The fate of microplastics in an Italian Wastewater Treatment Plant

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ABSTRACT

The emerged threat of microplastics (MPs) in aquatic ecosystems is posing a new challenges in environmental management, in particular the civil Wastewater Treatment Plants (WWTPs) which can act both as collectors of MPs from anthropic use and as a source to natural environments. In this study, MP fate was investigated in one of the biggest WWTPs of Northern Italy, built at the beginning of the 2000s and which serves a population equivalent of about 1,200,000, by evaluating their presence at the inlet (IN), the removal efficiency after the settler (SET) and at the outlet (OUT), and their transfer to sludge. Samples were collected in three days of a week and plastic debris were characterized in terms of shape, size and polymer composition using the Fourier Transform Infrared Microscope System (μFT-IR). The number of detected MPs was 2.5 ± 0.3 MPs/L in the IN, 0.9 ± 0.3 MPs/L after the SET and 0.4 ± 0.1 MPs/L in the OUT, indicating a total removal efficiency of 84%. However, considering that this WWTP treats about 400,000,000 L wastewaters/day, the potential release of MPs to the receiving aquatic system would be

approximately 160,000,000 MPs/day, mainly polyesters (35%) and polyamide (17%). Furthermore, a great amount of MPs removed from wastewater was detected in the recycled activated sludge, with 113 ± 57 MPs/g sludge dry weight, corresponding to about 3,400,000,000 MPs deposited in the 30 tons of sludge daily produced by this WWTP. Given the possible re-use of WWTP sludge in fertilizers for agriculture, our results highlight that WWTPs could represent a potential source of MPs also to agroecosystems.

Keywords: microplastics; wastewater treatment plants; effluents; sewage sludge; treatment efficiency

1 INTRODUCTION

Plastic materials have a pivotal role in the modern society and synthetic polymer production increased worldwide in the last decades, reaching 330 million tons in 2016 (PlasticEurope, 2017). Microplastics (MPs), particles smaller than 5 mm in size, are now recognized as an emerged worldwide issue in both marine and freshwater environments (Cole et al., 2011; Eerkes-Medrano et al., 2015; Avio et al., 2017). Ubiquitary distributed and with degradation periods of hundreds of years (Thompson et al., 2004), MPs are easily ingested and have the potential to accumulate in both biota (Browne et al., 2008; Avio et al., 2015a; Paul-Pont et al., 2016; Magni et al., 2018; Pittura et al., 2018) and aquatic food web (Avio et al., 2017; Carbery et al., 2018; Nelms et al., 2018).

Primary MPs, as plastic pellets, are produced directly with microscopic size to be used in air blasting technology, or as abrasive agents in personal care products (PCPs; Cole at al., 2011); their use in cosmetics has been recently banned or limited in some countries such as US, UK and Canada (Conkle et al., 2018). The large majority of environmental MPs are of secondary origin, deriving from degradation of plastic wastes (Cole at al., 2011) or from synthetic cloth washing (Napper and Thompson, 2016).

Land sources contribute to 80% of global MP pollution (Andrady, 2011), and debris collected from urban areas are expected to be treated by civil Wastewater Treatment Plants (WWTPs; Prata 2018), with some exceptions during periods of heavy rainfall for areas with combined sewers. However, WWTPs, being designed to remove organic matter and nutrients from wastewaters, are not efficient in the removal of other contaminants as pharmaceuticals, illicit drugs, heavy metals (Binelli et al., 2014, 2015; Magni et al., 2015) and also MPs that might be discharged in the aquatic environment with potential adverse effects on aquatic organisms (Magni et al., 2016, 2017, 2018). In this context, despite the toxic mechanisms of MPs need clarifications, especially on freshwater species, some studies reported the alteration of the oxidative status, neuro- and energy-related enzyme activity modulation, as well as intestinal damage in aquatic organisms after MP exposure (Avio et al., 2015b; Barboza et al., 2018; Lei et al., 2018; Magni et al., 2018).

The presence of MPs has been reported in the outlet of WWTPs in United States (Estahbanati and Fahrenfeld, 2016; Mason et al., 2016; Michielssen et al., 2016; Dyachenko et al., 2017), Australia (Browne et al., 2011; Ziajahromi et al., 2017), Finland (Talvitie et al., 2017a, b; Lares et al., 2018), Germany (Mintening et al., 2017), Netherlands (Leslie et al., 2017), Sweden (Magnusson and Noren, 2014) and UK (Murphy et al., 2016). Even when the efficiency of MPs removal is very high (72-98%; Murphy et al., 2016; Leslie et al., 2017), due to the great volume of treated wastes, a WWTP with a population equivalent of 650,000 may be responsible for a daily release of 65,000,000 MP debris (Murphy et al., 2016). Consequently, the presence of MPs has been reported in aquatic systems in Europe (Faure et al., 2012; Imhof et al., 2013; Sadri and Thompson, 2014; Wagner et al, 2014; Lechner and Ramler, 2015; Fischer et al., 2016; Guerranti et al., 2017; Imhof et al., 2018; Sighicelli et al., 2018), America, Asia and Africa (Free et al., 2014; Su et al., 2016; Wang et al., 2016, 2017; Anderson et al., 2017; Di and Wang, 2018; Nel et al., 2018).

In addition to the discharge of MPs in inland waters, WWTPs pose another potential threat for the high percentage of MPs (up to more than 90%) which settle on the bottom of WWTP tanks, accumulating in the recycled activated sludge (Carr et al., 2016). This aspect poses a potential threat

78 also for terrestrial pollution, considering that sewage sludge is widely re-used in agriculture as
79 fertilizer worldwide (Mahon et al., 2017) with a request of 50% of the total sludge production in
80 Europe and America, and approximately 125-850 tons of MPs/million inhabitants added every year
81 in European soils (Nizzetto et al., 2016).

82 To provide other information regarding MP input in the European inland waters from
83 WWTPs, the aim of our study was to evaluate the abundance and physical/chemical characteristics
84 of MPs in one of the main WWTPs of Northern Italy, characterizing these particles in wastewaters
85 at different treatment steps, as well as in the recycled activated sludge. To our knowledge, this is the
86 first study aimed to identify the fate of MPs through the entire waste treatment process from an
87 Italian WWTP, to evaluate the efficiency of various treatments in removing these particles, and to
88 assess the overall release of such emerging contaminants.

