Assessment of Microplastics in a Municipal Wastewater Treatment Plant With Tertiary Treatment: Removal Efficiencies and Loading Per Day Into the Environment

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Abstract

This study investigates the removal of microplastics from wastewater in an urban wastewater treatment plant located in Southeast Spain, including an oxidation ditch, rapid sand filtration, and ultraviolet disinfection. A total of 146.73 L of wastewater samples from influent and effluent were processed, following a density separation methodology, visual classification under a stereomicroscope, and FTIR analysis for polymer identification. Microplastics proved to be 72.41% of total microparticles collected, with a global removal rate of 64.26% after the tertiary treatment and within the average retention for European WWTPs. Three different shapes were identified: i.e., microfiber (79.65%), film (11.26%), and fragment (9.09%), without the identification of microbeads despite the proximity to a plastic compounding factory. Fibers were less efficiently removed (56.16%) than particulate microplastics (90.03%), suggesting that tertiary treatments clearly discriminate among forms, and reporting a daily emission of 1.6×10^7 microplastics to the environment. Year variability in microplastic burden was cushioned at the effluent, reporting a stable performance of the sewage plant. Eight different polymer families were identified, LDPE film being the most abundant form. Future efforts should be carried on source control, plastic waste management, improvement of legislation, and specific microplastic-targeted treatment units, especially for microfiber removal.

Highlights

- Isolated microplastics were found to be 72.41% of all collected microparticles.
- Selected sewage plant removes 64.26% of microplastics.
- Overall greater removal of particulate microplastics (90.03%) by tertiary treatment, compared to microfibers (56.16%).
- 16,000,000 microplastics are daily released to the environment.

Introduction

Plastic pollution is a widespread problem, mainly affecting the oceans but also human health, food safety and climate change, and its world production has been reported to grow from 359 million tons in 2018 up to 368 million tons in 2019 (PlasticsEurope 2020). Microplastics (MPs), first identified by Carpenter and Smith (1972) in the Sargasso Sea and defined as particles with a size below 5 mm in their longest dimension (Gatidou et al. 2019), can be originated by decomposition of macroplastics (secondary MPs) or intentionally manufactured by different firms with a microscopic size (primary MPs) (Estahbanati and Fahrenfeld 2016). Primary MPs are used in the form of spherules as precursors in the plastic industry, with a wide range of applications such as packaging, office equipment, and vehicle construction, or as scrubbing material in personal care products or air-blasting granules and pellets, among other uses (Mani et al. 2015). Secondary MPs are formed by means of chemical and physical mechanisms such as hydrolytic degradation, photolysis, weathering, ultraviolet radiation or abrasion (Ziajahromi et al. 2017; Arhant et al. 2019), or via biotic degradation, as biodeterioration (Jang et al. 2018;

Barlow et al. 2020). Microplastics are considered to be more prevalent in the environment than macro or mesoplastics, owning their larger quantities and small sizes (Karbalaei et al. 2018).

As reported by PlasticsEurope (2020), the European plastic industry, including plastics raw materials, producers, converters, recyclers and machinery manufacturers, gives direct employment to more than 1.56 million people in Europe, having plastic production and use an important role in delivering a more sustainable future because the unique properties of plastics. At the same time, its commitment as an industry is to avoid plastic waste, having taken important steps to understand the true nature of durable and degradable plastic materials and their behaviors in the environment (Sheavly and Register 2007).

Microplastic pollution is a hotspot presently, with a global concern both in marine and freshwater ecosystems; i.e., rivers, beaches, reefs, lakes, surface water, estuaries or lagoons (Vianello et al. 2013; Narciso-Ortiz et al. 2020; Mbedzi et al. 2020; Irfan et al. 2020). Furthermore, the sorption of toxic chemicals by MPs has been thoroughly reported, tending to be ingested by different organisms (Bayo et al. 2017).

Wastewater treatment plants (WWTPs) act as a sink of MPs from both domestic and industrial wastewater, but also as a source to the environment and freshwater (Gatidou et al. 2019; Bayo et al. 2020b), proven by the large amount of microfibers and secondary MPs reported close to wastewater effluents and mainly originating from laundry (Pirc et al. 2016). Several studies have shown an effective MP elimination from effluent, with a great variation of removal percentages; i.e. 53.6% (Lv et al. 2019); 57% (Akarsu et al. 2020); 64.4% (Liu et al. 2019), 72% (Leslie et al. 2017); 90.3% (Bayo et al. 2020c); 95.16% (Yang et al. 2019), or 99.9% (Carr et al. 2016), among many others.

Sewage plants are complex systems, with chemical, physical and biological processes taking place simultaneously, and the removal efficiency has been relied on different treatments, including skimming and settling processes, primary clarification, biological removal or tertiary treatments (Carr et al. 2016; Bayo et al. 2020a; Zou et al. 2020). However, synthetic microfibers are virtually found everywhere in the environment (Gavigan et al. 2020), and the ability of tertiary treatments to efficiently and significantly remove these microfibers is yet to be assessed. Domestic washing of textiles and garments is a constant and widespread source of plastic microfibers (Pirc et al. 2016) released from washing machines to wastewater treatment plants, especially during Autumn and Winter (Browne et al. 2011; Bilgin et al. 2020) and estimated over more than 1900 fibers per washing cycle and garment (Browne et al. 2011), that for polyester fleece fabrics could reach 7360 fibers m^{-2}·L-1 (Almroth et al. 2018).

The aim of this study was to monitor a full-scale WWTP in Southeast Spain, providing basic information on the abundance, shape, size, and type of MPs in influent (INF) and effluent (EFF) samples, after an oxidation ditch system, rapid sand filtration (RSF), and ultraviolet disinfection. We have assessed its commitment with European MP standards in wastewater systems, the importance of tertiary treatments in microfibers (FB) release, the influence of the surrounding environment in close proximity to the sewage plant; i.e., a plastic compounding factory, an expanding urban area, and greenhouse agricultural crops,

the matching between demanded and collected polymer types, and their possible sources. In addition, some solutions are proposed in order to improve the removal efficiency of these emerging pollutants.

Materials And Methods

Description of “La Aljorra” WWTP and sampling collection

“La Aljorra” is a full-scale WWTP treating both domestic and industrial wastewater, located in the Region of Murcia (Southeast Spain) (37º41’16”N, 01º03’13”W) (Fig. S1). It is designed with a maximum hydraulic flow of 677 m^3 h^{-1} and serving about 70,417 equivalent inhabitants. Pretreatment includes bar screens for both rough and fine solids, and grit and grease removal with aeration supplied by blowers. After pretreatment, wastewater is introduced in parallel into a two full-scale oxidation ditch system (8,502 m^3 each), equipped with an internal pre-anoxic zone. The mixed liquor leaves the oxidation ditch to secondary settlers, and clarified effluent ends into rapid sand filters with a total filtering surface of 320 m^2. For a detailed description of the sewage treatment line in “La Aljorra” WWTP and physicochemical and biological parameters, please refer to Supplementary Information (Tables S1 and S2).

