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Microplastic Accumulation in Sewage Sludge from Biological Wastewater Treatment Plants in Acapulco, Mexico: Implications for Sustainable Sludge Management

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Abstract

Wastewater treatment systems retain a significant proportion of microplastics (MPs) derived from domestic and industrial discharges; however, these emerging pollutants are not completely removed and tend to accumulate in the biological sludge generated during the treatment process. In this study, three biological-type wastewater treatment plants (WWTPs) located in Acapulco, Mexico, were analyzed. The concentrations of MPs in the biological sludge ranged from 830 to 9,300 items per liter. Using differential scanning calorimetry (DSC), the predominant polymers identified were high-density polyethylene (HDPE), polyethylene terephthalate (PET), and polypropylene (PP). It was estimated that the monthly concentrations of MPs in the sludge could reach up to 5.36×10^9 items/liter, while the annual concentrations could rise to 3.55×10^{10} items/liter. These findings highlight the urgent need to review and update the regulatory framework related to the use of residual sludge for agricultural purposes since high loads of MPs and their transfer pose a potential risk to soil quality, ecosystem health, and long-term environmental sustainability.

Keywords: microplastics; biological sludge; wastewater treatment plants (WWTPs); differential scanning calorimetry (DSC); synthetic polymers; sustainability; public policy

1. Introduction

Microplastics (MPs) are multidimensional emerging pollutants (< 5 mm) present in large quantities in natural and anthropogenic systems, posing an alarming challenge to sustainability [1]. The increase in plastic production and consumption in developed countries, as well as emerging economies, has generated an overload in waste management systems handling plastic until their degradation into MPs [2–5]. Due to their distribution and abundance in various environments such as air [6], water [7–10], beaches [11], and soils [12–16], MPs represent a significant threat as they can adhere to toxic elements such as heavy metals and hydrophobic additives, making them potential vectors for such contaminants [7,17,18]. In this regard, research has shown that MPs have a high affinity for these metals and additives, which increases the risk of toxicity and makes them a growing danger to environmental and human health [7,19].

Within different ecosystems, studies have revealed that microplastics can accumulate in marine organisms, causing damage and biological alterations, along with other adverse effects [19,20]. MPs also affect terrestrial ecosystems by interfering with the behavior of organisms such as collembolans and other insects and disrupting soil functions and associated biota [13,21]. Furthermore, it has been demonstrated that introducing MPs into various levels of the food chain can generate short- and long-term effects, including toxic risks such as oxidative stress and neurotransmitter alterations in various species, including humans [17,22–24]. Therefore, these impacts not only compromise food security but can also alter ecological dynamics, affecting biodiversity and ecosystem stability [10].

Concerning anthropogenic systems, wastewater treatment plants (WWTPs) significantly contribute to the removal of various contaminants and the production of biological sludge that can later be used as soil conditioner, fertilizer, and compost for agricultural use [25–29]. However, such treatment systems are not originally designed to eliminate emerging contaminants such as MPs [27,30–32]. Primary and secondary MPs have been identified through various analytical techniques (FTIR spectroscopy, Raman spectroscopy, Pry-GC-MS, SEM, and DSC) at different treatment stages, detecting considerable presence and variability [33–38].

In WWTPs in the United Kingdom, persistent MPs within the size range of 60–2800 μm have been found at all sampling points, with an average inlet of 8.1×10^8 particles per day, reduced by 96% during treatment stages; however, despite this removal, an average of approximately 2.2×10^7 particles per day are discharged into the environment [39]. Other studies have observed MP retention efficiencies between 94.9% and 99.7% [40–44]. Meanwhile, a significant number of MPs accumulate in the sludge generated during biological wastewater treatment processes [45–50]. For example, in Vancouver, Canada, it was estimated that up to 99% of MPs entering wastewater treatment systems annually are retained in primary and secondary sludge, with 1.28 trillion particles stored in the primary sludge [41].

In China [26], concentrations of 240.3 ± 31.4 ng/g of MPs were reported in biological sludge, with an average size of 222.6 μm. The predominant types of MPs—confirmed by their chemical composition—include polyethylene terephthalate (PET), polystyrene (PS), polyethylene (PE), polypropylene (PP), and polyvinyl chloride (PVC). Morphologically, fibers and fragments are the most common, and colors such as red, black, green, and blue are the most prevalent [30,33,42,43,51,52]. This accumulation of MPs in sludge poses additional risks, as they can be introduced into agricultural soils, altering terrestrial environments [43]. Despite various treatments applied to sludge—such as thermal pre-treatment, anaerobic digestion, and composting—they do not effectively eliminate MPs [53–55], which continue to persist in the sludge and may re-enter the environment, significantly affecting sludge quality for sustainable use and management [36,47,51,56]. In Mexico, the current environmental regulation establishing the maximum permissible limits for pollutants in the management and final disposal of biological sludges is NOM-004-SEMARNAT-2002 [57], but this standard does not consider microplastics (MPs) within its framework. Therefore, the current situation regarding the extent of microplastic contamination in biological sludge remains unknown, despite its growing relevance as an emerging pollutant. Consequently, it is essential to evaluate the problem associated with the presence of MPs in biological sludge generated by WWTPs.

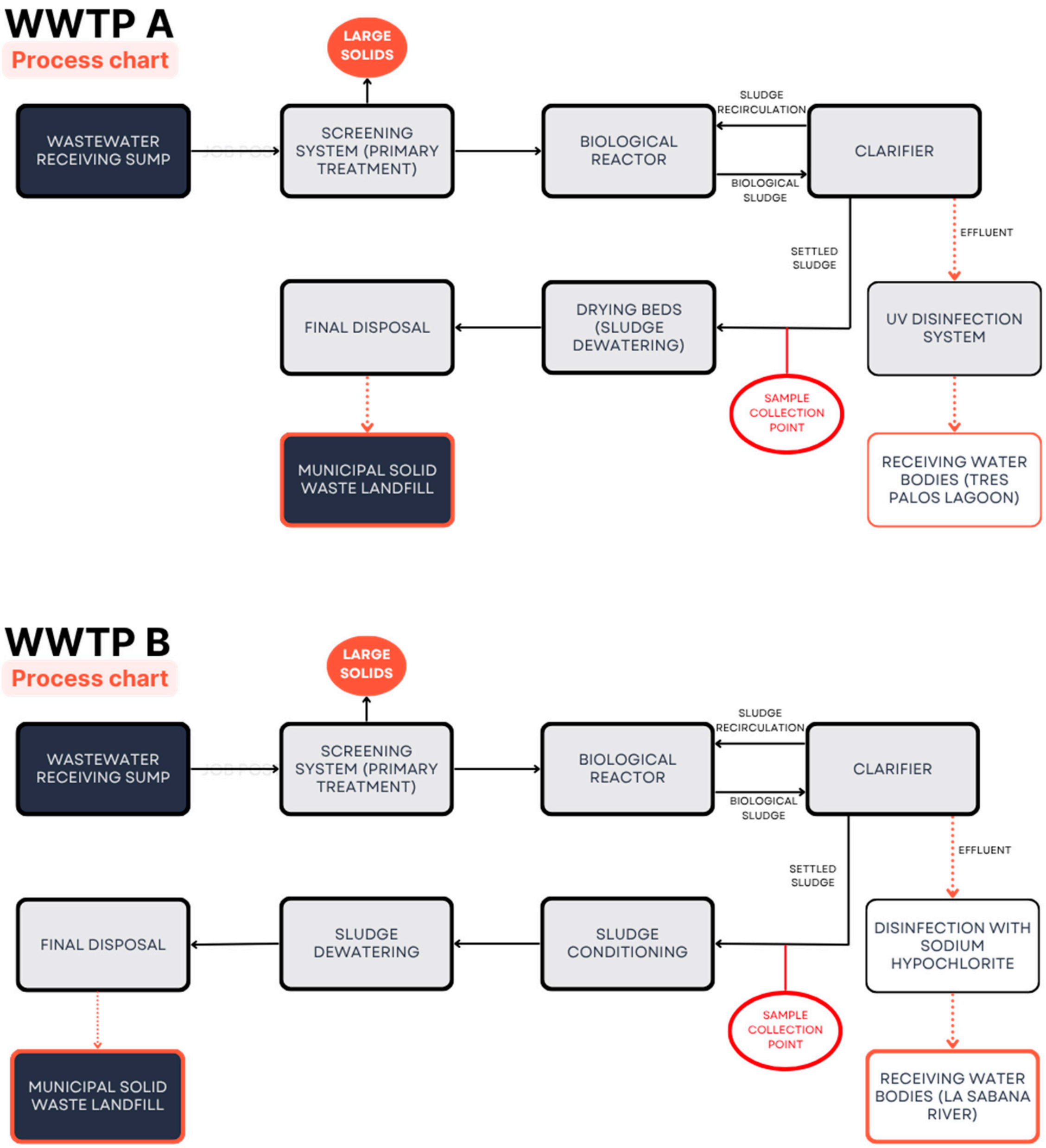
This study aimed to detect and quantify the presence of 300 μm MP particles in sludge from three biological WWTPs in Acapulco using density separation ($ZnCl_2$) and differential scanning calorimetry (DSC). Moreover, in this study, we aimed to estimate monthly and annual projections of MP concentrations.