89

90 ## 2 MATERIALS AND METHODS

91 ### 2.1 *Wastewater and sludge sampling*

92 The WWTP is located in Northern Italy and it represents one of the biggest station and more
93 recent Italian plants, built at the beginning of the 2000s and receiving waters from combined
94 sewers. It serves about 1,200,000 population equivalent and it is equipped with pre-, primary,
95 secondary and tertiary treatments, articulated in screening, grit and grease removal stages,
96 biological treatment, sedimentation (with recycled activated sludge), sand filter treatment and
97 disinfection. The average inlet flow rate of the plant is of about 400,000,000 L/day (the same value
98 of the outlet flow rate), with an average dry weather flow rate of about 18,000,000 L/h and a
99 maximum flow rate in wet weather conditions of 54,000,000 L/h.

100 To assess the presence, removal efficiency and release of floating MPs in the plant,
101 wastewaters were sampled at three different treatment steps: inlet (IN), after the settler (SET), and
102 outlet (OUT). In addition, since MPs can settle on the basis of polymer density (ranging from 0.01-
103 0.05 g/cm^3 for the expanded polystyrene, to 2.20-2.30 g/cm^3 for 25% glass filled

104 polytetrafluoroethylene; Crawford and Quinn, 2017), the recycled activated sludge with a
105 concentration of 7.5 g/L dry weight (dw) was sampled. To reduce the intrinsic variability associated
106 to weather conditions and/or possible changes in the urban release, the sampling was repeated for
107 three days in a spring week, without rainfall, at the same time (between 9 and 11 a.m.). In detail, 30
108 L of surface wastewater were collected every day from each treatment step using a steel bucket;
109 samples were subsequently filtered *in loco* with a suite of steel sieves (ISO 3310-1:2000) with a
110 mesh of 5 mm, 2 mm and 63 µm. Furthermore, 50 mL of recycled activated sludge were collected
111 using a glass beaker.
112
113 2.2 *MP separation from collected matrices*
114 Each wastewater filtered and collected sludge were put in glass bottles with 500 mL of
115 sodium chloride (NaCl) hypersaline solution (1.2 g/cm^3) to separate MPs from particulate matter,
116 exploiting the density gradient (Thompson et al., 2004). The use of NaCl hypersaline solution for
117 MP separation by sediments, similar to sewage sludge, is recommended by the Marine Strategy
118 Framework Directive (MSFD) and suggested when a huge number of samples needs to be
119 processed. Indeed, this method is cheap, widely available and eco-friendly, despite the extraction
120 performance of high density MPs, as plasticized polyvinylchloride (1.3-1.7 g/cm^3) or
121 polytetrafluoroethylene (2.1-2.2 g/cm^3), could be lower than other synthetic polymers (Crawford
122 and Quinn, 2017).
123 Samples were stirred and decanted overnight at 4 °C. Supernatants were then filtered on 8
124 µm cellulose nitrate membrane filters (Sartorius™ 50 mm) using a vacuum pump. In the same
125 filtration apparatus we put 500 mL of Milli Q®, to remove salt crystals, which were then filtered
126 using a vacuum pump across the obtained cellulose membrane filters with collected debris. The
127 organic matter was partially digested by 15% hydrogen peroxide (H_2O_2) for three days at room
128 temperature (RT), maintaining the filters under laminar flow hood to avoid atmospheric
129 contamination by microfibers (Avio et al., 2015a).

130

131 2.3 *MP quantification and characterization*

132 After the organic matter digestion, wastewaters and sludge filters were visually examined
133 under a stereomicroscope (SZM-D equipped with OPTIKAM B5, Optika). All particles suspected
134 to be plastics or particles whose nature was in doubt, as well as all fibers, were manually collected,
135 transferred onto a clean filter and then analyzed, that has allowed to distinguish between plastic and
136 *non*-plastic materials. Despite a rigorous visual protocol performed by experienced researchers, an
137 underestimation of smaller particles is possible, especially those between 30 µm and 10 µm that are
138 more difficult to notice. However, visual examination of particles prior their chemical
139 characterization represents a necessary step to isolate particles from filters, on which it is not
140 possible to completely eliminated organic matter and/or mineral components, without involving
141 more destructive or expensive methods. In addition, the validation of the original method on MPs-
142 spiked samples revealed an elevated yield of recovery, ranging from 78% to 98%, depending on the
143 particle size (Avio et al., 2015a).

144 On the basis of the different origin and abundance of microplastic particles (MPPs)
145 compared to microplastic fibers (MPFs - ribbon-like shape with frayed ends; Almroth et al., 2018;
146 Dris et al., 2018), we decided to consider separately these two types of MPs in the presentation and
147 elaboration of results. MPPs were categorized according to the shape (lines - same thickness in all
148 length with sharp ends, films and fragments; Figure 1) and measured (Optika Vision Lite 2.1,
149 Optika) to be classified into 4 size classes (5-1 mm, 1-0.5 mm, 0.5-0.1 mm, 0.1-0.01 mm).

150 All the collected MPPs and MPFs on membrane filters were characterized using the Fourier
151 Transform Infrared Microscope System (µFTIR; Spotlight 200i equipped with Spectrum Two,
152 Perkin Elmer) to confirm their plastic nature and to identify polymer typologies. FT-IR spectra of
153 individual MPs were acquired in attenuated total reflectance (ATR) mode (32 scans to produce
154 spectra with wavelengths between 600 and 4000 cm^{-1} and resolution of 4 cm^{-1}), analyzed using
155 Spectrum 10 software and compared with libraries of standard spectra. Similarity of measured