A total of 146.73 L of wastewater were collected for the study through 28 grab samples processed between February 28th 2019 and May 20th 2020, and distributed into 14 samples from influent (INF = 59.89 L) and 14 samples from effluent (EFF = 86.84 L) (Table S3). Sample volumes were accurately measured for each experiment, ranging from 2.40 to 6.23 L for INF (mean ± standard error) (4.28 ± 0.23 L), and 3.00 to 8.89 L for EFF (6.20 ± 0.42 L), always collected in the morning (9–11 a.m.) in glass bottles with metallic lid. Samples from EFF were directly vacuum filtered through a Büchner funnel using a 0.45 µm paper filter (Prat Dumas, Couze-St-Front, France, 110 mm Ø). INF samples were previously treated with an environmentally-friendly, cheap and inert salt-saturated solution 120 g L^{-1} NaCl (2.05M) (1.08 g cm^{-3}) (Panreac, Barcelona, Spain), in a methodology wholly reported in Bayo et al. (2020c) and detailed in Fig. 1. All experiments were carried out at room temperature (293K).

In order to prevent any risk of contamination, especially to minimize exposure to airborne MP, all equipment was covered with aluminum foil and filtered samples were placed into covered glass Petri dishes for further examination. All glassware was thoroughly washed with tap water and twice with deionized water after each experiment, and during the whole process, analysts only wore natural fabric clothes, nitrile gloves, clean cotton lab-gown, and face mask covering nose and mouth. Procedural blanks, consisting of 1.5 L of 0.45 µm filtered deionized water, were processed in parallel with each batch of wastewater samples, and placed into clean glass Petri dishes for further microscopic examination. Only one fiber was quantified and subtracted from results.

Microplastic analysis and dataset

Samples with possible MP were examined under a digital optical trinocular microscope (Olympus SZ-61TR Zoom, Olympus Co., Tokyo, Japan) coupled to a Leica MC190 HD digital camera and an image capturing software Leica Application Suite (LAS) 4.8.0 (Leica Microsystems Ltd., Heerbrugg, Switzerland),

used for the analysis and recording of color, shape and size of microparticles in their largest dimension. Analyzed microlitter (ML) was isolated in 40-mm glass Petri dishes for further study by FTIR. Microparticles were visually classified as: microfiber (FB), microbead or pellet (BD), fragment (FR), and sheet or film (FI), allowing a maximum length of 15 mm for fibers, as proposed by ECHA (2019).

FTIR was used for the identification of functional groups and molecular composition of polymeric surfaces. Samples were compressed in a diamond anvil compression cell, and spectra were acquired with a Thermo Nicolet 5700 Fourier transformed infrared spectrometer (Thermo Nicolet Analytical Instruments, Madison, WI, USA), provided with a deuterated triglycine sulfate, DTGS, detector and KBr optics. The spectra collected were an average of 20 scans with a resolution of 16 cm^{-1} in the range of 400–4000 cm^{-1}. Spectra were controlled and evaluated by the OMNIC software without further manipulations, and polymers were identified by means of different reference polymer libraries, as further explained. All statistical analyses were carried out with SPSS 26.0 statistic software (IBM Co. Ltd, USA), with a critical value for statistical significance set at $p < 0.050$.

Results And Discussion

General considerations and removal rates according to major shapes

A total of 319 microlitter particles (ML) were isolated from all wastewater samples, with an average concentration of 2.58 ± 0.40 items L^{-1} and minimum and maximum values corresponding to 1.33 and 6.37 items L^{-1} for INF, and 0.10 and 7.00 items L^{-1} for EFF, respectively. Average concentrations were 3.78 ± 0.48 items L^{-1} for INF and 1.38 ± 0.48 items L^{-1} for EFF, with a statistically significant removal rate of 63.41% (*F-test* = 12.509, $p = 0.002$). Among non-polymeric microparticles, chipboard and glass fragments, silica, cellulose from toilet papers and calcium stearate, stearic acid and surfactants (Kwaśniewska et al. 2019) from soap residues, cosmetics and personal care products were mainly identified. Furthermore, Zn/Ca PVC stabilizer was also identified as an additive in the particulate fraction. It is a heat stabilizer, incorporated during PVC synthesis in order to rein back the polymer degradation during thermal processing (Fang et al. 2009). Additives within the polymer matrix may leach into the environment during the lifetime of the product, causing alterations in freshwater microorganisms (Karbalaei et al. 2018). Figure 2 shows ML and MP images from samples collected at “La Aljorra” WWTP, proving both are optically similar and an additional spectroscopic technique is necessary to further minimize overestimation of suspected microplastics.

Every single isolated microlitter particle was analyzed by FTIR, and 72.41% of these microparticles (n = 231) were identified as MP, with an average concentration of 1.86 ± 0.32 items L^{-1}. All these results reinforce the need of additional spectroscopic techniques to objectively differentiate microplastics from non-plastic microparticles in the whole sample; i.e., FTIR or Raman spectroscopy (Zou et al. 2020). Table 1 shows the microplastic content in both INF and EFF of “La Aljorra” WWTP. Statistically significant

differences were observed between average MP concentration collected from INF (2.74 ± 0.49 items L^{-1}) *versus* those collected in the EFF (0.98 ± 0.27 items L^{-1}) (*F-test* = 9.998, $p = 0.004$), indicating a removal rate of 64.26% for all microplastic forms, within the average retention rate in Europe for WWTPs of between 53% and 84% (Hann et al. 2018). Lares et al. (2018) reported a concentration of 1.0 ± 0.4 items L^{-1} in the final effluent of a Finish WWTP with a daily average flow of 10,000 m^3, and Yang et al. (2019) reported 0.59 ± 0.22 items L^{-1} in the effluent from the largest water reclamation plant in China (1,000,000 m^3 day^{-1}). Minimum and maximum values corresponded to 0.65 and 6.37 items L^{-1} for INF, and 0.17 and 3.33 items L^{-1} for EFF, respectively. Concentrations in the EFF are consistent with those previously reported by our research group in another WWTP with RSF technology; i.e., between 0 and 5.28 items L^{-1} (Bayo et al. 2020a). Reported concentrations of MPs in final effluents from different WWTPs are highly variable according to wastewater treatment technology applied, but also because different sampling strategies or digestion procedures, among others. Talvitie et al. (2017) reported 97% MP removal by RSF (from 0.7 to 0.02 items L^{-1}), Hidayaturrahman and Lee (2019) a 73.8% after using an Al-based coagulant, while Ben-David et al. (2021) revealed a slight reduction after RSF treatment in an Israeli WWTP, with a final concentration of 1.9 items L^{-1}.

Table 1
Total count, percentages and average concentrations (± SE) of microplastics in the influent (INF) and effluent (EFF) of "La Aljorra" WWTP. The concentrations are presented in items per liter of wastewater.

