pollutants in surface water (such as pharmaceutical products) and microplastics have small particle size, large specific surface area and are hydrophobic (Rochman et al., 2013; Teuten et al., 2009). Recent studies have shown that microplastics can adsorb pollutants such as perfluorochemicals (PFCs) (Wang et al., 2015), drugs and personal care products (PPCPs) (Wu et al., 2016), and polybrominated diphenyl ethers (PBDEs) (Wardrop et al., 2016). The release of environmental pollutants adsorbed by microplastics can produce a series of toxicological effects on organisms. The toxicity of the release of individual pollutants would be insufficient to reflect the real risk that they entail once in the environment and in contact with water environmental pollution; toxicological studies need to define the combined effects of microplastics with a range of other common environmental pollutants. At present, research on microplastic composite pollution has just started, mainly focusing on the combined effects of heavy metals, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) on marine organisms. Recent investigations indicate that microplastics can alter the bioavailability of heavy metals in aquatic environments (Brennecke et al., 2016), PAHs

(Karami et al., 2016; Oliveira et al., 2013), and PCBs (Sleight et al., 2017), thereby causing complex changes in physiological processes such as protein synthesis, energy storage, and biotransformation (Karami et al., 2016; Oliveira et al., 2013). At the same time, the inhibition of microplastics on metabolic enzymes can weaken the metabolic transformation of PAHs and increase their accumulation in organisms (Paul-Pont et al., 2016).

The potential threat of traditional and new pollutants on the freshwater environment are higher than those in the marine environment because of the nearer proximity of human activities. However, reports on the ecotoxicological effects of microplastics on freshwater organisms, such as Mahon et al.'s (2017) research on those of microplastic compound pollution in the Irish freshwater system indicate that the thread also exists away from densely populated areas (Horton et al., 2017).

7 Policy development

Current international standards are not unified and regional test methods are too expensive and time-consuming in their ability to monitor and test the effects of microplastic pollution (even biodegradable microplastic fragments) within wastewater, freshwater (rivers, streams, and lakes), and marine environments. This is because this area of research is relatively recent, the non-availability of relevant reference materials, and a paucity of broader research into the biodegradation of plastic materials within these environments (Harrison, et al., 2018). There is also lack of knowledge on how the emission of microplastics could be reduced at potentially contaminating sites such as wastewater treatment.

Current legislation has serious flaws. The Austrian Ordinance on Waste-Water Emission classifies plastic as a filterable substance (Lechner and Ramler, 2015). Correspondingly, the upper limit of plastic discharge into running waters is specified as 30 mg L^{-1}. Assuming a hypothetical discharge of 100 L s^{-1} at the Borealis drain, one could legally release 3.0 g of industrial microplastics (Sutherland et al., 2010) per second and 259.2 kg within a 24h period, which is in the range of emission during heavy rainfalls. This yields a mass of 94.5 tonnes per year, which approximately equals 2.7 million PET bottles. According to their official statement, Borealis emitted approximately 200 g of industrial microplastic (IMP) per day under normal operating conditions over the monitoring period during 2010, while an estimated 50–200 kg of IMP was lost during a heavy rainfall event (Lechner and Ramler, 2015).

On a positive note, the European Commission launched a series of research projects on microplastics during January 2016 to standardize analytical methods for microplastics in the water environment and conduct baseline surveys of microplastics in European waters (Xanthos and Walker, 2017). The Marine Waste Project of the National Oceanic and Atmospheric Administration (NOAA) was approved under the Marine Waste Action Act (Xanthos and Walker, 2017). It covers research, on the distribution, abundance, and impact of microplastics and promotes attentiveness towards microplastics through public education programs. Some countries have issued pertinent research strategies and projects to inform regulations and policies focusing on gathering information on the pressures, fate and effects of microplastics in freshwater systems and pathways to the ocean (i.e. Environmental Protection Agency in

Ireland and Sweden), and measures in the field of cosmetics. In 2015, the United States promulgated the *Microbead-Free Waters Act* (McDevitt et al., 2017), which stipulated that no cosmetics containing plastic beads shall be produced starting July 1, 2017. Great Britain forced the elimination of cosmetics containing plastic beads by the end of 2017 (Xanthos and Walker, 2017). South Korea banned the sale of cosmetics containing plastic beads in July 2018 (Burton, 2015). Canada's *Regulations on Plastic Beads in Cosmetics* came into effect on January 1, 2018 (Xanthos and Walker, 2017). With the development and validation of monitoring technologies, establishment of standards for the analysis of microplastics in environmental samples, promulgation of relevant regulations with an impact on their release, and promotion of public education projects, the problem of microplastic pollution can be effectively controlled during the next few years.

8 Conclusions, next steps, and opportunities

Studies on the occurrence and distribution of microplastics in freshwater environments remain very scarce, especially in Africa, South America and North Asia. Additionally, there is currently no standardized reporting of microplastic concentrations, and as a result, information gained concerning microplastic pollution in freshwater environments cannot easily be compared – this may be limiting further understanding of microplastic pollution and development of measures to control it.

At present, research on the origin of microplastics is relatively mature. However, methods to extract microplastics, particularly fibres, from environmental samples such as freshwater and

555 sediments, need further study. Moreover, the processes that transform primary microplastics
556 into secondary particles as well as methods that prevent their decomposition and diffusion also
557 need to be further understood.
558
559 Research on pollution of different types of microplastics, and microplastics with other
560 substances in the freshwater environment is required given that environmental samples present
561 a mixture of pollutants. Because of their special physical and chemical characteristics,
562 microplastics are likely to adsorb micropollutants. Whether this will produce joint toxic effects
563 on freshwater organisms or change the bioaccumulation and food chain transmission of other
564 pollutants are among the key research questions to be studied.
565
566 In addition, there is no qualitative and quantitative method for detection of microplastics
567 suitable for real time monitoring in wastewater treatment plants. For example, techniques such
568 as μFTIR are expensive, while lower cost methods such as visual inspection are time
569 consuming. Therefore, there is a large need for research that develops novel cost-effective
570 qualitative and quantitative methods for accurate microplastic determination.
571
572 Regarding the effects of microplastics on organisms and humans, the process of ingestion from
573 freshwater, and the harm caused by the various types and sizes of microplastics remains unclear;
574 although it is accepted that the <100μm fraction of microplastics are the most hazardous.
575
576 Finally, it is of great importance to establish criteria for the assessment of ecological risk posed

by microplastics. As it is concluded by Pico et al., (2018), only through the joint efforts of legislation, public enrolment, engineering tools and biotechnological tools (such as production of biodegradable plastics), the issue of microplastic pollution can be properly solved.

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