Microplastics and Nanoplastics

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Microplastics in an advanced wastewater treatment plant: sustained and robust removal rates unfazed by seasonal variations

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Abstract

Microplastics (MP), fragments of plastic generally defined as, less than 5 mm in size, originating from various urban sources, have become a significant environmental concern due to their widespread presence and potential impacts on ecosystems. This study investigates the efficiency of an advanced wastewater treatment plant discharging into the Mediterranean Sea in removing MPs from wastewater. The plant processes wastewater through a series of treatment stages, including screening, desanding, coagulation/flocculation, biological filtration, and sludge incineration. Samples were collected and analysed during three distinct campaigns (dry, rainy, and touristic seasons) to assess the plant's performance under varying conditions. Using matrix-representative sampling methodologies and Focal Plane Array micro Fourier-Transform Infrared Spectroscopy (FPA-μFT-IR) for MP quantification, the study measured MP concentrations and removal rates. The treatment plant demonstrated high removal rates of microplastics across different periods. Using a mass balance approach, the removal efficiency during the dry sampling period was 99.85%. In the rainy campaign, the efficiency slightly decreased to 99.11% due to increased runoff, while during the touristic period, the efficiency peaked at 99.95%. Polyester was identified as the predominant polymer type. The primary treatment stages, particularly coagulation/flocculation and lamellar settling, are most effective in MP removal. The majority of MPs are retained in the sludge, which is subsequently incinerated, preventing environmental discharge. This research demonstrates that a WWTP employing advanced treatment processes is not a source of MP to the environment but rather a sink. Despite variations in influent MP concentrations across different seasons, the plant consistently maintained high removal rates, effectively mitigating MP pollution. In this study, sludge incineration further ensured that MPs were prevented from entering the environment.

Keywords μFT-IR, WWTP, Wastewater, Removal rate, MP, Microplastics, Seasonal variation

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Introduction

Microplastics (MP), tiny plastic fragments less than 5 mm in diameter, have emerged as a significant environmental concern [1]. These particles, originating from various sources, including cosmetic products, synthetic textiles, and the breakdown of larger plastic waste [2], have infiltrated nearly all ecosystems. Given their persistence in the environment and the potential impacts on wildlife, human health, and ecosystem functioning, the extent of MP pollution and its mitigation have become growing concerns [3].

Urban areas are key contributors to MP pollution, as noted by [4] .Human activities lead to MPs entering wastewater systems, while stormwater and combined sewer overflow (CSO) can also carry them into aquatic environments. Wind dispersal is another pathway for MPs to reach water bodies. In developed countries, wastewater undergoes treatment at wastewater treatment plants (WWTP), playing a significant role in MP abatement.

WWTPs exhibit variable removal efficiencies for MPs. Studies like [5] reported a 98.8% removal rate for small MPs (10–500 µm) in a Swedish plant, while [6] and [7] documented 98% and 97% removal rates for Danish and German WWTPs with tertiary treatment, respectively. Conversely [8] and [9], noted that certain MPs could bypass the treatment process, with removal rates of 75–99% and 84%, respectively, potentially leading to a significant discharge into the environment. MPs retained in wastewater treatment processes predominantly accumulate in sludge, as highlighted by [10] and [5]. This sludge is often repurposed for agricultural land application, presenting a potential route for reintroducing MPs into the environment. To entirely eliminate this risk, sludge incineration has been identified as an effective method to prevent MPs from entering the environment, as noted by [11].

The concentrations of MPs in wastewater can vary due to a range of complex factors, including catchment area size, population served, surrounding land use, combined sewer systems, and the nature of wastewater sources (residential, commercial, or industrial) [11]. Seasonal variations are also important to consider, as rainfall in the autumn can increase runoff and introduce additional MPs into the wastewater system. Similarly, population surges in touristic areas during the summer lead to greater wastewater production and potentially higher MP concentrations. Moreover, the type of treatment process, such as secondary or tertiary treatment, plays a crucial role. Additionally, the methodology employed in MP analysis is significant. Different analytical methods can lead to varying results, especially in terms of MP size, shape, and polymer type identification. The targeted size range of MPs is another critical factor. Studies focusing on different size ranges may report different removal efficiencies, as smaller MPs have been argued to be more challenging to capture. For example, using two different methodologies [9], found, on average, 2.0 counts L^{-1}, while [6] found a much higher median concentration of 7216 counts L^{-1} in influent wastewater [12] and [13]. have highlighted that relying solely on visual inspection for MP analysis is inadequate and subject to human bias. They advocate that MP analysis should always be complemented with chemical identification techniques. A prominent method in this regard is Fourier-Transform Infrared Spectroscopy (FT-IR). This method offers a powerful means of chemically characterising MPs. When combined with a focal plane array (FPA) attached to an FT-IR microscope, it allows for the imaging and chemical identification of samples without any manual sorting of particles. This automated approach enhances accuracy and reduces the likelihood of human error in MP identification. Numerous studies have effectively utilised this methodology, for example [5–7].

Sampling techniques also significantly impact the results. Factors like the frequency, duration, and location of sampling within the WWTP can influence the perceived concentration of MPs. Inconsistent sampling can lead to underestimating or overestimating MP levels [11].

Weather patterns, population density, and human activity can influence MP pollution in wastewater. However, due to the demanding sample collection and preparation requirements, many studies investigating MPs in treatment plants have focused on single-time-point sampling, not considering temporal variations in MP removal rates. However, research that has accounted for these variations over time, such as the studies by [14] and [15], indicate that MP removal rates remain consistent and unaffected by seasonal changes.

