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Microplastics occurrence and fate in full-scale treatment wetlands

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ABSTRACT

Treatment wetlands (TWs) are an efficient technology for removing microplastics (MPs) from wastewater, according to previous studies. This study investigates the dynamics and fate of MPs in two wastewater treatment plants (WWTPs) using TWs, one with horizontal subsurface flow (HF) and another with a floating plant system (FS). Special attention is paid to the retention produced in the sludge and the role of macrophyte roots. The abundance of MPs in the influent to the WWTPs was on average 20.3 ± 0.85 MP/L and 8.4 ± 1.13 MP/L in HF and FS respectively, while the effluent had 0.58 ± 0.07 MP/L and 0.17 ± 0.06 MP/L, thus giving overall efficiencies of 97.42% and 98.13%, respectively. In the HF wetland, sludge samples near the inlet and the outlet were taken, distinguishing between sludge adhered to gravel and sludge attached to roots. In the floating macrophytes, sludge samples from secondary and tertiary treatments were taken. The results indicate that roots play a significant role in MPs retention. In the HF wetland, the complex formed by roots and gravel attached more MPs than gravel alone in the final zone of the wetland. In the FS, roots retained a significant quantity of MPs, both in the secondary and tertiary treatments, thus giving rise to a sludge less concentrated in MPs. This study aims to improve the knowledge of MPs behavior and fate in full-scale TWs, providing valuable information to enhance retention efficiency.

1. Introduction

Plastics have been recognized as a worldwide environmental problem for decades (Carpenter and Smith, 1972). However, plastic materials are very advantageous, being more malleable, durable and cost effective (Andrady and Neal 2009). This is why millions of tons of plastic are produced annually and have been increasing over recent years (Plastic Europe, 2021). As a consequence, a wide range of plastic debris are accumulating in the biosphere (terrestrial and marine), which can be fragmented into smaller pieces due to the influence of different agents (sunlight, wind, mechanical, biological, or chemical forces) (Silva et al., 2018). Plastic particles smaller than 5 mm are considered microplastics (MPs) and are classified into primary and secondary MPs according to their origin (Thompson et al., 2004). Once in the environment, MPs can act as a means of transportation for other pollutants, such as heavy metals (Sarkar et al., 2021) or persistent organic pollutants (POPs), among others. Furthermore, due to its small size, MPs can be consumed by a variety of aquatic fauna. Indeed, MPs have been detected in more than 150 different plant or animal species (Jabeen et al., 2017), infiltrating the food chain. Additionally, some studies have shown that the presence of MPs in organisms can cause reduction of growth and photosynthesis, hinder reproductive ability, damage intestines, and even cause death (Lu et al., 2016; Wang et al., 2019; Yang et al., 2020).

Nowadays, researchers have found that one of the main sources of MPs to the marine habitats are wastewaters (Conley et al., 2019). MPs coming from cosmetics or toothpaste that contains microbeads, fibers from synthetic textiles that are released in washing machines (Boucher and Friot, 2017), and other articles like glitter, tires and cleaners or painting products (Sheavly and Register, 2007), reach the wastewater treatment plants (WWTP). Once there, most are removed from wastewater, given that the efficiency varies between 80 and 99% (Prata, 2018). Despite this, even if the MP output concentrations are low, the high discharge flow rates of WWTPs result in a significant number of MPs being released into the natural environment, causing considerable pollution throughout the day. Moreover, if the WWTPs removal of MPs are effective, it means that MPs are transferred into sewage sludge (van

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der Berg et al., 2020). Indeed, the concentration in the sludge is higher than influent wastewaters (Xu et al., 2020). When the sludge is then applied in farmlands, it represents a major input to the environment (Hernández-Arenas et al., 2021), for instance MPs may reach water bodies through runoff from the farmlands.

For a long time, treatment wetlands (TWs) have been investigated as a method to remove pollutants in wastewater. TWs are designed systems to treat wastewater and other pollution sources with low maintenance and operation cost (Vymazal, 2010). They represent a nature-based solution for wastewater treatment in small villages, for tertiary treatment in bigger plants and for dealing with stormwater (Rousseau et al., 2008). TWs have demonstrated to be competent in the reduction of MPs with an efficiency between 88%−95% when they are used as a tertiary treatment, adding to its already known benefits (Wang et al., 2020; Zhou et al., 2022; Bydalek et al., 2023). Previous researchers have found a good interception of MPs by vegetation in natural wetlands, whether in leaves, roots or attached in form of biofilm (Yin et al., 2021; Duan et al., 2021). Macroinvertebrates found in TWs, mainly worms, can also play a significant role in the distribution of the MPs (Wang et al., 2020). Furthermore, other studies have shown that worms may transport MPs and break them down even further. Bacteria present in the gut of the earthworms digest the MPs into their simpler, more volatile compounds (Lwanga et al., 2018).

In addition, there is a need to focus on further improving the legislative and policy system to eliminate MPs at source (Xu et al., 2022). Although the literature about microplastics is growing exponentially (Zhou et al., 2021), TWs are still a domain that needs to be investigated to gain a better understanding of the processes and the benefits that they can provide in this field.

The general objective of this study is to shed more light on the potential of TWs to remove MPs from wastewaters and evaluate the role of vegetation roots, as well as the fate of microplastics along the TWs, to better understand the complex mechanisms involved. To this end, two different WWTPs with TWs have been evaluated, including horizontal subsurface flow wetlands and floating wetlands. To the best of our knowledge, this is the first study addressing these issues in full-scale TWs. Additionally, this study looks at the degradation of MPs from a qualitative point of view. The two main degradation processes for MPs under research are biodegradation and photocatalysis (Pan et al., 2022). It can be hypothesized that MPs in TWs may be degraded by biodegradation processes due to the long residence time of solid particles retained inside TWs and because of the activity of their biological community, including bacteria, protozoans and worms, among others.

2. Materials and methods

2.1. Sampling sites

2.1.1. Carrícola's WWTP

Carrícola's WWTP (Valencia, Spain) serves a community of 115 population equivalents (P.E.). The scheme of this plant is a pretreatment (grids), followed by two Imhoff settlers (18 m^3) connected in series with pipes to the inlet of two horizontal subsurface flow TWs, operating in parallel, of 200 m^2 each and a depth of 0.35 m. They are filled with coarse gravel ($\Phi = 40$ mm) from the inlet to a distance of one meter and then are followed by a mix of coarse and fine gravel (10–25 mm). They are filled with common reed plants (*Phragmites*). There is a second TW of the same configuration (TW2) following TW1 to extend the exposer time of the water (Fig. 1).