	INF	EFF	TOTAL
Microplastic (MP)	161 (69.70%)	70 (30.30%)	231
	2.74 (± 0.49)	0.98 (± 0.27)	1.86 (± 0.32)
Fibes (FB)	119 (64.67%)	65 (35.33%)	184
	2.09 (± 0.47)	0.92 (± 0.26)	1.50 (± 0.29)
Microplastic particles (MPP)	42 (89.36%)	5 (10.64%)	47
	0.66 (± 0.14)	0.07 (± 0.03)	0.36 (± 0.09)
Film (FI)	21 (80.77%)	5 (19.23%)	26
	0.35 (± 0.10)	0.07 (± 0.03)	0.21 (± 0.06)
Fragment (FR)	21 (100%)	0 (0%)	21
	0.31 (± 0.09)	0	0.15 (± 0.05)

Three different shapes were isolated in wastewater samples: microfiber (FB) (79.65%; 1.50 ± 0.29 items L^{-1}), film (FI) (11.26%; 0.21 ± 0.06 items L^{-1}), and fragment (FR) (9.09%; 0.15 ± 0.05 items L^{-1}), similar to that described by Lares et al. (2018) in a pilot-scale membrane bioreactor, Bilgin et al. (2020) in a municipal WWTP in Turkey, or by Zou et al. (2020) in 6 WWTPs in Guangzhou (China). Our results are

also coincident to those previously reported in another WWTP from our Region, with also rapid sand filtration as a tertiary treatment (Bayo et al. 2020a), but different to those reported in a conventional activated sludge sewage plant, where fragment form was mainly isolated (Bayo et al. 2020c). In the present study, the only rounded or microbead form isolated was for a calcium stearate from soap residues, as shown in Fig. 2d. This is consistent with the prohibition in most countries of personal care products with microbeads, in addition to a change in the trend and public awareness to use cleansing products with natural scrubbers (Irfan et al. 2020; Zou et al. 2020). Besides, preventive tools developed by the plastic factory close to the WWTP, such as the installation of filters at drains or immediately cleaning spilled pellets, have proven to be decisive and very efficient, being included within the firms having signed the PlasticsEurope OCS (Operation Clean Sweep®) pledge since 2017, a voluntary industry initiative on plastic pellet loss (PlasticsEurope 2019). Anyway, there is no clear relationship between MP abundance in wastewaters and factories (Zou et al. 2020).

Particulate forms of microplastics (MPPs), including both fragments and films, proved to statistically significant decrease from INF (0.66 ± 0.14 items L^{-1}) to EFF (0.07 ± 0.03 items L^{-1}) (*F-test* = 17.364, *p* = 0.000) (Table 1), accounting for a removal percentage of 90.03%. On the contrary, FB displayed a low removal efficiency of 56.16%, also significantly decreasing from INF (2.09 ± 0.47 items L^{-1}) to EFF (0.92 ± 0.26 items L^{-1}) (*F-test* = 4.798, *p* = 0.038) and confirming better settling rates for MPP than for FB. In fact, there was an increase in the abundance of microfibers in EFF (92.86%) compared to INF (73.91%), similar to that reported by Ben-David et al. (2021) after a tertiary treatment (91%) relative to raw wastewater (74%). Conley et al. (2019) also reported a higher prevalence of fibers than particles across three WWTPs in the Charleston Harbor (USA). The average amount of microplastics released with the EFF was calculated in 1.6 x 10^7 MP per day, similar to that reported by Murphy et al. (2016) (6.5 x 10^7 MP per day); Ziajahromi et al. (2017) (3.6 x 10^6 – 1 x 10^7 MP per day); Lares et al. (2018) (1 x 10^7 MP per day); Blair et al. (2019) (1.2 x 10^7 MP per day) or Pittura et al. (2021) (4.15·10^7 MP per day) in different WWTPs.

Meanwhile FR totally disappeared in the EFF of the WWTP, after the RSF and UV disinfection, some film forms, less dense than fragments, still appeared, decreasing from INF (0.35 ± 0.10 items L^{-1}) to EFF (0.07 ± 0.03 items L^{-1}) and showing a statistically significant removal rate of 81.35% (*F-test* = 7.722, *p* = 0.010). Bilgin et al. (2020) reported similar results by the overall plant operation, with removal rates decreasing from fragments to films and fibers.

Nevertheless, the number of microplastics trapped between the sand grains or adhered to their surfaces by interception, the main mechanism underlying rapid sand filtration (Nakazawa et al. 2018) could be substantially enhanced with the use of coagulant (Talvitie et al. 2017; Hidayaturrahman and Lee 2019). Although “La Aljorra” WWTP is prepared for that procedure, no coagulant additions were used.

The global removal percentage for this study (64.26%) proved to be similar to that reported by Tang et al. (2020) in two WWPTs located in Wuhan (China), 62.7% and 66.1%. The removal rate for FB (56.16%) was similar to that reported in our previous study (53.83%) (Bayo et al. 2020a), suggesting that tertiary

treatments are overall more efficient in removing particulate than fiber forms (Michielssen et al. 2016). In fact, while the average ratio MPP:MP decreased from INF (0.28 ± 0.05) to EFF (0.14 ± 0.08), the FB:MP relationship increased after the treatment process, from 0.72 ± 0.05 (INF) to 0.86 ± 0.08 (EFF).

Figure 3 shows the annual mean variability of MPP and FB *versus* MP ratios for INF and EFF of "La Aljorra" WWTP. A statistically significant variation in both ratios could be observed for the influent, with an increase in MPP:MP ratio from 2019 (0.21 ± 0.05) to 2020 (0.45 ± 0.07) (*F-test* = 6.801, $p = 0.023$) and a decrease in FB:MP ratio from 2019 (0.79 ± 0.05) to 2020 (0.55 ± 0.07) (*F-test* = 6.804, $p = 0.023$). This sewage plant is located in an expanding urban area undergoing rapid urbanization within the last year, and the pronounced temporal variation in those ratios appears to reflect this fact. As described by Huang et al. (2020), higher levels of urbanization lead to a greater MP pollution. However, when the ANOVA was implemented for the effluent, no statistically significant differences were observed for MPP:MP ratio (*F-test* = 0.857, $p = 0.373$) or FB:MP ratio (*F-test* = 0.814, $p = 0.385$), indicating a similar and stable performance of the sewage plant efficiency during the studied period, despite a higher load of MPPs during 2020.

Microfiber form was also the dominant shape in all seasons, decreasing during the Summer (0.77 ± 0.28 items L^{-1}) with respect to Autumn (1.06 ± 0.28 items L^{-1}), Winter (1.34 ± 0.44 items L^{-1}) and Spring (2.72 ± 0.90 items L^{-1}), but without statistically significant differences (*F-test* = 2.331, $p = 0.100$). This absence of variations across seasons, together with a similar fiber length by seasons (*F-test* = 0.775, $p = 0.509$) should be associated to the important amounts of synthetic fibers released from washing machines, depending on textile properties, washing conditions, type of detergent and softener, and garment weathering (Browne et al. 2011; Almroth et al. 2018; De Falco et al. 2018). Modifications on washing machine filters would be an effective and simple way of preventing FB from entering sewer, as well as using washing bags that act as a microfiber filter between the synthetic clothing and the drain (Lv et al. 2019; Okoffo et al. 2019).