While the majority of studies typically report concentrations in terms of particle counts and size, these parameters, although crucial for eco-toxicological evaluations as outlined by [16], do not account for the shifting behaviour of MPs. Due to continuous fragmentation, MPs increase in number and decrease in size [17]. This process can potentially skew particle count and size-based assessments [6]. suggested that measuring MP concentration by mass could offer a more stable and consistent metric. Mass, as a conserved base quantity, remains unaffected by the physical and chemical processes MPs undergo. Hence, while particle count and size provide valuable information, mass-based measurements could offer a more reliable assessment of MPs in environmental studies, particularly in tracking their long-term fate and impacts.

This study seeks to enhance understanding of MP removal in wastewater treatment plants. It achieves this by conducting a detailed mapping of an entire plant,

employing a rigorous sample collection methodology coupled with an FPA-μFTIR analysis. A key aspect of the study is accounting for temporal variations reflected in three distinct sampling seasons: dry, rainy, and touristic. The chosen wastewater treatment plant, located in southern France, discharges its effluent into the Mediterranean Sea, making this study particularly relevant for coastal MP pollution dynamics. This approach aims to provide comprehensive insights into MP removal efficiencies under varying environmental conditions and operational capacities.

Materials and methods

Description of the WWTP

The Amphitria Wastewater Treatment Plant is located in Cap Sicié, La Seyne-sur-Mer, France, and discharges treated wastewater into the Mediterranean Sea. The plant is integrated into the environment with a discreet and compact architecture. It has a capacity for 500,000 population equivalents (p.e.) and processes a daily flow of 103,000 m^3.

The treatment commences with two screening stages, at 25 mm and 6 mm, followed by desanding and de-oiling pre-treatments. It then leads to a physico-chemical stage involving coagulation/flocculation and lamellar decantation. Afterwards, the wastewater continues to the biological filters and then gets discharged via the first outlet. The biological treatment involves regular backflushing of the biofilters. The backflush wastewater then undergoes an additional round of coagulation/flocculation and lamellar settling before environmental discharge via the second outlet. The final extracted sludge is centrifuged for dewatering and then incinerated in a fluidised bed furnace.

Sampling

This study collected samples mapping the entire treatment plant under distinct conditions, referred to as "campaigns." Each sample point was sampled in duplicates on two consecutive days. A total of 54 samples were collected – 18 for each campaign. The sample collection points can be seen in Table 1. An illustration of the WWTP can be seen in Fig. 1. The initial campaign, denoted as the "dry" campaign, spanned from 7-10-2019 to 10-10-2019. During this campaign, the prerequisite for sample collection was that no rainfall had occurred in the week preceding the sampling period. The second sampling campaign took place between 28-04-2021 and 30-04-2021, and it took place shortly after periods of significant rainfall and shall be referred to as the "rainy" campaign. The third campaign, known as the "touristic" campaign, was carried out from 7-07-2021 to 9-07-2021. This campaign was named as such due to the notable increase in population equivalents due to tourist activity during this period.

Wastewater samples were collected according to their solids concentrations. Samples with high suspended solids concentrations were collected in aluminium bottles (3 L) as 24-hour composite samples using an autosampler (Hydreka, Sigma SD 900). More diluted samples, such as the effluent, were taken using the Universal Filtering Unit (UFO) system [18, 19] which can filter large quantities of treated wastewater through a 10 μm stainless steel filter (Ø167 mm) in a few hours. The goal was to filter 1 m^3 of the diluted water or until four filters had become clogged, typically indicating sufficient material had been collected. Except for one sample at the second outlet, where only 300 L of wastewater was collected, all other UFO samples slightly exceeded 1 m^3.

Sludge samples were obtained as grab samples using a steel hand trowel and placed in empty aluminium paint cans (1 L), which were filled completely. Each sample container was rinsed three times with Milli-q water before use. Samples were refrigerated until further processing.

Sample preparation

At the beginning of the extraction process, each type of collected sample was handled differently before the protocols converged. The aluminium containers containing the more concentrated wastewater were shaken vigorously to ensure proper resuspension of the settled particles. Afterwards, two litres were measured in a glass cylinder and filtered through a 10 μm (Ø47mm) stainless

Table 1 Description of the samples, indicating at which point they were collected. Sample ID is a short version of the description

Sample ID	Description	Sampling method
Inlet	Inlet of the WWTP	Autosampler
After_grit_and_grease	After grit and grease removal	Autosampler
After_coag_flocc	After coagulation/flocculation	UFO
Outlet_1	Outlet 1 – After biofilters	UFO
Backflush_biofilters	Backflush of the biofilters	Autosampler
Outlet_2	Outlet 2 – After the 2nd lamellar settling	UFO
Physico_chemical_sludge	Physico-chemical sludge	Grab
Biological_sludge	Biological sludge from the 2nd lamellar settling	Grab
Dewatered_sludge	Dewatered sludge – final sludge product	Grab