156 sample and reference spectrum was accepted only after visual examination of spectra characteristics
157 and with a *Hit Quality Index* (*HQI*) ≥ 0.7 (Klein et al., 2015; Lusher et al., 2015).
158
159 2.4 *Contamination prevention during samples processing*
160 To prevent contamination with other MPPs or MPFs, samples were kept covered as much as
161 possible using glass lids; lab coats, cotton clothing and gloves were worn, both during samplings
162 and laboratory operations. Work surfaces, equipment and manipulation instruments were cleaned
163 with Milli Q® water and alcohol, and checked under the microscope before use. All solutions were
164 filtered twice on 1.2 μm glass fiber filters (Whatman® GF/C 47 mm) to eliminate impurities. In
165 addition, 2 filters were processed as blanks with the same procedure for each experimental
166 condition; during visual sorting, blanks were left exposed to the air the same amount of time as the
167 samples. Despite Simon et al (2018) presented a mass-based assessment of MPs, a similar approach
168 (requiring the precise determination of both minor and major dimensions of irregularly shaped
169 MPs) was considered not reliable in this study. We thus preferred to express our data as MPs/L,
170 similarly many other studies (e.g. Magnusson and Noren, 2014; Talvitie et al., 2015, 2017b;
171 Murphy et al., 2016; Leslie et al., 2017; Mintening et al., 2017; Lares et al., 2018).
172
173 2.5 *Statistical approach*
174 To evaluate the significant differences (*p < 0.05; **p < 0.01) about MP content between
175 the three different treatment steps (IN, SET and OUT), we performed the one-way analysis of
176 variance (one-way ANOVA); each difference, treatment *versus* treatment, was evaluated using the
177 Fisher LSD post-hoc test. For these analyses we used the STATISTICA 7.0 software package.
178
179 3 RESULTS
180 3.1 *Contamination control*

Analyses of blanks showed 1.6 ± 1.0 (mean value ± standard deviation; SD) microfibers of cotton/filter (only natural microfibers were detected in the blanks), corresponding to 30 L of wastewater and 50 mL of sludge; no MPPs were detected in the blanks. These values are much lower than 10% of the overall microfibers average throughout all samples, indicating a good contamination control as suggested by Lusher et al. (2015).

3.2 *MPs in wastewaters*

The characterization by μFT-IR has shown that, of all the particles collected during the visual sorting, the 72% from the IN, the 67% from the SET and the 55% from the OUT, were plastics. The number, shape (lines, films, fragments), size (mm), polymer composition (example of spectra in Figure S1, Supplementary Material) and library matching score (*HQI*) of individual MPPs detected in each of the three sampling days are reported in Table S1 (Supplementary Material). The same results are summarized below as mean value ± SD of the three days.

MPP quantification showed a value of 2.0 ± 0.3 MPPs/L in the IN wastewaters, reduced to 0.6 ± 0.2 MPPs/L after the SET and 0.3 ± 0.1 MPPs/L in the OUT (Figure 2A); similarly, MPFs decreased from 0.5 ± 0.1 MPFs/L in the IN, to 0.3 ± 0.2 MPFs/L after the SET and 0.10 ± 0.03 MPFs/L in the OUT (Figure 2A), for a total amount of detected MPs of 2.5 ± 0.3 MPs/L in the IN, 0.9 ± 0.3 MPs/L after the SET and 0.4 ± 0.1 MPs/L in the OUT (Figure 2A, B). In this context, we observed a significant effect of treatment steps on MP content in wastewaters ($F_{2,6} = 50.3$; $p < 0.01$), with a significant difference in MP concentration between IN and SET ($p < 0.01$) and IN and OUT ($p < 0.01$; Figure 2B); no significant difference has been observed between SET and OUT ($p = 0.07$; Figure 2B).

The total percentage of MP decrease between IN and OUT was 84%, with the greater removal (64%) occurring among IN and SET (Figure 2A). Since this WWTP treats an average of 400,000,000 L of wastewaters/day, the daily inlet and release in surface waters would correspond to about 1,000,000,000 MPs and 160,000,000 MPs respectively. Films were the main shape of MPPs

(73%) which enter in the plant (Figure 3A), followed by fragments (21%) and lines (6%). These ratios change during the wastewater treatments, with more similar percentages of films (36%), fragments (36%) and lines (28%) after the SET, while a greater ratio of lines (41%) and films (38%) followed by fragments (21%) was measured in the WWTP OUT (Figure 3A). The predominant size range of MPPs was 0.5-0.1 mm in all samples, accounting for 36% of total particles in the IN, 58% after the SET and 52% in the OUT (Figure 3B). In this regard, we observed a MP removal, on size basis, of 94% for 5-1 mm and 1-0.05 mm MPs, 77% for 0.5-0.1 mm MPs and 65% for 0.1-0.01 mm MPs.

Table 1 shows that the main MPP classes in the IN were represented by the co-polymer of acrylonitrile-butadiene (40%), followed by polyethylene (17%) and ethylene-propylene (14%). The other two typologies of wastewaters revealed a high concentration of polyesters (23%), polyethylene (13%), polyurethane (13%), polyamide (11%), and polypropylene (11%) after the SET, while the main polymers in the OUT were polyesters (35%), polyamide (17%) and polyethylene (10%).

Regarding microfibers, the ratio of natural microfibers *versus* MPFs was 66 and 34% in the IN, 72 and 28% after the SET, 81 and 19% in the OUT (Table S2, Supplementary Material). Natural microfibers were mainly made of cotton (Table S2, Supplementary Material) and were excluded from the values of MPFs and MPs. Among MPFs, polyesters represented the main polymer class, accounting for 83% of synthetic polymers in the IN, 79% after the SET and 89% in OUT; remaining polymers were polyacrylates (12%, 8% and 11% in the three steps) and polyamide (5% and 13% in the IN and after the SET respectively; Table 2).

3.3 *MPs in recycled activated sludge*

The number, shape, size and polymer composition (example of spectra in Figure S1, Supplementary Material) of MPPs detected in active sludge during the three sampling days are individually given in Table S1 (Supplementary Material) and presented below as average value ±

SD. Among all the particles collected in the visual sorting phase, the 81% resulted to be plastics after μFT-IR characterization.

The number of observed MPs was 59.5 ± 21.6 MPPs/g sludge dw, and 53.3 ± 48.9 MPFs/g sludge dw (Figure 2C), accounting for a total value of 113 ± 57 MPs/g sludge dw (Figure 2C, D). Considering that the investigated WWTP produces about 30 tons/dw of sludge daily, we can derive an estimate of about 3,400,000,000 MPs accumulating each day in the sewage sludge.

As reported in Figure 3A, shapes of MPPs were films (51%), fragments (34%) and lines (15%), while the main size class was 0.5-0.1 mm (54%; Figure 3B). Co-polymers of acrylonitrile-butadiene were the more abundant chemical typologies detected in the sludge (27%), followed by polyethylene (18%) and polyesters (15%, Table 1). Looking at the distribution of polymers among different shapes, acrylonitrile-butadiene represented 32% of films, 26% of fragments and 10% of lines; for this last shape, the main polymers were polyesters (60%), while fragments were mainly constituted by polyethylene (35%; Table 3).

