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**Title: Beyond the ocean: Contamination of freshwater ecosystems with (micro-) plastic particles**

Rachid Dris<sup>1δ</sup>, Hannes Imhof<sup>2δ</sup>, Wilfried Sanchez<sup>3</sup>, Johnny Gasperi<sup>1</sup>, François Galgani<sup>4</sup>, Bruno Tassin<sup>1\*</sup>, Christian Laforsch<sup>2\*</sup>.

1. Université Paris Est, Laboratoire Eau Environnement Systèmes Urbains (LEESU), 94010 Créteil, France.
2. Department of Animal Ecology I and BayCEER, University of Bayreuth, Universitätsstr. 30, 95440 Bayreuth, Germany.
3. Rovaltain Scientific Foundation for environmental toxicology and ecotoxicology, 1 rue Marc Seguin, Batiment INEED - Alixan, BP16110, 26958 Valence cedex 9, France.
4. Institut Français pour la Recherche et l'Exploration de la Mer (Ifremer), Immeuble Agostini, ZI Furiani, 20600, Bastia, Corsica, France.

δ shared first authorship

\* Corresponding authors:

Bruno Tassin: [bruno.tassin@enpc.fr](mailto:bruno.tassin@enpc.fr), Tel : +33 1 45 17 13 28

Christian Laforsch: [christian.laforsch@uni-bayreuth.de](mailto:christian.laforsch@uni-bayreuth.de), Tel.: +49 921 55 2651

## 1 **Environmental context**

2 Microplastics in freshwater ecosystems are an increasing issue, with the few available studies  
3 suggesting high contamination worldwide. Reliable data on concentrations, fluxes and polymer types  
4 in continental aquatic environments, including urban water systems, are needed. High-risk polymers  
5 and associated or adsorbed chemicals have to be identified, as well as their effects on both organisms  
6 and ecosystems. Therefore, numerous challenges arise to assess possible adverse effects.

## 7 **Abstract**

8 Massive accumulation of plastic particles has been reported for marine ecosystems around the world,  
9 posing a risk to the biota. Freshwater ecosystems have received less attention despite the majority of  
10 plastic litter being produced onshore and introduced into marine environments by rivers. Some  
11 studies report not only the presence of microplastics in freshwater ecosystems, but show that  
12 contamination is as severe as in the oceans. In continental waters microplastics have been observed  
13 in both sediments (predominantly lakeshores but also riverbanks) and water samples (predominantly  
14 surface water of lakes and rivers). This review highlights recent findings and discusses open  
15 questions, focusing on the methodology of assessing this contaminant in freshwater ecosystems. In  
16 this context, method harmonization is needed in order to obtain comparable data from different  
17 environmental compartments and sites. This includes sampling strategies (at spatial and temporal  
18 scales), sample treatment (taking into consideration high levels of organic matter and suspended  
19 solids) and reliable analytical methods to identify microplastics.

## 20 **Keywords:**

21 plastic debris, microplastics, freshwater ecosystems, emerging contaminants, plastic separation,

## 23 **1. Introduction**

24 Artificial polymers are lightweight, durable, display excellent thermal and electrical insulation  
25 properties and can be formed into almost any shape. These characteristics render them suitable for a  
26 huge variety of applications in almost every sector of our everyday life. Consequently, the worldwide  
27 production of plastic has increased from 1.5 million tons in 1950 to 288 million tons in 2012 <sup>[1]</sup>. A  
28 large proportion of plastic is used by the packaging industry for solely disposable use <sup>[1]</sup>. Given the  
29 extensive use of these materials, post-consumer plastic waste has dramatically increased while the  
30 percent recycled remains low. For instance, only 26.3% of all plastic waste in Europe was recycled  
31 in 2012 <sup>[1]</sup>. Plastic waste can enter the environment, for example, from poorly managed landfills or  
32 by carelessly discarded post-consumer products. Since plastic debris can often be transported by  
33 wind or direct runoff after rain events, a large proportion of this waste inevitably reaches aquatic  
34 ecosystems where it then accumulates. This contamination not only includes plastic debris  
35 characterized by a large size but also so called microplastics. This term was first used in 2004 to  
36 describe very small fragments of plastic observed in sea samples (20  $\mu\text{m}$  in diameter) <sup>[2]</sup>. The  
37 definition has since been broadened to include all particles  $< 5 \text{ mm}$  <sup>[3, 4]</sup>, although a subdivision into  
38 large (L-MPP: 1-5 mm) and small microplastic particles (S-MPP: 1  $\mu\text{m}$ -1 mm) has been introduced  
39 by several authors (e.g. <sup>[5-7]</sup>). Microplastics can be further classified into two kinds based on their  
40 origin. Primary microplastics are specifically engineered for various applications such as personal  
41 care products or can be in the form of pre-production pellets. So-called secondary microplastics  
42 result from degradation of macroplastics caused by UV radiation, mechanical abrasion, biological  
43 degradation and disintegration <sup>[8]</sup>. This seems to be a continuous process, most likely leading to very  
44 small particles on even the nanoscale <sup>[9, 10]</sup>. Synthetic clothing can be regarded either as a source of

45 primary or secondary microplastic fibers.

46 Detectable amounts of small plastic debris were documented in the open ocean as early as 1972.

47 Neuston net samples of surface plastic concentrations in the Sargasso Sea contained an average of

48 3,500 pieces/km<sup>2</sup> <sup>[11]</sup>. Concurrent studies reported plastic debris in Western Atlantic and North

49 Pacific surface waters <sup>[12-14]</sup>, although distribution was extremely variable. From the 1960's to the

50 2000's, many studies using direct surface debris measurements or seabird ingestion as a proxy

51 reported the massive occurrence of microplastic in marine environments around the world <sup>[2, 15, 16]</sup>.

52 Both primary and secondary microplastics can enter the continental aquatic environment through

53 several pathways (Figure 1). One of the main sources is inadequate end-of-life treatment of plastic

54 debris. This debris enters aquatic systems directly by water run-off or via stormwater and wastewater

55 treatment plant (WWTP) outlets.

56 Additionally, granulated polyethylene (PE), polypropylene (PP) or polystyrene (PS) particles, used

57 for example in skin cleaners, can be introduced into wastewater <sup>[17]</sup>. Furthermore, it has been shown

58 that laundry washing machines discharge a large amount of plastic fibers into wastewater, with one

59 study estimating that a single wash can produce 1,900 fibers <sup>[18]</sup>. Industrial activities also contribute

60 to the amount of microplastics in freshwater/aquatic ecosystems. High amounts of microplastic

61 particles and fibers have been detected in the vicinity of industrial plants involved in paper

62 production <sup>[19]</sup>. Synthetic fibers are also known to contaminate sewage sludge <sup>[20]</sup>. This observation

63 suggests that WWTP at least reduce the amounts of synthetic fibers in sewage effluents. However,

64 the use of sewage sludge for agricultural fertilization can still contribute to environmental

65 microplastic contamination. Moreover, plastic mulching could be another terrestrial source of

66 microplastics but to our knowledge, these pathways have not yet been sufficiently documented <sup>[21]</sup>.

