

# Microplastics transfer from a malfunctioning municipal wastewater oxidation pond into a marine protected area in the Colombian Caribbean

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## ABSTRACT

Wastewater is an important source of microplastics (MPs) in aquatic ecosystems, threatening their biodiversity and environmental services. This study examines the contribution of MPs from the effluents of a municipal wastewater treatment pond system (WTS) to a stream that flows into the Cispatá marine protected area (MPA) in the Colombian Caribbean. Samples of influent and effluent wastewater and sludge in the WTS were analyzed to determine the abundance and types of MPs. Additionally, the wastewater flow rate was measured using a volumetric method, and its MP load calculated. The abundance of MPs in the influent and effluent of the WTS was 15 and 5 items L<sup>-1</sup> of wastewater, and 11 and 4 items Kg<sup>-1</sup> dw of sludge, respectively. Fibers (67–75%) prevailed in the wastewater, while fragments (56–67%) were predominant in the sludge. Whereas the removal of MPs entering the WTS was 67%, the wastewater effluents still contribute approximately 1.3 million items day<sup>-1</sup> to the stream flowing into the MPA. Our study highlights that a poorly functioning WTS causes substantial MP discharges into sensitive aquatic coastal ecosystems. These WTS require ongoing maintenance and improvements to ensure their long-term optimal operation and function of reducing pollutant loads and environmental risks in recipient aquatic ecosystems.

## 1. Introduction

Wastewater refers to water that has been used in a set of human activities, including domestic, urban, and industrial usages, which contains a wide variety of contaminants, embodying microplastics (MPs) (Deblonde et al., 2011; Cowger et al., 2019; Sáenz-Arias et al., 2023). The latter constitute a subcategory of marine litter, with sizes ranging from 5 mm to 1 µm, and are classified as primary MPs when they are manufactured as components of other products, and as secondary MPs when they originate from the degradation or fragmentation of larger plastic items (GESAMP, 2019). In a recent study in Germany, Barkmann-Metaj et al. (2023) show similar MPs abundance in both municipal and industrial wastewater effluents, though this remains to

the proved in a broader context.

The discharge of MPs into aquatic ecosystems poses a significant threat to the sustainability of these environments, due to the negative impacts this type of pollution has on species and ecosystem services, namely the provision of healthy habitats and food (Antão-Barboza et al., 2018; Garcés-Ordóñez and Bayona, 2019; Huang et al., 2021). Often, MPs pollution affects densely populated areas, eventually industrialized, which are exposed to higher wastewater and sludge discharges into the aquatic environments (Uddin et al., 2020; Garcés-Ordóñez et al., 2022; Sáenz-Arias et al., 2023).

According to environmental regulations, wastewater must undergo treatment before being reused or discharged into the surrounding environment, i.e., in receiving water bodies. The purpose of this

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treatment is to eliminate or reduce the levels of certain contaminants to permissible levels to protect water resources and allow further uses of treated waters if needed or deemed convenient (MinAmbiente, 2015; Ehalt Macedo et al., 2022). To achieve this, wastewater is conveyed through pipelines to wastewater treatment systems or plants (WTS), which apply a sequence of treatment stages involving a combination of physical, chemical, and biological processes in ponds or tanks (Crini and Lichtfouse, 2019).

These treatment stages are classified as follows: (1) preliminary treatment (e.g., sedimentation, coagulation); (2) primary treatment (e.g., further coagulation, flocculation, precipitation); (3) secondary treatment (e.g., biodegradation, filtration, adsorption); (4) tertiary or advanced treatment (e.g., advanced oxidation, membrane filtration); and (5) treatment of the sludge generated as byproduct, which accumulates on the bottom of the ponds or tanks (e.g., controlled disposal, recycling, or incineration) (Crini and Lichtfouse, 2019).

When MPs enter WTS, partial deposition usually occurs, which is more pronounced for MPs with higher specific density, which concentrate in the sludge. Simultaneously, a portion becomes trapped in flocs, while less dense MPs remain floating in the upper layer of the wastewater (Carr et al., 2016; Gatidou et al., 2019). The number of MPs reaching the natural environment through wastewater and sludge discharges from WTS depends on the treatment employed and its efficiency, the operational conditions of the WTS, sludge management, and weather-driven events such as heavy rainfall causing excess influent volumes and wastewater overflows (Horton et al., 2017; Gatidou et al., 2019; Uddin et al., 2020).

WTS with complete and well-functioning stages manage to significantly reduce the number of MPs in wastewater and sludge (Gatidou et al., 2019; Uddin et al., 2020). Carr et al. (2016) observed that WTSs with tertiary treatment in Los Angeles, USA, removed all MPs > 40 µm from the effluents. In contrast, a WTS with secondary treatment in same city released an average of 0.001 items L<sup>-1</sup> into the effluent, which is equivalent to 930,000 MPs day<sup>-1</sup>. Magni et al. (2019) reported an 84% reduction in MPs > 63 µm in a WTS with tertiary treatment in northern Italy. However, their findings also revealed that the WTS was still discharging ~160 million MPs day<sup>-1</sup> and accumulated 3.4 billion of MPs in the sludge, which could enter the environment when using it as fertilizer. Gies et al. (2018) noted a 97–99% reduction of MPs > 65 µm in a WTS with secondary treatment in Vancouver, Canada, yet that system released ~30 billion MPs annually.

In Colombia, a developing country, discharges of untreated wastewater in coastal waters are one of the most significant sources of coastal and marine pollution (INVEMAR, 2020; Sáenz-Arias et al., 2023). Out of the 47 coastal municipalities in Colombia, only 43% have some form of WTS providing preliminary, primary, or secondary treatments based on facultative biological processes (Garcés-Ordóñez et al., 2016). However, most of these WTS were designed without adequate population assessment and forecast, which over time can lead to insufficient treatment capacity that can add to operation and maintenance problems (Vargas et al., 2020). Consequently, these infrastructures can rather easily become outdated and be forced to operate under precarious conditions (Garcés-Ordóñez et al., 2016).

