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HAL Id: hal-01134553
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Submitted on 11 May 2018

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Microplastic contamination in an urban area: a case study in Greater Paris

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**Introduction**

Plastic production has increased rapidly from 1.7 to 288 Tg annually within the last 60 years.\textsuperscript{11} In the early 70s, the presence of millimetre-sized plastic debris in the marine environment was highlighted.\textsuperscript{12, 13} More recently, in 2004, the term microplastics was used to describe millimetre and sub-millimetre-sized particles.\textsuperscript{14} Since 2008, plastic particles smaller than 5 mm have been defined as microplastics.\textsuperscript{15}

These microplastics cover a large and continuous spectrum of sizes and shapes including 1-D fibres (with one larger dimension), 2-D fragments (flat particles) and 3-D spherules. More recently, several authors\textsuperscript{16-17} have noted that the presence of nanoplastics in the marine environment is likely to be of increasing significance in the coming years. In the present study, as in most studies, microplastics with a characteristic dimension between 100 and 5000 µm are considered, providing an accurate overview of the microplastics distribution.

Microplastics are either primary, manufactured at a microscopic size for various applications such as personal-care products (facial and hand cleaners), cosmetic products and airblast cleaning,\textsuperscript{18, 19} or secondary, resulting from the breakdown of larger plastic debris and litter, largely owing to the effect of ultraviolet rays or mechanic abrasion.\textsuperscript{20} Particular attention should be paid to synthetic clothes. During their life cycle, they release millimetre and sub-millimetre-sized fibres, especially during laundry procedures, and can be considered as secondary microplastics.

Two significant environmental concerns related to plastics are raised in the present paper: (i) the ingestion of microplastics by various species, and (ii) the interaction between microplastics and other pollutants. The ingestion of plastics (micro and macro) has been reported in at least 267 marine species (turtles, seabirds, marine mammals, etc.).\textsuperscript{11} Microplastics ingestion may lead to various effects, including intestinal obstructions\textsuperscript{21} and false indication of satiation causing less food intake.\textsuperscript{12} A study mentioned a possible translocation of small microplastics (<10 µm), showing that these particles translocate from the gut into the circulatory system of the mussel.\textsuperscript{13} Microplastics can also act as ‘passive samplers’ and adsorb hydrophobic and persistent organic pollutants.\textsuperscript{14} Because of their low density, settling of plastic particles is reduced compared with usual suspended solids and they may travel overlong distances along with the adsorbed pollution.

![Fig. 1. System representation of potential microplastic sources and pathways in an urban context.](image-url)
Although plastic pollution in marine environments has been well documented, there has been limited focus on plastic pollution in continental environments.\textsuperscript{[15]} In fact, numerous studies assume that 80\% of the plastic pollution found in the marine environment originates from a terrestrial origin.\textsuperscript{[6,16]} To date, the dynamics of the continental inputs into the marine environments are poorly documented. Similarly, knowledge on the sources, fate and transfer of microplastics in continental environments is extremely limited. Some preliminary studies show the ubiquitous contamination of continental aquatic environments by plastics, either lentic: the Great Laurentian Lakes,\textsuperscript{[17]} Lake Geneva\textsuperscript{[18]} and Lake Garda beaches,\textsuperscript{[19]} or lotic. Studies carried out on rivers show high plastic flow in two urban Californian rivers,\textsuperscript{[20]} the Thames river,\textsuperscript{[21]} the Danube river,\textsuperscript{[22]} and four rivers flowing into the SE Pacific.\textsuperscript{[23]} These studies identify the abundance of plastics and microplastics in lakes and running water but their sources and dynamics remain largely unknown.

Special attention should be paid to the sources of plastic in catchments exposed to severe anthropogenic impacts, especially urban areas. A systemic approach to microplastic dynamics and the different pathways in an urban context is conceptualised here (Fig. 1). The city can be considered as a closed system and only primary plastics and microplastics may enter the system. During their life cycle, secondary plastics and microplastics that are produced may reach the three compartments of the environment: atmosphere, water and soil. Industrial emissions, particle resuspension and other anthropogenic causes (traffic, building, urban infrastructures, etc.) are potential sources of plastics in the atmosphere. Plastics may also reach the aquatic compartment either directly because of careless behaviour, or through the urban water system, ‘combined’ or ‘separate’. Finally, because sludge originating from wastewater treatment, which may contain plastics, is often used on agricultural soils, the soil compartment is also potentially contaminated.\textsuperscript{[24]} Additionally, atmospheric fallout of plastic particles may also contribute to soil contamination. Almost nothing is known about either the behaviour of plastics in these compartments or the dynamics within or between them, so as a first approach, it was decided to focus on the fluxes directly interacting with the receiving water system, namely:

- The microplastics discharged into the rivers from the urban water system and especially from wastewater treatment plants;
- The microplastics originating from the atmosphere.