Size, color, and polymer distribution

Figure 4(a) depicts a total of 10 different colors identified in all samples, being blue the most common one for fibers (67.39%), while MPP were mostly white (48.94%) followed by beige (19.15%), brown (8.51%), blue (6.38%), and black (6.38%), similar to that reported in another WWTP with rapid sand filtration (Bayo et al. 2020a). White, blue and clear colors have proven to be similar to those of plankton, a primary food source for fish (Boerger et al. 2010). Fibers were by far the most common type in both size groups; i.e., mini-microplastics (< 1mm) and microplastics (1–5 mm), accounting for 88.07% and 72.13%, respectively. The main size of MPs, both in INF and EFF samples, was between 1–2 mm (Fig. 4b). The minimum size corresponded to a 210 µm polypropylene film collected in an INF sample, as depicted in Fig. 2j, and the maximum dimension corresponded to a white transparent 12 mm fiber also collected in an INF sample. The average size increased from fragments (633.33 ± 79.19 µm) to films (1012.71 ± 235.78 µm) and fibers (1487.11 ± 104.64 µm), which implies that fibers are likely from domestic washing (Yang et al. 2019).

As previously reported by Long et al. (2019), the removal rate of MP proved to be related to their size, increasing with a decreasing size, as fragments, with the lowest average size, totally disappeared in the EFF, and films and fibers were removed at 81.35% and 56.16%, respectively. Several mechanisms have been proposed; i.e., a lower residence time for smaller MP than for larger plastic debris (Enders et al. 2015), small MP fragment in much higher abundance than large ones (Wang et al. 2018) and aggregation and settling into the sludge for the lowest sizes (van den Berg et al. 2020). Furthermore, the MPs percentage smaller than 500 μm increased from the INF (18.63%) to the EFF (21.43%), after UV disinfection, similar to those results reported after chemical disinfection, with a degradation level depending on contact time, temperature, and disinfectant concentration (Dris et al. 2015; Pittura et al. 2021).

Eight different polymer types were identified across wastewater samples, as depicted in Fig. 4c. The identification of polymer composition of MP was carried out by means of different reference libraries, containing spectra of all common polymers; i.e., Hummel Polymers and Additives Library (2,011 spectra), Polymer Additives and Plasticizers (1,799 spectra), Sprouse Scientific Systems Polymers by ATR Library (500 spectra), and Rubber Compounding Materials (350 spectra). Standard criteria reported by Frias *et al.* (2016) was applied, regarding a percentage match over 60% between sample and reference spectrum. In this sense, 78.35% of MP corresponded to unidentified microfibers that did not match this percentage. Their small size and thick, as well as the presence of additive compounds, pigments and dyes, could mask the FTIR signal, together with pollutants closely adhered to their surface (He et al. 2020). Also, photo-degradation and weathering are two factors which could alter the polymer spectra, hindering comparisons with reference libraries (Gatidou et al. 2019). Difficulties in visual classification under a stereomicroscope have been previously reported by Prata et al. (2020) in airborne samples, with 62.3% of unidentified fibers because of their small sizes or light colors, or by Ben-David et al. (2021) in wastewater samples, with 63.9% of fibers with low percentage match or plastic pigments that masked the Raman signal. Fig. S2 shows images of unidentified microfibers across the study. From the identified ones, 14.43% corresponded to polyethylene terephthalate (PET) and 7.22% to HDPE. The absorption bands for PET (Fig. 5a) at 3100–3400 cm^{-1} correspond to aromatic C-H stretch, 1730 cm^{-1} identifies the carbonyl group (C = O), 1300–1600 cm^{-1} the aromatic ring and 1027 cm^{-1} the C–H in plane stretch (Chen *et al.*, 2012; Cincinelli *et al.*, 2017). Because PET was only found in microfiber forms, a synthetic textile source from domestic washing should be suggested.

Figure 5 and 6 depict distinctive absorption peak band examples for 8 identified polymers. The wide range of applications and relatively low cost of polyethylene renders its prolific use, being identified as the predominant polymer in many wastewater studies (Mintenig et al. 2017; Gatidou et al. 2019; Akarsu et al. 2020). Absorption bands for LDPE (Fig. 5b) include an asymmetric vibration of CH_2 group between 2930 – 2850 cm^{-1} wavenumbers, 1450–1470 cm^{-1} for bending C-C bond between methylene carbons, and 700–750 cm^{-1} band due to rocking in plane of CH_2 groups. The distinctive peak near 1715 cm^{-1}, marked with a blue arrow, would be related to C = O stretching of non-ionized carboxylic group because an oxidation of the polymer (Matsuguma et al. 2017). It is important to take into account the aggressive

and severe conditions surrounding microplastics in a wastewater environment, together with a warm and arid climate that will facilitate oxidation of polymers. The vast majority of film was identified as LDPE polymer (63.16%), probably due to the proximity of the sewage plant to agriculture crops under plastic mulching, a major source of MPs in the terrestrial environment (Piehl et al. 2018). LDPE is the most inexpensive plastic film and the dominant covering material in the Mediterranean region, with high transmissivity to thermal radiation (Papadakis et al. 2000), reaching the WWTP as a MP by atmospheric transportation.

Polypropylene (Fig. 6a) has some spectral bands similar to polyethylene, although the absence of peaks between 700 and 750 cm^{-1} clearly distinguish them (Castillo et al. 2016). Again, a blue arrow marks a distinctive peak close to 1715 cm^{-1} wavelength due to PP oxidation. After the tertiary treatment, only PP significantly disappeared in the EFF (*F-test* = 5.819, $p = 0.023$), the removal rates for LDPE and HDPE being 98.72% and 86.71%, respectively.

Acrylate copolymers; i.e., ethylene/ethyl acrylate copolymer and poly (t-butyl acrylate) (Fig. 5d) were also identified, previously reported by Liu et al. (2020) in atmospheric microplastics deposited on terrestrial plants, and used in commercial shower gels, peelings, waterproof sunscreen or as a gallant for lipstick (Liebezeit and Dubaish 2012).

Copolymers as poly (dimer acid-sebacic acid) copolymer (PDASA) (Fig. 6c) and poly (ethylene:propylene:ethylidene norbornene) copolymer (PEP) (Fig. 6d) were also collected. PDASA biopolymer has been reported to be degradable both *in vitro* and *in vivo* studies, leaving an oily dimer acid residue after hydrolysis (Xu et al. 2001). Because of a quick and extensive degradation, this biopolymer totally disappeared in the EFF. PEP was also identified and also reported by Magni et al. (2019) in WWTPs, mainly used in automobile parts and in the production of pipe seals.