Previous studies report an average daily inflow of 16 m^3/day, a total suspended solids (TSS) removal efficiency of 98%, with an average value of 3.1 ± 3.7 mg/L in the final effluent, and chemical oxygen demand (COD) removal of 94%, with an average value of 42± 19 mg/L in the final effluent. The global hydraulic retention time (HRT) is 9 days, on average (Hernández-Crespo et al., 2022b).

2.1.2. Monasterios WWTP

Los Monasterios WWTP (Valencia, Spain) is serving a population of 1500 PE. The pretreatment consists in a screening and grit chamber. The secondary treatment is an aerated biological reactor with floating helophytes (176 m^3, 4 m depth). The plants are a mixture of *Typha* and *Phragmites* situated upon a buoyant structure. This biological reactor works in extended aeration mode with sludge recirculation and is followed by a sedimentation tank (48 m^3). Aeration is intermittent and controlled by dissolved oxygen probes. Finally, it has a tertiary treatment for disinfection formed by a floating TW (348 m^2 of area and a volume of 557 m^3), composed of vegetation growing on a buoyant surface. The buoyant or floating structure is placed into a pond and planted with helophyte (cattails and reed) to create the floating wetland. The floating structure is the same as in the bioreactor and with the same plant species. To illustrate the system, two pictures of the structure alone and the structure planted inside the floating TW are included in SI (Fig. S1). Followed by a horizontal subsurface flow TW (68 m^2 and 18 m^3 of area and volume respectively) with coarse gravel (2–3 cm) and planted with common reed (Fig. 2). It treats a flow of 100–120 m^3/day and 100% of the treated water is reused for irrigation of gardens. It has a COD and TSS removal efficiencies of 97% and 94%, respectively. The mean HRT is 1.6 days in the biological reactor, 5.1 days in the floating TW, and 0.2 days in the horizontal flow TW.

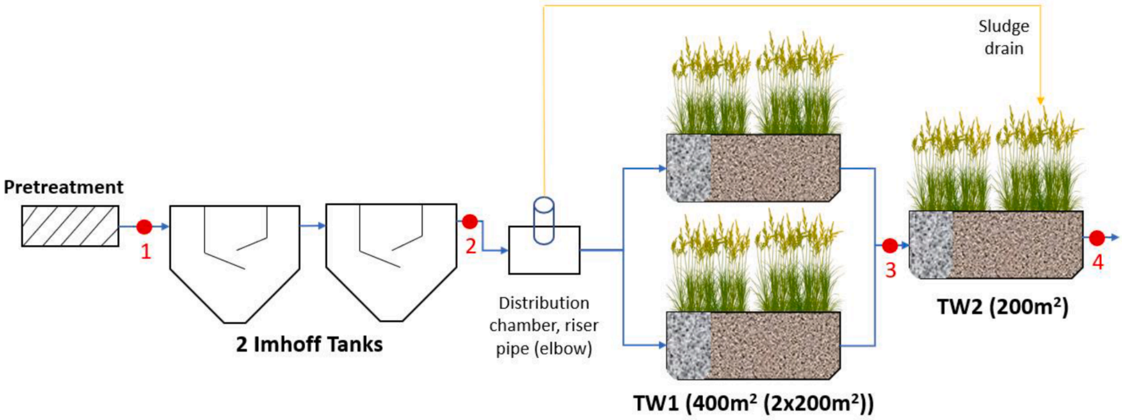

Fig. 1. Scheme of Carrícola's WWTP, with two horizontal flow TWs in parallel the third in series. With the points to be studied marked with red numbers (Figure adapted from Hernández-Crespo et al., 2022b).

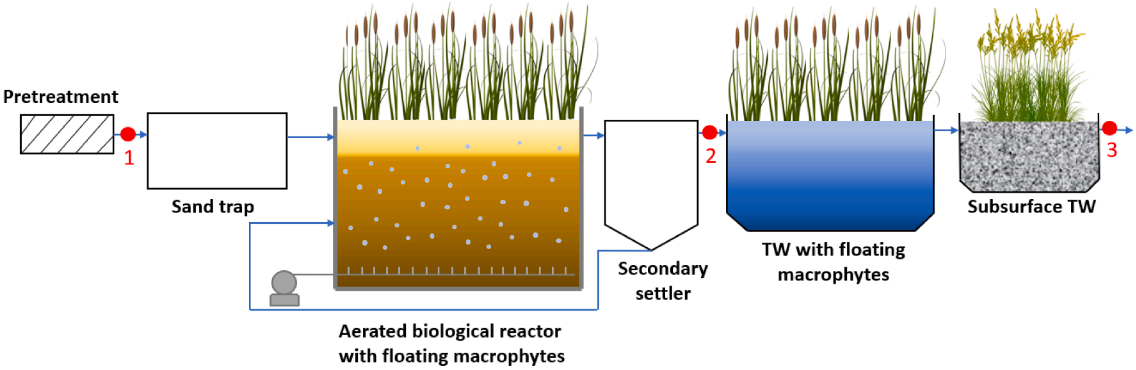

Fig. 2. Scheme of Los Monasterios WWTP, with the points to be studied marked in red numbers.

2.2. Wastewater and sludge sampling

Water samples were collected from different points in each treatment plant. In Carrícola WWTP four sites were sampled: raw wastewater (Point 1), the effluent of the primary treatment (Point 2), the outlet of TW1 (Point 3) and finally the outlet of the treatment plant (Point 4) (see Fig. 1). In Los Monasterios WWTP, three different locations were sampled: the influent to the treatment plant (Point 1), the outlet of the secondary treatment (Point 2), and the outlet of the subsurface TW and thereof, the treatment plant (Point 3).

