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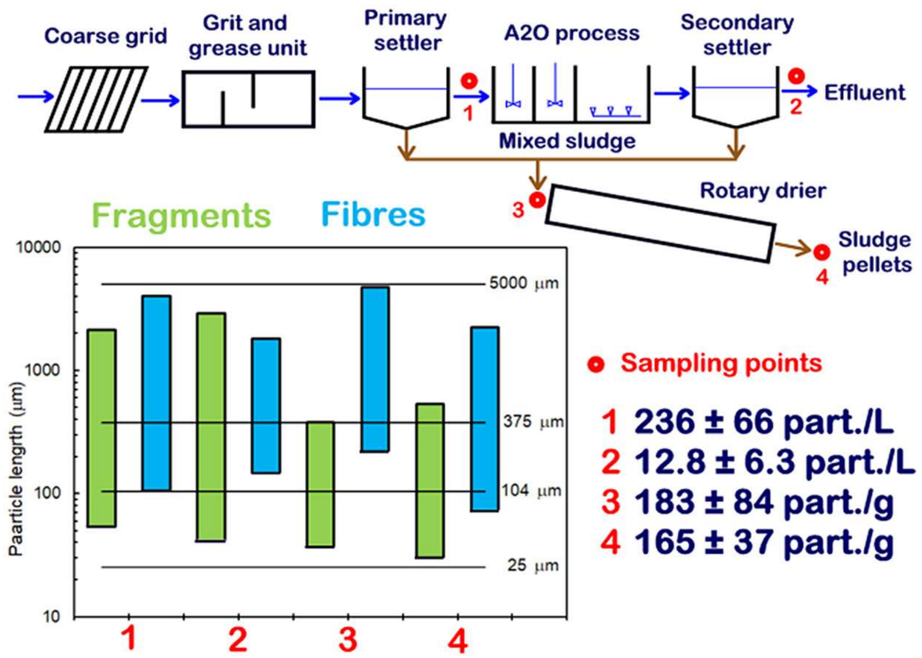

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Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge

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Abstract

This work studied the occurrence of microplastics in primary and secondary effluents and mixed sludge of a WWTP as well as in processed heat-dried sludge marketed as soil amendment. Sampled microparticles were divided into fragments and fibres, the latter defined as those with cylindrical shape and length to diameter ratio >3. We showed the presence of 12 different anthropogenic polymers or groups of polymers with a predominance of polyethylene, polypropylene, polyester and acrylic fibres together with an important amount of manufactured natural fibres. The smaller sampled fraction, in the 25–104 µm range, was the largest in both primary and secondary effluents. Fibres displayed lower sizes than fragments and represented less than one third of the anthropogenic particles sampled in effluents but up to 84% of heat-dried sludge. The plant showed a high efficiency (> 90%) in removing microplastics from wastewater. However, the amount of anthropogenic plastics debris in the 25 µm - 50 mm range still released with the effluent amounted to 12.8 ± 6.3 particles/L, representing 300 million plastic debris per day and an approximate load of microplastics of 350 particles/m^3 in the receiving Henares River. WWTP mixed sludge contained 183 ± 84 particles/g while heat-dried sludge bore 165 ± 37 particles/g. The sludge of the WWTP sampled in this work, would disseminate 8×10^{11} plastic particles per year if improperly managed. The agricultural use of sludge as soil amendment in the area of Madrid could spread up to 10^{13} microplastic particles in agricultural soils per year.

Keywords: Microplastics; Wastewater treatment plants; Sewage sludge; Wastewater effluent; Removal efficiency

Introduction

Microplastics are in the spotlight and a subject topic of continuous press releases reporting their presence in the most diverse environments (Zhang et al., 2019). Initially considered a local, and mostly aesthetic issue, scientists have now recognized plastic pollution as a major global pollution threat, and a key priority for research (Napper and Thompson, 2019). Plastic pollution has been largely studied in marine environments where plastic debris are ubiquitous in surface water and sediments (Clark et al., 2016; Ling et al., 2017). The presence of microplastics has also been reported in essentially all freshwater ecosystems (Dris et al., 2018; Li et al., 2018a). In addition to aqueous environments, the presence of microplastics has been reported in agricultural soils with potential risks for food chains (Corradini et al., 2019; Ng et al., 2018). Finally, both indoor and outdoor air have been proved to bear microplastics, mostly fragments or fibres, which may travel long distances transported by winds (Dris et al., 2016; Gasperi et al., 2018). The experimental evidence forced to consider microplastics as a new type of emerging contaminant potentially threating environment and human health. The issue reached government authorities and the European Parliament recently issued a resolution proposal (TA/2019/0071) stressing the need for addressing microplastics pollution in the context of wastewater treatment.

It is well-known that most plastic debris originate from land sources essentially due to improper waste management (Andrady, 2011; Yan et al., 2019). Even considering the marine environment, it has been estimated that 80 % of plastic debris originate inlands (Li et al., 2016). In Europe, 64 million tonnes of new plastics were marketed for new uses, but only 8.4 million tonnes (13 %) were recycled in 2017, the rest constituting a potential pollution source (PlasticsEurope, 2018). Once released, plastics undergo complex degradation processes leading to their progressive disintegration into smaller pieces (Eerkes-Medrano and Thompson, 2018; González et al., 2016). Due to the marine origin of plastic debris research, there is an almost consensus between researchers in using NOAA guidelines to classify plastics. Plastic fragments below 5 mm are commonly defined as microplastics in what turned to be an international standard (Edo et al., 2019; Gago et al., 2016). The lower size limit is not clearly established as the boundary with nanoplastics is still unclear. Gigault et al. proposed to define nanoplastics as fragments < 1000

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nm with colloidal behaviour if coming from the degradation of larger particles (Gigault et al., 2018). Although the experimental evidence is limited, it is generally considered that lower sizes, including the smallest fractions of microplastics and nanoplastics may constitute a major threat for the environment (Andrady, 2011; González-Pleiter et al., 2019). It has been estimated that environmental samples contain much less small microplastics than expected, suggesting a possible accumulation in the biota (GESAMP, 2016). Additionally, small particles can cross lung or gastrointestinal epithelia and translocate to different tissues, although the experimental evidence for it is still limited (Ribeiro et al., 2019).

Plastics reach the environment through point sources or diffuse pollution. Diffuse or non-point sources include escapes from industrial plastic production facilities, runoffs from urban, agricultural or industrial areas, and atmospheric deposition (Vermeiren et al., 2016). It has been estimated that rivers transport between 1.15 and 2.41 million tonnes of plastic to worldwide oceans every year (Lebreton et al., 2017). Wastewater treatment plants (WWTP) have been identified as an important point source for microplastics emission, particularly regarding fibres (Browne et al., 2011). The sources of plastic debris reaching WWTP are cosmetics and personal care products, the wearing of plastic products like textiles, and car tyres or road paints. Through domestic wastewater or drainage systems, microplastics reach WWTP and may end up either discharged into waterbodies or dispersed with sludge (Ngo et al., 2019). Some studies showed that microplastic removal rates in WWTP are high, typically over 95%, but even if most microplastics are removed with sludge the remaining fraction still represents a huge amount (Lv et al., 2019; Sun et al., 2019). Moreover, the sludge produced in WWTP is frequently reused in agriculture as soil amendment because of its good properties as fertilizer (Gherghel et al., 2019). Both water and sludge reuse practises, although responding to the concept of circular economy, reintroduce microplastics into the environment and may constitute an important environmental threat (Gatidou et al., 2019). Overall, there is still a considerable knowledge gap about the role of WWTP in the cycle life of small plastic particles and fibres and a debate exists trying to elucidate the extent to which water discharges and sludge management and use contribute to the accumulation of microplastics in environmental compartments (Carr et al., 2016).