The obtained results provide relevant information for the development of technical criteria that allow for the inclusion of MPs in Mexican environmental regulations, thereby contributing to the establishment of a broader regulatory framework that promotes the sustainable management and safe use of biological sludge.

2. Materials and Methods

2.1. Sampling

Sampling was conducted at specific points during June, July, September, and October 2023, at the outlet of the sedimentation tanks of the three WWTPs (Figure 1). Samples were collected in triplicate in 100 mL Teflon® bottles from the brand Nalgene®. Once collected, the samples were stored in the laboratory at 15 °C.



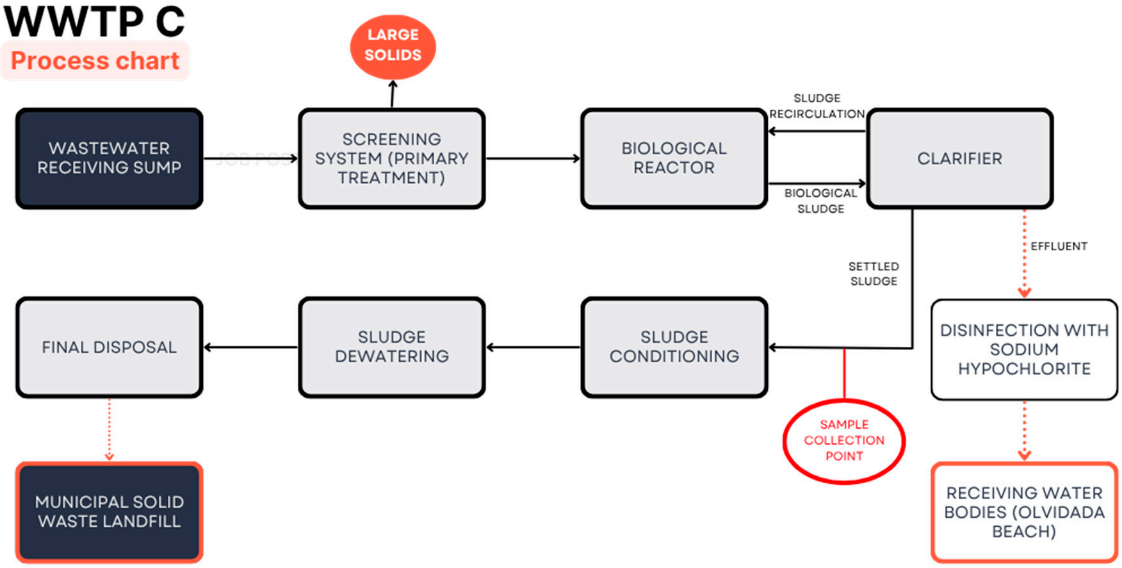


Figure 1. Process stages in the three WWTPs monitored in this study. In WWTP A, the sludge is disposed of in drying beds. In WWTPs B and C, the sludge is preliminarily conditioned and dewatered using filter presses.

2.2. Sample Treatment

Extraction of MPs from the sludge was performed using density separation, with modifications based on methods reported in other studies [26,43,58–64]. Each 100 mL sludge sample was dissolved in 1000 mL of distilled water for homogenization. Subsequently, it was sieved using 710 and 300 μm mesh sieves to remove large solids. After sieving, the 300 μm sieve was carefully rinsed with distilled water, which was collected in a 250 mL beaker. The rinse water was then dried at a controlled temperature of 80 ± 1 °C for 8 to 10 hours. Next, digestion was carried out by adding 40 mL of 30% H_2O_2 at 60 ± 1 °C for two hours. The process was repeated with an additional 20 mL of H_2O_2 under the same conditions for another two hours. Once cooled to room temperature, MPs were separated based on density using 60 mL of $ZnCl_2$ solution with a density of 1.62 g/cm^3 [58]. Finally, the supernatant was vacuum-filtered using glass fiber filters and stored in Petri dishes for identification [58]. Each sample was analyzed in triplicate. Throughout the process, an exhaustive cleaning protocol was followed to prevent cross-contamination. Blanks and filters were included to detect possible MPs from the atmosphere, with no MPs observed in the quality control samples, thus guaranteeing that the MPs detected originated exclusively from the analyzed samples.

2.3. Characterization of MPs

For MP identification, an adaptation of Flores-Munguía et al. [58] was used based on the classification criteria proposed by Hidalgo-Ruz et al. [65]. Quantification and physical characterization of MPs according to their color and morphology were performed using optical microscopy with 4× and 40× objectives. Identification of different MP types was carried out via differential scanning calorimetry (DSC), as performed in other studies [66–71], using TA Instruments DSC 250 model DSC. About 2.1 mg of each sample was weighed in an aluminum tray; an empty aluminum tray served as a reference. The sample was then subjected to a heating program in the temperature range of 25 to 290 °C with a heating rate of 10 °C/min in a differential scanning calorimeter. The melting temperature (Tf) and enthalpy (ΔHf) were obtained directly from TA Instruments Trios software version 4.

For the polymeric characterization of the MPs detected in sludge samples, fragments of commercially used plastics (PE, PP, PET, and PVC) were used as reference materials.

2.4. Daily and Monthly Projections

Projections were based on the report by Flores-Munguía et al. [58]. The daily MP concentration was determined by multiplying the MP concentration per 100 mL by the total volume of wastewater treated daily (m^3/day). The monthly concentration was estimated by integrating daily MP values and the average mass flow rate of sludge generated monthly. Finally, the annual concentration was calculated considering MP concentration and average annual sludge production during the ten months of WWTP operation (January to October 2023).

2.5. Statistical Analysis

An evaluative analysis of the results obtained from the three WWTPs was conducted to understand differences in their operation and the influence of seasonality regarding the presence and concentration of MPs. This was performed using one-way ANOVA considering a significance level of $p < 0.05$.

3. Results and Discussion

3.1. MP Concentration

The amounts of 300 μm MPs identified in this study's analyzed sludge samples ranged from 537 to 9,300 particles/L across the three WWTPs (Table 1). These figures show similarities and differences with previous studies [43,48,61,62,72,73]. The reported concentrations in other studies include ranges of 28–12,000 particles/L [72], 280 particles/gram [61], 22.7×10^3 particles per kilogram of dry sludge [05], and between 4,196 and 15,385 particles/kg of dry sludge [73]. However, some studies have documented even higher concentrations, such as Liu et al. [43], who quantified an average of 240.3 particles/gram in dehydrated sludge.