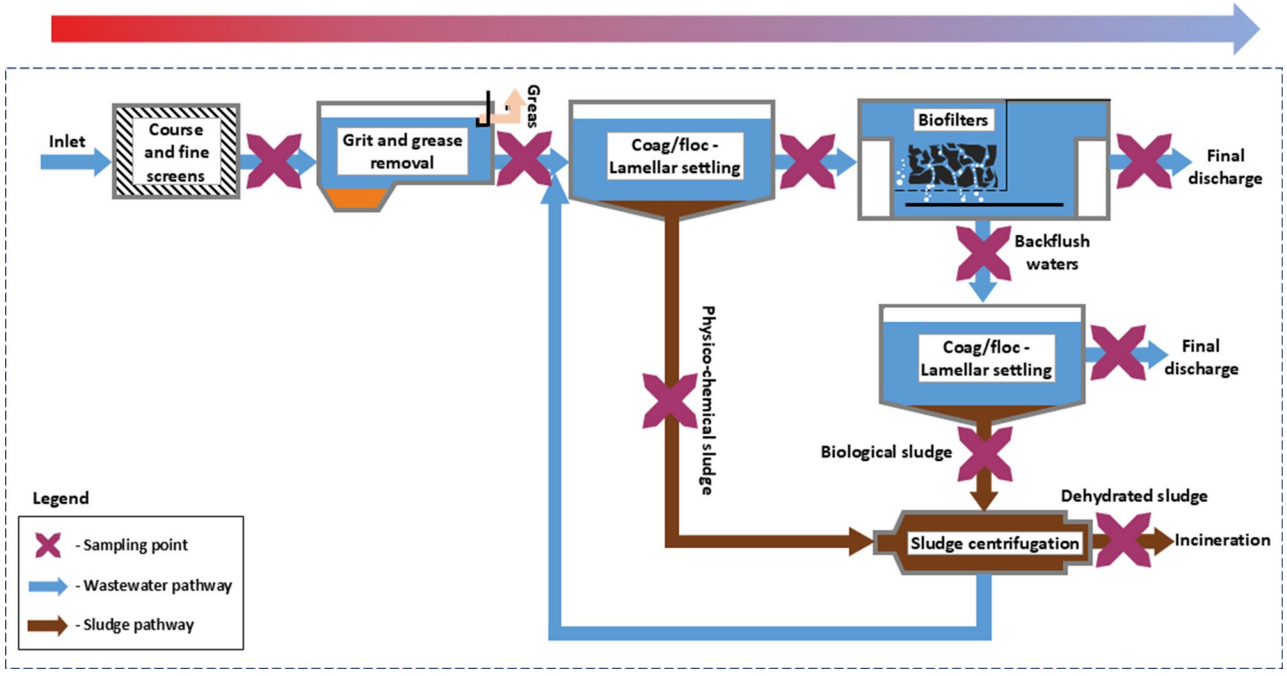

Fig. 1 Illustration of the Amphitria WWTP. The sample collection points are marked by an X. The wastewater pathway is illustrated in blue while the sludge pathway is illustrated in brown

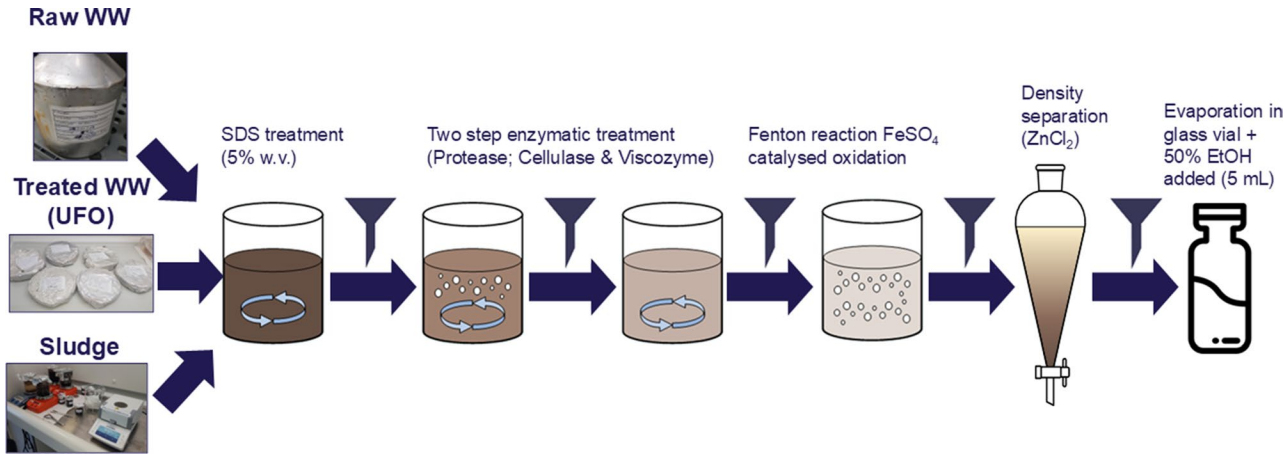

Fig. 2 The sample preparation procedure

steel mesh, which was saved in a glass Petri dish for subsequent sample preparation. The samples collected on the filters with the UFO system were placed into a crystallisation dish containing 5% w/w sodium dodecyl sulfate (SDS), and then the filters were sonicated in a sonification bath. Three sub-samples were extracted from the aluminium cans, and the water content of the sludge samples was determined using a Mettler Toledo Moisture Analyzer HE73 [5]. Based on the water content measurements, 5 g of dry sludge equivalent was processed further. The sludge samples were suspended in 200 mL of Milli-Q water, followed by the careful and gradual addition of hydrogen peroxide (H_2O_2) until a concentration of 10% was achieved.

Afterwards, all the sample types underwent the same extensive treatment following a slightly modified protocol from [6]. The analytical train of the sample preparation can be seen in Fig. 2. Firstly, the filters were incubated in 250 mL of 5% w/w sodium dodecyl sulfate (SDS) solution for at least 24 h. Next, the samples were transferred into 250 mL of tris(hydroxymethyl)aminomethane buffer solution at pH 8.2, and 500 µL of protease (Protease from Bacillus sp.®, Sigma-Aldrich) was added. The samples were filtered again and then placed in an acetate buffer at pH 4.8. To the sample solution, 500 µL of cellulase