Two sampling campaigns were performed in each WWTP. Different volumes were taken for analysis: 2.5 L for the raw wastewater, 7.5 L for the TW influent, 100 L for the TWs effluent. In Carrícola, just 60 L from the effluent of TW2 were taken (Point 4) because of some difficulties in collecting the sample. In the first sampling campaign, all the samples were taken in duplicate with a metal bucket and filtered by a stack of sieves (5600 μm, 425 μm, 75 μm, 40 μm) in situ. In the second sampling, one sample was taken at each point because the variability in the duplicates in the first sampling was low and only the smallest sieve was used, because these samples were taken for examination with Fourier-transform infrared spectroscopy (FTIR). The residue remaining on the sieves was transferred into glass beakers covered with aluminum foil and transported to the laboratory for further processing.

Sludge samples were also collected from different sites. In Carrícola WWTP one sampling point was at the beginning of the TW1 (2 m from the inlet distribution channel) and another site at the end of the TW1 (3 m before the outlet). In both sites, a depth between 10 and 15 cm was excavated and samples were taken in duplicate. Samples with and without roots were taken from the same depth where roots were most developed. At each sampling point, one sludge sample was collected from the gravel and a second was collected from sludge attached to the roots excavating in the lower area where a plant was found.

In Los Monasterios WWTP, two kind of sludge samples were taken from the biological reactor (secondary treatment): suspended sludge and sludge attached to the roots of the floating macrophytes. The sludge samples were collected during the aeration process to ensure an optimally mixed sludge. Roots from the floating TW (tertiary treatment) were also sampled. In all cases, two replicas were extracted.

A shovel and a pick were needed for sludge samples. All tools were made of metal and wood to avoid plastic and furthermore, samples were transported in glass jars covered with aluminum foil to process them in the laboratory. Once in the laboratory, both the gravel and roots samples were placed on a 5 mm sieve and cleaned with distilled water, placing another sieve of smaller size (40 μm) under the 5 mm sieve to collect the sludge samples. In the case of the roots, we dug around a plant and collected all the roots in the area, along with the sludge adhered to them.

2.3. Microplastics extraction procedure

For the extraction of MPs from the water samples the same procedure as Wang et al. (2020) was followed. In summary, the beakers were dried below 70 °C (Munno et al., 2018), once dried a digestion with H_2O_2 30% solution was applied and the residue was dried again, and then a two-step density separation was performed. First step was done with $CaCl_2$ (1.34 g/cm^3) and a second step was done with a higher density solution of KI (1.72 g/cm^3) to ensure that all the high density microplastics were recovered (Zhang et al., 2020). Otherwise, MPs with higher density than that of the solution could not be counted (Prata et al., 2019). The vials containing the density solution were centrifugated (5 min, 3500 rpm) and the supernatant was filtered through glass-fiber filters (pore size of 1 μm and a diameter of 47 mm). Then, 5 ml of Rose Bengal was applied to stain natural fragments or fibers.

In the case of the sludge samples, the samples were washed over the sieve of 40 μm to collect the sludge adhered to the roots or the gravel. After the samples were obtained, the rest of the process is the same as the water samples, except for the digestion process which required more volume of H_2O_2 30% and took more time due to the large amount of organic material.

2.4. Microplastics counting and characterization

The filters obtained in the previous step were examined following an orderly route: starting at the top from left to right, down and back in a right to left direction under stereomicroscopes with augmentations between x20 and x40 (SZ30, Olympus, Japan). Following the guidelines marked by Hidalgo-Ruz et al. (2012), the MPs were identified, also with help of the hot needle test for some particles that were unclear. Upon being touched with the needle the plastic items should curve or melt. Otherwise, the organic particles usually break under the pressure of the hot needle. During the counting, MPs were classified into fibers, particles, and films. Additionally, they were categorized by size according to the sieve from which the filter came.

As mentioned above, in the second sampling campaign the samples were sieved only through the 40 μm sieve, subjected to the same digestion and density-separation process, and analyzed with micro–Fourier Transform Infrared Spectroscopy (μFTIR) (Thermo Scientific Nicolet iN 10). The data base used included the Nicolet polymer and common materials set in addition to the Hummel polymer library.

2.5. Quality assurance and contamination prevention

To avoid contamination during the sampling and experimentation, quality control measures were adopted. The use of plastic materials was avoided as much as possible; the beakers were covered by aluminum foil after sampling and every time that they were stored, another aluminum foil was placed to protect. During all the steps, including sampling, laboratory cotton coat and latex gloves were used. All the working space and equipment were cleaned thoroughly before and after use.

At each sampling point, an in situ blank was prepared, and two laboratory blanks were conducted during the observation of filters to quantify MPs pollution in the lab environment. The blanks went through the same steps as the other samples and were also counted under a stereomicroscope. In the end, nine particles were found in the Carrícola field blank, and fifteen were found in Los Monasterios in a 10 L distilled water sample. These MP quantities were not subtracted from the sample results due to uncertainty regarding whether all samples were equally contaminated, as most of the contamination in the blanks comes from airborne sources. Instead, the blank concentrations were used as a detection limit (Smyth et al., 2021), as similar results were found in previous studies (Conley et al., 2019; Wang et al., 2020).

In addition, a recovery test was performed. For this, MPs were artificially prepared from plastic utensils of a different color (clothes, bags and bottle lid) by scraping with a knife or scissors. The test was performed four times with the following quantity of MPs: ten fibers, ten particles and five films with a medium size of 350 μm. The whole procedure was applied, including sieving, extraction, density separation and visual examination. The following average recovery percentages were obtained: 97.5% for fibers, 87.5% for particles/fragments and 75% for films. As blanks concentrations, this process is carried out to quantify the imperfections of the method, but not to alter the results since it cannot be ensured that it affects all samples equally.

2.6. Statistical analysis

An abundance of MPs were measured as MPs/L in water samples and MPs/g dry weight of sludge in the sludge samples. Statistical analysis was performed using Statgraphics software Version 18.0 (Statgraphics Technologies, Inc., The Plains, Virginia). The concentration, size and shape distributions were compared using parametric tests (ANOVA) if normality was satisfied and nonparametric otherwise (Kruskal Wallis). In all tests, the results were only considered statistically significant when p value was below 0.05.