67

68 Finally, atmospheric inputs cannot be ignored. Since plastic fragments are transported by the wind,

69 this must be also the case for microplastics. As for some organic micropollutants, atmospheric inputs

70 should be investigated since it could represent an important transfer vector <sup>[22]</sup>.  
71 Elucidating sources and pathways of microplastics in freshwater ecosystems will be a major  
72 challenge for future research. This information will be the basis for management strategies to tackle  
73 problems arising from this emerging environmental contaminant. In the light of available data, a  
74 specific regulation was adopted by the European Union in 2008. The Marine Strategy Framework  
75 Directive (2008/56/EC, MSFD) <sup>[23]</sup> aims to more effectively protect marine environments across  
76 Europe, aiming to achieve good environmental status for European marine waters by 2020. Among  
77 the MSFD qualitative descriptors for determining good environmental status, indicator 10 is related  
78 to marine litter properties and quantities, including criteria to assess trends in amount, distribution  
79 and, where possible, composition of microparticles with a focus on microplastics. Similarly to the  
80 Marine Strategy Framework Directive, the Water Framework Directive (2000/60/EC, WFD) <sup>[24]</sup> aims  
81 to achieve good chemical and ecological status of all water bodies, including rivers and lakes. Until  
82 now, contamination with plastic debris (from micro- to macroplastics) has not been considered. This  
83 gap could be explained by the lack of i) data related to the occurrence and associated effects of  
84 microplastic contamination in freshwater ecosystems, and ii) robust and accurate methodologies to  
85 assess concentrations of microplastics in freshwater (environment and biota). The aim of the present  
86 critical review is to summarize available data on microplastics in freshwater ecosystems, and to  
87 identify and discuss scientific challenges surrounding this issue.

## 88 **2. Microplastics in freshwater environments**

89 In contrast to the large amount of literature describing marine environment contamination with  
90 plastic waste, only a few studies have addressed the issue of microplastic contamination in lakes and  
91 rivers. Estuary microplastic abundance has also received little attention <sup>[25-27]</sup>, but given the strong  
92 influence of salinity gradients and tidal movements in these systems, only freshwater ecosystems

93 were considered in this review.

## 94 **2.1. Lakeshore and riverbank sediment samples**

### 95 *Microplastic occurrence in lakeshore and riverbank sediments*

96 Several studies reported microplastic contamination of lakeshore and riverbank sediment samples  
97 from continental aquatic systems in Europe, both North and South America, and Asia. To our  
98 knowledge, no studies have focused on lake bottom sediments.

99 Reported levels of microplastics in lakeshore sediments vary by a factor of 1,000 across the reviewed  
100 studies (Figure 2). In lakeshore sediments of Lake Garda (Italy), the authors reported that the north  
101 shore contained  $1,108 \pm 983$  microplastic particles/m<sup>2</sup> whereas only  $108 \pm 55$  microplastic  
102 particles/m<sup>2</sup> were observed on the south shore <sup>[9]</sup>. Spatial distribution of microplastics suggested that  
103 wind, lake morphology and the consequent currents are responsible for the observed pattern. The  
104 study showed that the most abundant polymer (45.6%) was PS, while PE was still highly abundant  
105 (43.1%). Polyamide (PA) and polyvinylchloride (PVC) were also identified down to a size of 9  $\mu$ m.  
106 This study highlighted that the particles observed were fragments originating from the breakdown of  
107 larger particles (most likely post-consumer products), given that the scanning electron microscopy  
108 analysis revealed distinct signs of degradation.

109 Another study assessed microplastic contamination of lakeshore sediments along Lake Geneva  
110 (Switzerland) <sup>[28]</sup>. The results of the study are indicated in particles per liter of sediments.  
111 Concentrations varied from 1 to 7 particles/L <sup>[9]</sup>, the predominant polymer was PS. Microplastic  
112 concentrations in a second study of Lake Geneva lakeshore sediments <sup>[29]</sup> varied from 2,656.25 to  
113 5,018.75 particles/m<sup>2</sup>, far greater than the highest concentration reported in lakeshore sediments of  
114 Lake Garda. A predominance of textile fibers, representing more than 90% of identified  
115 microplastics, was observed in the latter study.

116 In North America the distribution of particles along the lakeshores of one of the Laurentian Great

117 Lakes (Lake Huron Canada, USA) has been studied<sup>[10]</sup>. In this work, particles were directly  
118 collected at the shoreline, then separated into three groups: [ $< 5$  mm plastic pellets], [ $> 5$  mm broken  
119 plastic fragments], [all size PS particles]. The predominant microplastic form on Lake Huron  
120 lakeshores was dependent upon sample location. In one of the sampled sites, over 94% of observed  
121 plastic particles were industrial pellets, while at a different site pellets made up only 15% with the  
122 largest fraction consisting of PS foam. The majority of pellets, predominantly PE, were observed  
123 proximal to an industrial sector.

124 In a second publication<sup>[30]</sup>, the abundance of plastics on the lakeshores of Lake Huron, Lake Erie  
125 and Lake St. Clair (Canada, USA) was assessed and compared with those previously determined for  
126 Lake Huron. As in the first study, high numbers of pellets were reported in comparison to fragments  
127 and PS foam. Especially at Lake Huron 92% out of 3,209 particles were industrial pellets. The same  
128 was true for Lake Erie with 39% out of 1,576 particles. The abundance of industrial pellets was  
129 highest next to industrial areas and decreased along the shoreline. Along Lake Erie, PA was one of  
130 the main polymers observed, making up 33% of the total plastic and coming mainly in the form of  
131 pellets (47% of all pellets). Compared to other studies, the observed abundance of plastic debris is  
132 rather low (Lake Huron:  $4.75 \pm 11.83$  particles/m<sup>2</sup>, Lake Erie  $1.54 \pm 1.01$  particles/m<sup>2</sup>, Lake St. Clair  
133  $1.72 \pm 2.64$  particles/m<sup>2</sup>). This might be mainly due to restricted sampling of visible fragments and  
134 pellets, allowing microplastics invisible to the naked eye to be overlooked. Nevertheless, the Great  
135 Lakes display a high degree of contamination with plastic debris which is mainly due to industrial  
136 pre-production pellets making up 66% of the plastic load.

137 In general, the lakeshores of Lake Huron, Lake Geneva and Lake Garda contain lower concentrations  
138 than marine beaches known to be highly contaminated<sup>[4]</sup>. Lake Huron displays more ocean like  
139 characteristics and contains a similar concentration of plastic pellets as marine systems<sup>[31]</sup>; this is in  
140 contrast to Lake Garda or Lake Geneva where only low concentrations of pellets were reported.  
141 Local conditions/sources might explain these differences.



142 Studies of microplastics in river sediments are rare. A sediment study of St. Lawrence River  
143 (Canada) showed high microbead abundance (similar to those used in consumer products) with sizes  
144 between 0.5 and 2 mm <sup>[32]</sup>. Although the presence of microbeads was ubiquitous in all sediment  
145 samples (mean 13,832 particles/m<sup>2</sup>), some sites presented much higher concentrations (maximum  
146 10<sup>5</sup> particles/m<sup>2</sup>). This spatial distribution is potentially explained by environmental factors affecting  
147 sedimentation.

148 One single study investigated riverbank abundance and composition of macroplastics [ $> 1.5$  cm]  
149 from four rivers flowing into the south east pacific, starting at the headwaters down to the river  
150 mouth <sup>[33]</sup>. Plastics were the prevailing litter items at most sampling sites, their number varying  
151 between 15 to 73% of total collected litter items. Total abundance of plastics (including PS)  
152 remained below 4 items/m<sup>2</sup>. No specific pattern was observed along the river from headwaters to  
153 river mouth. Thus hydrology did not seem to be a key factor determining plastics abundance; their  
154 variability was more closely linked to land use and river shore accessibility, which facilitates such  
155 activities as illegal waste dumping.