The 35% of the total microfibers collected in the sewage sludge was synthetic and represented only by polyesters (Table 2); the remaining 65% of microfibers were of natural origin (cotton, Table S2, Supplementary Material), and excluded from the count of MPFs and MPs.

4 DISCUSSION

The pivotal result of this study was to demonstrate that millions of MPs both in the OUT and sewage sludge can be released in aquatic and terrestrial ecosystems being used for different purposes such as irrigation and fertilization. These evidences confirm the role of WWTPs as collector of MPs from anthropic use toward natural environment, as recently observed in other WWTPs around the world (Prata 2018).

Analyzing step by step the route of MPs through the WWTP, the mean value of MPs found in the IN (2.5 ± 0.3 MPs/L) was a much lower than those recently reported at the IN of other European WWTPs in UK (15.7 ± 5.2 MPs/L; Murphy et al., 2016, sampling performed with a

"lower size limit" -LSL- of wastewater filtration at 65 μm, the same size of sieve used in this study) and Finland (57.6 ± 12.4 MPs/L; Lares et al., 2018; LSL of 250 μm). Since the MPs found in civil wastewaters derive primarily from PCPs and synthetic textiles (Prata 2018), different habits, weather and season conditions can contribute to the variability of MP concentration in WWTP inlets in different countries. Another explanation to this difference could be the intentional infiltration of waters, e.g. groundwaters, that enter in the sewage system thus diluting the MP concentration in the wastewaters. This phenomenon is particularly present in Italy where this infiltration can reach the 30% of the entire flow rate: in addition, more than 70% of the national sewage system is made by combined sewages that collect domestic wastes with rainwater runoff and industrial wastewaters all in the same pipe (Autorità per l'energia elettrica, il gas e il sistema idrico, 2017). On the basis of these evidences, it is important to take into account that the observed differences in MP concentration can also be related to the use of different MP detection methods, highlighting the importance to establish common standardized protocol(s) for MP monitoring, as well as a uniform unit to express MP abundance to facilitate the data comparison between different sampling sites.

One of the most interesting results of this study was the abundance of co-polymers entering in the WWTP, in particular acrylonitrile-butadiene (40% of total MPs; Table 1). This is called nitrile-butadiene rubber (NBR), it is generally used in automotive seals, gaskets and pipes, but also in textiles, where its application to woven and *non*-woven fabrics improves the finish and waterproofing properties. Characterization of WWTP IN also revealed polyethylene (17%), one of the most common plastic material, and co-polymer of ethylene-propylene (14%), mainly used in automobile parts and in the production of pipe seals (Table 1). To complete the description of MPs entering in the WWTP, it is important to consider that 83% of MPFs were polyesters (Table 2), probably released by synthetic cloth washing (Prata 2018), since a 6 kg wash load release about 700,000 MPFs (Napper and Thompson, 2016).

284 The passage through the oxidative tanks and settler changed the polymer ratio percentage of
285 MPPs after the SET with a decrease of co-polymers and an increase of polyesters (23%),
286 polyurethane (13%), polypropylene (11%) and polyamide (11%); polyesters further increased in the
287 OUT (35%), along with polyamide (17%) and polyacrylates (7%), used in PCPs and paints as
288 adhesive agents. In this context, some MP classes, as epoxy resin (3%), polyvinylchloride (3%),
289 polyoxymethylene (3%) and styrene-isoprene-styrene (3%), were found only in the OUT
290 wastewaters (Table 1). These results could suggest the equipment used in WWTP processes as a
291 potential direct source of polymers towards the aquatic environment, which should thus be carefully
292 considered in future assessments of MP generation and fate. However, this aspect need
293 clarifications considering that the abovementioned MP classes were detected only in the OUT and
294 at a very low concentration of 0.01 MPs/L (Table 1). In the OUT, the MP removal efficiency from
295 wastewater was of 84%, with a 94% of removal between IN and OUT of 5-1 mm and 1-0.5 mm
296 MPs, 77% for 0.5-0.1 mm MPs and 65% with 0.1-0.01 mm MPs. After such removal efficiency, a
297 mean value of 0.4 ± 0.1 MPs/L was still detected, corresponding to 160,000,000 MPs released daily
298 in the receiving water-body. In this context, the large release of MPs in surface waters by WWTPs
299 could provoke adverse effects to aquatic species, considering that under laboratory conditions a
300 mixture of 16,000,000 MPs *per* tank (4,000,000 MPs/L) induced a significant alteration of
301 dopamine level on freshwater mussel *Dreissena polymorpha* (Magni et al., 2018). The release of
302 MPs observed in this study is within the range reported for other American and European WWTPs
303 (see Table 3 in Lares et al., 2018), with the lower value of 0.005 MPs/L observed in Finnish
304 WWTPs (Talvitie et al., 2017b) and the higher concentration of 91 MPs/L in 7 Dutch WWTPs
305 (Leslie et al., 2017). Relating the quantity of MPs in the OUT with the population equivalent of the
306 selected WWTP (1,200,000), we calculated a release of 133 MPs/equivalent inhabitant (*per capita*),
307 comparable with that reported by Murhpy et al., (2016) of 100 MPs/equivalent inhabitant. More in
308 detail, it would seem that the LSL of wastewater filtration influences the quantity of detected MPs
309 in the effluents of various European WWTPs: 0.00825 MPs/L were found in Swedish WWTP with

a LSL of 300 µm (Magnusson and Noren, 2014), 1.05 MPs/L in Finnish WWTP with a LSL of 250 µm (Lares et al., 2018), 0.25 MPs/L in Scottish WWTP with a LSL of 65 µm (Murphy et al., 2016), 0.1-10.05 MPs/L in German WWTPs with a LSL of 20 µm (Mintening et al., 2017), 0.005-13.5 MPs/L in Finnish WWTPs with a LSL of 20 µm (Talvitie et al., 2015, 2017b), and, lastly, from 9 to 91 MPs/L in Dutch WWTPs with a LSL of 0.7 µm (Leslie et al., 2017).