Conclusions

This paper presented the results of the occurrence, analysis, and removal of microplastics from a full-scale WWTP located in Southeast Spain. An environmentally-friendly, reproducible and cheap method for the purification and isolation of microplastics based on a density separation was used, avoiding handling with chemicals that may alter their properties and composition. FTIR spectroscopy allowed us to detect a 72.41% of microplastics within all microparticles isolated, with average concentrations of 2.74 ± 0.49 MP L^{-1} in the influent and 0.98 ± 0.27 MP L^{-1} in effluent samples. The removal rate was 64.26%, within the average retention for European WWTPs, contributing to a daily calculated emission of 1.6×10^7 microplastics. The main collected shapes were microfiber (79.65%), film (11.26%), and fragment (9.09%), without microbeads identified in wastewater samples. The dominance of microfiber in all seasons, mainly identified as PET fibers, should be associated to textiles and garments made of synthetic materials, shedding fibers during their washing process. The removal efficiency for particulate forms of microplastics (90.03%) proved to be higher than for microfibers (56.16%), indicating that tertiary treatments are overall more efficient in removing particulate than fiber forms and suggesting an

improvement in sand filtration mechanisms by means of coagulants. Differences in annual variations of microplastic burden in the influent, probably due to the proximity to an expanding urban area, were safely cushioned in the effluent, reporting a stable performance of the sewage plant. Lower microplastic size in effluent than influent samples could be related to UV disinfection, and a massive identification of LDPE film could be due to the proximity of the sewage plant to greenhouse agricultural crops. Future efforts related to microplastics in WWTPs should be devoted to follow four different strategies: (1) a source control, avoiding a massive and indiscriminate use of plastic items and exploring alternative materials to efficiently reduce marine plastic litter; (2) an effective waste management for the recovery of resources from plastics, preventing their entry into the food chain and water bodies in the form of microplastics; (3) an improvement on international policies legislation, public initiatives and regulations for the use of plastics; and (4) microplastic-targeted treatment processes in WWTPs to reduce their emission to the environment.

Declarations

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Ethics approval and consent to participate.

Not applicable.

Consent for publication.

Not applicable.

Availability of data and materials.

The datasets generated and analyzed during the current study are not publicly available due to data belongs to the company operating the sewage plant, but are available from the corresponding author on reasonable request. Operational data and sampling volumes are included in “Supplementary Information”.

Competing interests.

Competing interests have been included in “Acknowledgements” as follow: This work was financed by Project 5245/18IQA (Cetenma and Hidrogea). Analyses carried out by Sonia Olmos were supported by a grant from Fundación Séneca (20268/FPI/17). Authors gratefully acknowledge the work and cooperation of personnel of “La Aljorra” WWTP with wastewater samples collection.

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Author's contribution.

Javier Bayo: Methodology, Formal analysis, Writing, Investigation, Data curation, Visualization. **Sonia Olmos**: Data acquisition, Investigation, Resources, Supervision. **Joaquín López-Castellanos**: Conceptualization, Data acquisition, Methodology, Supervision.

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Figures

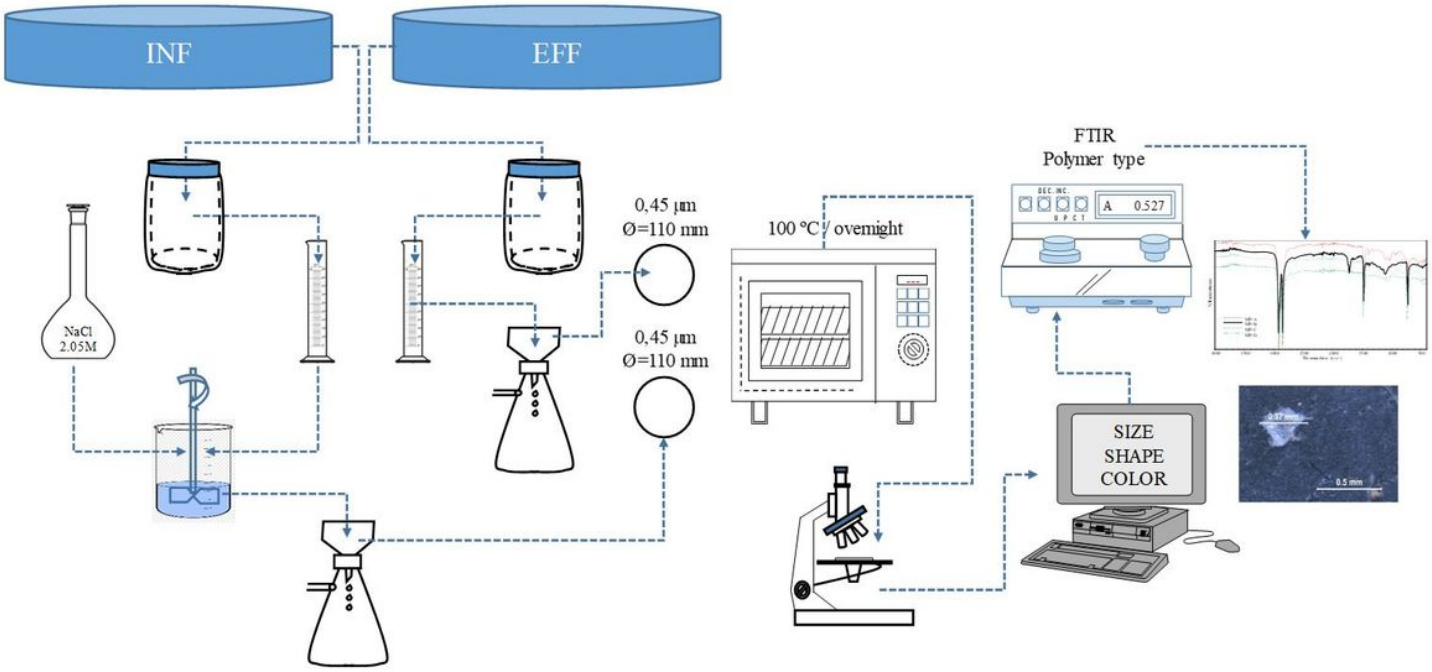

Figure 1

Flow diagram for the analysis of microplastics in “La Aljorra” WWTP.