The similarities and differences observed could be related to the specific treatment conditions at each WWTP. Additionally, the operational capacity of each treatment system might influence MP accumulation in biological sludges. Furthermore, the complexity of contaminant degradation processes, combined with the efficiency of biological reactors and primary and secondary sedimentation, can also affect the variability in ranges reported in different studies. Different sampling protocols, sample treatment methods, and detection techniques for MPs are also important factors [29,69].

The three WWTPs analyzed in this research all used the same secondary treatment process (biological sludge), and according to their operational systems, no significant differences in MP concentration variation trends were observed during the four sampling months, as confirmed by ANOVA analysis ($F = 0.29$, $p > 0.05$) (Table 2).

Table 1. Concentration of MPs in three WWTPs in 2023.

	MP (Items/L)			
	June	**July**	**September**	**October**
WWTP A	830 ± 33.60	537 ± 11.85	7650 ± 144.42	6583 ± 126.02
WWTP B	1207 ± 58.18	1357 ± 33.71	9300 ± 240.95	8803 ± 81.45
WWTP C	1470 ± 6.00	1640 ± 58.13	4813 ± 182.50	5180 ± 46.13

Note: Sampling conducted during dry periods (June and July) and rainy periods (September and October).

Furthermore, these same WWTPs were previously studied by Flores-Munguía et al. [58], who reported removal efficiencies between 82.5% and 98.7%. Based on our results, this indicates that most MPs tend to accumulate in the biological sludge. Similar removal efficiencies have been reported elsewhere, and these studies conclude that high removal rates are due to the accumulation of MPs in the biological sludge [44,48,54,59,63,74].

Although not originally designed to retain this type of contaminant, WWTPs demonstrate high MP removal efficiencies, preventing mass release of these pollutants into aquatic ecosystems [29,32,69]. The vast majority of MPs remained in the sludge generated during the treatment processes [34]. Thus, high MP concentrations in biological sludge pose challenges for sustainable management and utilization [44,47]. Kong et al. [50] indicates that MPs in sludge can inhibit microbiological processes in anaerobic treatments, potentially affecting contaminant removal efficiency in wastewater treatment.

On the other hand, high MP concentrations in sludge could affect crops if the sludge is used as soil conditioner or fertilizer, as MPs are often associated with other pollutants such as heavy metals [36,47,53]. Additionally, MPs can interfere with composting processes and increase the contaminant load in leachates from municipal landfills, potentially leading to soil infiltration and groundwater contamination [45].

Table 2. According to their treatment systems.

Groups	*Count*	*Sum*	*Average*	*Variance*
WWTP A	4	15600	3900	13998552.67
WWTP B	4	20667	5166.75	20166628.25
WWTP C	4	13103	3275.75	3975238.917

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7426966.17	2	3713483.08	0.29	0.754	4.26
Within Groups	114421260	9	12713473.3			
Total	121848226	11				

The variability in the results from this study highlights seasonality as a key factor influencing MP concentrations in sludge. According to the one-way ANOVA (F = 15.55, $p < 0.05$) (Table 3), significant differences were found in MP presence over the four sampling months.

Table 3. According to their sampling periods.

Groups	*Count*	*Sum*	*Average*	*Variance*
June	3	3507	1169	103483
July	3	3534	1178	328183
September	3	21763	7254.33	5150706.33
October	3	20566	6855.33	3337156.33

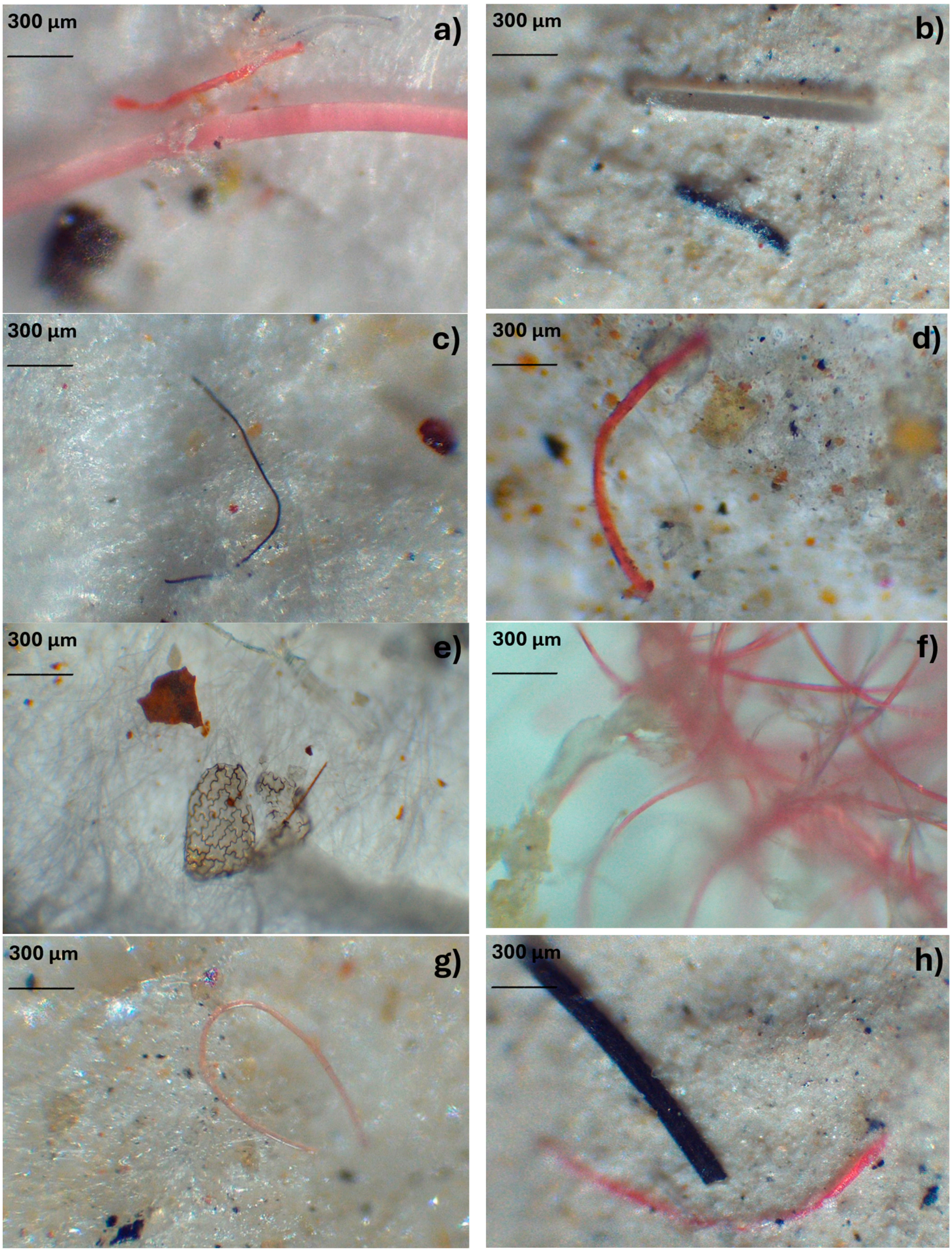
ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	104009168	3	34669722.8	15.55	0.001	4.07
Within Groups	17839057.3	8	2229882.17			
Total	121848226	11				

It was also observed that during rainy months (September and October), MP concentrations were considerably higher compared with during dry months (June and July). This may have been due to increased contaminant loads in influents linked to higher rainfall runoff. These findings align with reports by Li et al. [62] and Dronjak et al. [36], who documented seasonal variations in MP concentrations in biological sludge. Therefore, seasonality and weather events likely influence MP influxes into WWTPs, impacting primary physical treatment processes and increasing pollutant loads in biological systems [58].