Also the MP removal efficiency of 84% observed in this study is in the same order of other European WWTPs, ranging from 72% to 98% (Murphy et al., 2016; Leslie et al., 2017; Lares et al., 2018). Since the main removal of MPs occurred in the first steps of treatment (64% of MP retention from IN and SET), the grease removal and sedimentation processes are confirmed the pivotal steps involved in the reduction of floating and settling of MPs from wastewaters respectively (Murphy et al., 2016). However, also the sand filters at the end of the WWTP contributed to the treatment performance, decreasing by almost 50% the MP content from the SET (0.9 ± 0.3 MPs/L) to the OUT (0.4 ± 0.1 MPs/L). This is another crucial result in the attempt to define simple and cost-effective treatments to reduce MPs in wastewaters. In this context, Talvitie et al. (2017b) tested the performance of different final stage technologies, observing a MP removal of 97% after sand filters, and a higher activity for membrane bioreactor (99.9%). The great performance of sand filters should, however, be further validated in future studies, since the daily washing water is generally carried in counter-flow, potentially recirculating also MPs.

The description of the MP route through the WWTP cannot ignore the recycled activated sludge produced between IN and SET. The MPs density is not the only factor driving their sedimentation, since also low-density polymers were found in the sludge, as shown for polystyrene (density from 0.01 to 1.05 g/cm^3; Crawford and Quinn, 2017). Fouling by bacteria and other physical/chemical processes can modulate the mechanism of MP floating/sedimentation. In particular, for granular-like MPs, the sedimentation process could be explained by the Stokes law, considering the regime of wastewater flow (laminar or turbulent) around the particles, described by the Reynolds number (Re); for larger MPs with high Re, the shape seems the main factor

influencing the sedimentation (Khatmullina and Isachenko, 2017). Also flocculation phenomena are not negligible in wastewaters, explaining the high presence in the collected sludge of low density polymers and MPs without granular sizes; on the other hand, the aggregation, coupled with the potential occlusion of 63 μm mesh sieve during wastewater filtration, can justify the detection of MPs with 0.1-0.01 mm size. The value of 113 ± 57 MPs/g dw (3,400,000,000 MPs/day) observed in the recycled activated sludge is comparable to other European WWTP sludges, with 8.2-301.4 MP/g dw (Leslie et al., 2017), 186.7 MP/g dw (Talvitie et al., 2017a) and 4.2-15.4 MP/g dw (Mahon et al., 2016) in Dutch, Finnish and Irish WWTPs respectively. Worthy to note, 47% of MPs detected in sludge were characterized by MPFs (53.3 ± 48.9 MPFs/g dw). A recent study (Sillanpää and Sainio, 2017) calculated an annual emission of 154,000 polyester MPFs by washing machines with a number of polyester MPFs released in the first wash that varies from 2.1×10^5 to 1.3×10^7. The release of MPs from the washing machines will be one of the main challenges in the early future to decrease fibers in domestic wastes. Since the ban of production and use of synthetic clothes would be utopic, considering the pivotal role of *non*-disposable plastics in our lifestyle, there are already feasible solutions based on the use of filters for MPF retention in the washing machines (Napper and Thompson, 2016), the recourse to the labelling that certify the good practice in the clothes manufacture and the use of laundry soaps, softeners and the washing cycles more conservative. As previously observed in wastewater samples, also the sludge revealed the presence of MPs polymer classes not detected in the IN wastewaters, reinforcing the hypothesis of the possible release of some polymers, as polystyrene, directly by WWTP structures.

On the basis of these results, we observed a *surplus* of 2,560,000,000 MP/day from the final concentration balance: [(160,000,000 MP/day release by final effluent + 3,400,000,000 MP/day deposited in the sludge) – 1,000,000,000 MPs/day in the inlet]; this difference could be related to the intrinsic variability of wastewaters, to the relatively low volume (30 L) of filtered wastewaters, or to other unaccounted sources like fragmentation of MPs in smaller particles or environmental deposition of MPFs from air.