This work aims at shedding light on the fate of microplastics in a conventional wastewater treatment facility operating under Anaerobic-Anoxic-Oxic (A2O) technology. Samples were taken from the outlet of primary and secondary settlers and from sludge as well as from the pellets of heat-dried sludge marketed as soil amendment. We assessed the presence of microplastics in wastewater and sludge, compared the results with previous reports, and discussed the potential risks of microplastics to soil and freshwater ecosystems. In this work, we paid attention to manufactured natural polymers, a type of anthropogenic pollution with important similitudes with plastic microfibres. We also studied sludge, which wet or in the form of heat-dried pellets, constitute a way for microplastics dispersion into the environment that could require more stringent regulatory measures.

2. Materials and methods

2.1. Wastewater and sludge

Sampling was conducted during three different days in three different months during the Spring of 2019 at a WWTP located near Madrid (Spain). The installation is designed to treat 45 000 m^3/day and consists of a primary clarifier followed by A2O biotreatment. The effluent discharges to the Henares River in the Tagus basin. The mixed sludge from the clarifiers is dewatered prior to anaerobic digestion to produce biogas used to generate electricity and heat in the WWTP. Digested sludge is further heat-dried to 300 °C in a rotary drier and sold for agricultural use, mainly in neighbouring areas. During the sampling period, the average flow rate of untreated wastewater reaching the plant was 28 400 m^3/day. In the same period, the plant generated an average of 851000 m^3 of treated wastewater and 560 t of stabilized sludge per month. Heat-dried sludge is marketed in small pellets with a diameter of about 5 mm. The analysis of pellets yielded 92.4 % dry matter and 60.8 % organic matter. Heat treatment eliminates all biological activity so that no colony forming bacteria were detected in dry sludge. The content of metals allows its use as fertilizer up to 5 t per hectare and year according to the current local regulations.

2.2. Sampling

Water samples were directly collected from settler effluents, immediately transported to the laboratory and filtered through a sequence of three stainless steel meshes with 25 µm, 104 µm and 375 µm, opening sizes. Filters were then placed in glass beakers and put in contact with H_2O_2 (33 % w/v) at 50 °C for 20-24 hours to remove organic matter and prevent microorganism growth after which they were rinsed with Milli-Q water to remove residual H_2O_2 (Gies et al., 2018). The digestion time was chosen as optimum to ensure the complete removal of organic matter in the most difficult samples without affecting the integrity of microplastics. The samples were filtered through the same sequence of 375-104-25 µm meshes. All filters were dried and stored in previously cleaned glass Petri

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dishes prior to use. Wet sludge was collected from the anaerobic digestor. Dry sludge pellets, as marketed for soil amendment, were collected from the storage facilities. Samples of wet sludge and dry pellets (1 g) were treated with 30 mL H_2O_2 (33 %) at 50 °C as indicated before. After H_2O_2 treatment the suspension was diluted with NaCl, 1.2 kg/L, kept under stirring for 24 h and allowed settling for another 24 h, after which, both supernatant and sediment, without any loss or particles during the process, were inspected filtered and inspected as indicated before.

Glass material was used whenever possible and controls were taken before and during sampling. Two-litre Pyrex glass bottles were used to collect water from the primary settler. For the outlet of the secondary settler, 25 litre white high-density polyethylene containers were used. All recipients were thoroughly cleaned with 10 % HCl at least three times. Glass beakers, glass Petri dishes and steel tweezers were also cleaned in the same way to ensure the absence of plastic contamination. All material was covered with aluminium foils until use. The filtering system consisted of a Millipore Stainless 47 mm pressure holder. The stainless steel filters used were cleaned and heated to 500 °C prior to their use to remove all possible rests of organic matter. The integrity of steel mesh was checked by optical microscopy.

Controls to assess possible cross-contamination were performed by rinsing all used material and glassware elements with Milli-Q water, which was subsequently filtered through 25 µm opening size meshed and checked for the possible presence of microplastics. During sampling, filtering, observation, and measurement tasks, open mesh filters were kept open to quantify possible contamination from the surrounding environment. Clothing was also controlled by using non-typical bright colours like blue or orange preferable 100 % cotton.

2.3. Analytical procedure

Visual inspection and the counting of microparticles were performed using a stereomicroscope Euromex-Edublue equipped with USB digital camera and ImageFocus 4 software. The whole set of particles included plastic materials, natural fibres with evidence of anthropogenic process and natural materials as well as a residual category of non-identified fragments of fibres. Based on visual evaluation, a subsample of each typology was selected and derived for identification by means of Fourier-transform infrared spectroscopy (FTIR). FTIR spectra were recorded using a micro-FTIR a Perkin-Elmer Spotlight 200 Spectrum Two apparatus with mercury cadmium telluride (MCT) detector, which allowed high sensitivity measurements in the mid-infrared region. Samples were placed on KBr pellets and measuring parameters for the micro-transmission mode were as follows: spot 50 µm, 20 scans, resolution 8 cm^{-1}, spectral range 4000-550 cm^{-1}. The spectra were compared with a built-in database or with reference spectra created on purpose during this study. The spectra were compared in the Omnic 9 software obtained from Thermo Scientific with a built-in database and with reference spectra created on purpose during this study. Positive matching between samples and database or standards was assessed when a minimum of 70% similitude was obtained.

2.4. Statistics

Confidence intervals (CI) were computed at 95 % level with at least three replicates for each typology. For FTIR identification, Pearson correlation was used to assess matching between samples and database or standards.

3. Results

3.1. Occurrence of fragments and fibres in wastewater and sludge

According to typology, microparticles were first divided into fragments (small particles, films or beads) and fibres. For the purpose of our study, we defined fibres as microparticles with cylindrical shape and length to diameter ratio > 3 according to the definition of ECHA proposal to restrict intentionally added microplastics (ECHA, 2109). The samples showed a diversity of plastic fragments of different shapes identified as secondary plastics. There were also many white or transparent fibres, further identified as cellulosic material and abundant coloured fibres. Fig. 1 shows a selection of fragments recovered from wastewater and sludge. A significant feature of these samples is the wide variety of colours, consequence of their anthropogenic origin. A total of 14 different colours were found, and, as explained below, some were clearly identified as the product of dyeing natural fibres during manufacturing processes. This represents a wider range compared with other reported results (Bayo et al., 2020; Liu et al., 2019; Talvitie et al., 2017).