Moreover, surface water contamination was also monitored. This monitoring aimed at providing an integrated overview of the microplastics contamination at a city scale, contributing to an understanding of their dynamics in the urban water cycle. Greater Paris is located on the River Seine basin. This basin drains an ~32,000-km\(^2\) area from its headwaters to Paris and can be considered as representative of river basins exposed to the impacts of intense human activity (Parisian conurbation: 12 million inhabitants). This basin combines strong anthropogenic pressures with a very limited dilution factor due to the low flow rate in the River Seine (median flow at Paris: 350 m\(^3\) s\(^{-1}\))\textsuperscript{[25]} and is a relevant site for estimating microplastic pollution.

The present article delivers innovative knowledge on sources of microplastics in an urban area and is one of the first to estimate contamination in an urban river.\textsuperscript{[20]} Such information is useful and contributes to establishing the continental inputs into the marine environment and to better understanding microplastic dynamics in an urban environment. This article also represents a contribution to the debate on microplastics sampling and analysis strategies in fresh water.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Sampling sites: P1, Charenton; P2, Choisy le Roi; P3, Suresnes; P4, Sartrouville; P5, Poissy. M1, Asnières; M2, Sartrouville; M3, Conflans.}
\end{figure}
Materials and methods
The general procedure used follows the successive steps of: sampling, filtering, identification, accounting and finally estimation of concentration and flux.

Sites and sampling procedures
Total atmospheric fallout
Total atmospheric fallout (wet and dry deposition) was collected through a funnel in a 20 L glass bottle on the rooftop of Paris-Est Créteil University (48°47’17.8”N, 2°26’36.2”E, 11 km east from the centre of Paris), located in a dense urban environment. Monitoring was carried out during a 3-month period from 26 February 2014 to 22 May 2014. The surface of sampling was 0.325 m² and allowed collection of total atmospheric fallout (dry and wet deposition). The samples were collected at various frequencies ranging from 7 days (wet-weather periods) to 30 days (dry-weather periods). The funnel was rinsed with distilled water three times (3 x 1 L) in order to recover all microplastics that might be adhering to its walls. Rainfall intensity was recorded continuously at the same location. Microplastic fallout is expressed as number of particles per square metre per day.

Urban wastewater, settled and treated water
Wastewater was collected at the Seine-Centre wastewater treatment plant (WWTP, Fig. 2). This plant is located downstream of Paris and is supervised by the Syndicat Interdépartemental pour l’Assainissement de l’Agglomération Parisienne (SIAAP, the Parisian public sanitation service). The plant treats 240 000 m³ of wastewater daily. The water treatment is conducted in the following steps: the water is pretreated (screening, then grit and oil removal), then settled in a primary settling tank before undergoing biological treatment that consists of biofilters.[28] Three sampling campaigns on 3 consecutive days (8, 9 and 10 April 2014) were carried out. Raw wastewater after pretreatment, settled wastewater and treated water were collected conventionally, i.e. using an automatic sampler and 24-h averaged samples were analysed. An aliquot of 0.05 L was analysed for each sample and results are provided in particles per cubic metre.

Surface water
In order to cover the 100–5000-µm microplastic size under study, two different but complementary sampling approaches were followed: (i) sampling with a plankton net at five stations upstream and downstream from Paris (mesh size of 80 µm), and (i) sampling with a manta trawl (mesh size of 330 µm).