3. Results and discussion

3.1. Wastewater microplastics concentrations and removal efficiencies

MP concentrations presented a high degree of variability at the inlet, between 17.2 and 23.4 MPs/L in Carrícola WWTP and between 4.0 and 12.8 MPs/L in Los Monasterios WWTP (see Fig. 3). That variability was highly attenuated in the effluent, highlighting the buffering capacity of wetlands, a property noted in numerous studies (Hernández-Crespo et al., 2022a, b). The effluent concentrations varied between 0.18 and 0.98 MPs/L in Carrícola and from 0.04 to 0.29 MPs/L in Los Monasterios, being significantly lower than the influent concentrations ($p<0.05$). Moreover, it should be noted that the variability of the samples during the same sampling campaign was significantly lower than the variability between samplings, as reflected by the error bars of the points in which a duplicate was taken. In connection with the characteristics of both influents, it should be noted that the results of the second sampling could have been influenced by rainfall events during previous days, which could have provoked a flush of the sewage network (Zhou et al., 2022), causing to a reduction in MPs arrivals on subsequent days.

The MP removal efficiency for Imhoff tanks resulted, on average, in 17.4% for Carrícola. Furthermore, the removal efficiency of TW1 was on average, 98.6%. It can be seen that in Carrícola the concentration increases slightly after Point 3, in TW2, which may be caused by a sludge drain operation of the pipe system, that is carried out monthly, and the effluent of this operation is taken to the final part of TW2, which can cause a transport of MPs previously sedimented inside the pipes. On the other hand, the average MP removal efficiency after the secondary treatment in Monasterios was 53.3%. The removal efficiency of the TW system was on average, 96.2% in Los Monasterios. In terms of global removal efficiency, Carrícola presented an average of 97.4% and Los Monasterios 98.1%. These global efficiencies are higher than those obtained in small standard (pre-treatment, primary, secondary) WWTPs, where efficiencies lower than 90% are typical (Sun et al., 2019). Furthermore, in comparison with other studies where TW based technology is also used, especially those operated as a tertiary treatment, the removal efficiencies reached in this study are similar and sometimes higher, as can be noted in Table 1. Moreover, it has been demonstrated that full-scale TW, both working as secondary and tertiary treatment, can efficiently reduce the number of MPs in wastewater.

At first, it was hypothesized that, in a modern and more urban population such as Los Monasterios, the concentration of MPs in the influent to the WWTP would be higher. However, the influent concentrations to Carrícola WWTP were substantially higher. This may be due, among other causes, to the fact that it is common to find higher concentrations of pollutants in the wastewater of small villages, as they

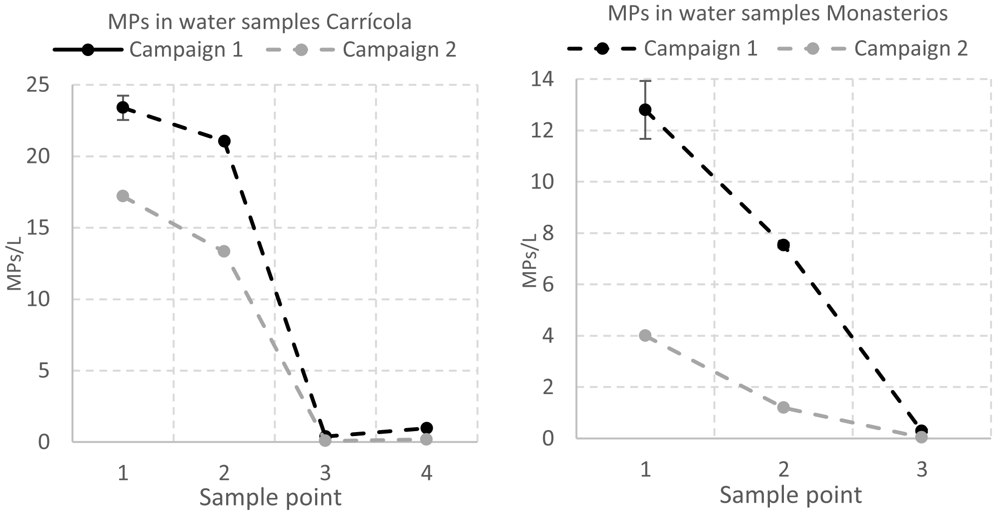

Fig. 3. Results of MPs concentration measured in the different points of both WWTPs. For Carrícola: Point 1 influent, Point 2 outlet of the sedimentation tanks, Point 3 outlet of the parallel TWs and Point 4 final effluent. For Monasterios: Point 1 influent, Point 2 outlet of the secondary treatment and Point 3 final effluent.

Table 1
MP removal efficiency (%) in other WWTPs with TWs. SFTW for surface flow TW; HSFTW for horizontal subsurface Flow TW; VFTW for vertical subsurface flow TW.

Study type	Design type	Removal efficiency (%)	Reference
Laboratory	SFTW	81.61	Chen et al. (2021)
Laboratory	HSFTW	100	Chen et al. (2021)
Field	VFTW	83–89	Zhou et al. (2022)
Field	SFTW	27.3–60	Zhou et al. (2022)
Laboratory	VFTW	100	Wang et al. (2021)
Field	HSFTW	88	Wang et al. (2020)
Field	HSFTW	45–100	Wei et al. (2020)
Field	HSFTW	72–68	Long et al. (2022)
Field	SFTW	95	Bydalek et al. (2023)

usually use a lower quantity of water and there are less uses (commercial, industry…) that can dilute these pollutants (Huertas et al., 2013). This can be corroborated with the COD and TSS concentrations of the influents of each WWTP: while in Los Monasterios we found average values of 357 and 82 mg/L respectively, in Carrícola values of 794 and 266 mg/L were registered. Similarly, this was confirmed in other studies such as Wei et al. (2020), who analyzed different treatment plants located in different areas: near cities, in rural areas and in mountainous areas far from large urban centers. Their results indicated that, wastewater concentration was higher in less urbanized areas. However, it should be noted that other studies in WWTPs in the city show very wide ranges (Qiu et al., 2020). Therefore, MPs concentration does not depend on the served population (Mahon et al., 2017), greater attention should be paid to the lifestyle of each community.