Microparticles were sorted in three size categories by means of steel meshes of 25 µm, 104 µm, and 375 µm size opening as follows: 25-104 µm; 104-375 µm and > 375 µm, < 5 mm). Both in primary and secondary effluents, size distributions were dominated by lower sizes: 54% (25-104 µm), 34 % (104-375 µm), and 12 % (> 375 µm, < 5 mm) for the primary and 48 %, 28 % and 23 % respectively for the effluent of A2O settler. The results indicate that most microparticles corresponded with the smallest measured fraction. In the primary effluent fragment length (larger dimension as measured from microscopy images) ranged from 53

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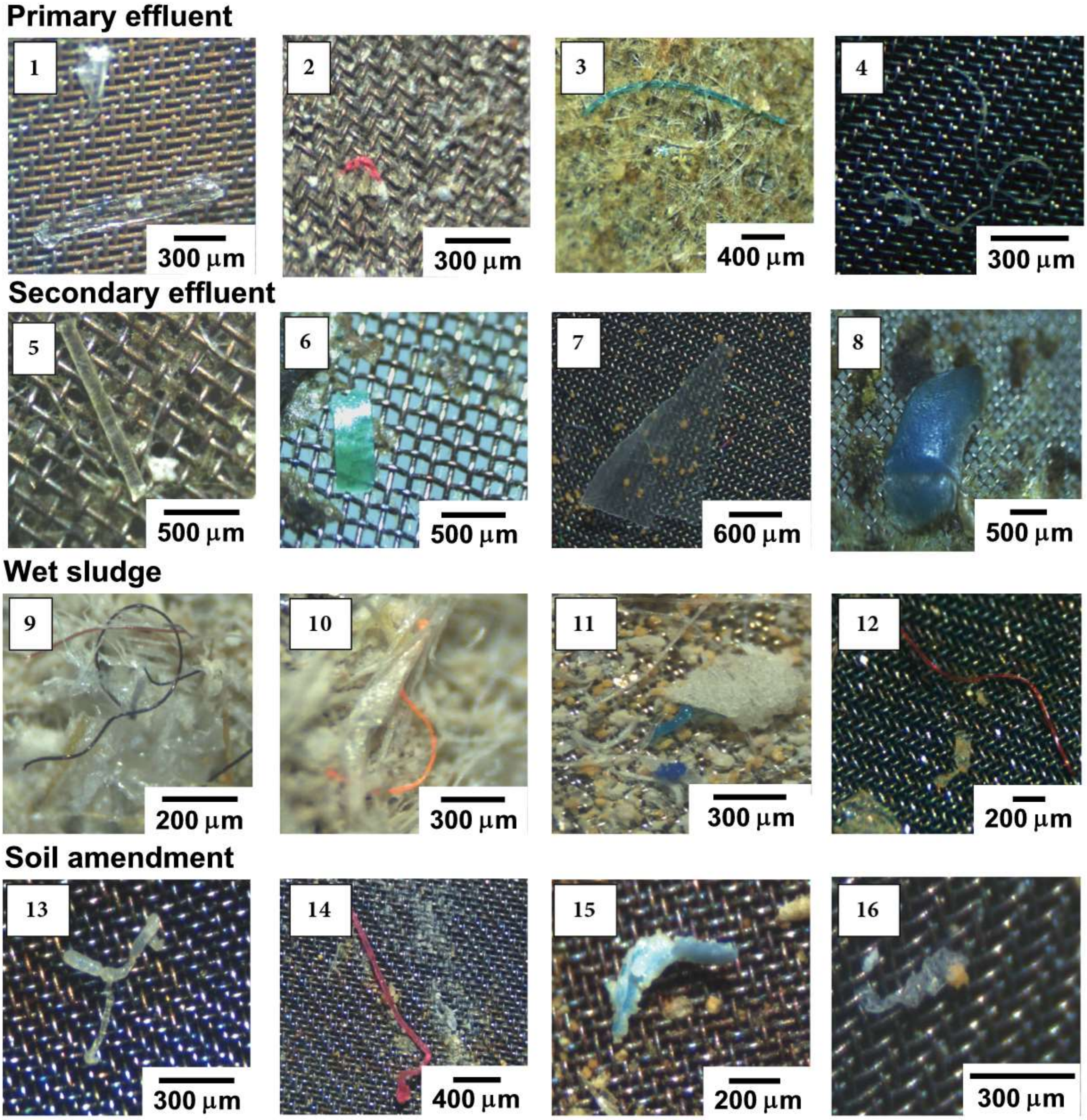

Figure 1. Microplastic particles from visual sorting (before FTIR analysis). 1-4: Samples from primary effluent (1: Transparent fragment and film, 2: Red fragment, 3: Blue fibre, 4: Transparent fibre); 5-8: Samples from secondary effluent (5: Transparent filament, 6: Green fragment, 7: Transparent film, 8: Blue fragment); 9-12: Samples from wet sludge (9: Black and red fibres on a white mass of cellulose fibres; 10: Orange fibre, 11: Blue fragments, 12: Red fibre); 13-16: Samples from soil amendment (13: Transparent fibre, 14: Red fibre, 15: Blue fragment, 16: Transparent fragment)

µm to 2100 µm (0.21 mm) whereas width (second dimension from projected images) were in the 18-900 µm range. Projected sizes in fibres range from 104-4000 µm (length) and 5-34 µm (width). In the secondary effluent, fragment length was in the 41-2890 µm range, while width varied from 34-1230 µm (0.33 mm). Size for fibres ranged from 144-1824 µm (length) and 8-89 µm (width). Fragments in wet sludge were in the 36-377 µm length range and 22-36 µm width range, like those found in heat-dried sludge use as soil amendment (29-533 µm length and 11-369 µm width). Fibres in wet sludge were in the 213-4716 µm (length range) and 5-34 µm (width range), while the figures for heat-dried sludge were 71-2224 µm (length range) and 7-58 µm (width range). Fig. 2 summarizes these results with relative abundances calculated for the larger dimension. Aspect ratio defined as the ratio between length and width for projected images for fragments

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was 2.0 and 1.9 for primary and secondary effluents, and 1.7 and 2.1 for wet and heat-dried sludge, respectively. For fibres the average values were 59 and 58 for wastewater (primary and secondary) and 101 and 46 for sludge (wet and heat-dried). This difference made it possible to unambiguously classify particles as fragments or fibres.

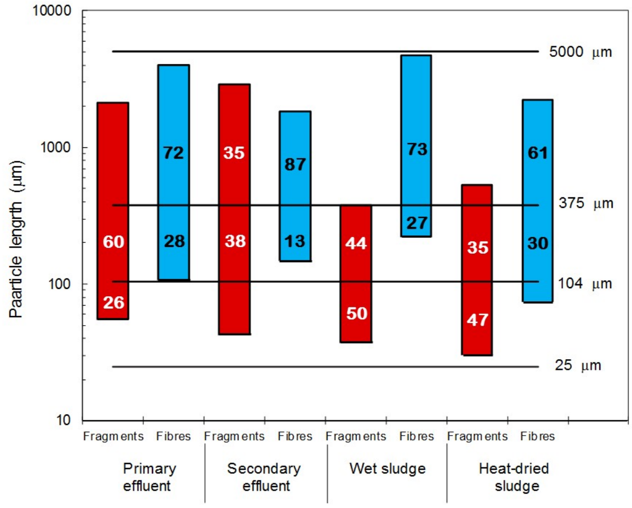

Figure 2. Range of sizes (length, µm) in primary and secondary effluents and in wet and heat-dried sludge. (The numbers represent percentages of abundance within each category.)