The WTS in the municipality of San Antero, in the Colombian Caribbean, faces maintenance issues leading to clogging, unmet hydraulic retention times, and improper sludge disposal (Vivas-Aguas and Franco-Angulo, 2022). This malfunctioning makes this WTS a source of pollution for the Cispatá marine protected area (MPA), a deltaic lagoonal system with well-preserved mangroves in the Colombian Caribbean (Garcés-Ordóñez et al., 2020a; Garcés-Ordóñez, 2022). This situation prompted us to investigate the presence of MPs in wastewater and sludge in San Antero, which could represent a scenario that likely affects other WTSs globally. The impact on such valuable ecosystems emphasizes the importance of investigating the contribution of this pollution source while advocating for measures to improve the conditions of WTSs in coastal populations.

The aim of this study was to analyze the contribution of MPs from the effluents of wastewater and sludge from the WTS in the municipality of San Antero to the stream that flows into the Cispatá MPA, located in the Colombian Caribbean. The specific questions addressed were: (1) What is the abundance and types of MPs in influent and effluent wastewater and sludge from a malfunctioning WTS, and how such situation affects the removal capability of MPs by the WTS? (2) What is the estimated load of MPs in wastewater contributed by the WTS to the receiving water body? and (3) What are the risks posed by this pollution to the Cispatá MPA? This study provides key information to raise awareness about the effects of WTSs malfunctions on coastal populations and their impact on sensitive ecosystems such as lagoons and mangroves.

## 2. Materials and methods

### 2.1. Study area and location of sampling stations

Our study area corresponds to the secondary treatment oxidation pond in the municipality of San Antero, located at coordinates 9°22'47'' N and 75°46'10'' W, in the northern part of the Córdoba Department, in the Colombian Caribbean (Fig. 1). According to the Köppen climate classification system, the climate in the area is tropical rainy savanna with a dry winter, with January and February being the driest months, and May and September the rainiest (IDEAM, 2014). The multi-year averages (1981–2010) of temperature, relative humidity, and total precipitation range from 26 to 28 °C, 80 to 90%, and 1000 to 1500 mm, respectively (IDEAM, 2014).

The municipality of San Antero covers an area of 191 km<sup>2</sup>, hosting a total population of ~35,000 inhabitants in year 2020; 46% of this population resides in urban areas and 54% in rural areas (DANE, 2018). The sewerage service embraces ~31% of the population, which wastewater is transported to wastewater pumping stations (WPS) after collection, from where it is conveyed to the municipal WTS. The average daily flow rate ranges from 3.5 to 43.7 L s<sup>-1</sup> (Alcaldía de San Antero, 2021; Vivas-Aguas and Franco-Angulo, 2022). The population fraction (~69%) without access to sewerage services discharges their untreated wastewater directly into natural water bodies, mainly streams, which can eventually reach the Cispatá MPA (Alcaldía de San Antero, 2021; Vivas-Aguas and Franco-Angulo, 2022).

The San Antero WTS incorporates three artificial ponds (Fig. 2). The first two are 90 × 193 m and 90 × 121 m, and 1.5 m deep facultative ponds. The third pond, intended for maturation, is 88 × 116 m in size, and 1.3 m deep (Alcaldía de San Antero, 2021). Typically, wastewater is retained in facultative ponds during a period that is sufficient to remove suspended particles through sedimentation, and to treat organic matter and nutrients through a combination of aerobic and anaerobic microbial processes (Mahapatra et al., 2022). In the maturation pond, treated wastewater is allowed to settle, thus further facilitating the settling of remaining suspended particles. Additionally, it aids in the removal of fecal bacteria and leftover nutrients, resulting in water quality enhancement before final discharge (Mahapatra et al., 2022).

In San Antero's WPS entering wastewater reaches a bypass —the main collector of the WTS—, where an overflow chamber diverts untreated wastewater to the receiving water body when the system is clogged (Fig. 3a). Non-diverted wastewater flows to the primary treatment inlet structure, which encompasses a metal gate for wastewater inflow control, a metal grille with horizontal bars to retain large solids (Fig. 3b), a metal weir for flow regulation (Fig. 3c), and a metal gate allowing wastewater entry into the ponds (Fig. 2). Concrete weirs enable wastewater to move from one pond to another (Fig. 3d). Additionally, there is an external concrete rainwater channel paralleling the facultative ponds, which receives rainwater from capture chambers installed in San Antero municipality. This rainwater channel connects with the final treated wastewater effluent channel exiting from the maturation pond, which ultimately opens into the receiving water body, namely the Cardales stream (Fig. 2).