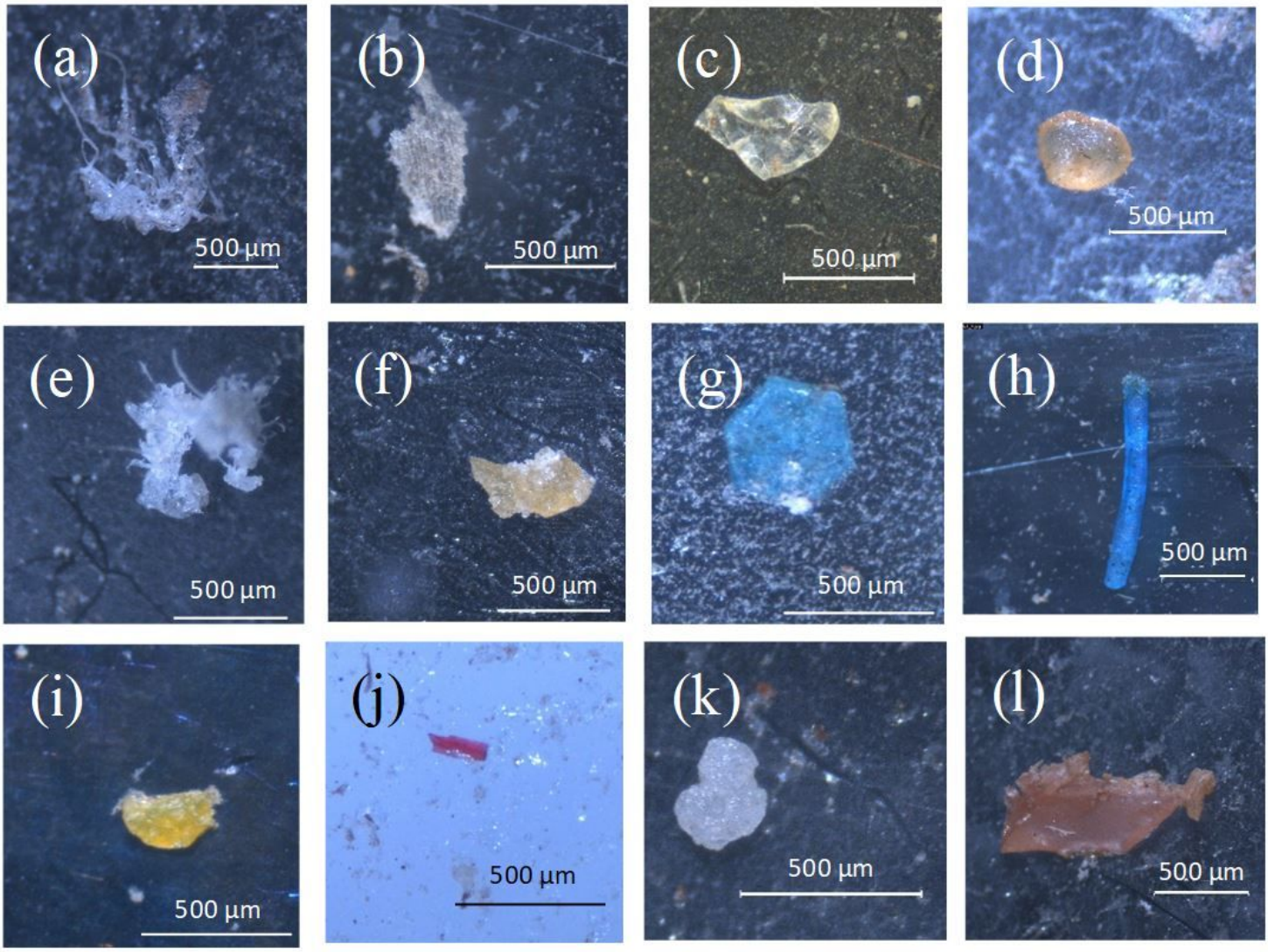

Figure 2

Microlitter (a-d) and microplastics (e-l) in “La Aljorra” WWTP: (a) cellulose (INF/1st.Aug.2019); (b) chipboard (INF/20th.Feb.2020); (c) silicic acid, sodium salt (EFF/20th.Feb.2020); (d) calcium stearate (INF/19th.Dec.2019); (e) poly(butyl acrylate (INF/12th.Mar.2020); (f) sebacic acid biopolymer (INF/20th.Mar.2020); (g) polyester (INF/19th.Sep.2019); (h) polyethylene filament (EFF/17th.Oct.2019); (i) polyester (EFF/19th.Sep.2019); (j) polypropylene (INF/1st.Aug.2019); (k) polyethylene wax (INF/20th.Feb.2020); (l) oxidized polyethylene (INF/20th.feb.2020).