### 156 *Sampling and separating microplastics from lakeshore and riverbank sediments*

157 At Lake Garda ,sediment sampling was performed using random grid samples” <sup>[6]</sup>. The separation of  
158 microplastics was performed using density separation with a solution of zinc chloride (ZnCl<sub>2</sub>). To  
159 analyze lakeshore sediment samples from Lake Geneva, two protocols have been applied <sup>[28, 29]</sup>. The  
160 first consisted of direct collection of coarse plastic fragments at the lakeshore, but the authors didn’t  
161 specify the size of fragments collected. In the second method, sand samples were successively  
162 separated using 5 and 2 mm sieves, after which water was added to collect floating particles.

163 At the sites on Lake Huron, Lake Erie and Lake St. Clair <sup>[10, 30]</sup>, plastic fragments [ $< 10$  cm] were  
164 sampled from sandy lakeshores using stainless steel trowels. Sampling was performed at each  
165 location using 1 m wide stripes running from the water to the vegetation line. These stripes were  
166 placed along a 60 m transect parallel to the shoreline in 10 m intervals. Larger items were counted

167 at each site but were not collected. The few studies of river sediment microplastics used either  
168 different types of grab samplers taken at depths varying from 10 to 15 cm<sup>[32]</sup>, or sampling was based  
169 on visual inspection within sampling circles at various locations within the river bed: i) river shore at  
170 the edge of the river, ii) mid bank between the river bank up to the high water mark and iii) upper  
171 bank outside the river bed<sup>[33]</sup>.

## 172 ***Identification***

173 Sampled plastic particles from lakeshore and stream sediments have been identified as polymers  
174 using a variety of methods. In three studies analysis was only performed by visual means<sup>[28, 29, 33]</sup>.  
175 Particle composition from the lakeshores of Lake Huron, Lake Erie and Lake St. Clair was  
176 determined with Fourier transform infrared spectroscopy (FT-IR)<sup>[10, 30]</sup>. Plastic particles observed in  
177 Lake Garda beach sediments have been analyzed using Raman microspectroscopy (RM)<sup>[34]</sup>. A rarely  
178 used method for plastic particle identification was employed in a study assessing the abundance of  
179 microbeads in St. Lawrence River beach sediments, where microbeads were analyzed using  
180 Differential Scanning Calorimetry<sup>[32]</sup>.

## 181 **2.2. Water samples**

### 182 ***Microplastic occurrence in freshwater***

183 Microplastic contamination of surface water has also been investigated, particularly in lakes. As seen  
184 in lakeshore sediment concentrations, those for lake surface water differ by a factor of 1,000 across  
185 different studies (Figure 2). In Lake Geneva (Switzerland)<sup>[28]</sup> authors reported an extrapolated  
186 density of 48,146 particles/km<sup>2</sup>. Similarly, surface water of the Laurentian Great Lakes (i.e. Lake  
187 Huron, Lake Superior, Lake Erie ) was sampled<sup>[7]</sup>. Plastic particles have been categorized in three  
188 groups: [0.355 mm – 0.999 mm], [1.00 mm – 4.75 mm], [> 4.75 mm]. Most plastic particles were  
189 observed in the smallest category, suggesting a similar risk for freshwater biota as reported for  
190 marine environments<sup>[8]</sup>. Several of the microplastic particles were green, blue and purple colored

191 spheres, likely stemming from facial cleaners and other personal care products. These were identified  
192 as PE and PP. The mean concentration on the studied lakes was 43,157 particles/km<sup>2</sup>, ranging from  
193 zero to 28,0947 particles/km<sup>2</sup>. Lake Erie alone accounted for 90% of the total plastics reported in all  
194 three lakes and contained the two most contaminated areas. The fact that two samples were highly  
195 contaminated compared to the rest of the samples is in concordance with other studies <sup>[9, 10, 30]</sup>. The  
196 authors suggest that this high abundance results from converging currents, proximity to several coal  
197 burning power plants and their downstream location from cities such as Detroit and Cleveland.

198 In a remote mountain Lake (Lake Hovsgol, Mongolia), an average density of 20,264 particles/km<sup>2</sup>  
199 (997 – 44,435 particles/km<sup>2</sup>, min-max values) was observed <sup>[35]</sup>. Particles were grouped into the same  
200 three size classes as for Laurentian Great Lakes. Although Lake Hovsgol is a large lake with a  
201 surface area similar to Lake Erie, its catchment is less densely populated. Despite this, contamination  
202 with microplastic particles is significant, which the authors attribute to aerial transfer from distant  
203 urban sources.

204 Concentrations of microplastics reported for rivers, is highly variable (up to a factor of 10<sup>9</sup>; Figure  
205 3), likely due to the different methodologies used. On the Greater London (Great Britain) rivers,  
206 microplastic pollution was examined in an unpublished study of four sampling sites <sup>[36]</sup>. This work  
207 reports a concentration between 3.3 and 9.9 particles/L in two sites. Two rivers in California, San  
208 Gabriel and Los Angeles Rivers (USA), were also investigated <sup>[37]</sup>. Plastic particle numbers ranged  
209 from 0.01 to 12.9 particles/L. After a rain event, smaller microplastics (1-4.75 mm) were 16 times  
210 more abundant in the Los Angeles River than larger plastic particles (> 4.75 mm). Under the same  
211 conditions in the San Gabriel River, small particles were only 3 times more abundant. In both rivers,  
212 71% of the plastic items recovered were comprised of foam. Extrapolation of these results estimated  
213 that 2.3 billion particles were introduced into the marine environment over a period of 3 days <sup>[37]</sup>.

214 A study of the Danube River in Central Europe <sup>[38]</sup> revealed a mean ( $\pm$  standard deviation) plastic  
215 abundance of  $316.8 \pm 4,664.6$  items/1,000 m<sup>3</sup> ( $0.00032 \pm 0.00465$  particles/L) during a two year

216 survey (2010, 2012). The corresponding plastic input via the Danube into the Black Sea was  
217 estimated at 4.2 t/day. Industrial raw material (pellets, flakes and spherules) accounted for substantial  
218 portions (79.4%) of the plastic debris.

219 Concentrations of microplastics in the North Shore Channel in Chicago (USA) were investigated  
220 upstream and downstream of a WWTP outlet (Terrence J. O'Brien Water Reclamation Plan) <sup>[39]</sup>. A  
221 mean concentration of 1.94 particles/m<sup>3</sup> (0.00194 particles/L) was observed upstream of the WWTP  
222 while downstream it was 17.93 particles/m<sup>3</sup> (0.01793 particles/L). These results highlighted that  
223 WWTP discharges might represent an important primary source of microplastics in freshwater.

224 Two different mesh size nets were used to collect surface water on the Seine River (France) <sup>[22]</sup>.  
225 Using an 80 µm size net, concentrations of microplastics were reported to vary between 3 to 106  
226 particles/m<sup>3</sup> (0.003 to 0.106 particles/L). In contrast, sampling using a 330 µm mesh size manta trawl  
227 yielded concentrations of only 0.28 to 0.45 particles/m<sup>3</sup> (0.00028 to 0.00045 particles/L). Most of the  
228 microplastics observed were fibers, and 52% of plastic fibers collected with the first method were  
229 smaller than 1,000 µm. Only 25% of plastic fibers collected with the second method were smaller  
230 than 1,000 µm.

231 In the Chesapeake Bay (USA), concentrations reached a mean of 246 g/km<sup>2</sup>, corresponding to  
232 260,000 particles/km<sup>2</sup>, in one of the four estuarine rivers studied <sup>[40]</sup>. Concentrations demonstrated  
233 statistically significant positive correlations with population density and proportion of  
234 urban/suburban development within watersheds. It should be noted that the greatest microplastic  
235 concentrations also occurred at three of four sites shortly after major rain events.