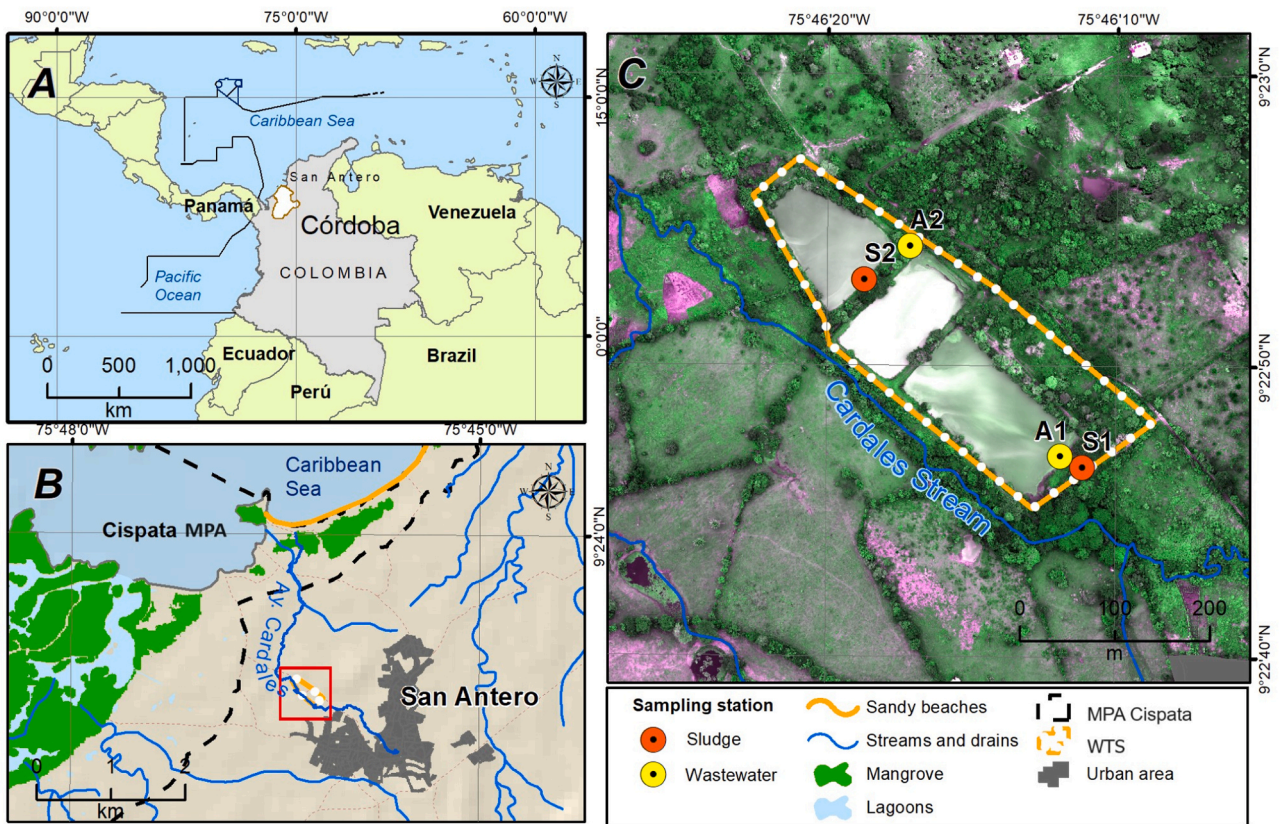


Fig. 1. A: General location of the study area in the municipality of San Antero, Córdoba Department, Colombian Caribbean. B: Map showing the urban area of San Antero in dark gray, the wastewater treatment system (WTS, red box; see Fig. 2), and the Cardales stream where treated wastewater and sludge are discharged, which flows into the marine protected area (MPA) of Cispatá. C: Aerial view of San Antero WTS showing the treatment ponds and the locations of wastewater (A1-A2) and sludge (S1-S2) sampling stations at the entry and exit of the system.

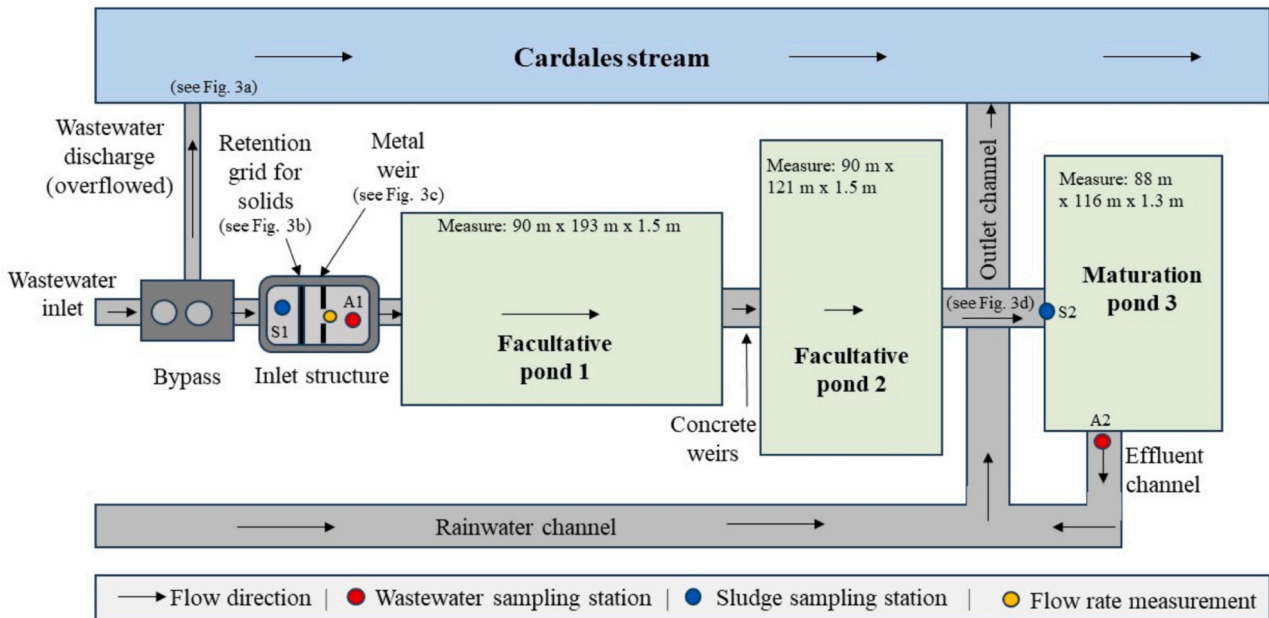


Fig. 2. Schematic diagram of the San Antero wastewater treatment system (WTS) operation. See legend for further explanation. Sampling stations as in Fig. 1 C. Photographs of the WTS are shown in Fig. 3.

Field work was conducted on May 17th 2022 before the rainy season, with the sampling points for wastewater and sludge established in the influent and effluent sections of the WTS (Fig. 1; Fig. 2). The wastewater sampling stations were set at the weir after the solid retention grid (A1)

and at the WTS effluent (A2), whereas the sludge sampling stations were in the wastewater inlet channel (S1) and the maturation pond (S2) of the WTS (Fig. 1c). The maturation pond was selected for sampling as it represents the final stage of the WTS, which facilitates correlating the



**Fig. 3.** Pictures of the wastewater treatment system (WTS) of San Antero municipality. A: Wastewater discharge from the WTS into the Cardales stream due to overflow. B: Inlet channel obstructed at the retention grid for solids. C: Wastewater inlet channel showing the concrete weir with hydraulic jump, where the inlet flow was measured. D: Concrete weir between ponds 2 and 3, below which is the outlet channel for the treated wastewater. See location of pictures in Fig. 2. Photographs courtesy of INVEMAR.

abundance and characteristics of MPs in both wastewater and sludge matrices, estimating the contribution of MPs in the effluents, and quantifying the effectiveness of MPs removal. An additional wastewater flow rate measurement point was placed at the weir located after the solids retention grid in the inlet channel (Fig. 2).