(i) Plankton net. Two snapshot campaigns (23 April, 14 May) were carried out at four stations (P2–P5) on the River Seine and one on the River Marne (P1, Fig. 2). In order to get an overall view of microplastic contamination, the sampling at the five stations was performed on the same day. Because the distance between the upstream and downstream points is ~30 km, a specific device was developed allowing rapid sampling from bridges. This device couples a plankton net (mesh size 80 µm) and a propeller-type current meter. It allows water sampling while measuring velocity simultaneously (Fig. 3). The net was immersed just below the water surface in the direction of flow, its opening perpendicular to the flow. Samples were collected within the superficial layer of the water column (0.1–0.35-m surface layer). The exposure time was 1 min, as the best compromise between: (i) sampling a sufficient volume to increase representativeness; (ii) limiting the volume sampled to avoid fouling and reduce the amount of mineral and organic matter; and (iii) enabling sampling between the passage of two barges. After collecting, the net was rinsed carefully three times from the outside with river water. Preliminary tests confirmed the good efficiency of such rinses (no particles were found on the fourth rinse whereas a few could still be found on the third rinse). The current meter measures the local instantaneous velocity of the water flow and allows estimation of the volume sampled. The water velocity ranged during the samplings between 0.1 and 0.4 m s⁻¹ and the collected volumes between 0.43 and 2 m³ (450 and 2000 L).
Knowledge of the spatial and temporal heterogeneity of the plastic fluxes in the river is necessary for estimating the uncertainty in the extrapolation of local fluxes to the river cross-section. Therefore, two specific sampling campaigns were carried out on 26 June 2014 and 3 December 2014 at P1 to assess microplastic lateral variability. The plankton net (80-µm mesh size) was used for these campaigns. Triplicates were sampled successively for the same duration (1 min) in each of the three following points: right bank, left bank and centre. The width of the River Marne at point P1 is 62 m. Right and left bank points are 7 m from the respective riverside. The duration of the entire test including sampling, rinsing, moving to and deploying the device at the next point was less than 10 min.

1.5 Manta trawl. Three different stations (M1–M3) were sampled on 17 July 2014, downstream from Paris (Fig. 2), close to the P3–P5 stations. Two WWTP discharges are located respectively between stations M1 and M2 (Seine-Centre, 240 000 m³ day⁻¹) and stations M2 and M3 (Seine-Aval, 106 m³ day⁻¹). The manta trawl was towed by a motorboat (~2 m s⁻¹) in the upstream direction for a period of 15 min, allowing the sampling of volumes ranging from 182 to 200 m³ of the 0–0.3 m surface layer. The manta trawl has a larger mesh size (330 µm) and a higher cut-off than the plankton net. As a consequence, fouling is reduced compared with the plankton net and the collection of higher volumes is possible, thus giving access to less abundant microplastics.27 However, this leads to collection of more organic debris (mainly plants and vegetation). After towing, the trawl was rinsed from the outside with river water. Samples were collected and sieved in the laboratory; the fraction ≥2 mm was sorted to remove large organic debris. For both methods, results are presented in particles per cubic metre.

Fig. 3. (a) Plankton net with a current meter that also serves as a ballast and a floater that can be moved along the rope to fix the depth sampling. (b) Manta trawl. (c) Plankton net in deployment. (d) Manta trawl in deployment. (e) Atmospheric fallout collector. (f, g, h) Fibres collected in different samples.
Histolab

Fig. — observation method: there is a tendency to overestimate brightly coloured fibres from atmospheric fallout. However, they have to be confirmed and other study sites are needed to assess the effect of local influences as well as spatial heterogeneity.

Table 1. Microplastic concentrations and fibre length distribution for each compartment

<table>
<thead>
<tr>
<th>Mean concentration (particles m⁻³)</th>
<th>Minimum-maximum concentrations (particles m⁻³)</th>
<th>100–500 µm</th>
<th>500–1000 µm</th>
<th>1000–5000 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>22%</td>
<td>29%</td>
<td>49%</td>
</tr>
<tr>
<td>30</td>
<td>3–106</td>
<td>21%</td>
<td>31%</td>
<td>48%</td>
</tr>
<tr>
<td>0.35</td>
<td>0.28–0.45</td>
<td>0%</td>
<td>25%</td>
<td>75%</td>
</tr>
<tr>
<td>293000</td>
<td>260 000–320 000</td>
<td>29%</td>
<td>26%</td>
<td>45%</td>
</tr>
<tr>
<td>90000</td>
<td>50 000–120 000</td>
<td>66%</td>
<td>27%</td>
<td>7%</td>
</tr>
<tr>
<td>35000</td>
<td>14 000–50 000</td>
<td>57%</td>
<td>43%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Sample treatment

All samples were filtered on glass fibre GF/A Whatman filters (Sigma–Aldrich, 1.6 µm). In order to limit contamination, the following precautions were taken:

- Samples were covered with aluminium foil to prevent airborne plastic particle contamination;
- Only glass vessels were used. The vessels and filters were heated at 500 °C for 4 h before use. Except for the nets used, which are made of nylon, the use of plastic materials was avoided. Tests were carried out to verify that the plankton net did not contaminate the samples. Cotton laboratory coats were worn during all laboratory procedures.
- Blanks were systematically run and followed the same treatments. Between 0 and 2 fibres per blank filter were observed, which is negligible compared with the number of particles observed on sample filters.