The shape and size distribution of MPs in wastewater are shown in Figs. 4 and 5. Regarding the shape distribution, the plastics found were divided into groups: fibers, particles, and films. As in other contemporary studies (Long et al., 2022; Wang et al., 2020), the predominant form was the fiber, in both influent and effluent. The removal efficiency was 97.5% for fibers in both WWTPs. Films were the second most typical form in both WWTPs, which is not in accordance with other studies, that show it is usually the smallest proportion (Bydaleck et al., 2023; Wang et al., 2020; Zhou et al., 2022). Regarding particles, their removal is complete, which may be because particles are more likely to be retained by the filtering effect of TW. Given that fibers are the most abundant fraction, it is advisable to carry out campaigns to raise awareness of this problem. Previous studies have proposed installing filters in washing machines and using natural fiber clothing, among other measures (Prata, 2018). Likewise, simple actions such as removing fibers from our feet before entering the shower can help to reduce the fiber content in wastewater. In this sense, treating greywater separately could help to reduce the MP load into wastewater, as it is considered a significant source of microfiber pollution (Sotiropoulou et al., 2023).

In addition, it is necessary to consider the important effect that the biofilm plays in the retention of MPs in TWs, since retention and adsorption are important mechanisms for pollutant removal in wetlands (Hernández-Crespo et al., 2017). The colonization of MPs by biofilm is a facilitator mechanism for the retention of MPs in wetlands, increasing their surface area and their density. Moreover, the biofilm adhered to the vegetation and the roots of the wetlands, also improves retention. This effect is of great importance in the retention of fibers. Due to their high surface area to volume ratio, the fibers are more likely to be colonized by microorganisms than other forms of MPs (Chen et al., 2021). This colonization also becomes very important when dealing with low density polymers (less than water) that might escape from settlers by flotation. During this study, fibers colonized by biofilm could be observed (Fig. S2), which could indicate a first symptom of potential biodegradation, a mechanism described as feasible by previous studies (Du et al., 2021). Signs of degradation in the MPs, such as bites, flakes, etc., were also observed during this study after passing through the wetlands (Fig. S3). In addition, the fibers are susceptible to entanglement, which is positive because it helps to improve their retention in the wetland gravels and their sedimentation, since their density and surface area increase (Fig. S4).

The results show a clear trend regarding the categorization by MPs size. This was one of the initial hypotheses: as MPs pass through the treatment system, especially in the TW, their size distribution shifts towards smaller dimensions. In both cases, as shown in Fig. 5, MPs collected in the larger sieve were reduced in proportion as they progressed through the system and in turn, the smaller MPs increase in percentage after passing through the TWs. This may be explained by a number of mechanisms: physical filtration, size reduction by earthworm digestion and size reduction due to the mechanical wear suffered by the MPs as they pass through the gravel. Therefore, a design recommendation that can be drawn from the results is the desirability of reducing the size of the filter material in the end zone of the wetlands, in order to retain these small size MPs, or design a multistage system varying the filter porous material. This will ensure that MPs are removed at different stages of the process, thereby increasing the overall efficiency of the CW.

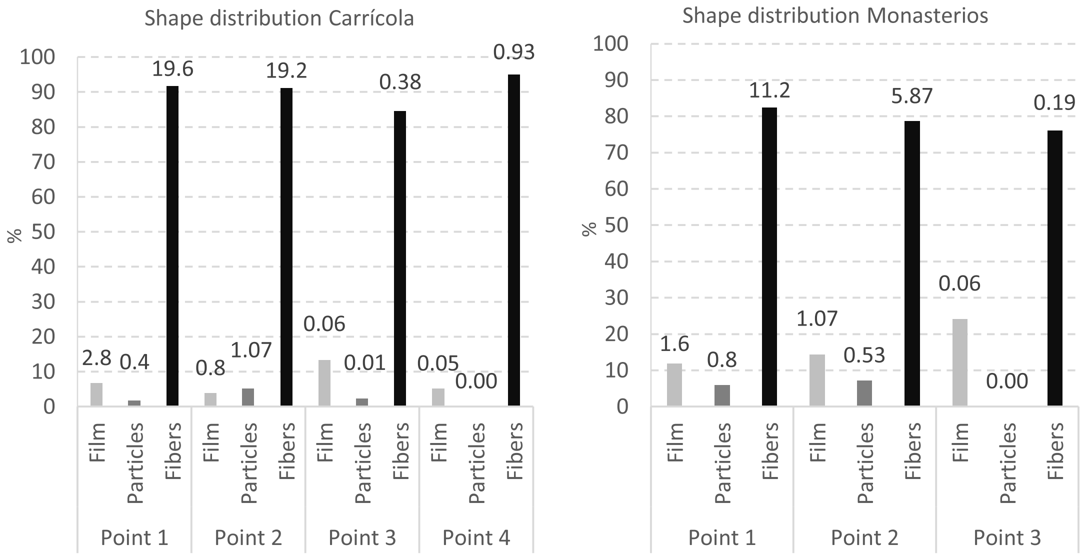

Fig. 4. Distribution by form (% in bars) and concentration (MPs/L) of each one (labels on bars) of the MPs in water sampled from different points in both WWTPs.

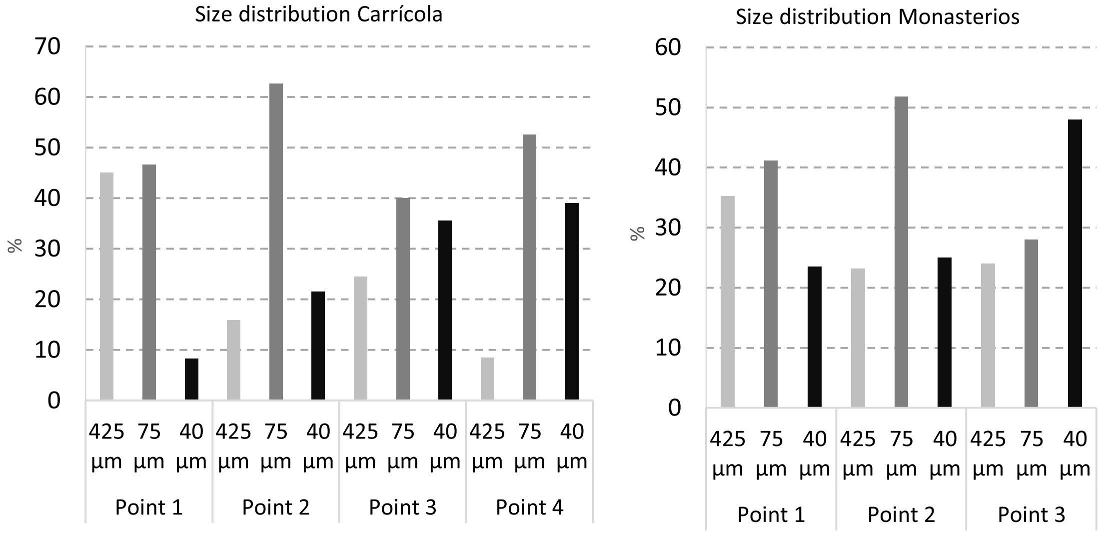

Fig. 5. Percentage of MPs classified by size in each point of both WWTPs.