It is worth noting that during the sampling days, the WTS was clogged, the wastewater inlet pipe was not visible, and some of the untreated wastewater was diverted to the overflow channel, flowing directly into the Cardales stream (Fig. 3a). This stream flows into the lagoon and mangrove of the Cispatá MPA (Fig. 1c). Furthermore, the area of the effluent channel was overgrown with dense vegetation and encompassed unstable terrain. Because of these unfavorable conditions, the sludge sampling station S2 could not be situated in the effluent channel area and had to be placed into the maturation pond instead (Fig. 2).

## 2.2. Sampling of microplastics

A point sampling of wastewater and sludge was conducted in the influent and effluent areas of the WTS to analyze MPs. At sampling stations A1 and A2, individual 800 mL wastewater samples were collected at a depth of 5 cm using glass containers previously rinsed with microfiltered water (Whatman™ glass fiber filters, 47 mm, 1.2 μm pore size). At sampling stations S1 and S2, individual ~900 g sludge samples were collected using a Van Veen grab and a metal scoop, and then deposited into pre-rinsed glass containers.

Subsequently, 200 and 250 mL of 30% H<sub>2</sub>O<sub>2</sub> were added, respectively, to the wastewater and sludge samples using a 100 mL graduated cylinder and a glass stirrer. Such an addition was carried out slowly to

avoid a violent reaction. This is a widely used method in the study of MPs in wastewater, aiming at reducing organic matter contents and also pathogenic microorganisms—as a sanitary control measure—through chemical digestion (Uddin et al., 2020). The samples were left partly uncovered for 30 min to prevent gas accumulation, then sealed, stored in a portable cellar, and preserved at room temperature for transportation to the laboratory. All samples underwent such chemical digestion process for eight days at room temperature under constant supervision to allow for a safe release—by slightly opening the lids of the glass containers—of the gases generated during the chemical attack. The chemical digestion process was checked at four and eight days by adding ~5 mL of 30% H<sub>2</sub>O<sub>2</sub> to the wastewater sample. The digestion was considered complete for our purposes after effervescence and heating reactions ceased.

## 2.3. Measurement of wastewater flow

The influent wastewater flow was calculated using the volumetric method, which involves obtaining a volume of water over a specific period (IDEAM, 2007). To this purpose, a wooden board was installed at the deteriorated metal weir located in the inlet channel (Fig. 3c) to create a jump, ensuring that all flowing wastewater was collected in a 12 L metal bucket. The time taken to fill this bucket was recorded using a manual stopwatch. Additionally, a 1000 mL graduated cylinder was employed to precisely measure the volume of wastewater collected in the bucket. This procedure was repeated three times. The influent wastewater flow rate was calculated by dividing the volume collected (L) by the elapsed time in seconds, expressed in L s<sup>-1</sup>, and then extrapolated to the daily flow rate. The three flow rate measurements were

averaged to determine a mean flow rate used for calculating the MP load in the wastewater.

Since the effluent flow rate from the WTS could not be measured due to unfavorable conditions during fieldwork (i.e., sedimentation, water-logging, and ground instability), the MP load in the effluent was calculated using the same rate than the influent flow, which implies assuming that the system is in dynamic equilibrium (i.e., outflow equals inflow). This approach is supported by [Vivas-Aguas and Franco-Angulo \(2022\)](#), who reported on the similarity of influent and effluent flow rates in San Antero WTS. These authors measured flow rates over a 24 h period between September 21st and 22nd, in year 2022, recording an average of  $3.55 \pm 0.4 \text{ L s}^{-1}$  for the influent and  $3.56 \pm 0.3 \text{ L s}^{-1}$  for the effluent.

#### 2.4. Sample processing in the laboratory

Each wastewater sample was transferred separately to a preceding 1000 mL beaker, vigorously stirred for 10 min using a glass rod, and subsequently filtered through a vacuum filtration system using Whatman™ 47 mm and  $1.2 \mu\text{m}$  pore size glass fiber filters. The beaker walls were rinsed with distilled and filtered water to prevent potential sample losses. The filters containing the retained material were carefully placed in Petri dishes and kept covered until the process of MP identification and characterization.

Every sludge sample was homogenized with a glass rod and two aliquots were obtained. These aliquots were placed separately in a pre-weighed aluminum container and dried in a Memmert oven at  $60^\circ\text{C}$  for 120 h until a constant weight was achieved. The weight of the dried aliquots was determined using an OHAUS Adventurer® analytical balance (0.1 mg readability). Once dry, the final weight of the sludge aliquots was determined after the difference between the weight of the sample and the one of the aluminum containers. In the sludge sample S1, the weights of aliquots 1 and 2 were 0.402 Kg and 0.396 Kg, respectively, whereas in the sludge sample S2, the weights of aliquots 1 and 2 were 0.410 Kg and 0.408 Kg, respectively.

Subsequently, the sludge aliquots were transferred individually to glass containers for density separation using hypertonic saline water, which was prepared by dissolving 1200 g of NaCl in 1 L of microfiltered distilled water at room temperature ( $\sim 25^\circ\text{C}$ ), resulting in a density of  $\sim 1.2 \text{ g cm}^{-3}$ . The sludge and the hypertonic saline water were vigorously mixed by stirring with a glass rod for 10 min and then allowed to settle for 15 min. The supernatant was then removed by tilting the container and letting it flow into another clean beaker. This procedure was repeated three times for each sample.