(Cellulase enzyme blend®, Sigma-Aldrich) and 500 µL of viscozyme (Viscozyme®L, Sigma-Aldrich) were added. Enzymatic treatment was carried out at 50 °C with gentle stirring of the samples. The sample was then transferred into 200 mL of filtered (0.7 µm) demineralised water and subjected to a catalysed oxidation (Fenton's reagent) [20] by adding 145 mL of 50% H_2O_2, 65 mL of 0.1 M NaOH, and 62 mL of 0.1 M FeSO4. The reaction temperature was kept between 15 and 30 °C to avoid iron precipitation and microplastic damage. The solution was then filtered through a 500 µm sieve, and the samples were placed in a $ZnCl_2$ solution ($\rho = 1.7$ g cm^{-3}) and transferred into separatory funnels. The particles > 500 µm were placed in aluminium trays and saved for later inspection. The samples were agitated using compressed air introduced from the bottom opening of the funnels for 15 min. After overnight settling, the denser inorganic particles were gradually removed. The supernatant was then filtered out and transferred into 50% v/v ethanol. The final sample concentrate was transferred into a 10 mL headspace vial. The ethanol was gradually evaporated in an evaporation bath (TurboVap® LV, Biotage) at 50 °C using a gentle flow of N_2. Finally, the final volume of the samples was fixed by adding 5 mL of ultra-pure HPLC grade 50% ethanol. All reagents employed for the sample preparation were filtered through a 0.7 µm glass fibre filter.

Contamination prevention and assessment

Several strict measures were taken to minimise the possibility of contamination throughout the entire sample preparation process. In order to avoid any potential airborne plastic-related contamination, plastic tools were completely avoided and substituted with either metal or glass whenever feasible. Glassware and any items that came in contact with the samples, such as spoons and spatulas, were thoroughly washed at least three times with filtered (0.7 µm glass fibre) deionised water. Moreover, the steel filters and the headspace vials were muffled at 500 °C. Furthermore, all lab personnel were required to wear cotton lab coats and t-shirts during the entire sample preparation procedure in the laboratory to reduce any potential contamination.

To further minimise the risk of contamination, most of the sample preparation was conducted in a fume hood, and the samples were always covered with aluminium foil while being taken out. The deposition process on the zinc selenide (ZnSe) windows was conducted in a laminar flow bench to maintain the cleanliness of the samples.

Lastly, the room housing the µFTIR machines was equipped with a high-efficiency particulate air (HEPA) filter (H14, 7.5 m^2) and continuously filtered by a Dustbox® (Hochleistungsluftreininger, Germany) to maintain a clean and controlled environment.

Despite the rigorous measures taken to prevent contamination during the sample collection and preparation process, the risk of contamination still exists, particularly during the sample collection process at the WWTP. An empty glass petri dish was placed close to the sampling spots and carefully opened each time the filters in the UFO system were exposed to open air, simulating the potential exposure of the samples to airborne MPs to assess this potential source of contamination. The petri dishes were then processed in the laboratory using the same procedure and reagents as the samples, ensuring that any potential contamination could be detected and accounted. A total of four blank samples were collected and processed.

Spectroscopic analysis

A subsample was deposited onto a ZnSe transmission window (Ø 13 mm × 2 mm) prior to analysis to identify the chemical composition of the concentrated particles. The window was placed in a compression cell (Pike Technologies), which reduced the active area of the window to Ø10 mm. Aliquots of 100 µl were added to the window using a glass capillary micropipette, and the windows were dried at 50 °C on a heating plate. This process was repeated until the window was well populated with particles while avoiding aggregation and overlapping. The chemical composition of the particles was determined using micro Fourier Transformation Infrared Spectroscopy (µFTIR) imaging, utilising a Cary 620 FTIR microscope coupled with a Cary 670 IR spectroscope (Agilent Technologies, USA). The entire active area of the ZnSe window was scanned (Ø 10 mm, area 78.5 mm^2) using a 15x magnification Cassegrain objective and mercury cadmium telluride (MCT) detector with a 128×128 focal plane array (FPA), yielding a pixel resolution of 5.5 µm. The scans were performed in transmission mode with a spectral range of 3750–850 cm^{-1} and a resolution of 8 cm^{-1} by co-adding 30 scans of each individual tile. A background scan was collected before each sample, co-adding 120 scans.

Data handling

The infrared images were analysed using siMPle software (previously known as MPhunter), as described by [21, 22]. This software minimises human bias in data analysis. siMPle quantifies polymer distribution in samples by matching each infrared (IR) pixel from spectral maps to a library of spectra comprising both synthetic and natural materials [23]. It calculates a particle's major dimension by identifying the longest distance between pixels in the particle's structure. Assuming an elliptical shape, the particle's minor dimension is inferred from the equivalent ellipse's area. The thickness is estimated at 67% of this minor dimension. Particle mass was then calculated from

its volume based on an ellipsoid shape and material density [6]. Particles were classified as "fibers" if their length-to-width ratio exceeded three and as "fragments" if this ratio was three or less [23].

The statistical analysis and graph creation were conducted using R version 4.3.0. The normality of the dataset was performed on the major dimensions of the particles using a Shapiro-Wilk test. When data were not normally distributed ($p<0.05$), differences between samples were evaluated using a Kruskal-Wallis non-parametric test.

Results and discussion

Contamination

The mean number of synthetic particles identified in the blank was only 4.3, whereas the average amount of MP per sample was 200. The predominant polymer types identified in the blanks were polyethylene and polyester. Since the levels of MP in the blanks were low and unlikely to have had a significant impact on the results, blank correction was not performed based on the the recommendation from European Commission & Joint Research Centre [24].

MP concentration and removal rates within the treatment plant

A total of 54 samples were collected and analysed (18 for each campaign), summing up to 18,054 L of filtered wastewater. In total, 7587 MPs, ranging between 10 and 500 µm, were identified. Particles exceeding 500 µm were excluded from further analysis due to their inconsistent occurrence, while many samples had none. Although the larger MPs were few, they would contribute disproportionately to the total mass, biasing the mass results. The summarised results can be seen in Table 2.