3.2. Physical Characterization of MPs

Optical microscopy analysis revealed a diversity of MPs based on their color and shape (Figure 2). The MPs observed in the sludge match the classification proposed by Hidalgo-Ruz et al. [65]. Moreover, the types of MPs detected are consistent with those reported in other studies [26,42,59,61,62,73,74].



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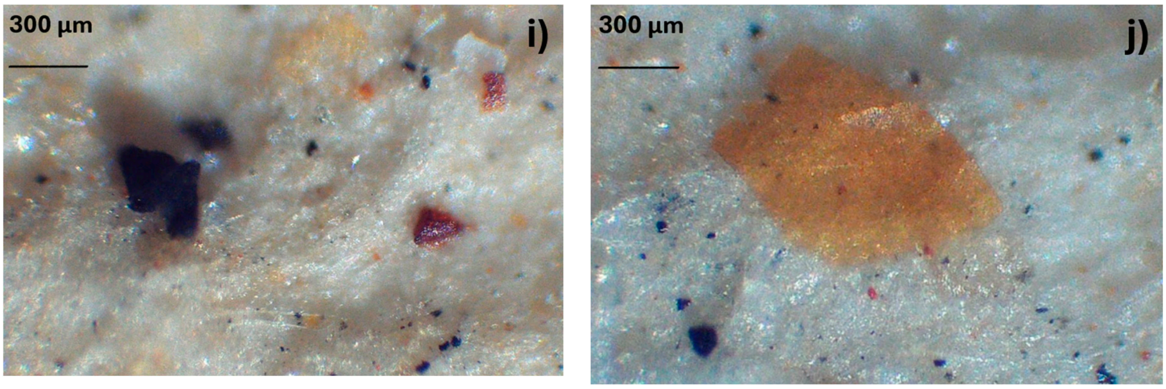


Figure 2. Filaments, fragments, and films of microplastics (MPs) identified in the biological sludge of the WWTPs. Samples correspond to plant A **(a, b, c)**, plant B **(d, e, f)**, and plant C **(g, h, i, j)**.

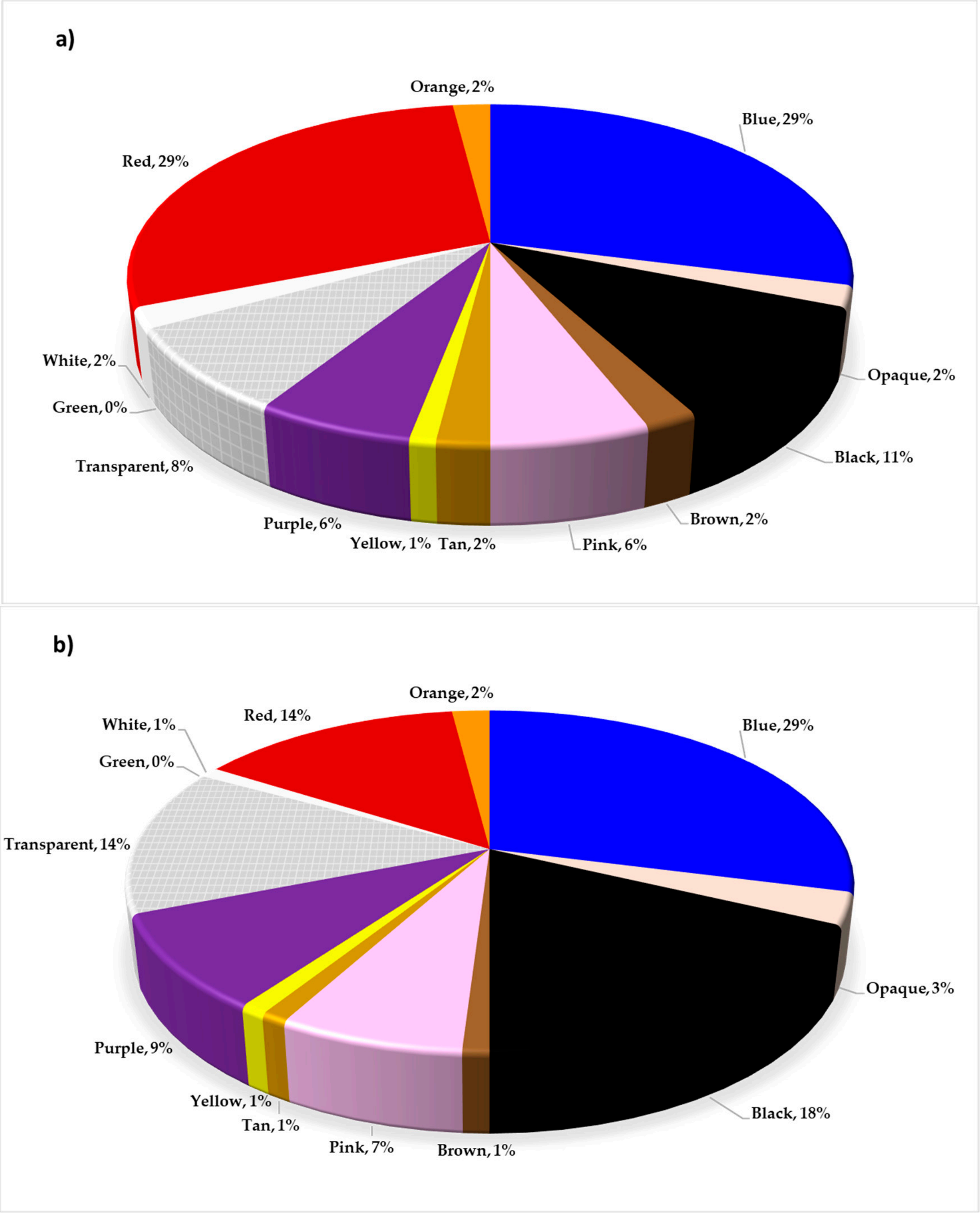
It should be noted that there were no observed differences in the physical characteristics of MPs compared with other investigations. The particle size reported in this study was 300 μm, which coincides with other studies identifying particles of the same size. Both studies, no significant morphological differences were found [63]. MPs originating from treatment systems with aerobic sludge show similarities in physical traits, with no apparent wear or color changes [45,46,48,50], despite differences in the physicochemical conditions during treatment and digestion. In contrast, studies on sludge from anaerobic processes have reported morphological and color alterations in MPs [29,49], suggesting that the type of biological treatment system may influence physical changes during wastewater treatment. The MPs detected in this study agree in physical appearance with those previously reported in influents and effluents of the same wastewater treatment plants (WWTPs) [58], demonstrating that larger and denser MPs tend to sediment during primary and secondary sedimentation processes. The diversity of shapes and forms of microplastics (MPs) suggests a complexity in their interactions once these biological sludges are disposed of for reuse or final disposal—whether for agricultural soils, landfills, or other environments. This complexity could influence their mobility, persistence, and potential release into the environment, as well as their effects on soil organisms and soil quality [29,49–51,54].

3.3. Color of MPs

In the three WWTPs, MPs of various colors were identified, with blue being the most prevalent, followed by black and red (Figure 3). These findings are consistent with those reported by Flores-Munguía et al. [58], who also observed this color distribution in the influents and effluents of these same plants. No significant differences (ANOVA F = 0.36; $p < 0.05$) were found in the variety of colors among the three WWTPs (Table 4), which could be attributed to the similarity in the characteristics of domestic discharges entering these treatment systems. The variety and frequency of the observed colors in this study differ from those reported in other studies; in several of them, the predominant colors were white, transparent, and black [11,26,29,34,48,52,59,62,75,76]. Additionally, other investigations in WWTPs from countries like China and England identified black, red, blue, and green as the most common colors [50,63]. Talvitie et al. [25] reported that yellow and transparent were the most prevalent in tertiary treatment systems of WWTPs in Finland. These differences may have been due to the specific discharge conditions in each city. Furthermore, the presence or absence of public policy regulations regarding the use of dyes and additives in plastic production (including primary MPs) could significantly influence the frequency and prevalence of certain colors.