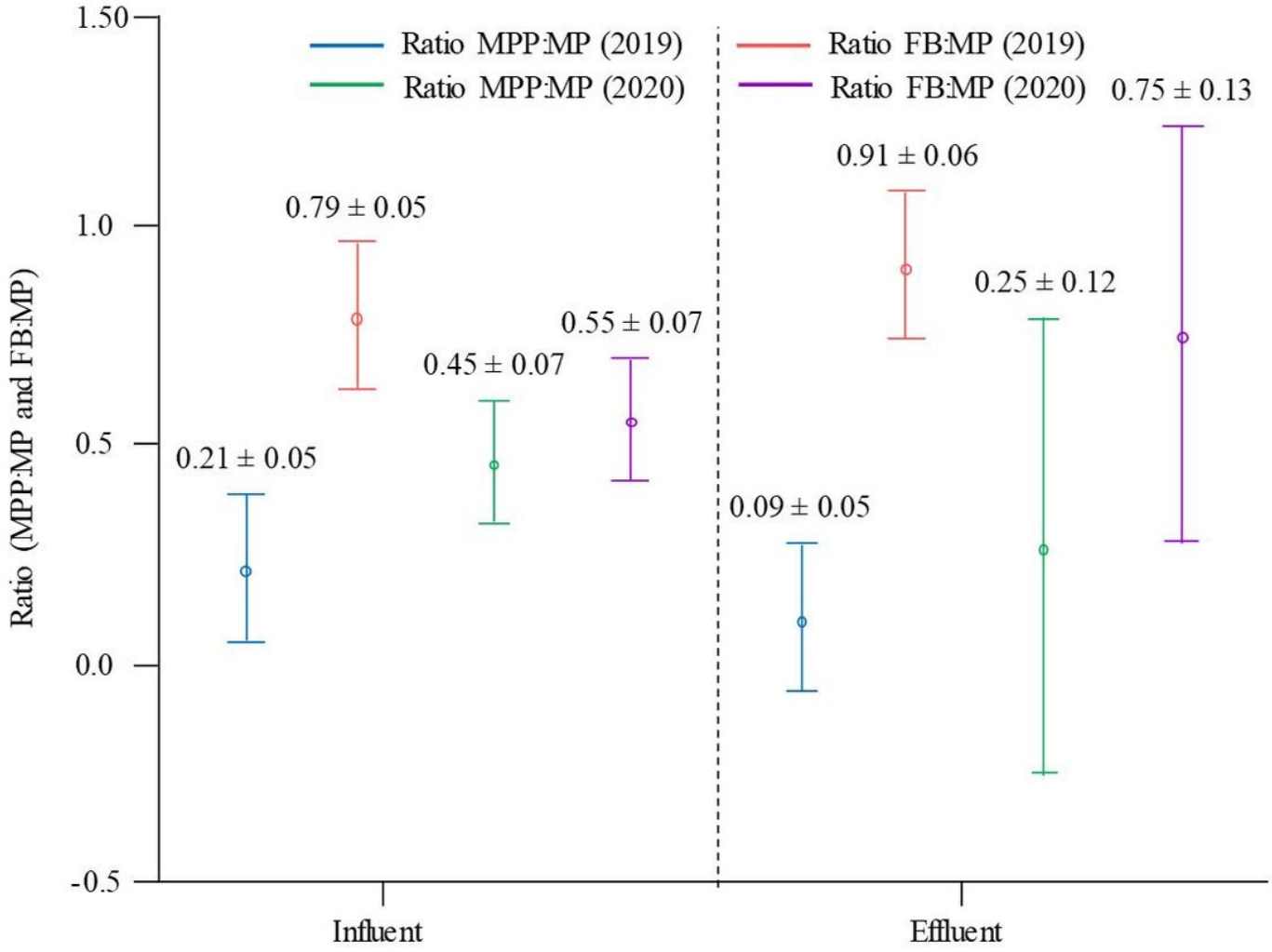

Figure 3

Annual variations of MPP:MP and FB:MP ratios (mean ± standard error).

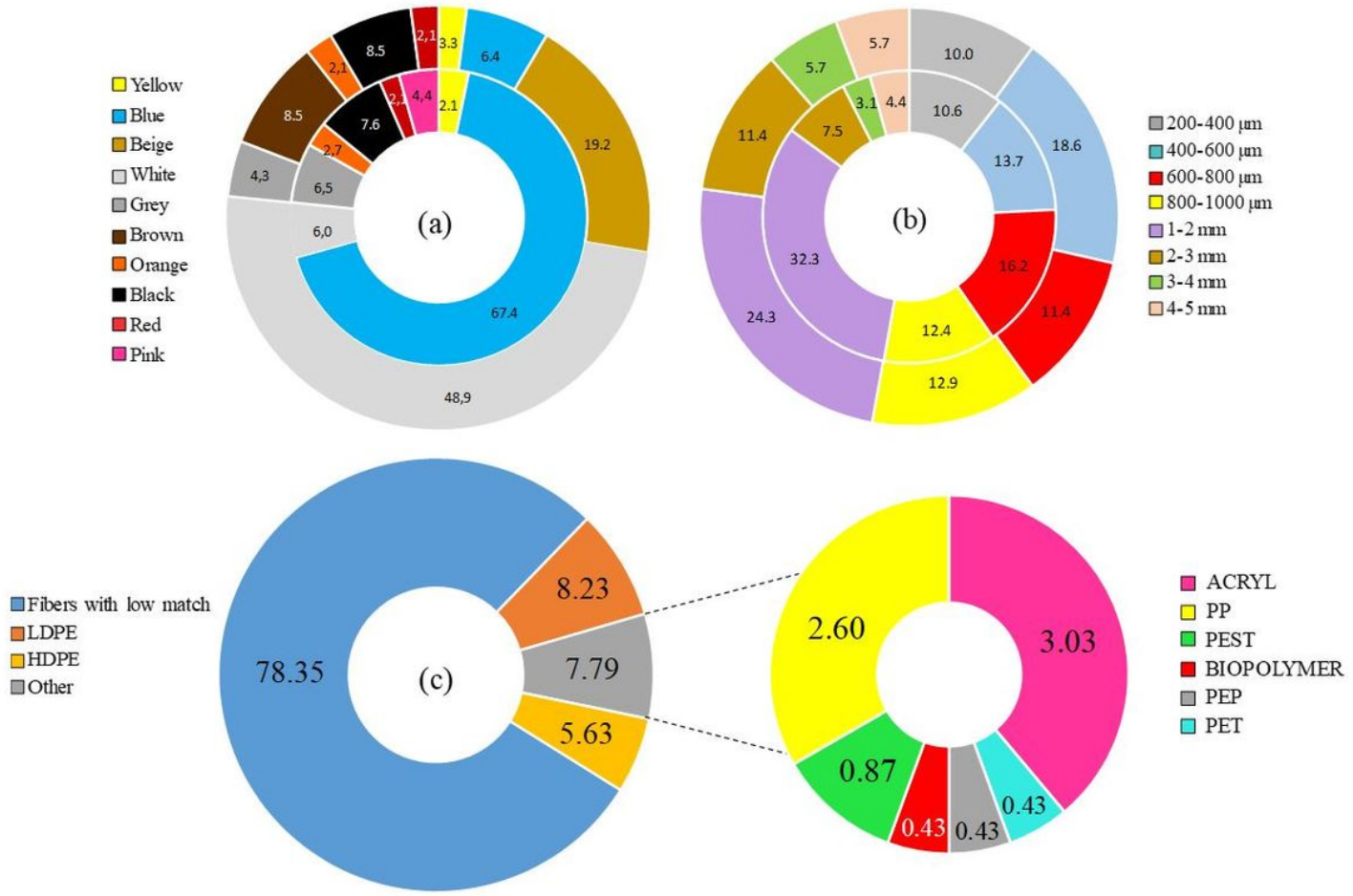

Figure 4

Accumulated percentages across the WWTP: (a) colors by microplastics shape: Inner ring means FB, outer ring means MPP; (b) size categories based on Spanish Environmental Ministry classification: Outer ring means EFF; (c) polymer types identified by FTIR, including fibers with low percentage match.