236 Large drifting plastic debris were documented on the bottom of the Thames River (Great Britain) <sup>[41]</sup>.  
237 For this study, a total of 8,490 submerged plastic items were intercepted during a 3 month sampling  
238 period.

239 The abundance and composition of floating plastic debris along the Seine River (France) was also  
240 investigated<sup>[42]</sup>. This study focused on macroplastic pollution. A significant proportion of buoyant  
241 plastic debris consisted of food wrappers/containers and plastic cutlery. PP, PE and, to a lesser  
242 extent, polyethylene terephthalate (PET) were the most abundant types of polymers observed. A first  
243 extrapolation estimated that 27 tons of plastic debris are intercepted annually by a regional network  
244 of floating debris retention booms.

### 245 *Sampling of surface water*

246 In marine environments, manta trawl sampling is the primary method used to sample lake surface  
247 waters or more recently in rivers. In Lake Geneva, a 333  $\mu\text{m}$  mesh manta trawl was utilized<sup>[28, 29]</sup>.  
248 Samples were then passed through a 5 mm sieve in order to separate macro- and microplastics. Other  
249 studies focusing on lake water contamination with microplastics also used a 333  $\mu\text{m}$  net to collect  
250 samples<sup>[7, 35]</sup>.

251 Manta trawls (333  $\mu\text{m}$ ) have also been used<sup>[22, 37, 39, 40]</sup> to sample river surface waters. Other types of  
252 nets/devices have been utilized in rivers: stream bed samplers and hand nets (sampling surface water  
253 < 1 mm mesh size)<sup>[37]</sup>, eel fyke nets (sampling water next to the riverbed - no details on mesh size)  
254<sup>[41]</sup>, and stationary driftnets (sampling the top 0.5 m of the water column - 500  $\mu\text{m}$  mesh size)<sup>[38]</sup>.  
255 One study combined the use of a manta trawl for particles down to 333  $\mu\text{m}$  and a plankton net for  
256 particles down to 80  $\mu\text{m}$ <sup>[22]</sup>.

### 257 *Organic matter removal*

258 Although organic debris hampers the identification of plastic particles and especially microplastic  
259 particles, only two studies applied a treatment to the samples prior to analysis<sup>[35, 40]</sup>. One was  
260 focusing on lake water and the other on riverine waters. Both applied a wet oxidizing protocol with  
261 hydrogen peroxide in presence of an iron(II) catalyst to remove organic material from surface water  
262 samples.

263 **Identification**

264 Plastic particles observed in surface samples from lakes and streams have been identified as  
265 polymers in ways similar to particles detected in lakeshore sediments. Although the identification by  
266 visual means is less reliable it was performed by 7 studies <sup>[22, 28, 29, 35, 37, 38, 41]</sup>. Spectrometric methods  
267 have been used in 3 studies to identify polymers, namely IR-spectroscopy <sup>[26]</sup>, FT-IR using the ATR  
268 (Attenuated total reflection) technique <sup>[42]</sup> and RM <sup>[40]</sup>.

269 **2.3. Biota samples**

270 Only two studies assessed freshwater biota ingestion of microplastic particles. The first was  
271 performed in Lake Geneva (Switzerland). The gut content of 21 adult northern pikes (*Esox lucius*),  
272 18 common roaches (*Rutilus rutilus*) and 2 common breams (*Abramis brama*) were analyzed but no  
273 plastic fragments were found <sup>[28]</sup>. The second study assessed microplastics in the gut of wild  
274 gudgeons (*Gobio gobio*) sampled in 11 French rivers <sup>[43]</sup>. For this purpose, fish guts were dissected  
275 and subjected to direct visual inspection under a dissecting microscope. Microplastics, defined as  
276 hard and colored fibers, were observed in fish from 8 rivers with an occurrence between 11 and 26%.  
277 The authors showed that fish from urban rivers were more contaminated with microplastics  
278 compared to those collected in rivers with low anthropogenic impact. This study confirms that  
279 freshwater fish do ingest microplastics and supports further studies to characterize microplastic  
280 contamination of river and lake biota. Both studies used visual inspection methods to identify the  
281 plastic particles and fibers.

282 **3. Challenges to and recommendations for the analysis of microplastics in**  
283 **freshwater environments – A crucial need for harmonization**

284 One of the major challenges in microplastic research is the need for general definitions and method  
285 harmonization. This will enable comparison of results between studies and sites, and should

286 encompass every step involved in the microplastic investigation, i.e. sampling, samples processing,  
287 identification and final statistics.

### 288 ***3.1 Definition of microplastics and units used***

289 Microplastics comprise a heterogeneous assemblage of pieces that vary in size, shape, color, specific  
290 density and chemical composition. The definition of microplastic size varies in previous marine  
291 studies. This heterogeneity is also found in studies concerning the continental environment. To avoid  
292 this issue, our recommendation is to provide a common definition of microplastics which should  
293 support the establishment of a standardized sampling method and improve concordance between  
294 future studies. In the marine environment, studies consider microplastics as particles smaller than  
295 5 mm in size <sup>[4, 44]</sup>. However, given possible uptake by different aquatic organisms as well as the  
296 handling during extraction and identification, we suggest a subdivision of the term microplastic in  
297 particles that can be optically identified with the naked eye as well as handled with tweezers and  
298 particles which are impossible to distinguish without optical tools and cannot be handled individually  
299 without optical tools (e.g. microscope, stereo microscope). This is in concordance with several  
300 authors of marine studies <sup>[4, 45]</sup> as well as limnetic studies <sup>[7, 9, 35]</sup>. Similarly the Technical work group  
301 defining suggestions for implementation of Monitoring of Marine Litter for the Marine Strategy  
302 Framework Directive suggested to separate between microplastic >1 mm and microplastic <1 mm  
303 due to the above mentioned reasons <sup>[46]</sup>.

304 In addition to a different microplastic definition, studies often give their result in different units,  
305 making comparability almost impossible (Table 1). This is mainly due to different sampling,  
306 extraction and identification methods.

307 Studies assessing lakeshore sediments give particles per volume <sup>[28]</sup> or per sampled surface <sup>[9, 10]</sup>.  
308 This is comparable to marine studies, although the latter also provide measurements of particles per  
309 sediment weight <sup>[4]</sup>.

310 For lake samples, concentrations are mentioned in particles per surface area <sup>[7]</sup>, again comparable to  
311 marine surface water sampling <sup>[4]</sup>. For the river data particle abundance is generally reported as  
312 particles per water volume <sup>[36, 39]</sup>. It is possible sometimes to calculate from one unit to the other, but  
313 the required information must be provided. The usage of particles per biomass is not helpful in an  
314 environment with seasonal changes (e.g. algal bloom in spring and summer, clear water phase,  
315 zooplankton blooms).

316 In order to identify plastic sources and characteristics, studies should also categorize plastics into  
317 different shape/size classes. Separating fibers (1 dimension larger than the two other dimensions),  
318 fragments (2 dimensions are large in contrast to a small third dimension) and spherules (similar  
319 extent of all 3 dimensions) would enhance comparability between different sites.

## 320 **3.2 Sampling methods**

### 321 *Sediment sampling*

322 Correct and representative sampling of an adequate matrix is the first step to assess environmental  
323 contamination. Unfortunately, the same methodological divergence seen in the marine system (for  
324 review see <sup>[4]</sup>) occurs in sampling methods used for lakeshore sediments. Sampling differs not only  
325 in the methodologies used and the volume sampled, but also in the sample location. In order to avoid  
326 local heterogeneities, we propose a combined sampling approach using sediment core samples  
327 (diameter 10 cm, depth 5 cm) taken along a 20 m transect (e.g. along the drift line at a lake system)  
328 at a distance of 2.5 m from one another, which would result in a sample volume of 4 - 6 liters. For  
329 rivers, a different methodology should be applied. Instead of running parallel to the waterline, the  
330 river bank could be divided into 3 short transects of 5 m stretched over the accumulation zone  
331 perpendicular to the waterline.