This data indicates that, by counts, on average, the wastewater treatment plant was highly effective in removing MPs (99.26%), with slightly higher efficiency observed when measuring by mass (99.6%). This slight increase in efficiency by mass at all stages suggests a trend where larger or denser particles, which contribute more to the total mass, were more effectively removed

Table 2 Averaged concentrations (duplicates) found in the sampled points. For wastewaters, the concentrations are expressed as per litre, and for sludge, per gram of dry weight

Campaign	Sample Name	MP concentration [counts L^{-1}] for wastewaters / [counts g^{-1}] for sludge	MP concentration [µg L^{-1}] for wastewaters / [µg g^{-1}] for sludge
Dry	Inlet	109.38	61.50
	After_grit_and_grease	61.89	4.76
	After_coag/flocc	0.61	0.06
	Outlet_1	1.12	0.09
	Backflush_biofilters	210.50	74.73
	Outlet_2	0.11	0.01
	Physico_chemical_sludge	1435.00	253.59
	Biological_sludge	87.70	6.36
	Dewatered_sludge	1281.67	318.09
Rainy	Inlet	745.83	100.71
	After_grit_and_grease	1083.33	2057.93
	After_coag/flocc	3.45	5.31
	Outlet_1	3.74	0.55
	Backflush_biofilters	240.63	113.44
	Outlet_2	3.39	0.42
	Physico_chemical_sludge	618.33	1217.21
	Biological_sludge	279.17	48.68
	Dewatered_sludge	561.67	3669.22
Touristic	Inlet	1583.33	62.24
	After_grit_and_grease	787.50	34.02
	After_coag/flocc	1.58	0.12
	Outlet_1	0.28	0.01
	Backflush_biofilters	77.60	3.56
	Outlet_2	1.56	0.02
	Physico_chemical_sludge	782.50	91.53
	Biological_sludge	905.14	240.94
	Dewatered_sludge	798.67	72.87

compared to smaller ones, a phenomenon that was also observed by [25].

The Inlet MP concentration exhibited significant variation across different campaigns, ranging from 109.38 to 1583.33 counts L^{-1} or 61.5 to 100 µg L^{-1}. Despite these fluctuations, the wastewater treatment plant demonstrated consistent removal rates, indicating its effectiveness in MP removal was largely unaffected by the variations in influent MP concentrations.

Table 3 shows a uniform overall MP removal rate across all campaigns in terms of mass and particle counts. This consistency aligns with findings from [14] and [15] who also reported that MP removal rates are independent of seasonal variations.

The initial step of grit and grease removal shows limited efficacy in MP removal, with average concentrations at the Inlet being 812.85 counts L^{-1} and 74.82 µg L^{-1}, compared to 644.24 counts L^{-1} and 698.90 µg L^{-1} after grit and grease removal. This results in a substantial variation in removal rates.

The majority of MPs are effectively removed in the coagulation/flocculation-lamellar clarifier step, with an average removal rate of 99.45%. This process reduces MP counts from an average of 644.24 P L^{-1} at "After_grit_and_grease" to 1.44 P L^{-1} at "After_coag/flocc". In terms of mass, the concentration decreases from 698.90 µg L^{-1} at "After_grit_and_grease" to 3.01 µg L^{-1} at "After_coag/flocc". These findings are very similar to what Talvitie et al. (2017) found in a Finish wastewater treatment plant. That study found that 97.4 −98.4% of microlitter was removed during mechanical and chemical pre-treatment. Similarly [8, 10], found that MPs are mainly removed in the primary treatment via skimming and sludge-settling.

Due to sampling uncertainty, it is challenging to accurately assess the biofilter removal rate. In several sampling campaigns, the removal rates appeared negative, which can be attributed to the already minimal MP concentrations after coagulation/flocculation, averaging 1.88 counts L^{-1} and 1.83 µg L^{-1}. The first outlet of the plant "Outlet_1" recorded similar concentrations of 1.71 counts L^{-1} and 0.22 µg L^{-1}, further complicating the evaluation of the biofilter's effectiveness. In the study of [26], the biofilters also did not show a decrease in MP concentrations. Nonetheless, at the backflush waters of the biofilters "Backflush_biofilters," the concentrations were substantially higher (176.24 counts L^{-1} and 63.91 µg L^{-1}). This significant increase indicates that the biofilters did retain some MPs over extended periods despite the challenges in measuring precise removal rates.

The sludge from the coagulation/flocculation-lamellar clarifier step had the highest MP concentration during both the dry and rainy campaigns, as expected due to significant MP removal at this stage. However, during the touristic campaign, the highest concentration was found in the sludge from the biological treatment. This result may be attributed to variations in daily plant fluxes or sampling uncertainties.

While there were some variations, including occasional negative values in the calculated removal rates for different treatment steps, the coagulation/flocculation-lamellar clarifier consistently demonstrated high removal efficiency. The same was observed for the second coagulation/flocculation-lamellar clarifier, which treats the backflush waters from the biofilters, showing similarly reliable performance.