Upon completing the density-based MP separation, the water volume in each sample was reduced using a vacuum filtration system and pre-weighed  $1.2 \mu\text{m}$  pore size Whatman glass fiber filters. The beaker walls were rinsed with distilled and filtered water to avoid potential sample losses. The filters containing the retained material were carefully placed in Petri dishes and kept covered until the process of MP identification and characterization, which is described below.

#### 2.5. Microplastic identification and characterization

The filters containing the material retained from wastewater and sludge samples were stained with 1% Rose Bengal using a pipette, applying it over the entire filter area. This technique colors organic components that were not fully digested in the samples, thus easing MP detection. This procedure represents an economical and useful option when vibrational methods (FTIR, Raman) for polymer identification cannot be accessed ([Gbogbo et al., 2020](#); [Garcés-Ordóñez et al., 2022](#)). Once stained, the filters were left to air dry at room temperature for 24 h.

The filters were then carefully examined under a Nikon C-LEDS binocular stereomicroscope for visual identification, counting, and characterization of MPs. The size range bound for MPs was between

5 mm and 0.2 mm to ensure proper identification after the application of the direct observation, Rose Bengal staining and hot needle techniques, altogether with a millimeter-scale size reference. The following combined criteria were considered for MPs identification: (1) absence of visible cellular or organic structures; (2) fibers have consistent thickness along their length and should not taper at the ends; (3) particles are not shiny; and (4) particles are not stained with Rose Bengal ([Hidalgo-Ruz et al., 2012](#)).

For possible MPs items that raised identification doubts, the hot needle test was performed, which consists of bringing a hot needle close to the suspected MP and observing if it adhered, bent, or generated smoke ([Kumar et al., 2018](#)). The occurrence of any of these reactions confirmed the MP nature of the particle. The hot needle test was applied, in particular, to some red/fuchsia particles since staining with Rose Bengal could make difficult to visually identify MPs with these colors ([Gbogbo et al., 2020](#)). Further, the identified particles were visually characterized, recording shape categories —i.e., fragment, fiber, film, foam, pellet, and granule— ([Kovač et al., 2016](#)), as well as colors.

#### 2.6. Prevention of sample contamination

Preventing external contamination of wastewater and sludge samples was ensured by using non-plastic materials and tools, which were washed with microfiltered water before and after each usage. Exposure of sample containers to the environment was limited for  $\text{H}_2\text{O}_2$  addition and gas release only. The laboratory was kept clean before and after each procedure. Used filters were covered in Petri dishes and only exposed to the environment during examination under the stereomicroscope. These precautionary measures allowed maintaining the integrity of the samples, thus ensuring the quality of the results.

#### 2.7. Data management

The identified MP quantities were standardized based on the volume of wastewater and the mass of analyzed sludge, expressing particle abundance in items  $\text{L}^{-1}$  of wastewater and items  $\text{Kg}^{-1}$  dry weight (dw) of sludge, respectively. MP loads in influents and effluents were calculated after MP abundance values and the influent flow rate. We used the formula (1):

$$\text{CL} = \text{MP abundance} * \text{Q}(1)$$

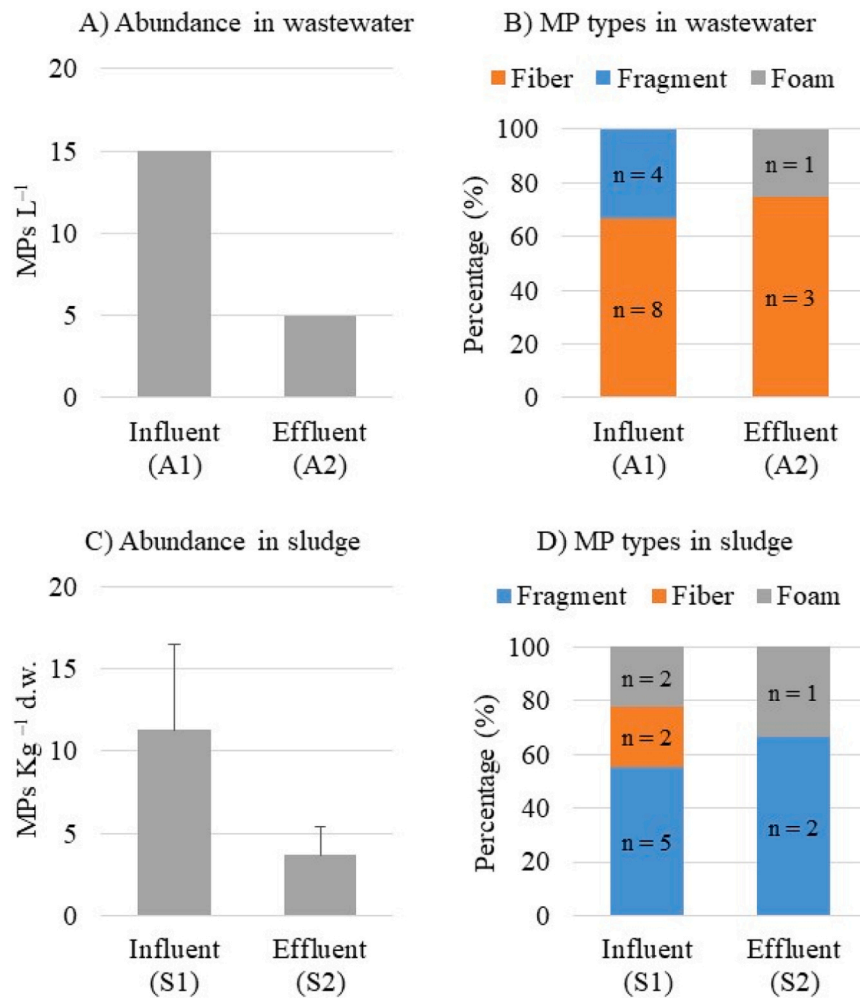
where "CL" represents the contaminant load, "MP abundance" is the MP abundance expressed in items  $\text{L}^{-1}$ , and "Q" is the flow rate expressed in  $\text{L day}^{-1}$ . The efficiency of MP removal in wastewater was determined by subtracting the number of MPs in the effluent from the one in the influent, then dividing the result by the number of MPs in the influent, and finally multiplying by 100 to express the result as a percentage.