Among other general recommendations to ensure the long-term effectiveness of TWs as optimize the hydraulic load, monitor and maintain the system. If smaller MPs are kept within the TWs, it may promote their biodegradation potential. Specifically, the MPs can be consumed by the macroinvertebrates inhabiting the wetlands, such as earthworms, as observed by Wang et al. (2021). The microorganisms present in both the earthworms and the wetlands could then facilitate the biodegradation process, as reported by Lwanga et al. (2018) and Yuan et al. (2020).

Furthermore, previous studies paid attention to finding a correlation between TSS and MPs, because if a strong correlation is found, the concentrations of TSS could be used as an indicator of MPs content. Currently, the TSS variable has not yet been used as an indicator, because there are a variety of opinions on the matter, and the relationships obtained are different in each study, hence there is still no agreed empirical equation. There are studies that found a strong correlation (Wang et al., 2020; Zhou et al., 2022), while others did not find a strong enough correlation to use as an indicator (Bydalek et al., 2023). In this study, the correlation is weak (Fig. 6) although statistically significant ($p<0.05$), which gives an indication of the interest in pursuing such relationships. A graph including the data from this study and previous ones has been made in order to further analyze this correlation. As can be seen in Fig. 6, the results of several studies fit this linear relationship in a statistically significant way ($p<0.05$) and with a considerably high goodness of fit (see R^2 in Fig. 6). Even those data for which the relationship was weak, when included in this graph, are within the point cloud formed by the set of values.

A very interesting parameter to quantify is the number of MPs that each treatment plant is releasing into the natural environment per inhabitant, which is known as emission of MPs per capita. In this study only for Carrícola this parameter could be calculated, in the case of Los Monasterios, there was no precise information available to calculate this parameter. With a flow rate of 14,000 L/d and a population of 115 PE., the emission rate would be 71 MPs/capita/day. The data obtained are lower than those showed in other studies, such as that of Wang et al. (2020), where emissions of MPs per capita were also calculated, resulting in 428 MPs/capita/day, for a population of 494 PE. These variations may be due to the different forms of consumption in the different societies studied.

3.2. *Sludge microplastics retention*

Different sludge samples were taken to check the role of the roots to capture MPs, both in TW and by introducing floating macrophytes in the bioreactor.

Regarding the fate of MPs in Carricola's TW, Fig. 7 indicates a higher accumulation of MPs in the inlet zone. This is an accordance with the

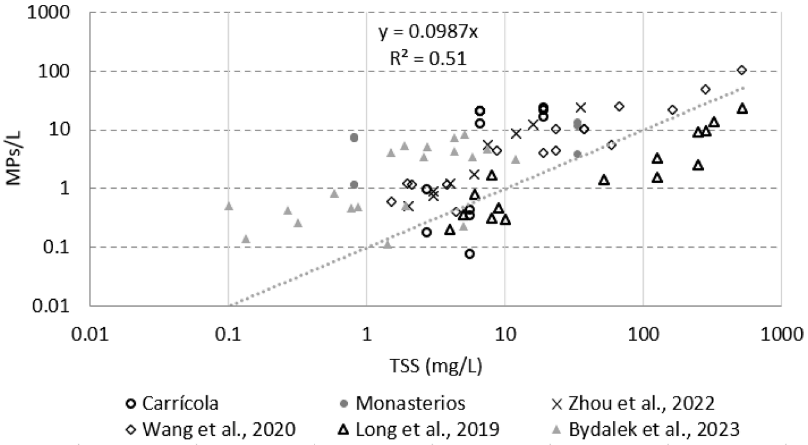

Fig. 6. Relationship between TSS and MPs from different studies. Data from Carrícola and Los Monasterios WWTPs obtained in this study. Data from other studies: Zhou et al., 2022; Wang et al., 2020; Long et al., 2019; Bydalek et al., 2023.

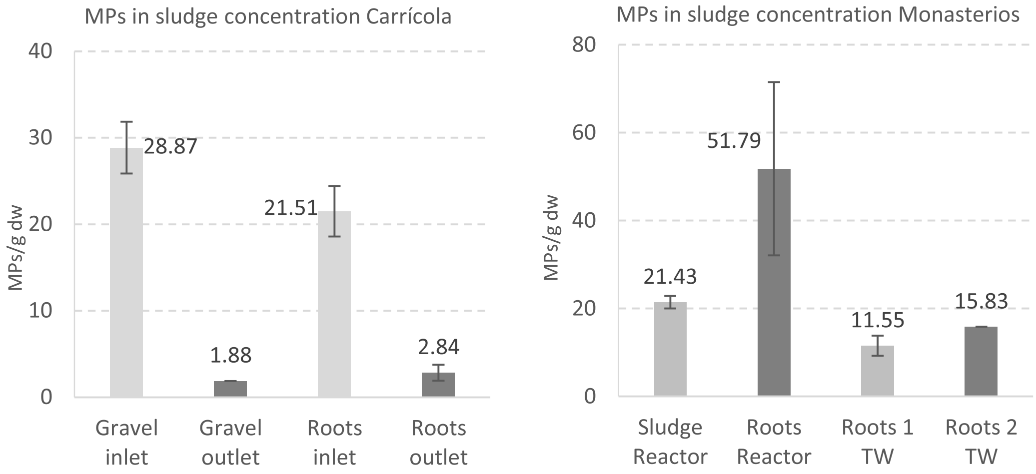

Fig. 7. Concentrations measured in Carrícola and in Los Monasterios sludge, for the different sampling points, measured in MPs per dewatered gram of sludge. Gravel inlet, for the average of the samples taken from gravels in the TW1 inlet. Gravel outlet, for the average of the samples taken from gravels in the TW1 outlet. Roots inlet, for the average of the samples taken from roots in the inlet. Roots outlet, for the average of the samples taken from roots in the outlet of the TW. Sludge Reactor, for the suspended sludge in the bioreactor; Root Reactor, for the sludge taken from the roots inside the bioreactor; Root 1 TW, for the sludge taken from the roots in one corner of the floating TW; Roots 2 TW, for the sludge taken from the roots in the other corner of the floating TW.

higher accumulation of TSS in the inlet zone of TWs (García et al., 2003, 2005).