Polymer composition

In the entire dataset, polyester is the predominant polymer type, comprising 47.21% of the identified MPs. Polyethylene (PE) follows at 19.45%, and Polypropylene (PP) makes up 12.18%. Polyurethane (PU) accounts for 5.26% of the MPs. The 'Other' category, which includes a variety of polymers, represents 4.51%. Polystyrene (PS) and Polyvinyl Chloride (PVC) have similar proportions, with 3.93% and 3.90%, respectively. Polyamide (PA) constitutes 3.56% of the total MP count. The category labelled 'Other' encompasses the less abundant polymers which include pan_acrylic fibre, cellulose acetate, acrylic, acrylic paints, ABS (acrylonitrile butadiene styrene), vinyl copolymer, PVAc (polyvinyl acetate), PU (polyurethane) paints, PVA

Table 3 Removal rates at each treatment step in the plant. The overall removal rate is calculated as the difference between the inlet (AW1) and the sum of the two outlets (AW4 + AW6)

Campaign	Removal Grit and grease step	Removal Coag/flocc step, lamellar settling	Removal Biofilters	Removal 2nd Coag/flocc step, lamellar settling	Overall removal
Particle counts					
Dry	43.41	99.02	-	99.95	**98.87**
Rainy	-	99.68	-	98.59	**99.04**
Touristic	50.26	99.80	82.00	97.99	**99.88**
Mass of particles					
Dry	92.25	98.82	-	99.99	**99.84**
Rainy	-	99.74	89.60	99.63	**99.03**
Touristic	45.34	99.64	90.60	99.35	**99.94**

(polyvinyl alcohol), alkyd, polycarbonate, epoxy, and PTFE (polytetrafluoroethylene).

Tracing the journey of wastewater MP counts through the treatment plant, Fig. 3, A illustrates a notable trend in polyester content. Initially, at the plant's inlet, the polyester levels were highest. A discernible decrease in polyester concentration was observed as the wastewater progressed to the coagulation/flocculation step. Interestingly, polyester re-emerged in significant quantities in the backflush waters from the biofilters and was found in even higher concentrations in the sludge.

When analysing the prevalence of polymers by campaign, polyester consistently emerged as the most prevalent polymer across different seasons. Nonetheless, a notable reduction in polyester levels was observed during the rainy campaign in comparison to both the dry and tourist campaigns. This trend suggests that introducing stormwater into the treatment plant introduced a varied array of polymers, notably PP, PS, PU, and a broader spectrum of diverse and less common polymers categorised under the "Others" group.

Numerous studies have pinpointed polyester [27–32], primarily sourced from textiles, as a leading type of MP in wastewater, aligning with its prevalence observed within the WWTP of the present study. This consistency underscores the significant contribution of textile fibres to MP pollution. Along with polyester, other research [6, 7, 26, 33] highlighted PE or PP as the predominant MP type in wastewater. These polymers also rank among the most frequently manufactured polymers within the European Union [34].

Size and shape of the MPs

A Shapiro-Wilk test conducted on the major dimension of MPs for each campaign indicated that the distributions were not normally distributed ($p<0.05$). Subsequently, a non-parametric Kruskal-Wallis test revealed significant differences ($p<0.05$) in the major dimensions of MPs between all campaigns.

The size distribution of MPs in sludge, wastewater and treated wastewater remained consistent across all campaigns, as depicted in Fig. 4. MPs in treated wastewater were notably smaller than those in sludge or within the wastewater system, with the smallest sizes predominantly observed during the dry campaign. Additionally, the sludge samples exhibited the broadest range in particle sizes, indicating a greater variability in MP dimensions in this medium.

The distribution of fibres and fragments remains consistent across seasons (Fig. 5). Within WWTP, fibre content decreased post grit and grease removal and was further reduced after the coagulation/flocculation step. An increase in fibre content was noted in the backflush from the biofilters. The lowest fibre concentrations were observed at both outlets. This pattern indicates that fibres, attributable to their elongated structure, are effectively removed by an advanced treatment plant.

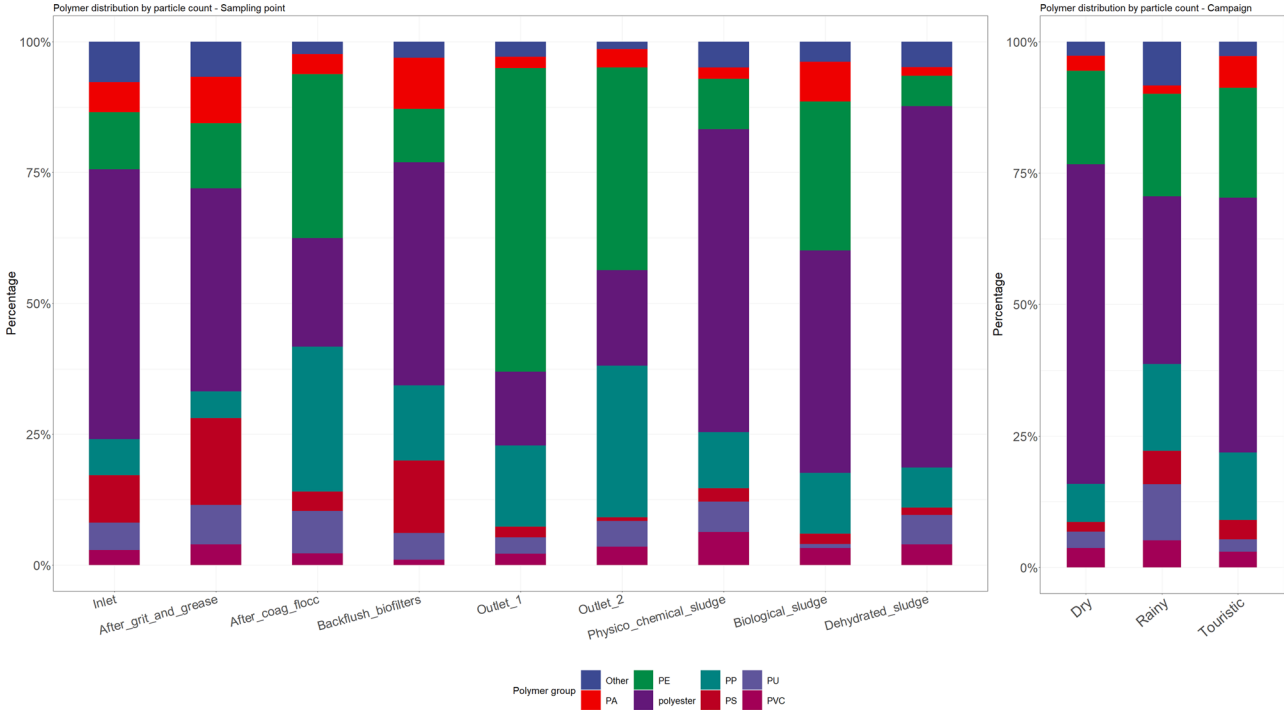

Fig. 3 The polymer distribution by MP counts grouped by the sampling point (**A**) and grouped by Campaign (**B**)