Particle and fibre counting in the effluent of the primary settler yielded 451 ± 106 microparticles (fragments and fibres)/L, the error indicating the standard deviation among samples. Clear (white and transparent) fragments and fibres represented 60 % and 28 % of the total amount of microparticles, while coloured fragments and fibres represented 9 % and 3 % respectively. The effluent from the secondary settler showed less fragments and fibres, with total amount of 26 ± 14 microparticles/L, which corresponded to 94 % removal efficiency in the secondary settler. They mainly consisted of clear (56 %) and coloured fragments (24 %), while fibres (15 % clear, 5 % coloured) were in lower amounts. WWTP sludge (mixed from primary and secondary settlers) showed an average of 314 ± 145 microparticles per gram of dry matter. In contrast to wastewater, mixed sludge was dominated by fibres both clear (white or transparent) and coloured. With respect to the total amount of microparticles, clear fibres represented 47 %, clear fragments 31 %, coloured fibres 15 %, and coloured fragments 7 %. Heat-dried pellets used as soil amendment carried a total amount of microparticulate particles (fragments and fibres) of 302 ± 83 microparticles per gram of amendment, very similar to WWTP sludge. Its distribution yielded clear fibres (67microparticles per gram of amendment, very similar to WWTP sludge. Its distribution yielded clear fibres (67 %), coloured fibres (17 %), white fragments (11 %) and coloured fragments (5 %).

3.3. Micro-FTIR identification

A subsample of 172 microparticles from wastewater and sludge were carefully inspected by micro-FTIR. The identification revealed plastic materials (n = 77), natural substances with evidence of anthropogenic manufacturing processes (n = 27), natural materials (mainly cellulose, n = 25), and non-identified substances (n = 43). Manufactured natural polymers refer to materials based on natural constituents like cotton or wool that display evidences of having been manufactured to modify their properties, notably the presence of dyes. Non-identified materials refer spectra clearly showing non-plastic materials or with correlation matching < 70%. Fig. S1 (Supplementary Material, SM) shows particle distribution among these categories FTIR characterization. In wastewater, both from primary and secondary settlers, the results were similar, with a percentage of plastics representing 35-40 % of the total amount of microparticles analysed. In sludge, either wet sludge or heat-processed pellets, the percentage of microplastic particles identified raised to about 60 %, with a considerably lower percentage of particles not identified with enough evidence.

A total of 12 different anthropogenic polymers and groups of polymers were identified in the samples which are listed together in Fig. S1. Among identified microplastic particles, 51 % were fragments (and 49 % fibres). In case of manufactured natural polymers 62 % were fibres. The main polymers found in the primary effluent were, in decreasing occurrence: polyester fibres, polyethylene (PE), dyed cotton, polypropylene (PP) and cellophane fibres. In the secondary effluent, PE outnumbered dyed cotton, polyester fibres identified as PET, PP, and cellophane. Polyester fibres prevailed in sludge followed by acrylic fibres, PE, dyed cotton and PP. Other polymers identified were polymethyl methacrylate (PMMA), polycaprolactone (PCL), polyurethane (PU), and polystyrene (PS). The density of the polymers identified is indicated in Table S2 (SM). Most of them correspond to buoyant particles or are manufactured as foams with lower density than pure polymers. Fig. 3 shows typical IR spectra of some sorted plastic materials, namely a PE fragment, a PP filament and a polyester fibre, together with the standards used for identification (coincident peaks are highlighted for the sake of clarity; spectra from other sampled polymers are shown in Fig. S2, SM).

4. Discussion

Particle counting and the results of the identification of plastics and manufactured natural materials were combined to calculate the amount of plastics and all artificial materials in wastewater and sludge (Table 1).

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The total concentration of microplastic particles was 171 ± 42 particles/L in the primary effluent that got reduced to 10.7 ± 5.2 particles/L at the outlet of the secondary settler (coincident in this case with WWTP final effluent). The microplastic particles in sludge amounted to 133 ± 59 particles/g (of dry matter), not significantly different from the figure obtained in heat-dried sludge used as soil amendment. Overall, FTIR analyses confirmed the presence of the most common

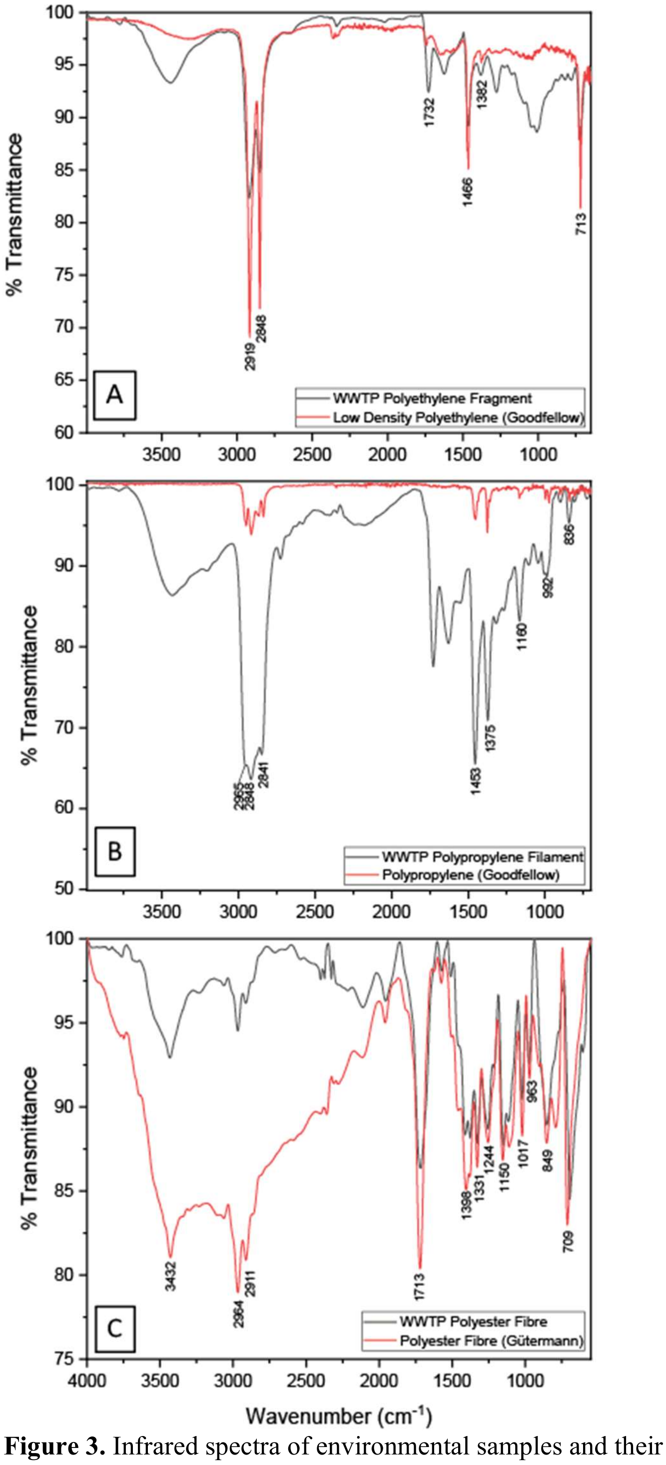

Figure 3. Infrared spectra of environmental samples and their corresponding reference standards for polyethylene (A), polypropylene (B), and a polyester fibre (C).

plastic materials including PE, PP, and polyesters and acrylic fibres as well as natural manufactured fibres in line with data published elsewhere (Magni et al., 2019; Zambrano et al., 2019). The variability observed in literature data is not generally high and can be mostly interpreted in terms of sociodemographic variables (Liu et al., 2019). The presence of low density polymers, like PE in sludge samples agrees with data reported elsewhere (Mahon et al., 2017; Mintenig et al., 2017). The reason may be that microplastics get trapped into flocs favoured by their los polarity and higher sorption potential, which favours their partitioning to the sediment phase.