Microplastic identification and counting

Filters were observed with a Leica MZ12 stereomicroscope coupled with software for image analysis (Histolab). Filters were observed with a magnification of 16x. A higher magnification was needed for some particles that were hardly identifiable (small particles, transparent fibres, etc.). The same operator performed all the observations. To distinguish synthetic from biological fibres, the following criteria were used[19,27]:

- The fibres have to be equally thick through their entire length;
- Fibres should not be entirely straight, which indicates a biological origin;
- No cellular or organic structures should be visible to consider a fibre as microplastic;
- Transparent fibres were examined with higher magnification to confirm their nature. Green fibres were also carefully observed because this colour is very widespread in natural particles.

A bias exists for the results obtained with this observation method: there is a tendency to overestimate brightly coloured fibres (blue, red) in comparison with other particles because they are more easily recognised. Further, uncoloured plastic particles are harder to identify and may be underestimated.

Great care was taken to avoid the overestimation of microplastics. Particles of uncertain nature were ignored. Microplastics with a size under 100 µm are hard to identify and difficult to count. Therefore, only microplastics with characteristic dimensions over 100 µm were considered. The software (Histolab) allowed fibre lengths to be measured and three size classes were taken into account: 100–500, 501–1000 and 1001–5000 µm. No chemical identification was performed for the identification of the polymers.

Results and discussion

Total atmospheric fallout

For the first time, microplastics were observed in atmospheric fallout. More than 90 % of the microplastics observed were fibres. Some 2-D fragments were occasionally observed (10 %). Approximately 50 % of the fibres were longer than 1000 µm. The remaining particle sizes were equally distributed into the 100–500 and 501–1000 µm classes (Table 1).

Microplastic atmospheric fallout ranged from 29 to 280 particles m⁻² day⁻¹, with an average of 118 particles m⁻² day⁻¹. Although no clear correlation was observed, it seems that rainfall influences the total atmospheric microplastic fallout (Fig. 4). For instance, during the 93 days of collection, the lowest atmospheric fallout level (29 particles m⁻² day⁻¹) was observed during a dry-weather period (from Day 23 to Day 30), but during the preceding period (Day 1 to Day 23) where almost daily rainfall occurred, highest fallout fluxes were observed (280 particles m⁻² day⁻¹). Such data may suggest that atmospheric fallout is a significant source of microplastics. Some studies have revealed the presence of fibres in remote lakes.[28] It might be possible that a fraction of these originates from atmospheric fallout. To our knowledge, this is the first experiment providing fluxes of microplastics in atmospheric fallout. However, they have to be confirmed and other study sites are needed to assess the effect of local influences as well as spatial heterogeneity.

Urban wastewater, settled and treated water

Microplastics were found in high concentrations (from 260 x 10³ to 320 x 10³ particles m⁻³) in raw wastewater. With the exception of one spherical particle, the microplastics observed were fibrous (Fig. 3, picture H). Fibres were mainly millimetre sized (Table 1) (45 % between 1000 and 5000 µm) whereas other fibre sizes were homogeneously distributed between the two other classes (100–500 and 501–1000 µm). The presence of numerous fibres may be explained by washing machine effluent because it has been reported that up to 1900 fibres per wash can be discharged.[29] Other sources of contamination also have to
be considered. After primary treatment (physicochemical lamellar settling), concentrations between $50 \times 10^3$ and $120 \times 10^3$ particles m$^{-3}$ were observed as well as a decrease in fibre size (Table 1). The amount of larger particles drastically decreased, attesting to their removal: the 1001–5000-$\mu$m size class only represents 7% of the total particles. The 100–500-$\mu$m size class is predominant at this step of the treatment (66%). In the final effluent, the contamination decreases to $14 \times 10^3$–50 $\times 10^3$ particles m$^{-3}$. None of the observed fibres was in the size class of 1001–5000 $\mu$m. Size distribution is globally similar to that after the primary treatment (Table 1). These first results suggest that WWTPs remove a large amount of the microplastic contamination (from 83 to 95%), which is probably transferred to sludge. The presence of fibres observed in sludge confirmed this hypothesis.\textsuperscript{[30]} As highlighted by the size pattern changes, water treatment seems to be more effective at removing longer fibres, which are absent from treated water (Table 1).