On the other hand, it should be noted that not all studies on MPs in biological sludge from WWTPs include data on the coloration of these particles, limiting comparisons between studies [41,45,73,74]. Characterizing MPs by color is relevant because it allows us to estimate their degree of toxicity [58], and these microplastic particles act as vectors for the emission and exposure of additives and dyes, which often contain metallic elements [77].

Due to their ability to bioaccumulate, these compounds can pose risks to living organisms in any ecosystem, including those present in biological sludge used for composting and as soil conditioner in agriculture [5,18,78].



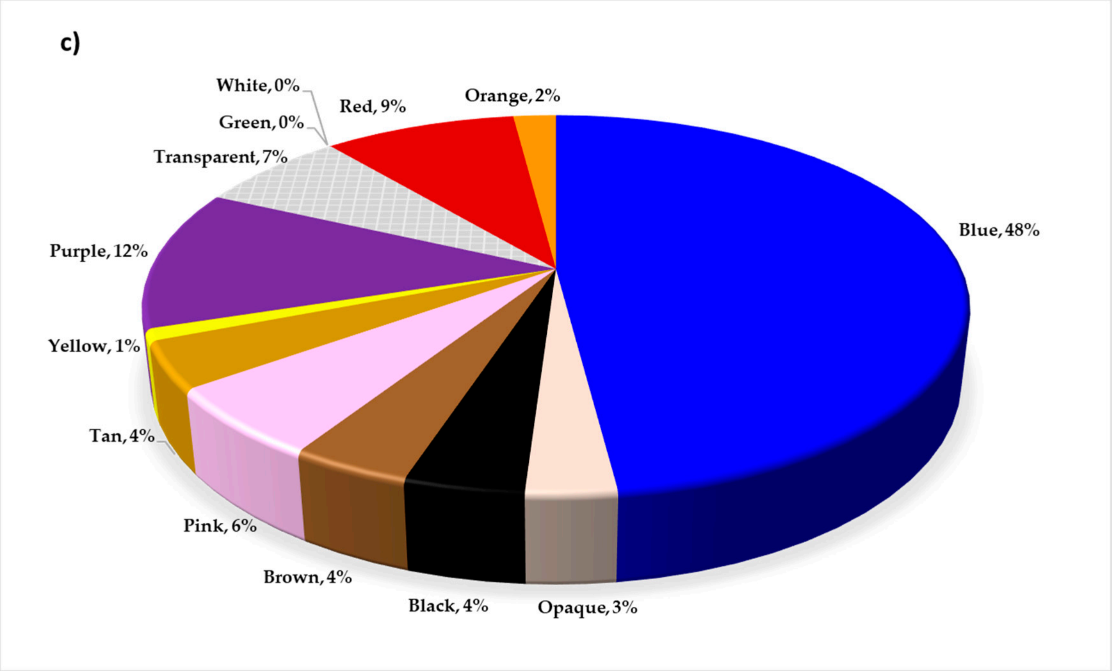


Figure 3. Color of microplastics (MPs) present in (a) WWTP A, (b) WWTP B, and (c) WWTP C. In all three wastewater treatment plants (WWTPs), blue was the most prevalent color among the MP particles identified in biological sludge.

Therefore, the colors of MPs can be used as indicators of the presence of associated contaminants, such as antimony (Sb), used in various plastics; aluminum (Al), common in PET and PE; arsenic (As) and bromine (Br), detected in PE and PP; and mercury (Hg) and lead (Pb), with the latter commonly found in red pigments used in different types of plastic. Similarly, cobalt (Co) has been identified in blue pigments, especially in PET bottles [1,17,75,79].

Regarding black MPs, these are mainly associated with the black pigments 7 (carbon black) and 11 (iron oxide), which, although they do not pose a significant environmental threat, account for approximately 2% of commercial plastics [77].

Table 4. ANOVA according to treatment systems.

Groups	*Count*	*Sum*	*Average*	*Variance*
WWTP A	13	795.00	61.15	5206.14
WWTP B	13	1135.33	87.33	9914.17
WWTP C	13	831.33	63.95	7213.11

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5373.41	2	2686.70	0.36	0.70	3.26
Within Groups	268000.99	36	7444.47			
Total	273374.40	38				

In addition, the results from this study suggest that seasonality could influence the variation in microplastic (MPs) presence, as reflected in the diversity of observed colors (Table 5). The ANOVA results show significant differences in MP concentration over the four months of sampling and analysis ($F = 3.96$, $p > 0.05$). So far, there are no reports in the literature specifically addressing the relationship between seasonality and color distribution in MPs, which is a relevant contribution. This information could be useful for implementing preventive strategies based on predominant coloration, especially those potentially associated with greater negative impacts on the quality of biological sludge and its subsequent use in agriculture [1,17,75,79].

Table 5. ANOVA according to sampling period and analysis time.

Groups	*Count*	*Sum*	*Average*	*Variance*
June	13	1055	81.15	7267.14
July	13	1060	81.54	14293.44
September	13	6529	502.23	377811.03
October	13	6170	474.62	327377.76

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2159209	3	719736.33	3.96	0.01	2.80
Within Groups	8720992.308	48	181687.34			
Total	10880201.31	51				

The coloration and pigments of MPs could represent a significant factor in the sustainability of wastewater treatment systems. The quality of biological sludge directly affects related processes, such as its use in agriculture as fertilizer or soil conditioner, as well as its disposal in landfills. However, high MP concentrations and the prevalence of certain dyes in MPs reveal the potential degree of contamination, as well as the vulnerability of the sustainable use of biological sludge. This highlights the need to evaluate and control the presence of these contaminants in domestic wastewater discharges to ensure responsible agricultural practices and the protection of ecosystems [17,75,79].

3.4. Morphology of MPs

In WWTPs A and C, fragments were the most common, followed by filaments and granules. Conversely, in WWTP B, filaments were the predominant MPs (Figure 4). Several studies on treatment systems with similar characteristics have documented a higher abundance of plastic fibers, followed by fragments, granules, and films, which aligns with the results of this study [26,29,32,34,36,61–63,69,73]. Conversely, another study found that films were the dominant form of microplastics, followed by fragments and fibers [45].

These similarities and differences could be attributed to the type of secondary process underway; for example, Schwinghammer et al. [45] reported that the detected microplastics came from anaerobic systems, which could explain differences compared with aerobic digestion processes. It is important to note that variations in the reported abundance of microplastic morphologies across studies might be influenced by the nature of influent discharges entering the WWTPs. Additionally, these differences can also stem from specific sampling and analysis methodologies employed to extract MPs, owing to the lack of standardized methods [32,69].

In this context, Flores-Munguía et al. [58] identified that in the same WWTPs, the predominant microplastics in influents and effluents were fragments, followed by fibers and granules. However, this study observed an increase in the presence of fibers in sludge samples compared with what was reported by the same authors [58] for influents and effluents.

This behavior could relate to the fact that filamentous particles are composed of polymers of higher density, such as polyethylene terephthalate (PET) [62], which favors their sedimentation and subsequent incorporation into biological sludge during sedimentation processes [32]. Furthermore, due to their particular morphology (characterized by a fine size and elongated structure), MPs can more easily evade conventional filtration systems in WWTPs, increasing their presence in sludge.