Manufactured natural polymers were identified in all cases, although in lower amounts than microplastic particles. It is interesting to note the difficulty to accurately identifying certain fragments or fibres as natural or manufactured. Fig. S3 (SM) shows the spectra of three samples identified as cellulose. The FTIR spectra shows the typical bands from cellulose based materials. Spectra are similar and the most common bands for all spectra are the broad band at about 3600-3200 cm^{-1} that corresponds to the OH stretching vibration, the absorption at 2900 cm^{-1} due to the C-H stretching of alkyl groups, and the intense absorption at 1000-1080 cm^{-1} that corresponds to C-O stretching vibration (Reddy et al., 2016). Once computed manufactured natural materials as anthropogenic litter, the total quantity of microparticles discharged by the WWTP amounted to 12.8 ± 6.3 particles/L with the effluent and 183 ± 84 particles/g with sludge.

The role of WWTP in contributing to river and marine pollution has been studied in the past and identified as a potential major driver of plastic pollution in aquatic environments (Mourgkogiannis et al., 2018). Some authors reported removal efficiencies for WWTP of up to 98-99 % for particles in the tens of micrometre range (Gies et al., 2018; Lares et al., 2018; Murphy et al., 2016; Ziajahromi et al., 2017). Other authors reported lower removal rates (Liu et al., 2019; Talvitie et al., 2017). Differences in sampling points and size ranges make it difficult to accurately compare results. A summary of recently reported data is shown in Table 2, which indicates the values concentrations of microplastics in raw wastewater, effluents from primary and secondary settlers and WWTP final effluent. Efficiencies are reported for the whole plant and, in brackets, comparing primary and discharged effluent. The results of removal efficiency obtained in the present study (93.7 %) compared the outlet the primary settler and the final discharged effluent and were reasonably aligned with other published data, particularly when the range of sample sizes is similar (Ziajahromi et al., 2017). Noteworthy, there is

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Table 1. Concentration of artificial microparticles in the samples.

	Primary Effluent	Secondary Effluent	WWTP Sludge	Soil amendment
	particles/L	particles/L	particles/g	particles/g
Plastic particles	171 ± 43	10.7 ± 5.2	133 ± 59	101 ± 19
Manufactured natural materials	66 ± 28	2.1 ± 1.1	49 ± 26	64 ± 20
Total anthropogenic particles	236 ± 66	12.8 ± 6.3	183 ± 84	165 ± 37

considerable dispersion in the reported results for the removal of microplastic particles in the primary screening and clarification stages, which range from 20-40 % to > 99 %. Table 2 shows reported concentrations in the final effluent, which range from < 1 particle/L to 28.4 particles/L with our figure, 10.7 particles/L, in between. The fact that microplastics are not completely retained with sludge, results in considerable emissions amounting to figures in the range of 106-108 particles emitted per day and per WWTP. Considering the average flow of raw wastewater during the sampling period (28400 m3/day), our data indicated a discharge of about 300 million microplastic particles (> 25 µm) per day to the Henares River. This value is comparable to other in which the particle size range was similar (Liu et al., 2019; Talvitie et al., 2017; Yang et al., 2019). Considering the historical average flow of Henares River, about 10 m^3/s, the discharge we measured (one of the hundreds of WWTP discharging to Tagus basin, 154 only in Madrid) represented a contribution of 350 particles/m3 of microplastics.

Microplastic particles concentrate in the sludge recovered from clarifiers. Therefore, any uses different from incineration inevitably result in their dissemination into the environment (Weithmann et al., 2018a). Our study showed a concentration of 133 ± 59 microplastics per gram of dry sludge and 101 ± 19 microplastics per gram in the heat-dried sludge used as soil amendment. It is noteworthy that processing sludge at temperatures reaching 300 °C did not significantly alter microplastic particles. A summary of recent research can be found in Table 3 that shows considerable variability among authors, with concentrations in sewage sludge ranging from a few to several hundred of particles per gram of dry sludge. Such high variations could be attributed to differences in the efficiency of the mechanisms involved in microplastics removal, essentially the skimming of floating low-density debris and their capture into settling flocs (Carr et al., 2016; Gatidou et al., 2019).

When sludge, either wet or heat-dried (biologically inactivated) is improperly managed or used as soil amendment, microplastics find a route towards the environment. Table 3 shows the estimated number of plastic microparticles potentially emitted by different WWTP though sludge. It has been estimated that 86 % of the 8 x 10^6 tons of sludge generated in China become released into the environment representing the emission of 1.6 x 10^{14} microplastic particles/year (Li et al., 2018b). Our results showed that the WWTP would emit 8 x 10^{11} plastic particles per year, within the broad range limited by the values of Mintenig et al. (2017) and Magni et al. (2019) for German and Italian WWTP respectively (Table 3).

The spreading of microplastics into agricultural soils as fertilizer is a cause for concern (Weithmann et al., 2018b). Microplastics can be found in agricultural soils that had undergone sludge applications in the past showing their persistence (Corradini et al., 2019). The production of sludge pellets in the area of Madrid accounts for roughly 100 000 t/year, all of them marketed for use in agriculture, mostly in neighbouring places and spread over a surface of about 14 200 ha (Comunidad-de-Madrid, 2018). Our results showed that more than 10^{13} microplastic particles are disseminated every year in agricultural soils only in Madrid, where the use of sludge is limited to 5 t/ha per year (dry sludge). Our results also showed that the size of particles in sludge was smaller than in wastewater effluent with almost all particles below 375 µm. Additionally, there was a predominant presence of fibres in sludge (31 % and 20 % of the anthropogenic particles in wastewater from primary and secondary settlers, and 62 % and 84 % in wet sludge and heat dried-sludge, respectively). The higher amount of fibres in sludge has been reported before and even proposed as indicators of historical spreading of wastewater sludge (Corradini et al., 2019). Zubris

Table 2. Overview of previous studies used for comparison with this work.