As it can be seen in Fig. 7 for Carrícola, the initial hypothesis cannot be accepted at the inlet of the TW. Comparing both inlets, gravel sludge had a higher concentration of MPs per dry gram of sludge. This may be due to the ease of flow through the lower part of the wetland, below the roots, which is why more MPs are dragged to that area. Moreover, in this zone a high quantity of solids and biofilm are present, so the gravel can be as efficient as roots. Conversely, in the outlet, the concentration of MPs of the sludge attached to the roots was higher than in gravel, so here the hypothesis is fulfilled. It must be highlighted that the difference between roots and gravel concentration is, in relative terms, greater at the outlet than in the inlet. This shows the relevant role of roots in enhancing the retention mechanism. (Gersberg et al., 1986).

In the case of Los Monasterios, Fig. 7 shows the high difference, more than double, that exists between the concentrations measured in the root sludge and the sludge suspended in the bioreactor. This highlights the positive role of vegetation, since the concentration of MPs in the sludge is being reduced thanks to the capture by macrophytes, thus helping to reduce the load of MPs introduced in the environment when sewage sludge is used in agriculture, apart from other additional benefits provided by the macrophytes in the reactor (Gersberg et al., 1986).

Regarding the root samples from the floating TW, the results indicate that roots continue playing a crucial role in the tertiary treatment, trapping a noticeable quantity of MPs. In the floating TW, intercepted MPs could remain attached to the roots for a long time, since roots are not removed when the macrophytes are harvested, this being a positive factor to facilitate their potential degradation.

A recently published study that characterized the sediment of five TWs in Australia (Lu et al., 2022), reported values of MPs in the sediments varying between 736 ± 335 and 3480 ± 4330 MP/kg dry sediment. It is clear that sediment and sludge are not directly comparable but serve as a reference. In this case study, higher values of MPs in the sludge were observed, but also the input loads to the TWs were significantly higher.

Within the research conducted, there is a wide range of results, also depending on many factors, such as the type of TW operation, population served, size of filter used, as mentioned by Hidalgo-Ruz et al. (2012), among others. Higher values than those obtained in this study can be found in Magni et al. (2019) and Liu et al. (2019). Both authors, reported values of 113±57 MPs/g dry sludge and 240.3 ± 31.4 MPs/g dry sludge respectively in one WWTP in Italy and another one in China. Similar values are reported by Li et al. (2018), where 79 sludge samples were collected from 28 different WWTPs in China, obtaining results ranging from 1.6 to 56.4 MPs/g dry sludge.

With regards to levels in roots, other authors (Yin et al., 2021; Duan et al., 2021), reported amounts of 4 MPs/g and 5.11±2.95 MPs/g of dry sludge respectively, although those studies were carried out in a natural environment, which is likely to be less polluted. However, the concentrations at the outlet do resemble those found in the natural environment in these studies and are even lower.

3.3. *Microplastic polymer types in treatment wetlands systems*

Seven different types of polymers were the most commonly found in both water and sludge samples, including polyethylene terephthalate (PET), polyethylene (PE), polypropylene (PP), Polyvinyl chloride (PVC), polyurethane (PUR), Polytetrafluoroethylene (PTFE), Polytrimethylene terephthalate (PTT), other types of polymers were also found to a lesser extent (OTHER) (e.g. polystyrene, polyester, polyamide) (see Fig. 8). The most dominant component in water samples were PET, which is in third place overall. PP and PVC were the most predominant polymers in the sludge, being significant in both WWTPs, as in other studies (Lu et al., 2022; Huang et al., 2020).

The higher proportion of PP and PVC in the sludge is in line with the production data of plastics, since the most produced plastics are, in this order, PP, PE, and PVC, mainly for their uses as packaging and in the construction and automotive industry (Plastic Europe, 2021), followed by PET. So, it is not surprising that they are the most common, as in other sediment studies where PP prevails (Yuan et al., 2020; Lu et al., 2022; Olesen et al., 2019).

In the water samples (Fig. 8), a large amount of PET is found, especially in Carrícola where it reaches almost 60% of the total polymers found in the influent. PET is a material widely used in textiles and packaging, so it is very common to find, in addition to PE, which is also very important in the Monasterios influent, which may be contributing a large amount of the fibers found in the water samples. It should be noted that both materials are the most predominant after passing through the primary treatments, as can be seen in points 2. A high percentage of PVC is also found in both treatment plants at the inlet, although this type of polymers is satisfactorily removed along the WWTP. It should be noted that point 3 is not very relevant due to the small quantities of MPs found