### 3. Results

#### 3.1. Abundance and types of microplastics in wastewater and sludge

The abundance of MPs in wastewater samples was 15 and 5 items  $\text{L}^{-1}$  in the influent (A1) and 4 in the effluent (A2), respectively ([Figs. 4A, 5](#)). These MPs were dominated by blue and white fibers in both the influent and the effluent, accounting for 67% and 75% representations, respectively. White fragments (33%) were found in the influent, and white foams (25%) in the effluent ([Fig. 4B](#)).

The abundance of MPs in the sludge samples was  $11 \pm 5$  and  $4 \pm 2$  items  $\text{Kg}^{-1}$  dw for the influent (S1) and the effluent (S2), respectively ([Fig. 4C, Fig. 5](#)). The most common types of MPs in the sludge were white and blue fragments, accounting for 56% and 67% in the influent and effluent, respectively. Fragments were followed by white foams, amounting 22% and 33% in influents and effluents, respectively, and also fibers in the influent with 22% ([Fig. 4D](#)).



**Fig. 4.** Abundance and types of microplastics (MPs) in the San Antero wastewater treatment system (WTS). A and B: MPs in influent and effluent wastewater. C and D: MPs in the sludge).

### 3.2. Flow rate and estimated microplastics load in wastewater

The average flow rate of wastewater entering the San Antero WTS during our sampling was  $3.20 \pm 0.21 \text{ L s}^{-1}$ , equivalent to  $276,048 \text{ L day}^{-1}$ . We assumed this same value for the effluent flow rate that is discharged into the Cardales stream. The estimated load of MPs in the influent wastewater was  $4,140,723 \text{ items day}^{-1}$ , while in the effluent it was  $1,380,241 \text{ items day}^{-1}$ . Therefore, the resulting removal of MPs from wastewater entering the San Antero WTS was 67%.

## 4. Discussion

### 4.1. Abundance and types of microplastics in wastewater and sludge

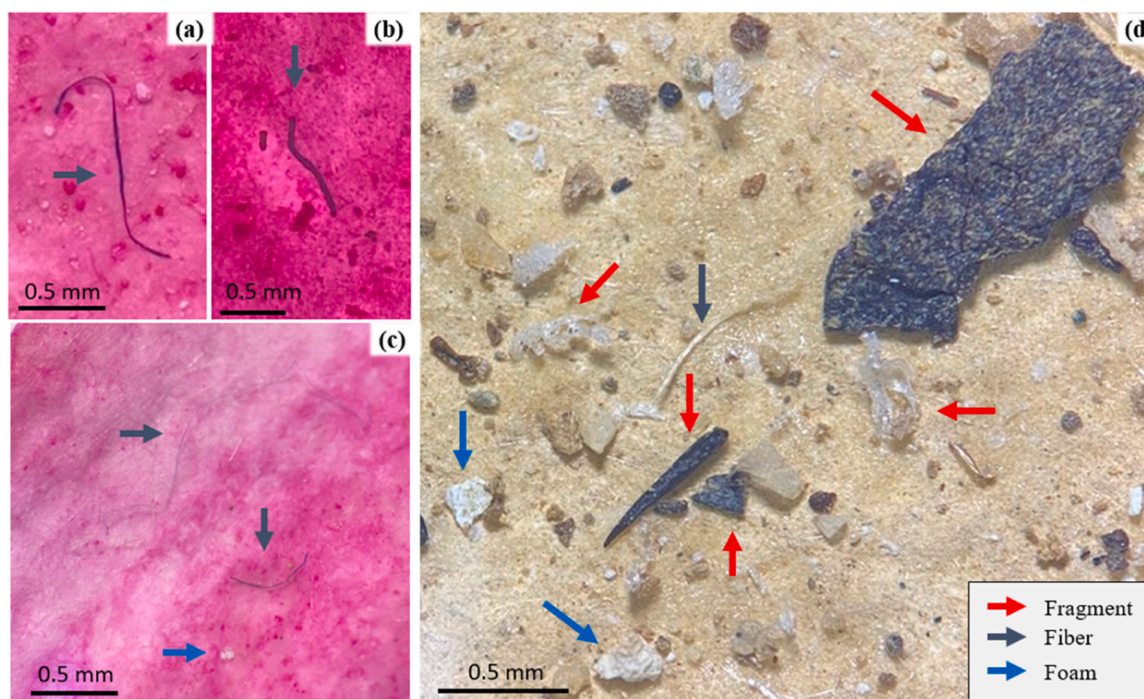
This study is one of the first to quantify MP pollution in wastewater and sludge from oxidation/stabilization ponds in a WTS in a tropical setting. The malfunctioning of the investigated WTS, due to the lack of maintenance (Fig. 3), makes it a significant source of MPs that reach the aquatic ecosystems receiving effluent wastewater and sludge, including the Cardales stream and the lagoon and mangrove ecosystem of the Cispata MPA in Colombian Caribbean. The deterioration of municipal WTS infrastructures is quite common in other coastal towns and villages in Colombia and, likely, beyond (Garcés-Ordóñez et al., 2016), which raises concern as this situation diminishes their effectiveness in removing pollutant loads that are being discharged directly into aquatic ecosystems (INVEMAR, 2020; Sáenz-Arias et al., 2023). Beyond MPs,

such loads include nutrients, heavy metals, and pathogenic microorganisms.

Oxidation/stabilization ponds are conventional biological wastewater treatment systems commonly used in rural communities due to their simple and cost-effective design (Mahapatra et al., 2022). The few studies on MPs in wastewater and sludge from this type of WTS include Fan et al. (2023), who assessed MPs with sizes  $> 25 \mu\text{m}$  in a conventional pond WTS in Victoria, Australia, reporting abundances of  $55.3 \text{ items L}^{-1}$  in the influent and  $7.0 \text{ items L}^{-1}$  in the effluent of the maturation pond. These values, which include smaller MPs than in our study, surpass the abundances we found in the influent and effluent of the San Antero WTS.