Reference	Daily flowrate, m^3, (Population served/equivalent)	Size rage sampled, lower, upper	Microplastics, particles/L	Removal efficiency, %*	MP discharge, particles/day	Type of facility	Location and discharge
(Murphy et al., 2016)	2.6×10^5 (650 000)	11 µm 65 µm	15.70 ± 5.23^a 3.40 ± 0.28^b 0.25 ± 0.04^d	98.4 (92.6)	6.5×10^7	Primary and secondary treatments	River Clyde, Scotland
(Mason et al., 2016)	ranges: 2.35×10^3-3.82×10^5 (3 500-1 400 000)	125 µm 335 µm (and higher)	n.a.	n.a.	5×10^4 - 1.5×10^7 Average: $(4.4 \pm 2.1) \times 10^6$	17 facilities not identified due to confidentiality, some including advanced filtration granular or biological	Discharges in San Francisco Bay, Lake Michigan and several lakes in New York area
(Talvitie et al., 2017)	2.7×10^5 (800 000)	10 µm > 300 µm	$< 0.651^d$	65-94** (> 99**)	1.7×10^6 - 1.4×10^8	Pretreatment, activated sludge and denitrifying biological filter	Gulf of Finland, Baltic Sea, Finland
(Ziajahromi et al., 2017)	3.08×10^5 (1 227 150) 1.7×10^4 (67 130) 6.1×10^4 (150 870)	25 µm 500 µm (and higher)	1.5-2.2^a 0.21-0.28^d	> 99 (> 90)	3.6×10^6 - 4.6×10^8	3 WWTP, (1) only primary treatment, (2) primary, secondary and disinfection and (3) with tertiary membrane treatment	Discharges to ocean and to an urban river in Sidney, Australia
(Lares et al., 2018)	10^4	250 µm 5 mm	57.6 ± 12.4^a 0.6 ± 0.2^b 1.0 ± 0.4^d	98.3 (-)	1.0×10^7	WWTP with primary, secondary with activated sludge, and disinfection	Mikkeli, Finland
(Gies et al., 2018)	4.9×10^5 (1 300 000)	> 1 µm	31.1 ± 6.7^a 2.6 ± 1.4^b 0.5 ± 0.2^d	98.3 (80.8)	8.2×10^7	Primary and secondary treatment & seasonal chlorination	Vancouver, British Columbia discharging to Fraser River near the Strait of Georgia
(Magni et al., 2019)	4.0×10^5 (1 200 000)	10 µm 5 mm	2.5 ± 0.3^a 0.9 ± 0.3^c 0.4 ± 0.1^d	84 (n.a.)	1.6×10^8	WWTP with primary, secondary and tertiary treatments (sand filter and disinfection)	WWTP located in Northern Italy; no details about discharge
(Yang et al., 2019)	10^6 (2 400 000)	50 µm 5 mm	12.03 ± 1.29^a 0.59 ± 0.22^d	95.2 ± 1.6 (72 ± 12)	$5.9 \pm 2.2 \times 10^8$	Primary and secondary treatments, A2O, membrane treatment, and disinfection.	Gaobeidian treatment plant in Beijing, discharges to Tonghui River
(Liu et al., 2019)	2.0×10^4	20 µm 5 mm	79.9 ± 9.3^a 47.4 ± 7.0^b 34.1 ± 9.4^c 28.4 ± 7.0^d	64.4 (40.1)	5.7×10^8	Primary and secondary treatments plus chlorination	Wuhan City, discharges into the Yangtze River via effluent pipe
(Bayo et al., 2020)	3.5×10^5 (210 000)	0.45 µm 5 mm	12.43 ± 2.70^a 9.73 ± 3.04^b 3.21 ± 0.50^c 1.23 ± 0.15^d	90.1 (87.4)	6.7×10^6	Primary and secondary activates sludge process plus chlorine disinfection	Cartagena, Spain, discharging to Mediterranean Sea
This study	4.5×10^4 (300 000)	25 µm 5 mm	171 ± 43^b 10.7 ± 5.2^d	- (93.7)	3.0×10^8	Primary and A2O (Anaerobic, Anoxic, Oxic) biotreatment.	Plant located near Madrid and discharging to Henares River, tributary to Tagus River

a: influent
b: primary effluent
c: after secondary settler
d: final effluent
* Removal efficiency from primary effluent to discharge
** Includes all microlitter (not only microplastics)
n.a.: not available

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Table 3. Some recent studies reporting microplastics emission with WWTP sludge.

Reference	**Source of data**	**Population served**	**Size sampled**	**Microplastics, particles/g**	**Microplastics emitted per year and WWTP**
(Mintenig et al., 2017)	6 German WWTP	-	< 500 µm	1-24	1.24×10^9- 5.67×10^9
(Mahon et al., 2017)	7 WWTP from Ireland	6 500- 2 400 000	250-4000 µm	4.2-15	-
(Lares et al., 2018)	1 Finish WWTP	55 000	250 µm-5 mm	170.9 ± 28.7	No Data
(Lusher et al., 2017)	8 WWTP in Norway	18 150 - 615 000	> 50 µm	1.7-19.8	2.2×10^9- 2.8×10^{11}
(Li et al., 2018b)	28 WWTP in China	51 900- 7 050 000	37 µm-5 mm	1.60-56.4	-
(Gies et al., 2018)	1 WWTP in Canada	1 300 000	> 1 µm	14.9 ± 6.3 (primary) 4.4 ± 2.8 (secondary)	1.64×10^{12}
(Magni et al., 2019)	1 Italian WWTP	1 200 000	10 µm- 5 mm	113 ± 57	1.24×10^{12}
(Liu et al., 2019)	1 WWTP in China	-	20 µm-5 mm	240 ± 31	-

et al. (2005) showed that fibers from sludge were detectable in soil even many years after application with the same characteristics they had when applied.

The presence of fibres in influents and effluents of WWTP has been extensively documented and mainly corresponds to the laundering of synthetic fibres (Zambrano et al., 2019). Other studies gave lower values, indicating a large seasonal variability. Browne et al. (2011), estimated > 1900 fibres/wash with potentially increasing up to three orders of magnitude in winter due to the higher usage of washing machines (Browne et al., 2011). It has been pointed out that washing procedures are subjected to culture habits, therefore influencing the amount of fibres that reach environmental compartments. The number of washing cycles per week, the different use of detergents, washing temperature, volume of water used, and type of clothes strongly influence the number of fibres released (Cesa et al., 2017; Kelly et al., 2019). Together with synthetic fibres, there also exist a large variety of fibres from natural polymers including cellulose derivatives or wool, which have been processed up to a certain extent and, therefore, their release to the environment constitute another kind of anthropogenic pollution. There are many types of manufacturing processes involved such as dying or bleaching or the blending with additives granting better mechanical properties, flame retardancy, and light stabilization among others (O'Brien et al., 2015). Manufactured natural polymers are not plastic materials, but in view of their anthropogenic character and the presence of additives, they should be considered for their possible risk if delivered into the environment. Cellulose fibres, for example, detach in huge quantities from toilet paper and may contain diverse substances like softeners (sometimes made of silicone derivatives), perfumes or metals like copper, magnesium or zinc, all of them added to improve certain properties of the final product (Abildgaard et al., 2003). Another risk associated with bleached fibres products is the presence of dioxins produced during manufacturing and that can be released during use and from detached fibres (Keenan et al., 1989). Besides, the obvious presence of a plethora of dyes is a well-known fact (Biermann and Wiggins, 2018).