362 The sludge is re-used in agriculture in many countries as fertilizer, and detection of MPs
363 poses a potential threat for terrestrial environments. The use of sludge in agriculture is actually
364 banned if they contain high levels of toxic pollutants, as heavy metals, but neither European (EU
365 86/278/EEC) nor U.S. (Code 503) legislations put limits for MPs (Nizzetto et al., 2016).
366 Considering the adverse effects reported for MPs on earth worms (Huerta Lwanga, 2016),
367 degradation from micro- to nanoplastics, and their leaching to groundwater (Hurley and Nizzetto,
368 2018), results obtained in this study highlight the need of future evaluations of economic and
369 ecological costs of sludge fate. An additional problem associated to release of MPs in the soils
370 could be their re-translocation in freshwater and marine environment, partially nullifying the
371 WWTP activity.
372
373 5 CONCLUSIONS
374 Our results highlight that, despite the high MP removal efficiency of selected WWTP of 84%, its
375 contribution to MPP and MPF pollution of freshwaters is worrisome, in accordance with results of
376 other European WWTPs. MPs were removed from wastewaters probably mainly in the grease and
377 sedimentation processes, but also the advanced final stage treatments with sand filters significantly
378 contributed to MP retention. Unfortunately, MPs were not completely eliminated by the final
379 effluent, considering that 160,000,000 MPs are released daily in freshwaters by selected WWTP,
380 and their route towards the sludge, a matrix often re-used in agriculture in which we calculated a
381 daily deposition of 3,400,000,000 MPs, provides new elements for regulation of the biosolid
382 disposal in the environment. Future studies are necessary to deeper investigate the distribution,
383 removal and release of MPs by WWTPs in the aquatic environment, considering that the links
384 among physical/chemical behavior of these pollutants and efficiency of various treatment steps still
385 remain to be fully elucidated.
386
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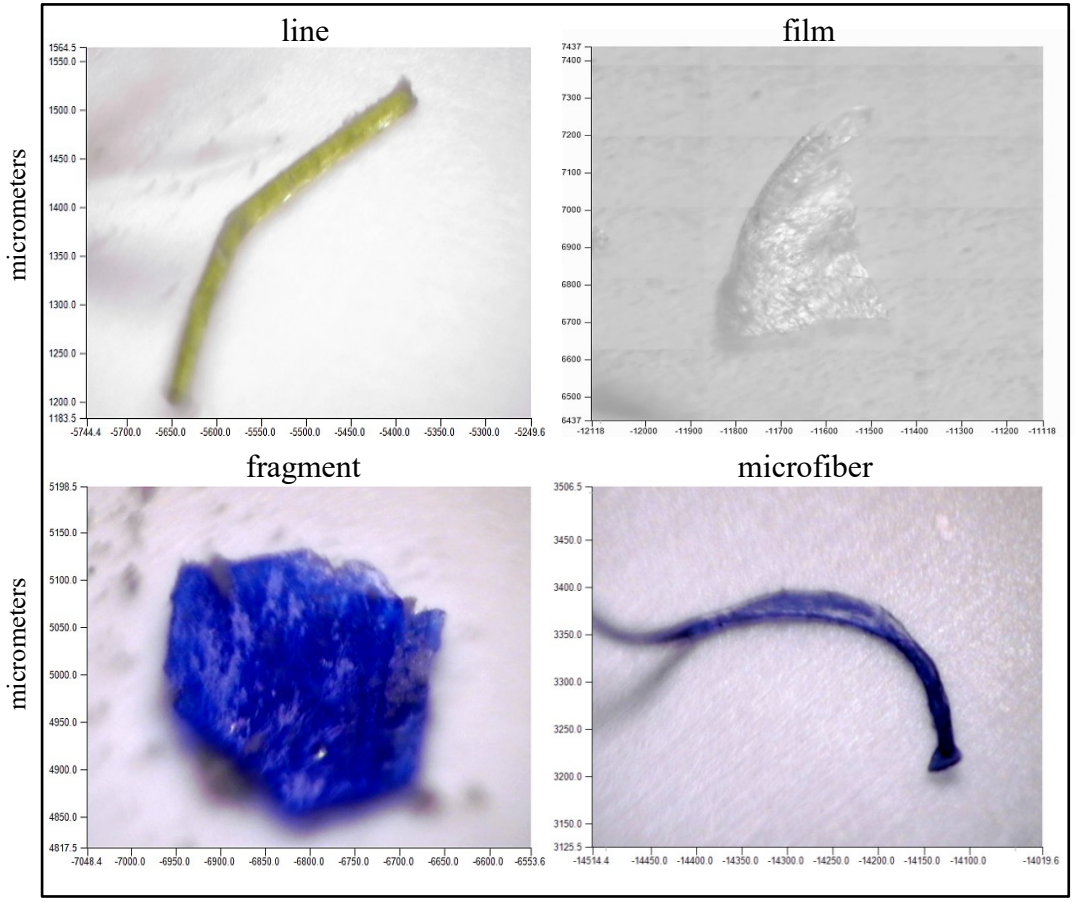
572
573 Captions:
574 Figure 1: MPPs (line, film and fragment) and MPF (microfiber) extracted from both wastewaters
575 and sludge and observed at μFT-IR.
576
577 Figure 2: Mean number of MPPs/L and MPFs/L (A) with the relative total MPs/L amount (B; mean
578 ± SD) in the three different steps of wastewater treatment (IN, SET and OUT); letters indicate the
579 significant differences regarding the MP content in the three different treatment steps (one-way
580 ANOVA, Fisher LSD post-hoc test). (C) Mean number of MPPs/g dw and MPFs/g dw in sludge
581 with the relative total MPs/g dw amount (D; mean ± SD).
582
583 Figure 3: Percentage of shapes (A; lines, films and fragments) and sizes (B; 5-1 mm, 1-0.5 mm, 0.5-
584 0.1 mm, 0.1-0.01 mm) of detected MPPs in both wastewaters (IN, SET and OUT) and sludge.