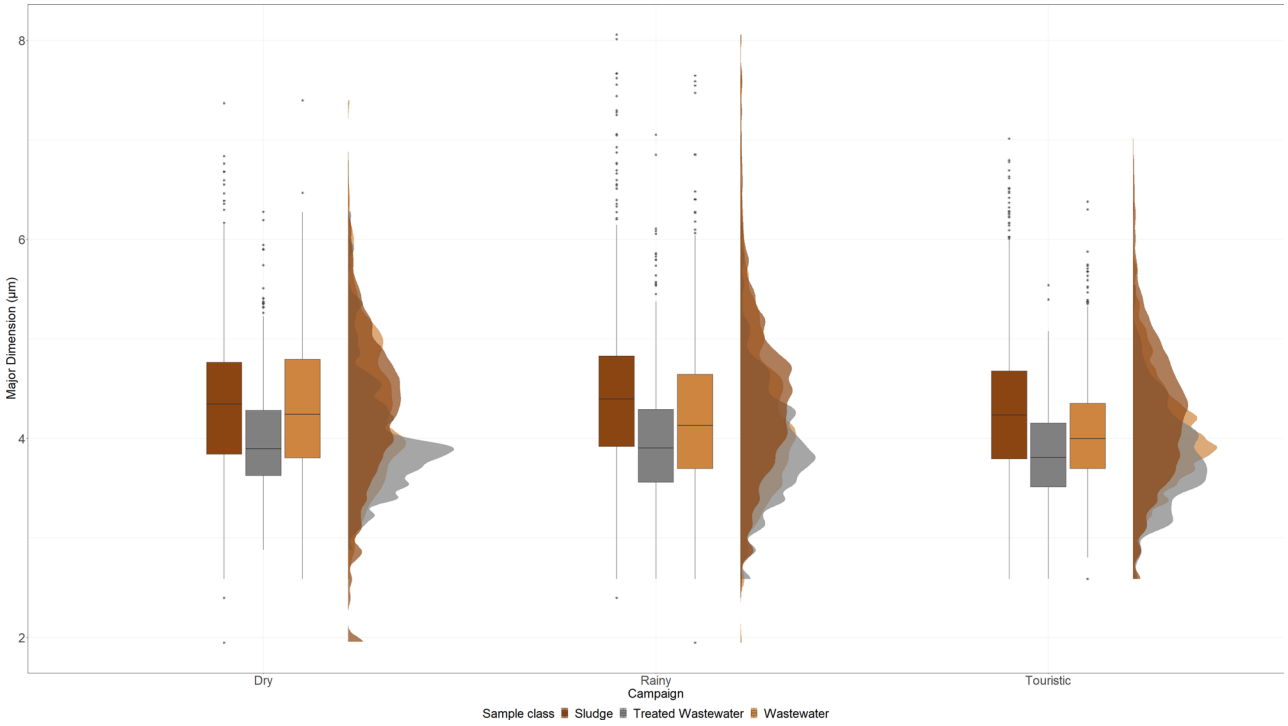

Fig. 4 Raincloud plots [35] of the major dimension of all identified MPs log-transformed. Data is grouped by sample type and campaign. Outliers are marked with an asterisk. The category "Wastewater" includes all the MPs from all the sampling points before the outlets