### 332 *Water sampling*

333 To date a comparison between results from different studies is nearly impossible because nets with



334 various mesh sizes are used. Moreover, river water is sampled from different positions both in  
335 sampling depth and distance from the riverbanks. The main challenges for method standardization  
336 are i) the spatial-temporal frame and ii) the utilized mesh size which controls the smallest particle  
337 size sampled. A mesh size of 300 - 333  $\mu\text{m}$  is common in marine plastic and plankton research, and  
338 offers a tradeoff between good handling and accumulation of larger fragments resulting in a blockage  
339 of the net. Using a mesh size of 300 - 333  $\mu\text{m}$  in the freshwater environment would enable  
340 comparison of data gathered from both marine and continental environments. However, the use of a  
341 manta trawl may lead to an underestimation of microplastics in a size range smaller than the mesh  
342 size <sup>[22]</sup>. In order to have a complete overview of plastic contamination, we suggest also using nets  
343 with a smaller mesh size or, when necessary, even bulk water sampling. This would be an important  
344 consideration given that especially the smallest particles present a greater risk to be ingested and  
345 subsequently translocated into an organism's tissues <sup>[47]</sup>.

346 Sampling microplastics on the surface of inshore waters may present some technical difficulties  
347 compared to marine ecosystems. In contrast to rather nutrient poor marine ecosystems, the amount of  
348 organic matter is generally much higher, especially during periods of algal blooms or leaf fall in  
349 autumn. Additionally, during periods of high water levels, suspended matter, clay minerals and  
350 allochthonous inputs of organic material increase the risk of clogging nets. This might limit the  
351 sampling duration, and thus reducing considerably volumes sampled. Therefore, while towing a  
352 manta trawl seems optimal for marine water sampling, it might be necessary to modify the net  
353 configuration in order to sample lakes and streams.

354 In contrast to lakes, rivers present a permanent flow of water and the current velocity has to be  
355 considered in order to facilitate surface sampling. The current velocity can be either too slow ( $< 0.1$   
356 m/s) or far too high, the latter resulting in a high ram pressure hampering net inflow. River water  
357 sampling should be preferable performed from a fixed position. A second problem arises from the

358 need for a reference parameter to give either particles per surface or per volume. While in standing  
359 waters, length of the performed manta tow can be used to calculate either the volume or surface  
360 sampled, but in running water, determination of current velocity must be estimated using a  
361 flowmeter.

362 Additionally, in order to determine fluxes of microplastics in running water, it is important to  
363 identify both the spatial and temporal variability of plastic particles as well as their dynamics in the  
364 river (distribution along the water column, sedimentation).

### 365 **3.3 Methods for sample processing**

#### 366 *Separation of plastic polymers from inorganic/mineral material*

367 If plastic particles are directly collected at the lakeshore or riverbank, it is very likely that especially  
368 microparticles will be overlooked. A crucial step in taking all plastic particles into account is  
369 therefore the extraction of microplastics from bulk environmental samples. Different density  
370 separation methods can be used to separate microplastics from sediments <sup>[4]</sup>. The used methodology  
371 can massively account for a large portion of uncertainty in the recovery success. Due to their surface  
372 properties, microplastics can attach to any surface they come in contact with. Thus, the amount of  
373 working steps should be reduced for density separation of microplastics. Since sample preparation  
374 and particle identification is time consuming, the procedure has to be optimized regarding the  
375 extraction method. A density separator was developed for this purpose. With the Munich Plastic  
376 Sediment Separator (MPSS), a sample volume of up to 6 L can be analyzed in one run <sup>[6]</sup>. It should  
377 be noted that for sediments including high amounts of organic particles or clay minerals, the sample  
378 volume should be reduced. For very small sample volumes (< 250 mL), a MPSS in smaller scale  
379 could be built. The MPSS offers a good recovery rate of microplastics, especially for S-MPP, and is  
380 commercially available <sup>[6]</sup>. However, other methods using elutriation followed by density separation  
381 <sup>[48]</sup> and fluidization, and finally followed again by density separation <sup>[49]</sup> were also efficient.

382 Protocols can be adopted to optimize microplastic recovery in different types of sediments (sandy or  
383 clay soils, biota-rich sediments, etc.).

384 Depending on the density of the solution used for the separation, the range of recovered polymers is  
385 highly different. Previous studies on marine or estuarine environments use mainly sodium chloride  
386 solution (NaCl - 1.2 kg/L) <sup>[4, 25]</sup>. If all commercially produced polymers should be recovered, the  
387 solutions used for future separations must have a density of at least 1.5 kg/L or higher <sup>[6, 50]</sup>. The use  
388 of a higher density solution takes into account both potential additives that may increase the density  
389 of the particles, and attached biota or organic particles. Recently suggested separation fluids are zinc  
390 chloride (ZnCl<sub>2</sub> - 1.6 to 1.7 kg/L) <sup>[6]</sup> or sodium iodide (NaI - 1.6 kg/L) <sup>[48, 49]</sup>. Both are relatively  
391 cheap but have the drawback of being moderately toxic for the biota. The separation fluid may be  
392 used more than once, being easily recovered for example with the use of candle filters. A more  
393 expensive method is the use of a non-toxic polytungstate solution that offers a density up to 2.0 kg/L  
394 <sup>[10]</sup>.

### 395 ***Removal of organic matter***

396 The separation of plastic particles from other organic materials (such as shell fragments, small  
397 organisms, algae or sea grasses, and tar) has been shown to be necessary for marine matrices and, to  
398 a greater extent, for continental environment matrices. These latter matrices contain more organic  
399 material, especially in the case of eutrophic lake and streams. Performing solely density separation to  
400 isolate plastic particles from sediment samples is not efficient in reducing natural organic debris.  
401 Hence, treatment methods have to be applied to facilitate identification of plastic particles. Several  
402 oxidation agents such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) <sup>[51]</sup>, strong acids such as nitric acid (HNO<sub>3</sub>) <sup>[52]</sup>,  
403 hydrochloric acid (HCl) and mixtures of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) with H<sub>2</sub>O<sub>2</sub> <sup>[34]</sup> were applied to remove  
404 organic material for limnetic but also for marine samples. However, methods utilizing strong acids  
405 have to be avoided since they affect and degrade plastic polymers <sup>[48]</sup>. Therefore alternative anti-

406 organic treatments need to be developed. A wet oxidizing protocol was recently used in a variety of  
407 studies, but the extent of degradation induced by the wet oxidizing protocol has not been analyzed  
408 yet. Some polymers are affected by H<sub>2</sub>O<sub>2</sub> at room temperature (e.g. PA & POM <sup>[53]</sup>).  
409 Enzymatic digestion methods may prove to be more useful to remove organic matter without  
410 affecting plastic polymers. In 2014, an enzymatic protocol for marine water samples was published  
411 revealing that 97% of the organic material can be digested without doing harm to microplastic  
412 particles <sup>[54]</sup>. The digestion step can be conducted on bulk samples or after the density separation of  
413 sediment samples.