Gao et al. (2021) studied MPs with sizes  $> 45 \mu\text{m}$  in wastewater from an aerated stabilization pond WTS in Oxford, United States, where they encountered average abundances from  $3.8$  to  $4.0 \text{ items L}^{-1}$  in the treated effluent. This WTS, where smaller MPs were analyzed, has lower abundances compared to those found in our study. On the other side, the abundances of MPs in the sludge from the stabilization pond of the same WTS ( $12,800 \text{ items kg}^{-1}$ ; Gao et al., 2021) significantly exceed the abundances in the sludge of San Antero WTS.

It should be noted that variations in the design and construction of WTSs, operating conditions, and study methods and procedures affect the calculation of MP abundances in wastewater effluents. Tertiary treatment systems show fewer microplastics in effluents than secondary and primary treatment systems (Carr et al., 2016; Krishnan et al., 2023). Using appropriate treatment technologies also plays a key role, with up



**Fig. 5.** Photographs of some of the microplastics (MPs) identified in the San Antero wastewater treatment system (WTS). A, B and C; MPs in wastewater samples. D: MPs in sludge samples.

to 99.9% reduction of MPs larger than 20  $\mu\text{m}$  in wastewater effluents in some cases (Talvitie et al., 2017; Krishnan et al., 2023). Lack of standardization in sampling and processing methods, including instrumentation, reagents, and the lower retention bound size of particles also influence results (Uddin et al., 2020), especially for < 50  $\mu\text{m}$  particles, which commonly show a low recovery rate (9%) in wastewater (Weber and Kerpen, 2023).

Fibers were the most prevalent type of MPs both in the influent and effluent wastewater from the WTS in San Antero. These findings align with those reported by Fan et al. (2023) regarding MPs in influents and effluents of the maturation pond in the above mentioned WTS in Victoria, Australia, where fibers represented 70% and 80%, respectively. In the wastewater of the WTS in Oxford, United States, fibers were also the most abundant MP type, accounting for 62% (Gao et al., 2021).

Fiber-type MPs are characterized by their small sizes, lightweight nature, and elongated shapes. Usually, fiber density is lower than the wastewater one, which facilitates their passage through various steps of the treatment process until being released into the receiving environment (Fan et al., 2023; Sheriff et al., 2023). This type of MPs usually originates from washed synthetic garments, wet wipes, and sanitary towels, among others, and are highly abundant in aquatic, terrestrial, coastal, and marine ecosystems receiving wastewater discharges (Briain et al., 2020; Garcés-Ordóñez et al., 2022; Sheriff et al., 2023).

Fragments were found only in the influent waters. These MPs usually arise from the breaking of packaging materials and commonly used consumer products (Hidalgo-Ruz et al., 2012; Garcés-Ordóñez et al., 2020b). Other studies have also been found fragments in WTS wastewater though with low prevalence, as they are more effectively retained during biological treatment processes (Vardar et al., 2021; Fan et al., 2023). In the San Antero WTS this type of MPs was dominating in the sediments of both the influent and the maturation pond (Fig. 4). Likely, their accumulation in the sludge was eased by ballasting processes such as bacterial colonization, flocculation, or heteroaggregation and, at least in some cases, by a relatively high density (Sun et al., 2019; Bydalek et al., 2023).

Foams were found in the wastewater effluent and in the sludge of both the influent and effluent. Their prevalence in both wastewater and

sludge was low, as reported in other WTS (Bayo et al., 2020; Ben-David et al., 2021; Sheriff et al., 2023). This type of MPs could originate from fragments of containers, disposable tableware, expanded polystyrene and other types of foam packaging materials (Hidalgo-Ruz et al., 2012; Garcés-Ordóñez et al., 2020b). They might enter the WTS through stormwater runoff carrying foams and other types of MPs from urban areas (Alcaldía de San Antero, 2021; Vivas-Aguas and Franco-Angulo, 2022). Small MPs, including low-density foams, can settle and incorporate in sludge deposits through coagulation-flocculation processes in wastewater. Further, they can also undergo heteroaggregation with natural particles occurring in the waters of artificial wetlands, such as stabilization and maturation ponds (Liu et al., 2019; Paduani, 2020).

#### 4.2. Estimated microplastic load in wastewater

The effluents from the WTS based on oxidation/stabilization ponds in San Antero contribute a similar load of MPs as the one found by Fan et al. (2023) for a conventional pond-based WTS in Victoria, Australia, which is around 1.3 million items  $\text{day}^{-1}$ . This figure is less than the value reported by Gao et al. (2021) for the effluents from an aerated stabilization pond-based WTS discharging into a receiving lake, which reached 786,000 items  $\text{day}^{-1}$ .

The MP load from the San Antero WTS is higher than in other types of WTS, such as a secondary treatment WTS in Los Angeles, United States, with an estimated MP load for 930,000 items  $\text{day}^{-1}$  (Carr et al., 2016). However, it is lower than the estimated MP load of the above-mentioned secondary treatment WTS in Vancouver, Canada, which yielded about 82 million items  $\text{day}^{-1}$  (Gies et al., 2018), and the tertiary treatment WTS in northern Italy, releasing nearly 160 million items  $\text{day}^{-1}$  (Magni et al., 2019). It's important to note that the two latter WTS have significantly larger flow rates, as they are in large cities with a population in the millions.

The efficiency of MP removal in pond-based stabilization WTS is directly influenced by water retention times (Fan et al., 2023), representing the period during which wastewater remains in the system before being released as treated effluent. In the pond-based stabilization WTS in Victoria, Australia, MP removals of 87% after treatment in the

maturation ponds and 97% in the final effluent were reported, thanks to a retention time in excess of 250 days (Fan et al., 2023).