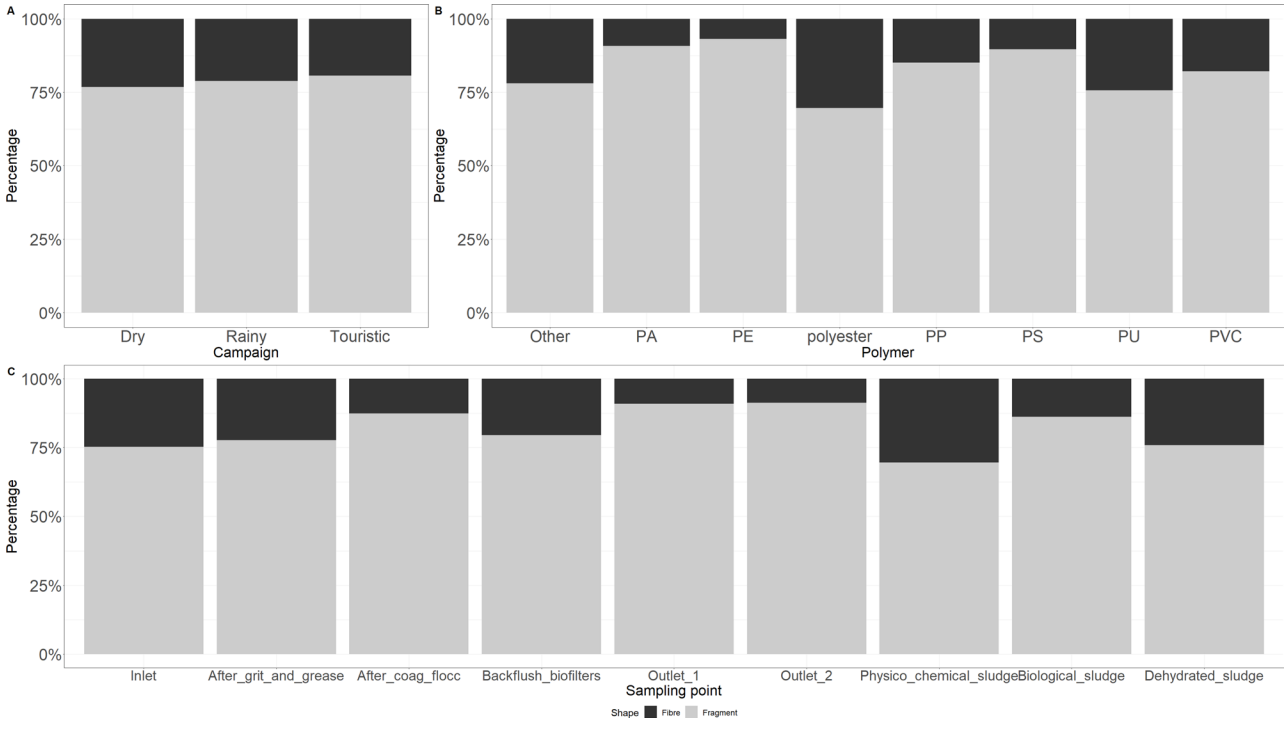

Fig. 5 Fibre/Fragment distribution in the entire dataset grouped by Campaign (**A**), Sample Class (**B**), and Sampling point (**C**)

[36] also found that polyester fibres are efficiently removed in a small lab-scale WWTP. While μFTIR faces challenges in analysing fibres due to their tendency not to remain in the focal plane on the Znse window, it nonetheless succeeds in detecting some of them. This partial detection ensures that the data remains comparable across the same dataset. This being said, Fig. 5B illustrates that polyester is the type of polymer that is found as fibres in the highest percentage (30%).

Mass balance at the Amphitria wastewater treatment plant

Applying a mass balance approach during the dry sampling period, the treatment plant processed an average daily inflow of 48,391 m^3 of wastewater, discharging 47,512 m^3 of treated water per day. The mass balance of small MPs was estimated to be 2.97 kg of MPs entering the plant each day, with only 0.0045 kg per day being released into the environment. The sludge retained 6.62 kg of MPs daily, and the calculated removal efficiency of the plant for MPs during this period was 99.85%.

During the rainy campaign, the plant processed an average daily inflow of 62,300 m^3 of wastewater, with 60,500 m^3 of treated water discharged per day. The MP inflow was 7.09 kg/day, with 0.0632 kg/day being released into the environment. The removal efficiency during this period was 99.11%, slightly lower due to increased runoff and stormwater inflows. Despite the greater MP inflow, the plant continued to retain the majority of MPs.

During the touristic period, the plant handled 55,400 m^3 of wastewater per day, discharging 54,300 m^3 of treated water daily. The inflow of MPs was 3.20 kg/day, with 0.0017 kg/day being discharged. The removal efficiency for this period was the highest at 99.95%, showing that despite the increase in wastewater production due to tourism, the plant effectively managed the MP load while minimizing environmental release.

Conclusion

The treatment plant demonstrated high removal rates of microplastics across different periods. Using a mass balance approach, the removal efficiency during the dry sampling period was 99.85%. In the rainy campaign, the efficiency slightly decreased to 99.11% due to increased runoff, while during the touristic period, the efficiency peaked at 99.95%. These results show the plant's consistently high performance in microplastic removal, despite varying seasonal conditions. The bulk of microplastics are eliminated during the primary treatment stage. The few microplastics that do slip through are typically small and non-fibrous. Moreover, the plant's strategy of incinerating sludge effectively prevents any reintroduction of microplastics into the environment, thus breaking the cycle of MP pollution.

While the biological active filter step shows limited impact on microplastic retention, the high concentration of microplastics in the biofilters' backflush water indicates some retention and warrants a deeper investigation to understand their role in microplastic containment fully.

Abbreviations

MP	Microplastics
CSO	Combined Sewer Overflow
WWTP	Wastewater Treatment Plant
FT-IR	Fourier-Transform Infrared Spectroscopy
FPA	Focal Plane Array
MCT	Mercury Cadmium Telluride
μFTIR	Micro Fourier Transformation Infrared Spectroscopy
PE	Polyethylene
PP	Polypropylene
PU	Polyurethane
PS	Polystyrene
PVC	Polyvinyl Chloride
PA	Polyamide
ABS	Acrylonitrile Butadiene Styrene
PVAc	Polyvinyl Acetate
PVA	Polyvinyl Alcohol
PTFE	Polytetrafluoroethylene
SDS	Sodium Dodecyl Sulfate
H_2O_2	Hydrogen Peroxide
HEPA	High-Efficiency Particulate Air
HPLC	High-Performance Liquid Chromatography

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Author contributions

Lucian Iordachescu: conceptualized the project, developed the methodology, performed the laboratory experiments, analyzed the data, and wrote the manuscript; Konstantinos Papacharalampos: performed the laboratory experiments, analyzed the data and contributed to the writing; Lauriane Barritaud: conceptualized the project, developed the methodology, analyzed the data, Marie-Pierre Denieul: conceptualized and managed the project, analyzed the data, contributed to the writing, Emmanuel Plessis: secured the funding and managed the project, Gilles Baratto: conceptualized and managed the project, organized the sampling, Veronique Julien: managed the project, organized the sampling, contributed to the writing. Jes Vollertsen: contributed to conceptualizing the work, supervised the study, and contributed to the writing.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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