#### 414 **3.4 Quality assessment/Quality control**

415 To achieve the development of a common protocol to sample, extract and identify microplastics in  
416 freshwater ecosystems, QA/QC (Quality assessment/Quality control) approaches will be very useful  
417 to ensure the quality of results, evaluate sources of variability and error, and increase confidence in  
418 the data collected. During the sample processing, sampling and laboratory blanks following the same  
419 analytical protocols should be performed. Caution should be exercised in wearing synthetic fiber  
420 clothing and should be avoided during sampling, extraction and further processing through until  
421 identification. Samples have to be covered in order to prevent airborne contamination. Plastic  
422 extraction recoveries also need to be validated using, for example, sediments spiked with artificially  
423 placed plastic fragments. The impact on artificial polymers of all organic matter removal methods  
424 have to be examined. In addition, identification methods should be used that corresponds to the size  
425 of the analyzed particles.

#### 426 **3.5 Identification of polymers**

427 Counting and identification are crucial steps to address microplastic contamination. Visual  
428 examination has been commonly applied to assess size and quantities of microplastics. However,  
429 pure visual examination using light or electron microscopy cannot be used to reliably distinguish

430 between polymers and other particles or to determine the polymer type. This method may lead to an  
431 overestimation of the plastic polymer contamination. In one study, nearly 20% of particles less than  
432 1 mm which were initially identified as microplastic by visual observation were later realized to be  
433 aluminum silicate from coal ash <sup>[7]</sup>. Hence, visual characterization and identification should be  
434 coupled systematically to a characterization technique. Reliable identification results are achieved  
435 with the use of spectrometric methods like FT-IR microspectroscopy and Raman microspectroscopy,  
436 or for some polymers by the use of SEM/EDS (scanning electron microscopy / Energy Dispersive X-  
437 Ray Spectrometer) <sup>[55]</sup>. Pyrolysis followed by GC-MS can also be applied <sup>[49, 56]</sup>, but information on  
438 particle shape/size is lost.

439 The visual identification of macroplastic for abundance estimates might be acceptable, especially for  
440 studies only sampling large fragments of obvious plastic products <sup>[33]</sup>. In this case, microscopic  
441 inspection of texture and surface characteristics can be accompanied by verification by a  
442 spectrometric method similar to that performed at Lake Erie and St. Clair <sup>[30]</sup>. L-MPP can be handled  
443 with tweezers and can therefore be easily placed under the ATR crystal of a FT-IR, put under a  
444 Raman or electron microscope, be inserted in Pyrolysis GC/MS, or prepared for Differential  
445 Scanning Calorimetry. S-MPP samples have to be captured on matrices (e.g. filters) and  
446 subsequently undergo either manual or automatized identification protocols using FT-IR or RM.  
447 Manual identification methods are highly time consuming.

### 448 **3.6 Microplastic contamination impacts in freshwater**

449 As documented in marine organisms <sup>[47]</sup>, freshwater fish <sup>[43]</sup> and invertebrates <sup>[9]</sup>, microplastics can  
450 be ingested by aquatic organisms. However, the effects induced by microplastics are poorly  
451 documented and major questions should be investigated to address this issue. Microplastic  
452 contamination of biota has been classically documented using gut contents. After ingestion,  
453 microplastics may be retained, excreted or translocated into other body tissues and fluids. Evidence

454 of translocation is available in rodents and humans where 150  $\mu\text{m}$  particles of PVC and PS were  
455 identified in the lymph and circulatory system <sup>[57, 58]</sup>. More recently, particles were proved to interact  
456 with mammalian cells in the intestinal system <sup>[59]</sup>. A translocation experiment was performed with  
457 mussels (*Mytilus edulis*) exposed to 3 and 9.6  $\mu\text{m}$  particles. After 3 days, translocation to the  
458 circulatory system was observed and consisted of a greater number of smaller particles compared to  
459 larger microplastics <sup>[60]</sup>. In concordance with this, another study detected microplastic occurrence in  
460 the soft tissues of cultured *Mytilus edulis* and *Crassostrea gigas* <sup>[52]</sup>. These studies confirm that  
461 microplastics can be translocated into tissues, but further studies are needed to address this aspect in  
462 freshwater organisms.

463 Ingested or translocated microplastics induce adverse effects according to their mode of action. The  
464 effects can be categorized as follows:

- 465 i) mechanical impairments through swallowed plastics mistaken as food
- 466 ii) polymers and plastic associated chemicals (additives) may be endocrine-disrupting or toxic
- 467 iii) polymers can adsorb toxic organic pollutants, nanoparticles or metals which may evoke  
468 adverse effects
- 469 iv) Finally, plastic debris may act as vector for alien species and diseases.

470 To improve our knowledge of the microplastic hazard, organic micropollutant contamination must be  
471 investigated. Acting as passive samplers, microplastics can indeed adsorb some pollutants. To date  
472 regarding to persistent organic pollutants, the contribution of microplastic to the total pollution in  
473 freshwater remains unknown, although numerous studies on marine beaches exist <sup>[61]</sup>. Freshwater  
474 systems present different conditions compared to marine systems (salinity, organic debris etc.), and  
475 concentrations of persistent organic pollutants in the continental environment are expected to be  
476 greater than in marine ecosystems. Hence, it is essential to address interactions between micro-  
477 pollutants and plastics in freshwater, focusing specifically on urban lakes and rivers.

#### 478 **4. Conclusions**

479 Although environmental contamination with microplastics is constantly in the media, almost no basic  
480 data on contamination in freshwater ecosystems exist. As summarized in this review, recent studies  
481 demonstrate an almost equal contamination as reported in the oceans. Therefore, it is of utmost  
482 importance to elucidate sources, fate, fluxes, and impact of microplastics and associated chemicals in  
483 freshwater ecosystems.

484 Sampling, separation and identification methods are key steps for an accurate characterization of  
485 microplastic contamination. However the methods described in the existing literature are very  
486 diverse and no common methodology has emerged. This is likely due to the novelty of the topic, and  
487 is not astonishing given that many of the studies were pilot projects. The imposed constraint,  
488 however, is the low comparability between studies using different methods. This methodological  
489 heterogeneity might be intrinsically more pronounced for freshwater (especially in rivers) due to  
490 many parameters related to the freshwater sampling (river flow, season, type of net, position of the  
491 net or manta trawl, water column height, dynamic or static sampling, time of exposure, presence of  
492 suspended matter, vegetal debris, etc.). This could introduce various constraints leading to the use of  
493 different methods. The development of an improved, automated and harmonized methodology for  
494 detection and identification of microplastics appears a real challenge, but should regardless be a  
495 priority in order to improve comparability between future studies (in both continental and marine  
496 environments). Comparable data on contamination of different habitats are important for a reliable  
497 risk assessment, which will be needed for adequate mitigation and prevention measures in the future.  
498 Guidance for monitoring microplastics have been recently proposed to support the monitoring of  
499 microdebris in the marine environment within the Marine Strategy Framework Directive <sup>[62]</sup> but gaps  
500 have to be identified as preliminary steps.

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## List of figures

Figure 1: Microplastic sources/pathways in a continental context. Atmospheric fallout, runoff and punctual discharges (wastewater treatment plants, combined sewer overflows) are proposed as the main freshwater sources of microplastics<sup>[22]</sup>.

Figure 2: Comparison of micro- and macroplastic abundance in lakeshore sediment and lake surface water environments. The units are expressed on a logarithmic scale of items per m<sup>2</sup>. Overlapping dots were separated to show all data points. Each point represents one data point from one study. Data points were taken whenever the units were given as items per surface and calculated to items per m<sup>2</sup>. In some studies numbers were given in items per volume, and these data points were calculated to items per surface if sufficient information was available. Studies represented here are marked with an asterisk (\*) in Table 1.