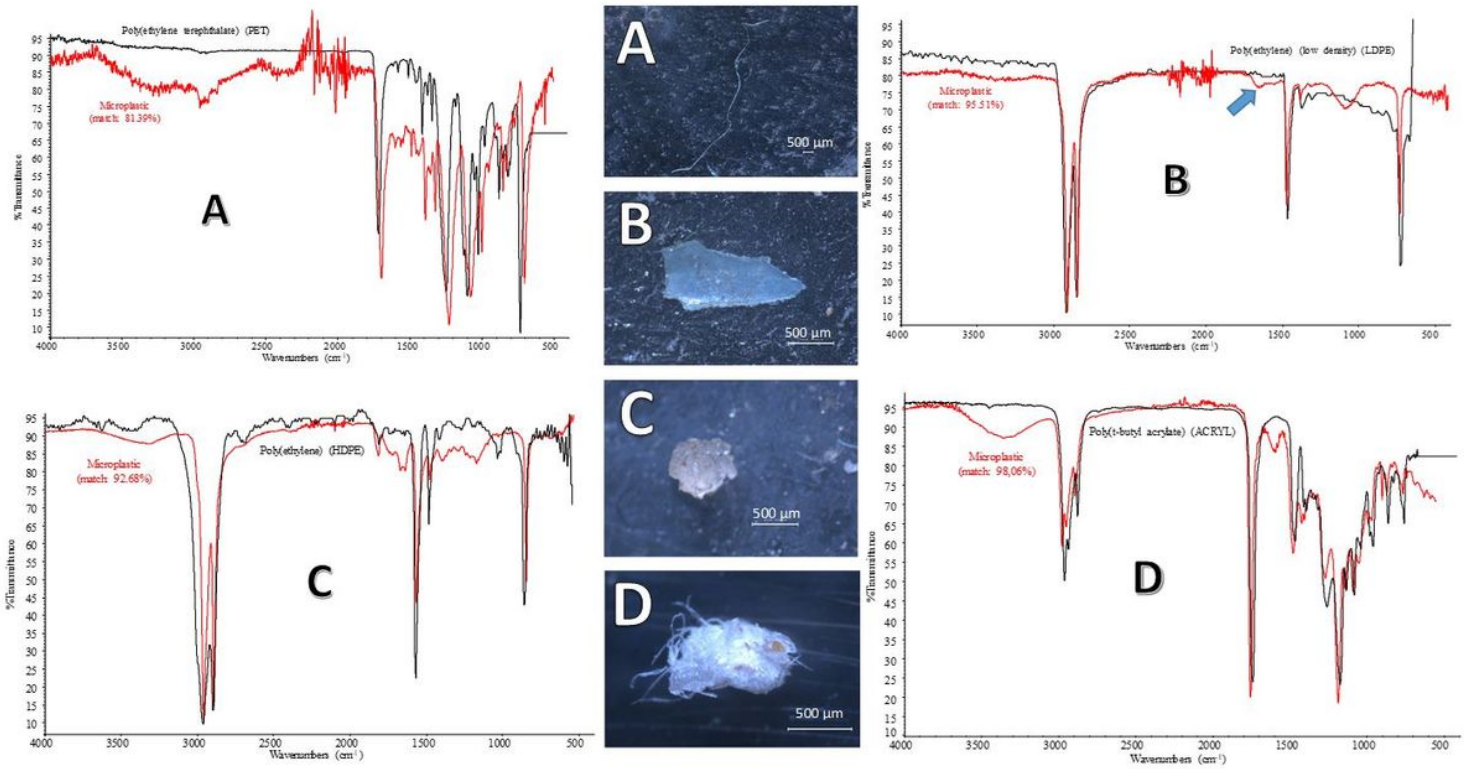

Figure 5

Infrared standard spectra (black) and microplastics (red) for: (A) Polyethylene terephthalate (PET) EFF 17th.Oct.2019 (81.39%) (Sprouse Scientific Systems Polymers by ATR Library); (B) Poly(ethylene) (low density) INF 28th.Feb.2019 (94.51% match) (Sprouse Scientific Systems Polymers by ATR Library); (C) Polyethylene INF 23rd.Jan.2020 (92.68% match) (Polymer Additives and Plasticizers). (D) Poly (t-butyl acrylate) EFF 19th.Dec.2019 (98.06% match) (Sprouse Scientific Systems Polymers by ATR Library).

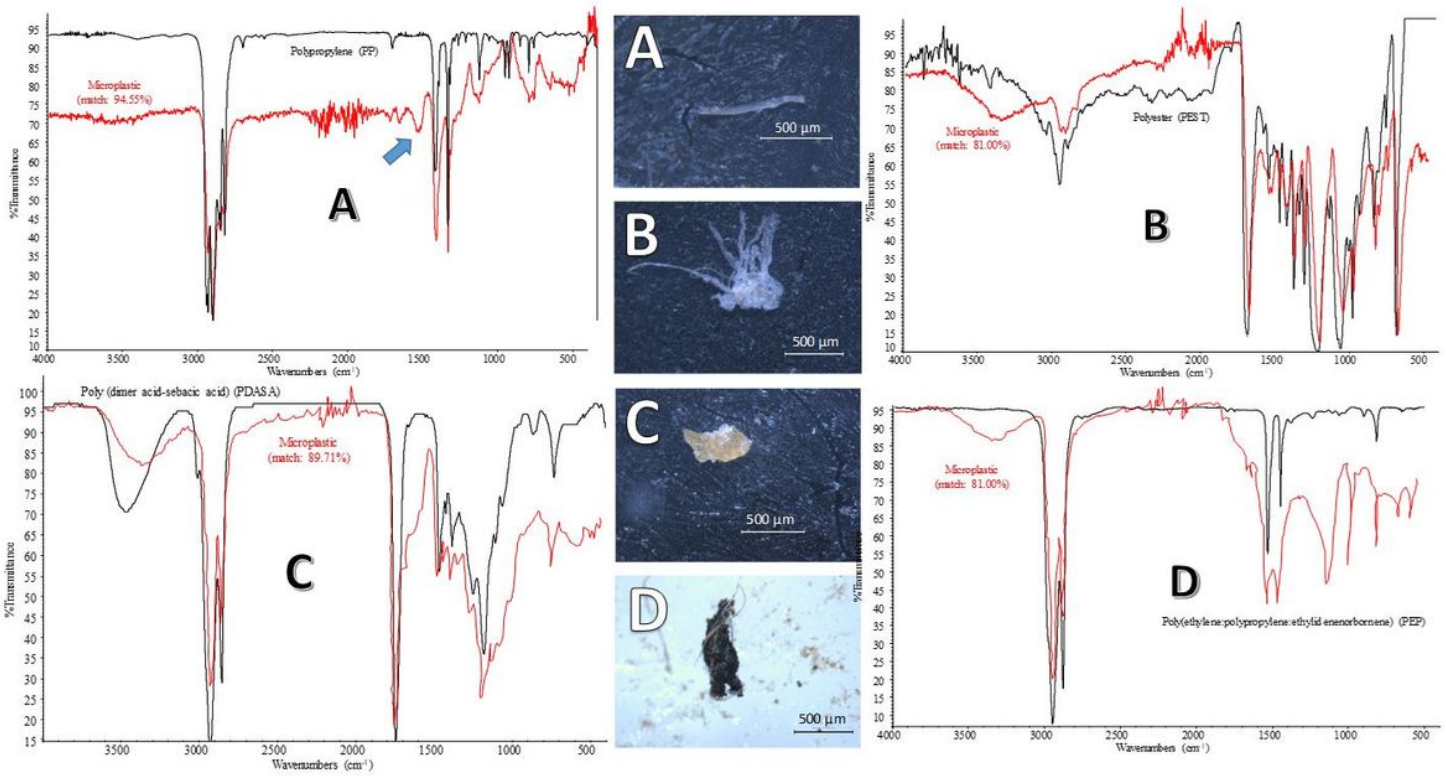

Figure 6

Infrared standard spectra (black) and microplastics (red) for: (A) Polypropylene (HDPE) INF 24th.May.2020 (94.55%) (Polymer Additives and Plasticizers); (B) Polyester (PEST) INF 19th.Sep.2019 (81.00%) (Synthetic Fibers by Microscope); (C) Poly (dimer acid-sebacic acid) copolymer (PDASA) INF 20th.Mar.2019 (89.71%) (Polymer Additives and Plasticizers); (D) Poly (ethylene:propylene:ethylidene norbornene) (PEP) INF 28th.Feb.2019 (81.00%) (Hummel Polymer and Additives).

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