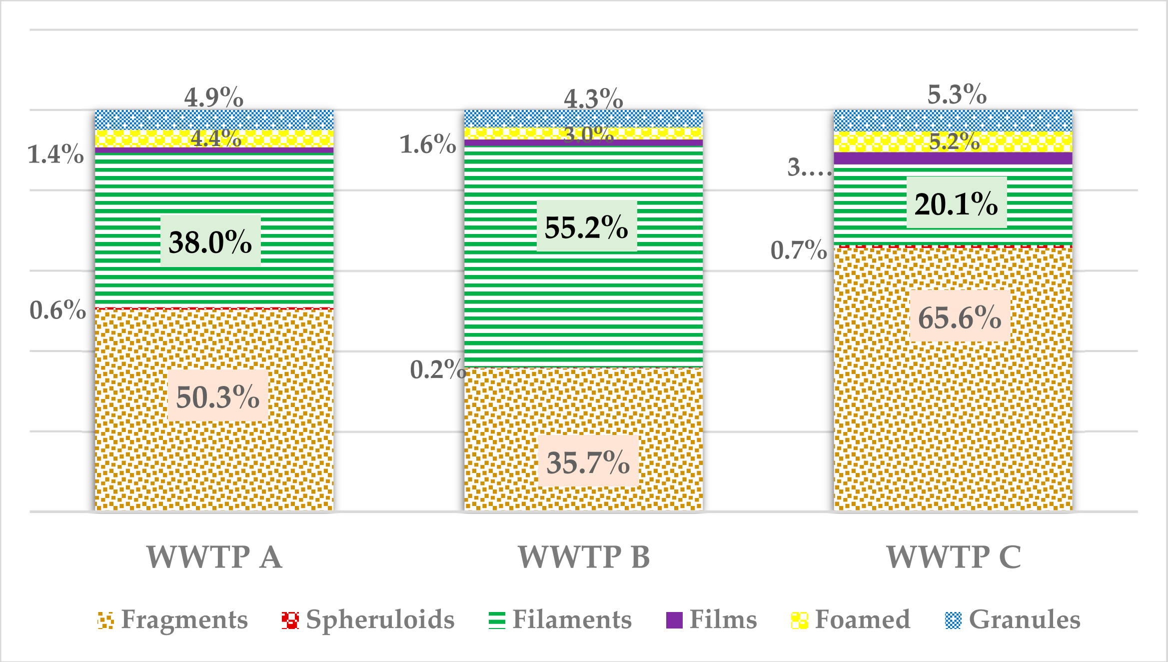


Figure 4. The color of microplastics (MPs) present in (a) WWTP A, (b) WWTP B, and (c) WWTP C. In all three wastewater treatment plants (WWTPs), blue was the most prevalent color among the MP particles identified in biological sludges.

In this article, the detected particle size in the sludge was 300 µm, whereas Flores-Munguía et al. [58] reported sizes of 150 and 38 µm in influents and effluents, respectively. This difference could be due to the progressive fragmentation of microplastics throughout the treatment process, especially in the case of fragments.

In the three WWTPs analyzed, no significant differences were observed in the proportion of the different MP morphologies detected in the biological sludge. According to the one-way ANOVA ($p > 0.05$) (Table 6), these results suggest that regardless of the treatment system, MP morphologies tend to behave similarly regarding their accumulation in biological sludge. The high presence of fragments could be associated with tire degradation [63], the fragmentation of larger plastics, and the use of cosmetic products containing exfoliating particles [69]. Regarding plastic filaments, their presence is mainly associated with laundry residues [32,62,63].

The consistency observed in the morphological distribution of MPs detected in biological sludge over the four months of sampling suggests the existence of a continuous pattern reflecting the nature of the domestic wastewater treated in these WWTPs. This regularity was confirmed through a one-way ANOVA ($p > 0.05$), which indicated no significant differences in the morphological distribution of MPs throughout the study period (Table 7).

Consequently, it can be inferred that regardless of the season, fragments and fibers are predominantly constant components in the microplastic composition of the biological sludge produced by these secondary treatment systems. It is important to note that the analysis of temporal stability in the morphological distribution of MPs in sludge has not been widely addressed in previous studies [26,29,32,34,36,61–63,69,73], representing a relevant opportunity to strengthen the characterization of these emerging contaminants in residual matrices.

The high and constant presence of fragments and fibers in biological sludge constitutes a significant environmental concern, especially considering their potential for reuse in agricultural practices such as soil conditioning or composting [80].

Table 6. ANOVA of MP morphology according to three treatment systems.

Groups	*Count*	*Sum*	*Average*	*Variance*
WWPT A	6	4680	780	1045434.8
WWPT B	6	5823	970.5	1819489.5
WWPT C	6	3868	644.67	931028.7

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	321545.4	2	160772.7	0.13	0.88	3.68
Within Groups	18979764.8	15	1265317.7			
Total	19301310.3	17				

Table 7. ANOVA according to sampling period and analysis time.

Groups	*Count*	*Sum*	*Average*	*Variance*
M1 (June)	7	124.77	17.82	782.49
M2 (July)	7	126.34	18.05	926.39
M3 (September)	7	892.68	127.53	24913.71
M4 (October)	7	685.53	97.93	16115.36

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	65965.06	3	21988.35	2.06	0.13	3.01
Within Groups	256427.65	24	10684.49			
Total	322392.71	27				

These MPs, in their various morphologies, when present in the organic matrix of sludge, may undergo additional fragmentation during handling, storage, or waste transformation processes, leading to even smaller particles like nanoplastics with greater mobility and transport potential in soils [34,36,51]. Furthermore, exposure to environmental factors, leaching processes, and physicochemical reactions inherent in the treatment or disposal of the sludge can facilitate the release of toxic additives and adsorbed pollutants on MPs' surfaces, such as heavy metals, polycyclic aromatic hydrocarbons, and persistent organic compounds [17,79].

This combination of factors not only threatens soil quality and biodiversity but also jeopardizes the principles of sustainability associated with the reuse of sludge as organic amendments [1]. Therefore, the presence of MPs in biological sludge poses a significant challenge for its safe valorization and should be considered in environmental risk assessments before its application in agricultural or urban soils [81–83]. This issue underscores the urgency of establishing regulatory limits and standardized protocols for the characterization of microplastics in residual matrices with potential use as soil conditioners or in agriculture [69].

3.5. Chemical Characterization of MPs

Figure 5 shows the variety of pure polymers that were used as references for the identification of MPs using differential scanning calorimetry (DSC) in biological sludges from the three WWTPs. Among the most common MPs identified in these WWTPs are HDPE, PP, and PET, as they are semicrystalline polymers. PVC, since it is an amorphous polymer, could not be identified due to detection difficulties in DSC. Several studies using DSC have also reported difficulties in identifying PVC, particularly due to the thermal interference of PET. Therefore, for this specific polymer, techniques such as FTIR, μFTIR, or gas chromatography coupled with mass spectrometry (GC/MS) can be used for confirmation [38,64,66–68,70,71,84,85].

For the pure or reference polymers, HDPE showed a melting temperature of 130 °C, while PP and PET showed melting temperatures of 166 and 250 °C, respectively. Majewsky et al. [67] reported similar values for the reference polymers, mainly for PP (164 °C) and PET (250 °C), but not for PE (101 °C). This difference is because the PE used as a reference in this study is of high density. Other studies have reported that HDPE exhibits a higher melting temperature [84].