The occurrence of microplastics in the environment is reasonably documented, and there is a growing evidence that they interact with many organisms. However, the extent to which they pose an ecotoxicological threat is controversial and a subject topic of active research (de Souza et al., 2018). Several groups studied the environmental impact of plastic microparticles to different aquatic invertebrates by means of acute and chronic toxicity tests. The concentrations that proved toxic or statistically significant effects were typically many orders of magnitude above environmentally relevant levels. Table S2 (SM) details some studies reporting median effects (LC50, EC50) or LOEC for microplastic particles > 1 □m to aquatic invertebrates. The reported values range from 7.1 x 104 particles/L (10-day mortality of Hyalella azteca) to 4.4 x 108 particles/L (120-h mortality of Daphnia magna adults), which are 4-to-8 orders of magnitude above usual concentrations in the effluents of WWTP (Table 2). Even using the conservative factor of 1000, applied for risk assessment if only limited data are available, no evidence of toxic risk can be appreciated. Concerning primary producers, most

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works did not find EC50 values due to the high concentrations required to induce toxic responses except if exposed to very low sizes (Prata et al., 2019; Wang et al., 2019b). Finally, no effect of PE and PS microplastics to the earthworm Eisenia foetida has been reported except for concentrations as high as 20 % (w/w in soil) (Wang et al., 2019a).

It has been suggested that human health could be threatened by microplastics because they are known to accumulate in certain wild or aquaculture species of fish and shellfish. The concern refers to physical toxicity, and to additives or adsorbed chemicals. However, there is still insufficient information to assess the exposure of humans to microplastics via food with estimations ranging from tens to tens of thousands of particles ingested per year and an almost absolute lack of toxicological and epidemiological data (Smith et al., 2018). There is an urgent need for assessing the risk of anthropogenic plastics including key aspects like the production of secondary nanoparticles due to ageing and the translocation of small plastic particles to food chains to accurately assess such risk. There is also a need for standardization in sizes and other methodological details that make results fully comparable among studies. Sufficiently comparable to at least allow precise estimates of the global plastics cycle and to perform sound risk assessment calculations. Clearly, microplastics escape in considerable amount to current wastewater treatment practices. Some specific sources of pollution like domestic microfibers, synthetic or anthropogenically modified, could be reduced in origin by introducing changes in washing machines. Concerning WWTP, attention should be paid to enhance technologies limiting the emission of microplastics with the effluent and, overall, on the use of sludge as soil amendment.

5. Conclusions

This work evaluated the presence of microplastics through the different steps of a WWTP including heat-dried sludge used as soil amendment. Our results showed that the efficiency of the WWTP in removing microplastics was high, with a removal rate of 93.7 % between primary settler and final effluent. The quantification of the particles released with the effluent yielded 12.8 ± 6.3 items/L including manufactured natural fibres, while sludge contained 183 ± 84 items/g (wet sludge) and 165 ± 37 items/g (heat-dried sludge).

FTIR identification revealed the existence of PE, PP, polyester and acrylic fibres and an important amount of natural fibres with evidence of anthropogenic processing.

Size distributions were dominated by the smaller particles, in the 25-104 μm range, which represented 54 % and 48 % of primary and secondary effluents. Fibres represented 31 % and 20 % of the anthropogenic particles in primary and secondary effluents and 62 % and 84 % in wet sludge and heat dried-sludge, respectively.

Our results showed that despite the high efficiency of conventional facilities, a huge number of particles escaped through the discharge of treated wastewater. The WWTP we studied releases about 300 million microplastic particles per day to the Henares River representing an approximate load of microplastics of 350 particles/m3. WWTP sludge contributes to microplastics pollution with 8 x 1011 plastic particles per year. Dried sludge used as soil amendment in the area of Madrid (100 000 t/year) would disseminate 1013 microplastic particles per year in agricultural soils.

There is no direct evidence that exposure concentration of microplastics due to WWTP effluent discharge and wastewater or sludge reuse results in direct toxicity to soil or aquatic organisms. However, the huge amount of debris released and the possibility of fragmentation to non-sampled sizes, with possible translocation to food chains, makes further research necessary.

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SUPPLEMENTARY MATERIAL

Fate of microplastics in wastewater treatment plants and their environmental dispersion with effluent and sludge

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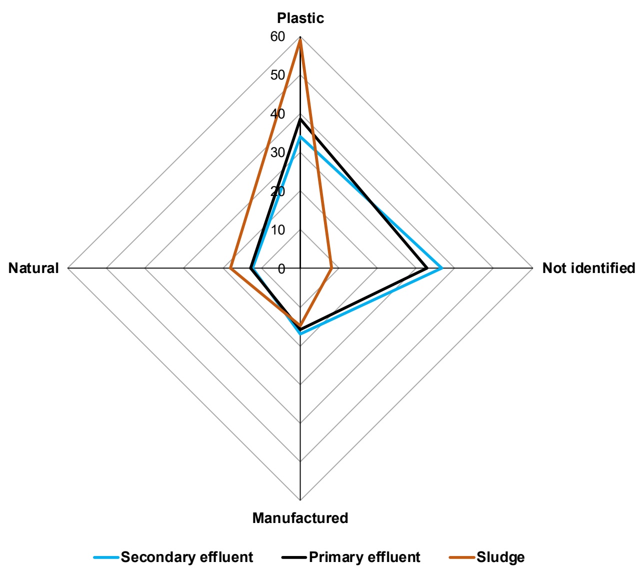
Contents:



Polymers identified by micro-FTIR
Cellulose polymers (cellophane, rayon/viscose, cellulose acetate, cotton)
Polymethyl methacrylate
Polyamide fibres
Polycaprolactone
Polyester fibres and resins
Polyethylene
Polyethylene terephthalate
Polypropylene
Polystyrene
Polyurethanes
Poly(vinyl acetate) and copolymers
Poly(vinyl chloride)

Figure S1. Distribution of materials present in the different samples after FTIR analyses. "Manufactured" refers to natural substances with evidence of anthropogenic modification and list of main polymers identified by micro-FTIR.