line
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fragment
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micrometers
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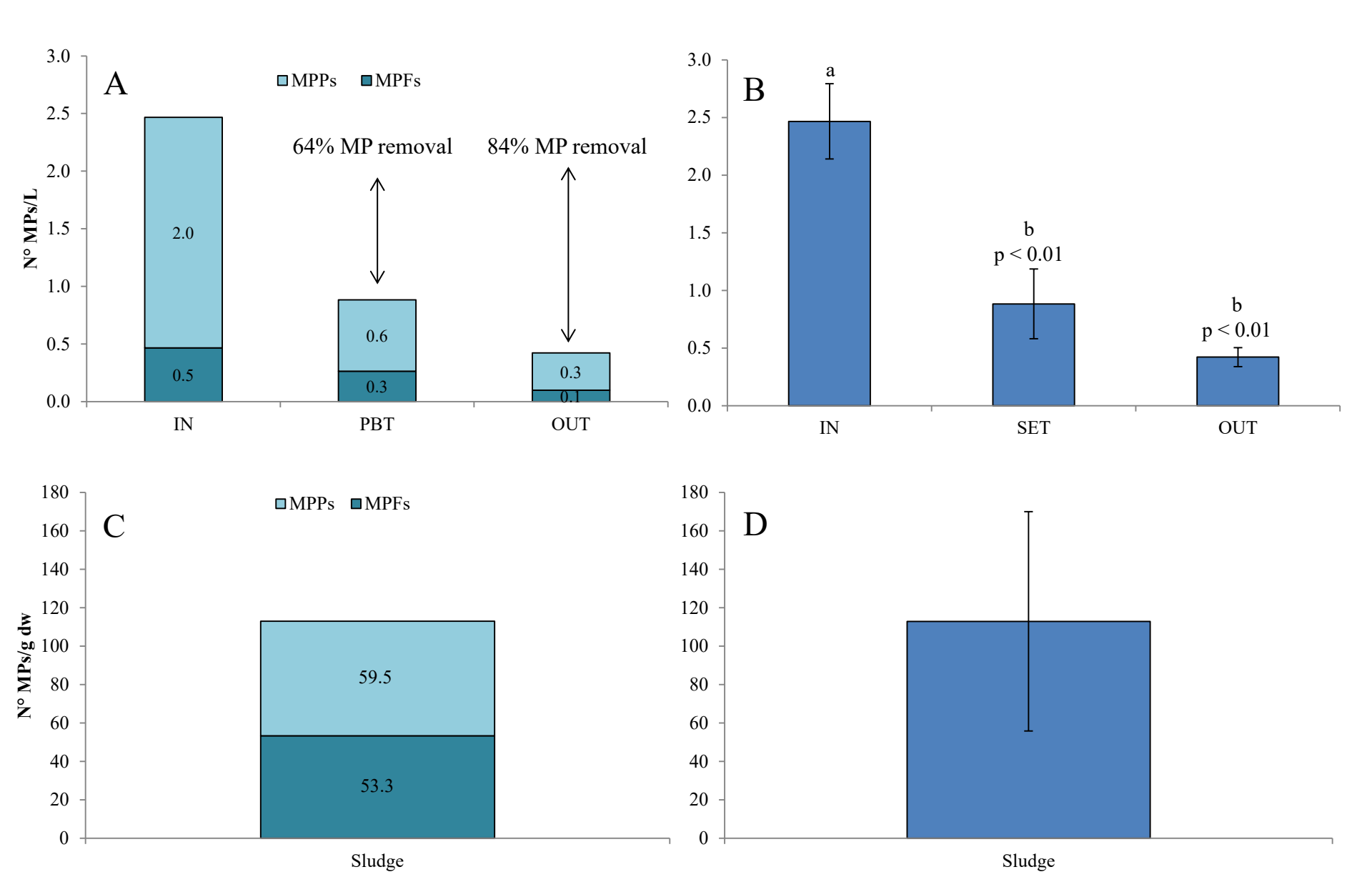
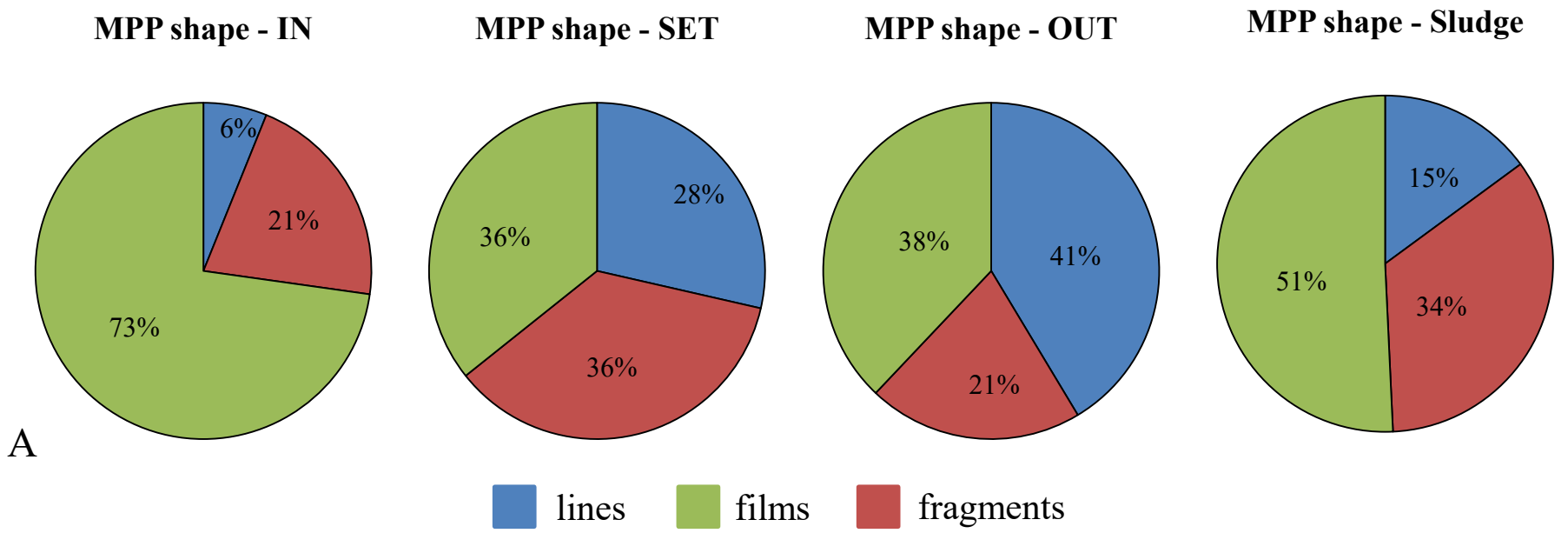
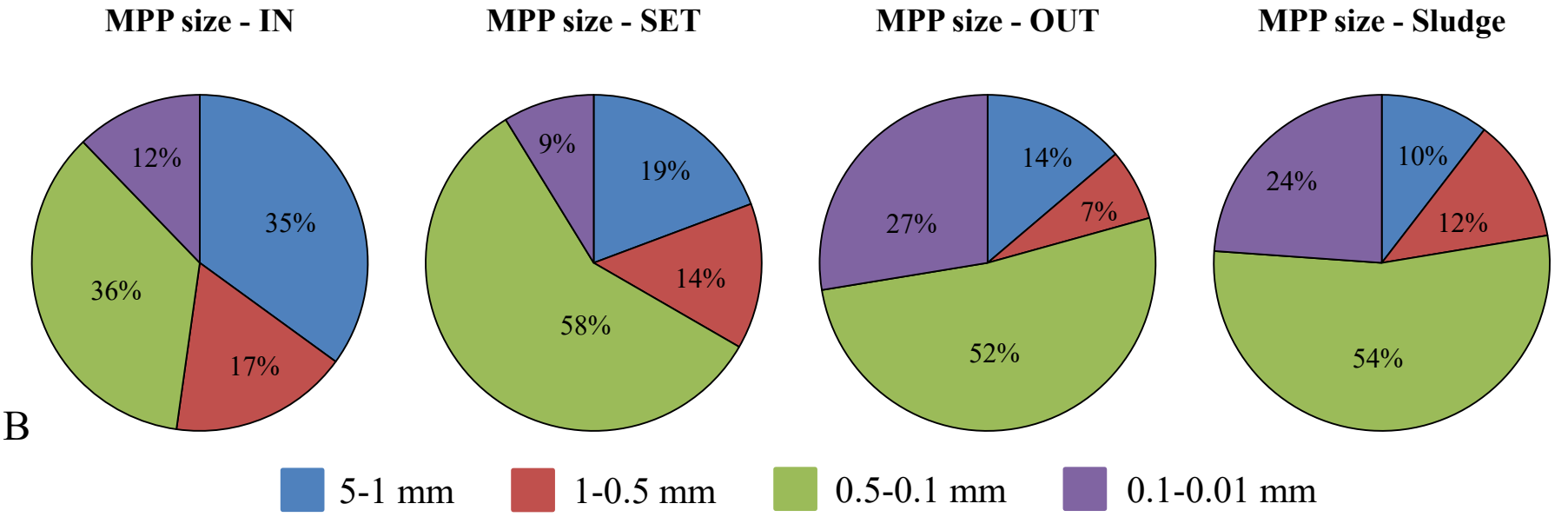