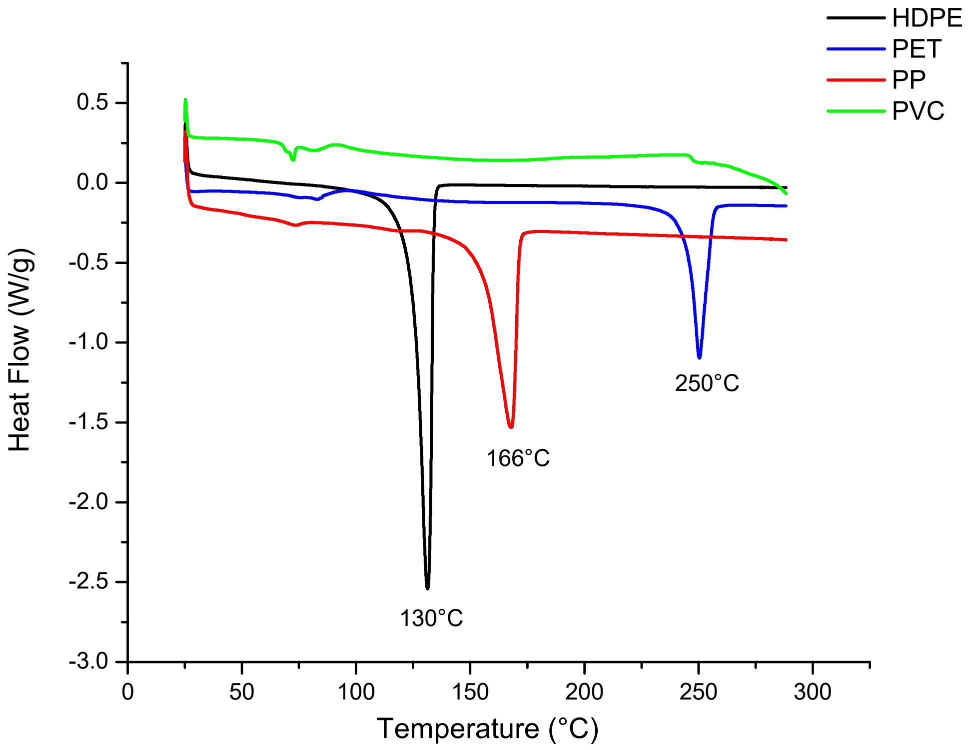


Figure 5. Melting temperature of pure polymers PE, PP, and PET.

Figure 6 shows the DSC thermograms of the sludge samples analyzed from the three wastewater treatment plants. The presence of PE, PP, and PET MPs can be observed, showing no significant differences in melting temperature among polymers of the same type. However, some MPs exhibit differences in the intensity of their melting peak; for example, sample WWTP-B-HDPE shows a less intense peak compared with WWTP-A-HDPE and WWTP-C-HDPE microplastics. These results indicate that the polymer chains of the MPs differ in size [67]. The same behavior is observed for PP and PET MPs.

Furthermore, thermal studies for MP identification determined the melting enthalpy of the reference polymers (HDPE, PP, and PET) and the MPs present in the wastewater treatment plants (Table 8 and Table 9). It can be observed that sample WWTP-C-HDPE showed a higher enthalpy (161.73 J/g) compared with WWTP-A-HDPE (145.20 J/g) and WWTP-B-HDPE (121.22 J/g) (Table 9). These results indicate that the crystalline regions of the MPs are degraded, consistent with the melting temperature of the PE samples. PET MPs showed similar behavior to HDPE. Bitter, Lackner, and collaborators [68,85] reported that PET microplastics in environmental samples can exhibit different degrees of crystallinity, which result in varying melting enthalpy values.

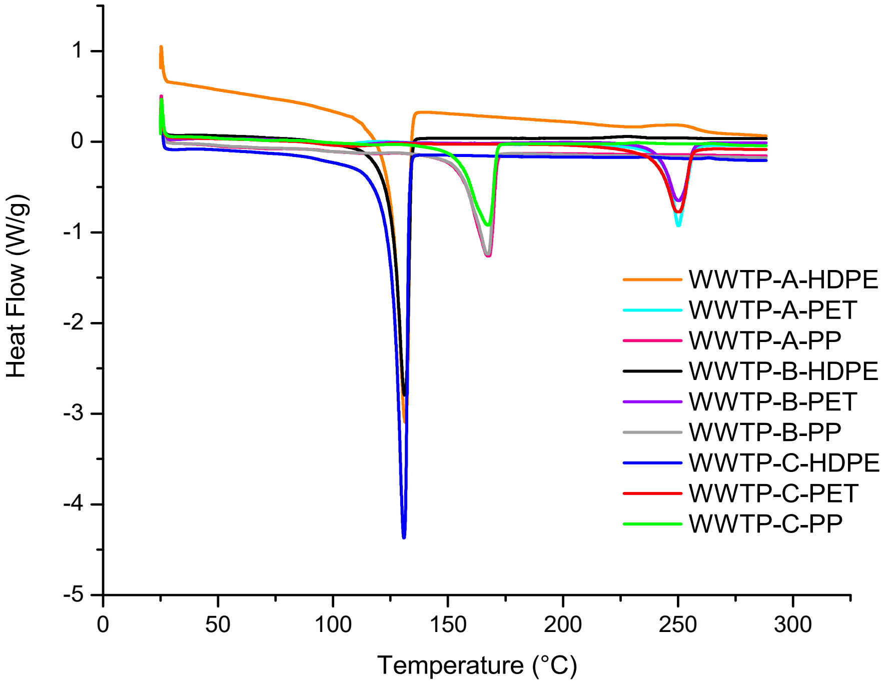


Figure 6. Melting temperature of pure polymers PE, PP, and PET.

In this study, the identification of synthetic polymers in microplastics (MPs) accumulated in biological sludge through DSC coincides with the presence of these emerging contaminants in other matrices where MPs tend to accumulate, such as marine sand [71], seawater [38], WWTP influents and effluents [64,66], and natural waters such as lakes and rivers [70]. It is worth noting that no polystyrene (PS) was detected in this study, unlike in other similar works [64,66,70,71]. This difference may have been because PS commonly occurs in foam form, and in this study, the presence of foams in the three WWTPs was very low compared with other MP morphologies found in greater proportion. Moreover, considering its density range (1.04–1.10 g/cm^3) [86], it is likely that PS entering the treatment plants concentrates in the treated effluents and is consequently released into receiving water bodies.

Table 8. Enthalpy of fusion of different synthetic reference polymers.

Polymers	Enthalpy (J/g)
HDPE	107.91
PET	42.59
PP	79.40
PVC	ND

Table 9. Enthalpy of fusion of synthetic polymers present in sludge from different wastewater treatment plants (WWTPs).

	Samples	Temperature (°C)	Enthalpy (J/g)
WWTP A	HDPE	131.39	145.20
	PET	250.10	45.28
	PP	167.18	75.19
WWTP B	HDPE	131.19	121.22

	PET	250.32	42.06
	PP	167.30	65.98
	HDPE	130.87	161.73
WWTP C	PET	250.0	47.77
	PP	167.45	63.06

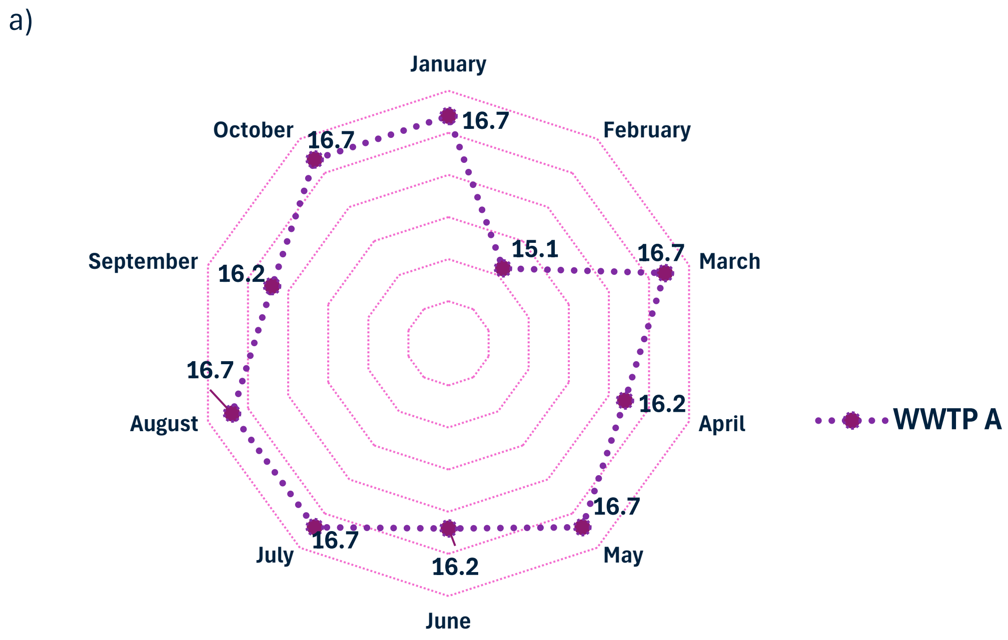
The presence of MPs in agricultural soils has been reported in recent studies, with the most abundant polymers being PE, PP, PET, PVC, and PS [87], suggesting that a possible source of these contaminants could be biological sludges applied as organic or inorganic substrates to improve or use as a soil conditioner.