Figure 3: Comparison of micro- and macroplastic abundance in river beach sediment and river surface environments. The units are expressed on a logarithmic scale of items per m<sup>2</sup>. Overlapping dots were separated to show all data points. Each point represents one data point from one study; standard deviations are given when available. Data points were taken whenever the units were given as items per surface and calculated to items per m<sup>2</sup>. In some studies numbers were given in items per volume, and these data points were calculated to items per surface if sufficient information were available. Studies represented here are marked with a hash (#) in Table 1.

Figure 1

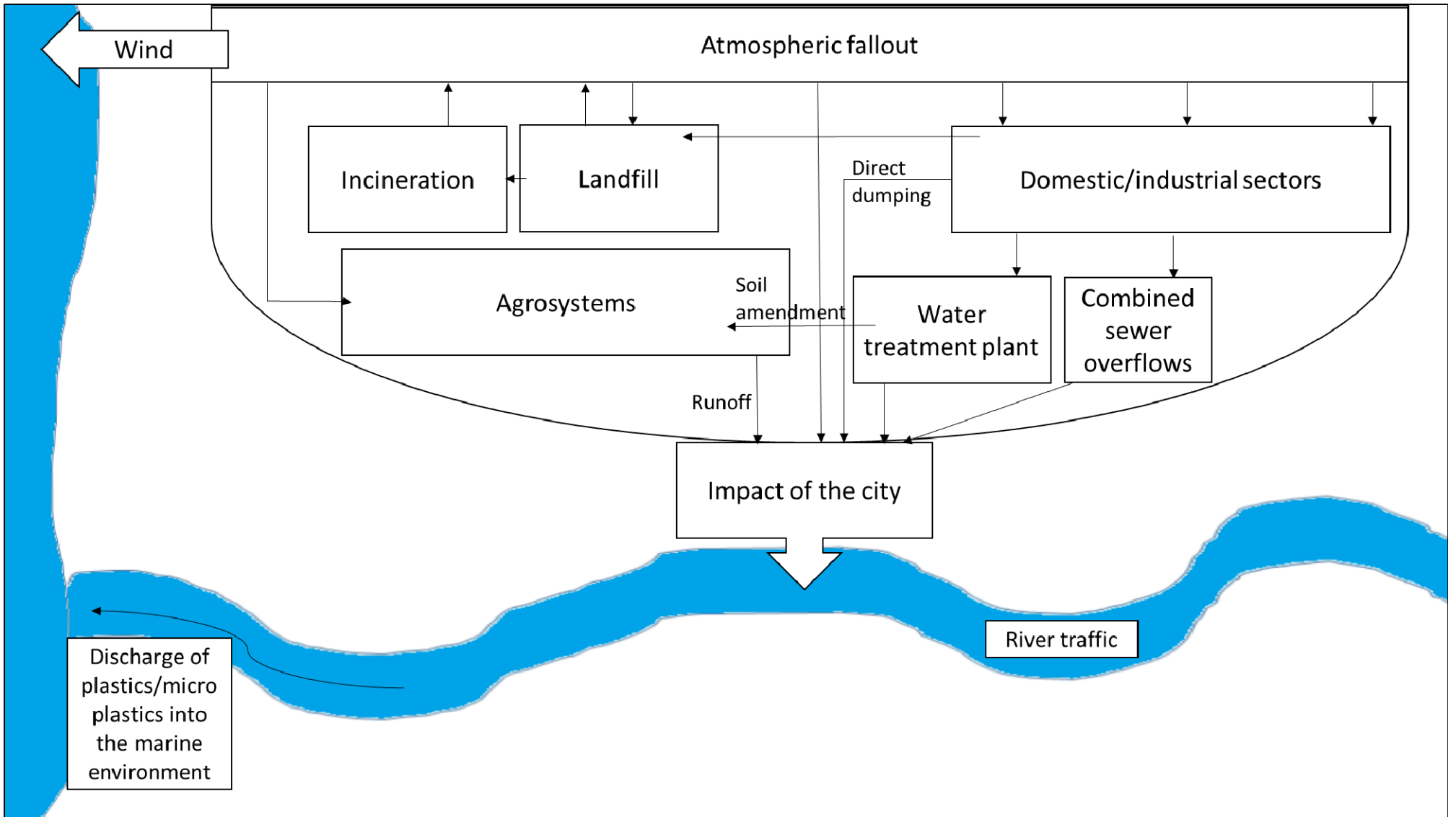


Figure 2

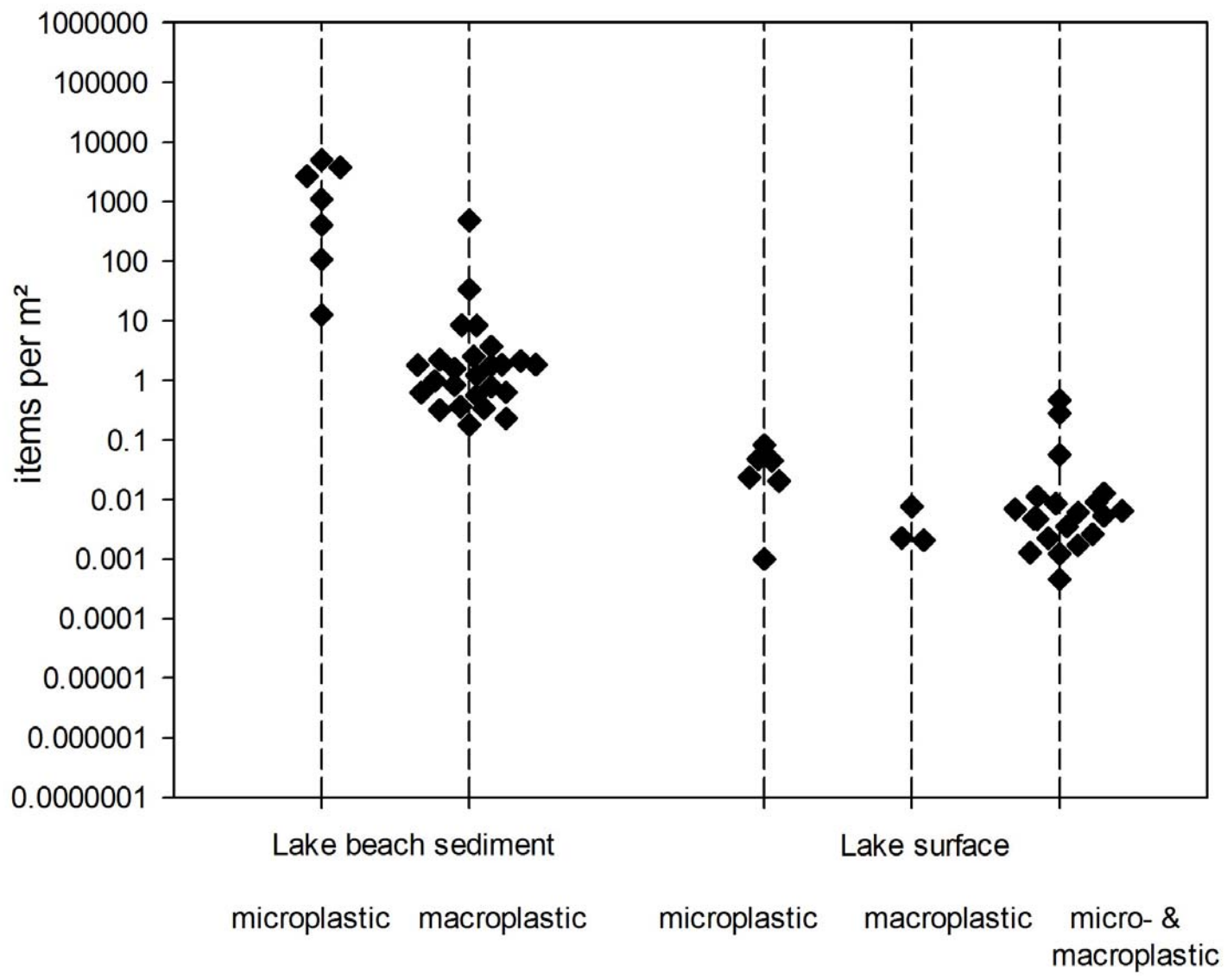
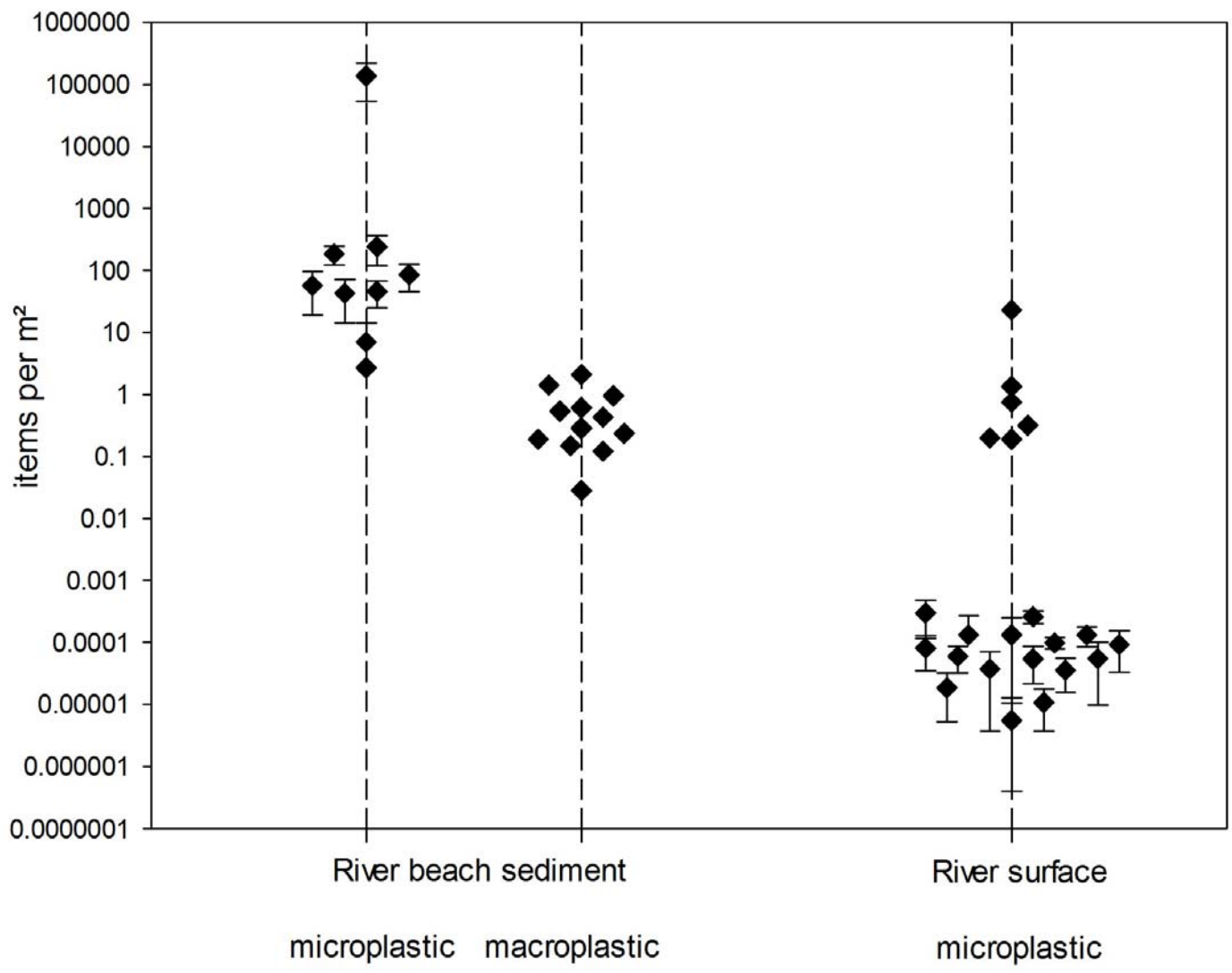


Figure 3



**List of tables**

Table 1: list of studies related to microplastic contamination on lakes and rivers indicating used methods for sampling and units to report contamination



**Table 1**

Environment	Year	Site	Compartment(s) studied	Sampling methods	Identification-method	Unit used	Reference
Lakes	2011*	Lake Huron (Canada, USA)	Lakeshore sediments	Visual inspection and plastic particles	FT-IR	Particles/m <sup>2</sup>	[10]
	2012	Lake Geneva (Switzerland, France)	Lakeshore sediments	Manual collection of sediment samples	Visual inspection	Number of particles in 1L samples	[28]
			Biota (fish and birds)	-	-	-	-
	2013*	Lake Garda (Italy)	Lake water	Manta trawl		Particles/km <sup>2</sup>	
			Lakeshore sediments	Random grid sediment sampling	RM	Particles/m <sup>2</sup>	[9]
	2013*	Lakes Superior, Huron and Erie (USA, Canada)	Lake water	Manta trawl	SEM/EDS	Particles/km <sup>2</sup>	[7]