![Fig. 4. Atmospheric fallout of microplastics and daily rainfall.](image)

**Surface water**

**Plankton net**

Both sampling approaches highlighted the contamination of the two rivers by microplastics. Campaigns with the plankton net showed that most of the microplastics are fibres. No fragments, beads or macroplastics were observed. This is probably caused by the short time of exposure and consequently the limited water volume sampled as well as by the low concentration of such particles. The distribution size of the fibres found is presented in Table 1. Most of the fibres were larger than 1000 $\mu$m (48%). Respectively 31 and 21% of the fibres belong to the 501–1000 and 100–500-$\mu$m size classes. This size distribution is different to the one described for treated water but similar to the one for atmospheric fallout. Atmospheric fallout on the catchment could be a potential source of the fibres found in the surface water after deposition, runoff and transfer to the receiving system. However, other sources also need to be considered.

Concentrations observed for both campaigns are depicted in Fig. 5. No significant upstream–downstream evolution of the contamination was observed. Concentrations lie in the 4–108 particles m$^{-3}$ range. Larger values were observed in April (sites P1 to P4, 14 to 108 particles m$^{-3}$) than in May (3 to 36 particles m$^{-3}$). The inverse behaviour was observed at station P5 with a higher concentration in May (13 particles m$^{-3}$) than in April (8 particles m$^{-3}$). These differences are probably related to (i) local variability, (ii) a water flow decrease of ~20%, leading to lower flow velocity, and (iii) visual identification. In fact, numerous natural debris (mainly organic debris and clays) were present in the May samples, leading to more difficult observation and possibly microplastic underestimation. The presence of a large amount of debris in May could be explained by meteorological conditions and much higher rainfall leading to an increase of suspended matter (0.6 mm of cumulative rainfall in the 10 days preceding the sampling in April and 11.4 mm in the 10 days preceding the sampling in May).

![Fig. 5. Microplastic concentration at the five points for April and May campaigns (net plankton used) (River Marne (P1) and River Seine (P2 to P5)).](image)

Other sources of variability should be considered. Besides, it is known on the River Seine that suspended matter concentration is highly related to traffic on the river.\textsuperscript{[31]} A similar effect on microplastics should be expected.

**Transverse variability**

Results from lateral variability tests (centre, right and left banks) are given in Fig. 6. Concentrations observed in the first
campaign are generally higher than concentrations observed on the second one and present a lower variability based on triplicates. This lower variability can be mainly explained by the higher number of microplastics collected during the first campaign (~40–60 fibres min\(^{-1}\)) in comparison with the second one (~15–30 fibres min\(^{-1}\)). For both campaigns, featuring contrasting river flow (17 v. 132 m\(^3\) s\(^{-1}\)), the orders of magnitude of concentrations are similar between the centre of the river section and near the banks, except for one sample of the second campaign, suggesting that some processes may lead to a high local and punctual variability. Further work is needed to confirm these preliminary results and identify the processes that occur.

![Image](58x598 to 246x755)