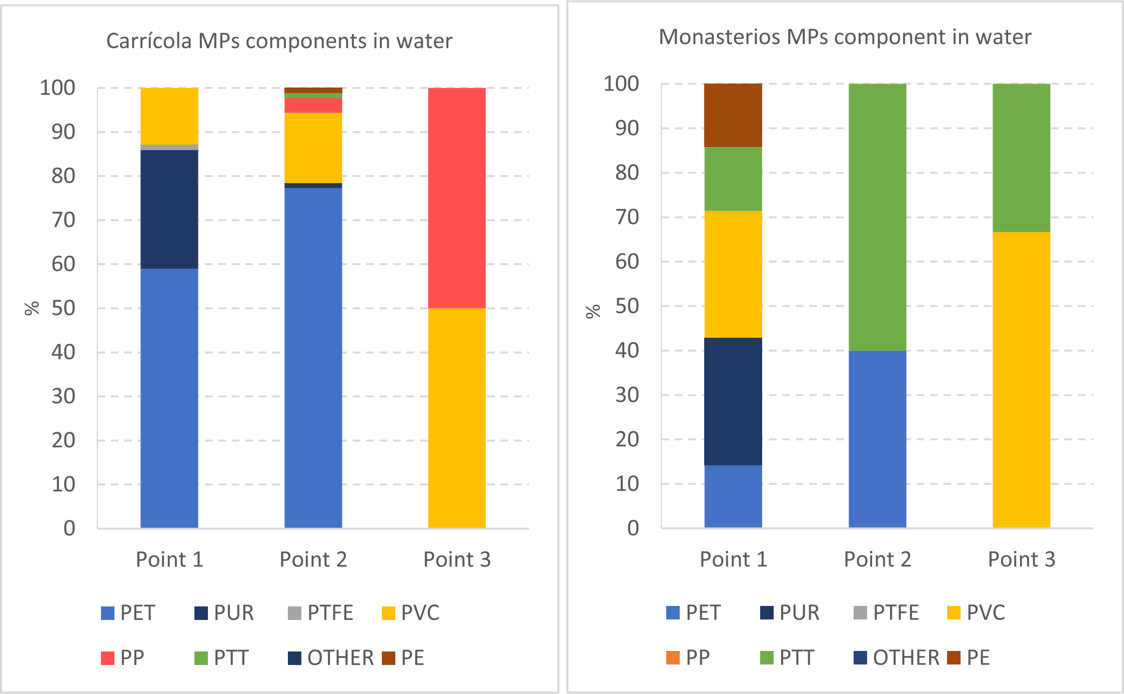

Fig. 8. Components of the MPs found, in percentage, in water samples at both monitoring locations.

there.

The polymer most adhered to roots, both in Carrícola's inlet roots and in the Monasterios TW roots (Fig. 9) is PP, the lowest density polymer registered. This highlights the important role of the roots in the retention of lower density MPs, which could otherwise be escaping from the WWTPs by flotation. In addition, the roots seem to play a key role in intercepting PET, which is usually referred to as polyester in textile products. This coincides with the higher proportion of fibers in this sludge samples (Fig. S5) as well as in the effluent samples (Fig. 4). On the other hand, in the suspended sludge the polymer compound with the highest presence is PVC, one of those with the highest density, in both WWTPs. Other studies reported different results, where the PE and PP fragments were predominantly recorded in the sediment, hence the density cannot be considered as the only factor in the distribution of MPs in the medium (Lu et al., 2022), although in this case it does seem to be relevant. Moreover, it should be noted that not all studies use the same extraction procedure, and this could lead to different results.

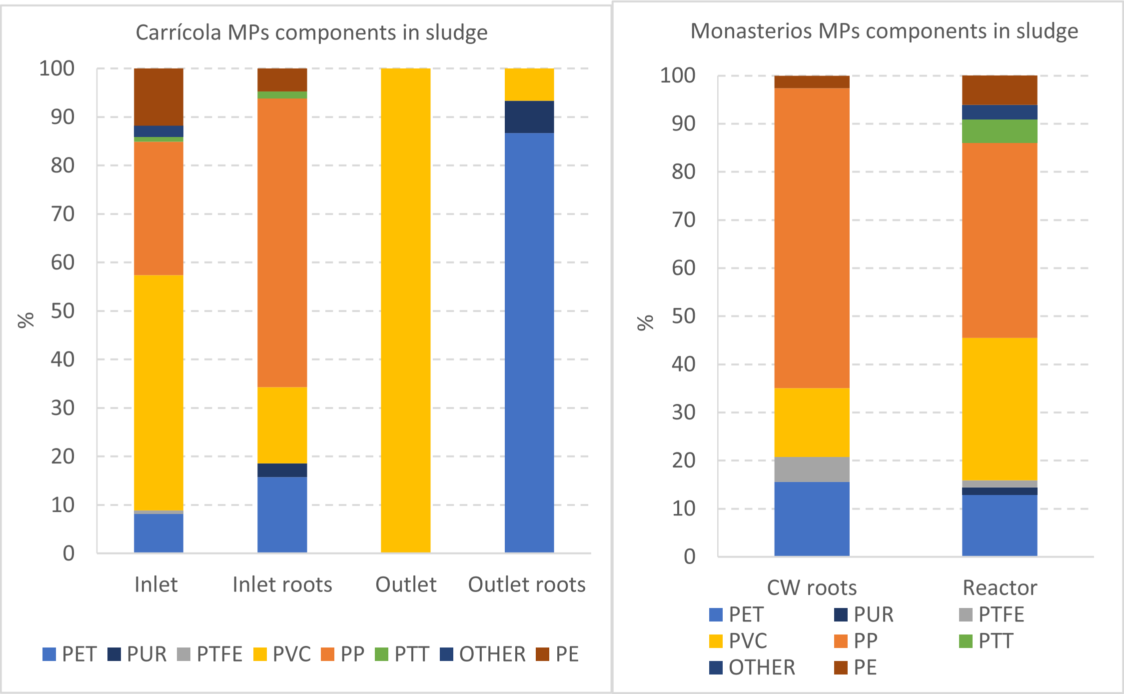

Fig. 9. Components of the MPs found, in percentage, in sludge samples at both monitoring locations.

Conclusions

The following conclusions can be deduced from the study carried out:

- Treatment Wetlands (TWs) have significant potential for reducing the discharge of MPs into the natural environment, functioning as both secondary and tertiary treatment.
- Fibers are the most common form of influent and effluent of both WWTPs, presenting the greatest challenge for MP pollution control. Actions aimed at reducing their arrival at WWTPs, such as washing machine filters and promoting the use of natural textiles, are highly recommended.
- Smaller particles predominate at the final stage, and it is recommended to have a final zone or wetland cell with a finer filtering material to further reduce MP emissions. Once these smaller MPs are retained inside the wetland, their biodegradation becomes more feasible.
- MPs are largely concentrated in the sludge from sewage treatment plants. Roots play a positive role in capturing more MPs, both in TWs and in floating macrophytes in the bioreactor. Therefore, it is important to maintain an adequate vegetation density in TWs and to install floating macrophytes in the biological reactors of WWTPs.
- MPs and TSS exhibit similar behavior in the TWs, being mostly retained in the initial zone, and are significantly correlated. Further study of their relationship can provide an estimation tool for MPs content. Pooling data from different studies can provide a more solid relationship.
- The importance of root retention in capturing lower-density MPs that could escape from WWTPs is once again demonstrated in polymer retention. Additionally, the correlation between the different materials observed (PET and PE) in the water samples and the main forms (i.e., fibers) can be appreciated.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2023.120106.

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