	2013*	Lake Geneva (Switzerland, France)	Lakeshore sediments	Manual collection of sediment samples	Visual inspection	Particles/m <sup>2</sup>	[29]
			Lake water	Manta trawl		Particles/km <sup>2</sup> and g/km <sup>2</sup>	
2014*	Lake Hovsgol (Mongolia)	Lake water	Manta trawl	Visual inspection	Particles/km <sup>2</sup>	[35]	
2014*	Lake Erie and St. Clair (USA, Canada)	Shorelines	Manual collection of sediment samples	Randomly selected samples with FT-IR	Particles/m <sup>2</sup>	[30]	
Rivers	2010	Rivers in Greater London (UK)	River water	No information	IR-spectroscopy	Particles/L	[36]
	2011 <sup>#</sup>	Los Angeles and San Gabriel Rivers (USA)	River water	Manta net	Visual inspection	Particles/L	[37]
				Streambed samples			
	2014 <sup>#</sup>	Rivers Elqui, Maipo, Maule and BioBio (Chile)	Riversides sediments	Hand net (0.8, 0.5 mm mesh size)	Visual inspection	Number of items	[33]
				Visual sampling (items >1.5cm)			
	2014	Thames river (UK)	Subsurface water	Eel nets	Visual inspection	Numbers of items sampled	[41]
	2014	Danube River (Germany, Austria etc.)	River water	Stationary driftnets (0.5 mm mesh size)	Density separation & Visual inspection	Particles/1000 m <sup>3</sup>	[38]
	2014	Seine River (France)	Floating plastics in river water	Floating booms	FTIR-ATR	Tons of plastic	[42]
	2014 <sup>#</sup>	St. Lawrence River (Canada)	Bank sediments	Grab samplers	Differential scanning calorimetric	Particles/m <sup>2</sup>	[32]
2014 <sup>#</sup>	Four Estuarine Rivers in the Chesapeake Bay (USA)	River water	Manta Trawl	RM	g/km <sup>2</sup>	[40]	
2015 <sup>#</sup>	Seine & Marne River (France)	River water	Manta Trawl (330 µm) & Plankton net (80 µm)	Visual inspection	Particles/m <sup>3</sup>	[22]	

RM = Raman microspectroscopy