Although the retention time in the San Antero WTS ponds remains non quantified, evidence suggests that inflow and outflow rates are similar (Vivas-Aguas and Franco-Angulo, 2022), which could indicate a too short water retention to achieve a higher purification of various contaminants, including MPs. Our results show that the San Antero WTS retains approximately 67% of the incoming MPs, implying that a considerable fraction is being released through the effluent into the Cardales stream, subsequently reaching the coastal lagoon and the mangroves within the Cispatá MPA.

It should be highlighted that, beyond the WTS effluent-mediated discharge, a substantial additional amount of MPs likely enters the receiving ecosystem because of the overflow of untreated wastewater before reaching the pond system. These overflows result from infrastructure deterioration, channel clogging, and lack of maintenance of the WTS (Vivas-Aguas and Franco-Angulo, 2022). The sludge produced at the San Antero WTS also contains a significant quantity of MPs. Improper management of the sludge, as observed in this WTS during fieldwork, may lead to deposition nearby the oxidation ponds and Cardales stream. With the arrival of rainfall, the MPs contained in the sludge could be easily washed into the surrounding aquatic ecosystems, thus adding even more to the overall discharge.

It should be also noticed that wastewater contains a variety of pollutants other than MPs, such as heavy metals, pesticides, pharmaceuticals, and hydrocarbons, which can be absorbed by MPs (Joo et al., 2021). Small-sized plastic particles may act as micro-carriers of these pollutants to the wastewater receiving aquatic ecosystems (Nikpay, 2022). A variety of microorganisms also occur in wastewater, including those with drug resistance genes and pathogens that are able to affect ecologically and commercially important species (Lai et al., 2022; Sáenz-Arias et al., 2023). Scientific evidence has demonstrated that these pathogenic microorganisms use MPs as a substrate for growth and propagation (Cheng et al., 2022).

Poorly known interactions between organic and inorganic pollutants, microorganisms, and MPs potentially poses a significant environmental risk to aquatic ecosystems such as coastal lagoons and mangroves, which are highly vulnerable to this pollution cocktail (Garcés-Ordóñez et al., 2022; Sáenz-Arias et al., 2023). Habitat pollution and ingestion of MPs by different fish species, which are a food source for local communities, have already been documented in the Cispatá MPA (Garcés-Ordóñez et al., 2020a; Garcés-Ordóñez, 2022). Consequently, such pollutant mixtures may involve a direct threat to biodiversity, ecosystem services and human health (Vivas-Aguas and Franco-Angulo, 2022).

#### 4.3. Limitations and future plans

The current study allowed determining the quantity and the physical characteristics (shape and color) of MPs with sizes  $> 200 \mu\text{m}$  in the wastewater and sludge of the San Antero WTS through point sampling. This provides a snapshot on MP pollution in this malfunctioning WTS, involving restricted sample volumes due to logistical and sampling constraints. It should be noticed that the conditions under which sampling was performed can vary through time—as is most often the case in fluctuating environments—due to a number of factors, such as weather state or further deterioration of the WTS. Other influencing factors could be the sampling methodology and future system maintenance if ever done. Sample size and representativeness is a debated topic, particularly in large WTSs (Uddin et al., 2020). On the other side, there is limited research on oxidation/stabilization pond systems, like the one in San Antero WTS. Despite these constraints, individual, composite, or continuous samples collected at various treatment stages still allow capturing MP loads (Uddin et al., 2020).

It should be reminded here that our research focused on the  $> 200 \mu\text{m}$  MPs size class, which represents a fraction of the overall

pollution by MPs, as smaller, and likely abundant, size classes were not addressed. Due to severe economic restrictions, lack of access to advanced technologies, and also time constraints, the polymer characterization of the MPs found in the wastewater and sludge of San Antero could not be conducted. Hopefully, international collaboration in the near future will allow extending the results achieved so far.

Similarly, it would be valuable to analyze, in subsequent investigations, how technical factors related to the WTS operation, and sampling methods, influence the calculated MP loads in the effluents. Such factors would be inclusive of—but not exclusive of—water retention times in the ponds, wastewater overflow events, and the dynamics of MPs in different treatment stages. We also consider relevant examining the potential influence of meteorological factors on MP dynamics, so that a more comprehensive understanding of how these oxidation/stabilization pond systems interact with MPs, and how management practices can be improved to mitigate their release into the receiving aquatic ecosystems, are achieved.

## 5. Conclusions

WTSs play a critical role in the global strategy to reduce the presence of contaminants in the vast volumes of wastewater flowing through these systems. However, some WTSs, such as those based on oxidation/stabilization ponds, lack efficiency in removing MPs and other pollutants in the treated waters and, thus, can become significant sources of plastics, which are ultimately released to the surrounding environment.

This study demonstrates that the treated wastewater and the sludge from a pond-based WTS in the municipality of San Antero, Colombian Caribbean, contain significant amounts of secondary MPs in the form of fibers, fragments and foams. These MPs enter the aquatic ecosystems receiving the effluents from the WTS, with an estimated load of at least  $1.3 \text{ million MPs day}^{-1}$ . This figure does not account for contributions due to overflows or inadequate management of untreated wastewater, neither for MP sizes smaller than  $200 \mu\text{m}$ . Could these aspects have been considered, the estimated load would have been substantially higher. Therefore, the figure provided above should be viewed as a minimum estimate.

The transfer of MPs from municipal wastewater to the surrounding aquatic ecosystems, including Cardales stream, coastal lagoons, and mangroves within the Cispatá MPA poses a high risk to its biodiversity and ecosystem services. This research serves as an urgent call to the Colombian government and environmental authorities to recognize this issue, which threatens ecosystem health and the provision of environmental services, and to take appropriate actions. We finally notice that similar situations to the one described in this paper could occur in other WTSs in developing countries and beyond.

#### CRediT authorship contribution statement

**Garcés-Ordóñez Ostin:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Vivas-Aguas Lizbeth-Janet:** Funding acquisition, Supervision, Writing – review & editing. **Arregocés-Garcés Roberto:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **Canals Miquel:** Conceptualization, Visualization, Writing – review & editing.

#### 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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