A
MPPs
MPFs
64% MP removal
84% MP removal
N° MPs/L
3.0
2.5
2.0
1.5
1.0
0.5
0.0
2.0
0.5
0.6
0.3
0.3
0.1
IN
PBT
OUT
B
a
b
p < 0.01
b
p < 0.01
IN
SET
OUT
C
MPPs
MPFs
N° MPs/g dw
180
160
140
120
100
80
60
40
20
0
59.5
53.3
Sludge
D
Sludge


MPP shape - IN
MPP shape - SET
MPP shape - OUT
MPP shape - Sludge
6%
21%
73%
28%
36%
36%
38%
41%
21%
15%
51%
34%
A
lines
films
fragments


MPP size - IN
MPP size - SET
MPP size - OUT
MPP size - Sludge
12%
35%
36%
17%
9%
19%
14%
58%
14%
7%
27%
52%
24%
10%
12%
54%
B
5-1 mm
1-0.5 mm
0.5-0.1 mm
0.1-0.01 mm

MPP polymer class	IN %	SET %	OUT %	Sludge %	IN MPPs/30L	SET MPPs/30L	OUT MPPs/30L	Sludge MPPs/50mL	IN MPPs/L	SET MPPs/L	OUT MPPs/L	Sludge MPPs/g
epoxy resin	-	-	3	-	-	-	0.33	-	-	-	0.01	-
polyacrylates	-	2	7	3	-	0.33	0.67	0.67	-	0.01	0.02	1.79
polyamide	2	11	17	6	1.33	2.00	1.67	1.33	0.04	0.07	0.06	3.55
polyesters	4	23	35	15	2.67	4.33	3.30	3.33	0.09	0.14	0.11	8.88
polyoxymethylene	-	-	3	-	-	-	0.33	-	-	-	0.01	-
polytetrafluorethylene	-	-	-	2	-	-	-	0.33	-	-	-	0.88
polyterpene	2	-	3	-	1.00	-	0.33	-	0.03	-	0.01	-
polyethylene	17	13	10	18	10.00	2.33	1.00	4.00	0.33	0.08	0.03	10.67
polypropylene	4	11	-	9	2.33	2.00	-	2.33	0.08	0.07	-	6.21
polystyrene	-	-	-	5	-	-	-	1.00	-	-	-	2.67
polyurethane	3	13	7	3	2.00	2.33	0.67	0.67	0.07	0.08	0.02	1.79
polyvinylchloride	-	-	3	-	-	-	0.33	-	-	-	0.01	-
silicone	-	-	-	2	-	-	-	0.33	-	-	-	0.88
acrylonitrile-butadiene	40	9	3	27	24.00	1.67	0.33	6.00	0.80	0.06	0.01	16.00
acrylonitrile-butadiene-styrene	-	2	-	-	-	0.33	-	-	-	0.01	-	-
ethylene-acrylate	7	7	3	5	4.33	1.33	0.33	1.00	0.14	0.04	0.01	2.67
ethylene-propylene	14	-	-	-	8.33	-	-	-	0.28	-	-	-
ethylene-propylene-diene	2	9	-	5	1.33	1.67	-	1.00	0.04	0.06	-	2.67
ethylene-vinylacetate	1	2	-	-	0.33	0.33	-	-	0.01	0.01	-	-
styrene-butadiene-styrene	1	-	-	-	0.67	-	-	-	0.02	-	-	-
styrene-ethylene-butadiene-styrene	3	-	-	-	1.67	-	-	-	0.06	-	-	-
styrene-isoprene	-	-	-	2	-	-	-	0.33	-	-	-	0.88
styrene-isoprene-styrene	-	-	3	-	-	-	0.33	-	-	-	0.01	-
styrene-vinyltoluene-butylacrylate	1	-	-	-	0.33	-	-	-	0.01	-	-	-

Table 1: Polymer (black labels) and co-polymer (red labels) classes of detected MPPs in both wastewaters (IN, SET and OUT) and sludge expressed as percentage (%), number of MPPs/volume of sampled wastewater (30L) or sludge (50 mL) and number of MPPs/L of wastewater and MPPs/g dw of sludge. The results are presented as mean value of the three days of sampling (see Table S1, Supplementary Materials).

MPF polymer class	IN %	SET %	OUT %	Sludge %	IN MPPs/30L	SET MPPs/30L	OUT MPPs/30L	Sludge MPPs/50mL	IN MPPs/L	SET MPPs/L	OUT MPPs/L	Sludge MPPs/g
polyacrylates	12	8	11	-	1.67	0.67	0.33	-	0.06	0.02	0.01	-
polyamide	5	13	-	-	0.67	1.00	-	-	0.02	0.03	-	-
polyesters	83	79	89	100	11.67	6.33	2.67	20.00	0.39	0.21	0.09	53.33

Table 2: Polymer classes of detected MPFs in both wastewaters (IN, SET and OUT) and sludge expressed as percentage (%), number of MPFs/volume of sampled wastewater (30L) or sludge (50 mL) and number of MPPs/L of wastewater and MPPs/g dw of sludge. The results are presented as mean value of the three days of sampling (see Table S2, Supplementary Materials).

MPP shape	MPP polymer class	Detection (%)
Line polymer class (15%)	polyamide	20
	polyesters	60
	silicone	10
	acrylonitrile-butadiene	10
Film polymer class (51%)	polyacrylates	6
	polyamide	6
	polyesters	6
	polytetrafluorethylene	3
	polyethylene	14
	polypropylene	15
	polystyrene	3
	polyurethane	3
	acrylonitrile-butadiene	32
	ethylene-acrylate	6
	ethylene-propylene-diene	6
Fragment polymer class (34%)	polyesters	9
	polyethylene	35
	polypropylene	5
	polystyrene	9
	polyurethane	4
	acrylonitrile-butadiene	26
	ethylene-acrylate	4
	ethylene-propylene-diene	4
	styrene-isoprene	4

Table 3: Percentage of shapes with the relative polymer (black labels) and co-polymer (red labels) classes of detected MPPs in sludge. The results are presented as mean value of the three days of sampling (see Table S1, Supplementary Materials).