**Manta trawl**

In contrast to the plankton net samples where only fibres were detected, different shapes of plastic including 2-D fragments and 3-D spherules were observed in the manta trawl samples. Macroplastics (>5000 \(\mu\)m) were also trapped in the trawl; these included packaging fragments, polystyrene macrofragments, lollipop sticks and cotton buds. Although the present study does not focus on macroplastics, these debris items represent an important issue to consider in order to assess the plastic pollution in an urban environment. For instance, a recent study estimated a total mass of 27 Mg of macroplastics trapped each year in the floating boom network on the Seine and Marne rivers in the greater Paris area. Fibres account for 40 to 45% of the microplastics observed on the manta trawl samples. The millimetre-sized fibres were predominant (75%) whereas the remaining 25% belonged to the 500–1000-\(\mu\)m size range. No fibres smaller than 500 \(\mu\)m were observed. Concentrations of plastic items varied from 0.28 to 0.47 particles m\(^{-3}\) (Table 1). These values are ~30 times smaller than those obtained with the plankton net. As previously underlined by a study, the higher cut-off due to a larger mesh size explains this difference. Another study using the same sampling methodology in surface water presented a similar size distribution, with more fragments in the size range 1000–3000 \(\mu\)m, than fragments in the size range <1000 \(\mu\)m. Station M3 had the highest concentration (0.47 particles m\(^{-3}\)) whereas stations M1 and M2 exhibited similar contamination (0.28 and 0.29 particles m\(^{-3}\) respectively). If confirmed, these results could be explained by the presence, between points B and C, of the discharge of the biggest WWTP in the Parisian region (Seine-Aval WWTP). With a different methodology but using a 500-\(\mu\)m net, a study reported the presence of microplastics on the Danube at levels of ~0.32 particles m\(^{-3}\) and observed that fragments and spherules were the most frequent shapes of microplastic found. Our results are in good agreement with results reported by these authors, although different mesh sizes were used (500 v. 330 \(\mu\)m).

**Comparison of both sampling techniques**

As indicated previously, the particles collected with the manta trawl are different from those collected by the plankton net in shape and size distribution: the larger mesh size of the manta trawl allows fibres, especially those belonging to the smallest size class (100–500 \(\mu\)m) to pass through it more easily. However, this methodology highlighted the presence of 2-D fragments and 3-D spherules. Such particles were not observed during plankton net campaigns, probably as a consequence of their low concentration and the low water volume sampled. In fact, only up to 2 m\(^3\) can be sampled with the plankton net whereas the manta trawl allows sampling up to 200 m\(^3\). The results presented are based on a limited number of field surveys and have to be confirmed.

From an ecotoxicological point of view, literature reports that risks of ingestion and ecotoxicological effects are higher with smaller microplastics. In this context, manta trawl sampling campaigns alone cannot characterise this risk but investigations on smaller microplastics are also required. Therefore, the two different but complementary sampling approaches developed in the current study could be implemented in future studies. Although analysing fibres needs the use of the plankton net owing to its small mesh size, sampling higher volumes is necessary to collect other shapes of microplastics.

**Conclusions**

The present study aimed to investigate the microplastic contamination of both urban matrices (wastewater, total atmospheric fallout) and surface water in a continental environment. These first investigations confirm the presence of microplastics in sewage, fresh water and total atmospheric fallout and provide knowledge on the type and size distribution of microplastics in the 100–5000-\(\mu\)m range.

For the first time, the presence of microplastics, mostly fibres, is also highlighted in total atmospheric fallout. Results suggest that atmospheric fallout could be a significant source of fibres in freshwater ecosystems but further work is needed to confirm these results and evaluate the effect of local influences. Rainfall is possibly an important factor for atmospheric fallout but other possible influencing factors, like the wind conditions or specific local conditions have to be investigated. All these results have to be confirmed over longer periods.

The present study has also highlighted high wastewater microplastic contamination. Fibres were predominant and no larger fragments were observed. The Seine-Centre WWTP appears to significantly reduce their concentrations. The efficiency of other treatment processes have to be evaluated.
The present article also contributes to the debate on microplastic sampling and analysis strategies in fresh water. The microplastic contamination survey in the rivers was performed with different sampling approaches, providing an overall view of microplastic contamination in the 100–5000-μm size range. This combined approach indeed enables the observation of all microplastic types from small fibres (plankton net campaigns) to 2-D fragments and 3-D spheres (manta trawl). Although the available studies focussing on microplastic contamination in fresh water mostly use the manta trawl, we observed much higher small fibre concentrations than other studies. Considering that the smaller microplastics present a higher ingestion risk and ecotoxicological impact, investigations on these are also required. Therefore, the two different but complementary sampling approaches developed in the present study could be implemented in future studies.

Acknowledgments

The authors thank the technical team of the SIAAP and especially Céline Briand, Erwan Garcia Gonzalez, Julien Pouillaude and Anthony Augé. The authors address sincere thanks to the members of the LISA (Laboratoire Interuniversitaire des Systèmes Atmosphériques), especially to Anne Chabas. The PhD of Rachid Dris is funded by the region Ile-de-France Research Network on Sustainable Development (R2DS Ile-de-France). The authors thank François Galgani from the IFREMER (Institut Français de Recherche pour l’Exploitation de la Mer) for his helpful contribution.

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