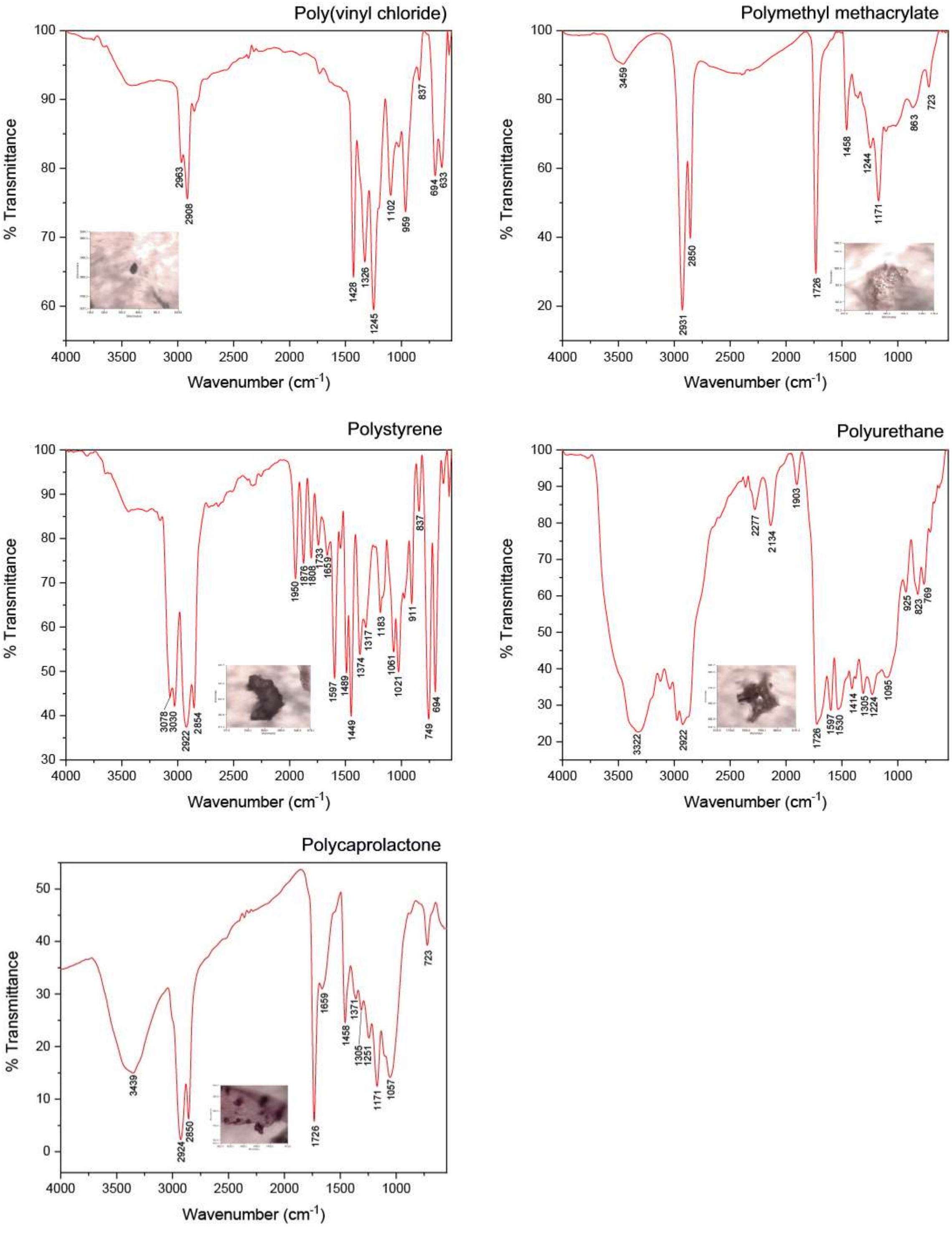


Figure S2. Infrared spectra of samples of poly(vinyl chloride), polymethyl methacrylate, polystyrene, polyurethane and polycaprolactone.

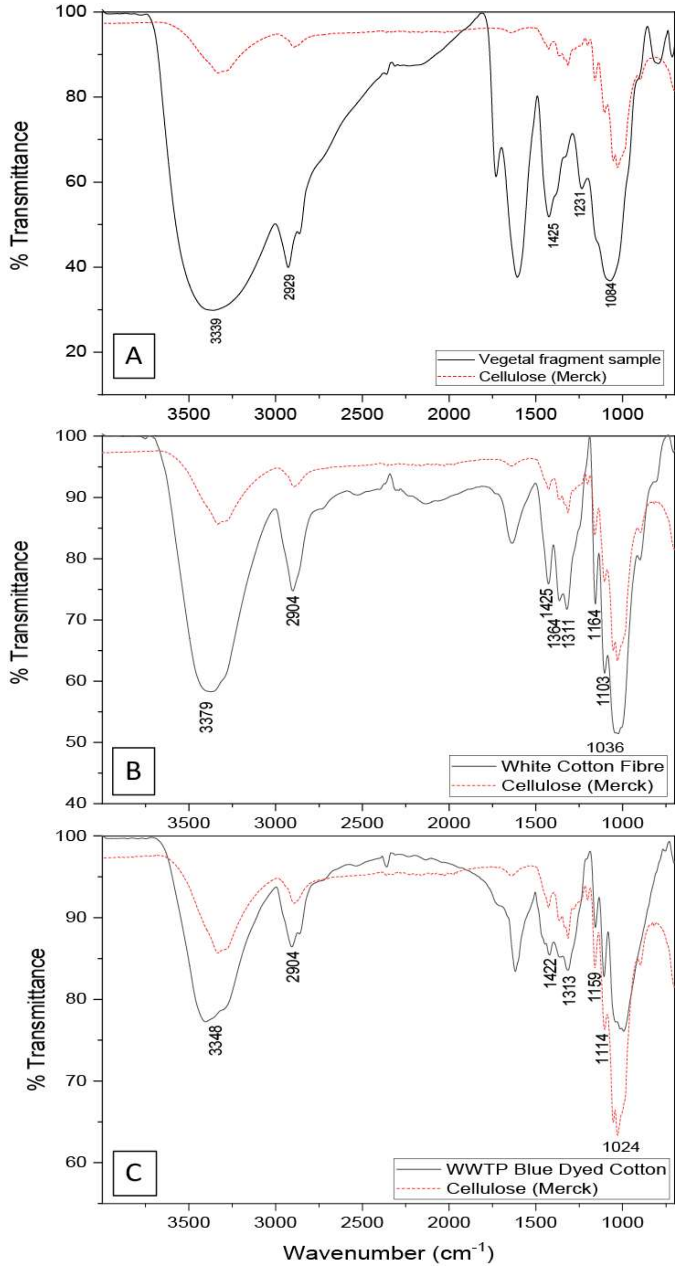


Figure S3. Infrared spectra of cellulose-based particles. A. Vegetal fragment. B. White cotton fibre. C. Blue-dyed cotton fibre.

Table S1. Toxicological data reported in the literature for microplastic particles > 1 mm.

Reference	**Polymer**	**Test organism**	**Endpoint**	**LC/EC$_{50}$ or LOEC, particles/L**
(Au et al., 2015)	10-27 µm PE microparticles	*Hyalella azteca*	10-day mortality	4.6×10^7
(Au et al., 2015)	20-74 µm PP microfibres	*Hyalella azteca*	10-day mortality	7.1×10^4
(Rehse et al., 2016)	1-4 µm PE microspheres	*Daphnia magna*	96 h immobilization	1.3×10^8
(Lee et al., 2013)	6 µm PS microbeads	*Tigriopus japonicus*	Fecundity	2.1×10^5 (LOEC)
(Cole et al., 2013)	7.3 µm PS microbeads	*Centropage s typicus*	Algal ingestion rate	7×10^5 (LOEC)
(Kaposi et al., 2014)	10−45 µm PE microspheres	*Tripneustes gratilla.*	Reduction of larvae body width	3×10^5 (LOEC)
(Eltemsah and Bøhn, 2019)	6 µm PS microbeads	*Daphnia magna*	120-h mortality in juveniles	2.9×10^8
(Eltemsah and Bøhn, 2019)	6 µm PS microbeads	*Daphnia magna*	120-h mortality in adults	4.4×10^8
(Ogonowski et al., 2016)	< 63 µm	*Daphnia magna*	Reproductive output	8.6×10^4

References (Table S1)

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