In this study, the polymers identified coincide with those found in agricultural soils, reinforcing the need to establish control mechanisms and proper management of biological sludge to prevent their contribution to terrestrial ecosystem pollution. Likewise, the use of such sludge for agricultural purposes without adequate regulation could compromise soil quality, highlighting the importance of having updated standards and public policies that define technical criteria and permissible limits for their proper and sustainable management within the context of secondary wastewater treatment systems.

3.6. Monthly and Annual Projections of MPs

The amounts of sludge generated in the three WWTPs analyzed during the period from January to October 2023 varied according to their design operating capacities. In WWTP A, recorded volumes ranged from 15.1 to 16.7 m³/month; in WWTP B, volumes ranged from 252 to 279 m³/month, while in WWTP C, biological sludge production ranged from 866 to 1319 m³/month (Figure 7).

The size and capacity of these plants are consistent with those of other facilities reported in various countries operating with secondary treatment systems based on biological sludge [28,29,69,88–90]. During primary and secondary sedimentation stages, approximately 75% to 99% of MPs present in the influents are retained [44]. In this context, the concentrations of MPs detected in the biological sludge analyzed in this study are consistent with the removal efficiencies previously reported by Flores-Munguía et al. for the same WWTPs [58], suggesting a high degree of consistency between removal data and the accumulation of MPs in the generated by-products.



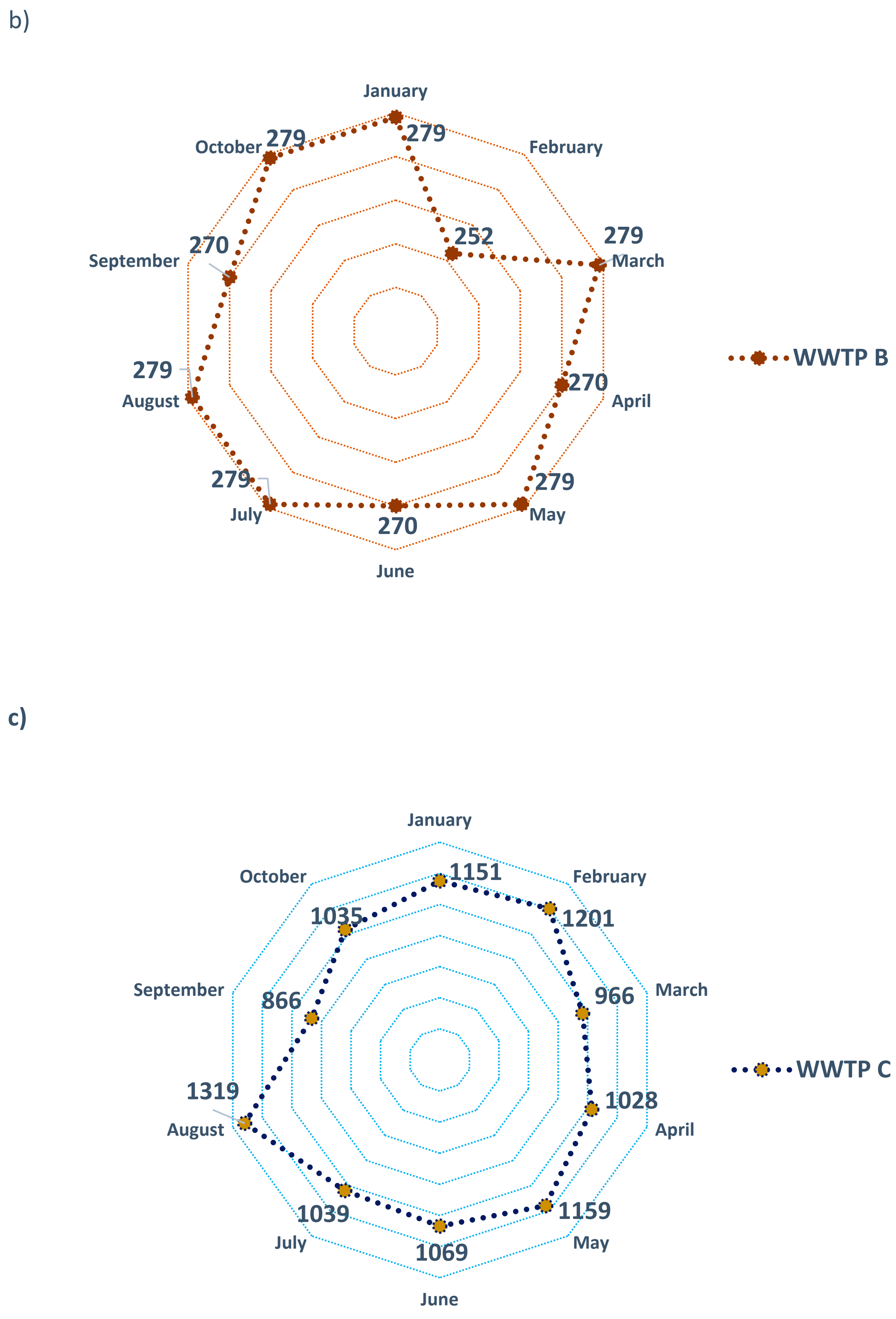


Figure 7. Monthly production of biological sludge (m³) in (a) WWTP A, (b) WWTP B, and (c) WWTP C.

The monthly estimates of generated MPs were calculated based on the values obtained from point sampling and the average monthly sludge volumes produced by the WWTPs. The results indicate ranges from 13.4×10^6 to 123×10^6 particles for WWTP A, 325×10^6 to 2511×10^6 for WWTP B, and 1517×10^6 to 5361×10^6 for WWTP C (Figure 8).

These figures show a notable increase during September and October, which coincides with the rainy season during the study period. This suggests that stormwater runoff may significantly contribute to the MP load entering the WWTPs. Consequently, the increase in MP concentrations in biological sludge appears to be directly related to the higher inflow of these contaminants in the influents, as sedimentation processes act as the main retention pathway.

While these figures provide an approximation of the magnitude of MP accumulation in sludges, it is important to note that the accuracy of these values could be improved through the implementation of composite sampling at different time points of the plants' daily operational cycles. Other studies have also reported monthly projections of MPs in biological sludge in the order of 1.38×10^{10} items [74], 1.37×10^{11} items [41], and even 1.30×10^{13} items [59,62]. Conversely, some studies did not provide monthly projections for these contaminants [61,66,73].

The differences observed between the estimates in this study and those reported in the literature can be mainly attributed to the specific characteristics and operational scales of the WWTPs analyzed. Despite these variations, the present study is consistent with previous research in confirming that the concentration of MPs in biological sludge is considerably high. Furthermore, variability in sampling and analytical criteria across studies contributes to the dispersion of results, highlighting the need to establish standardized protocols to ensure the comparability and robustness of data generated in this field.

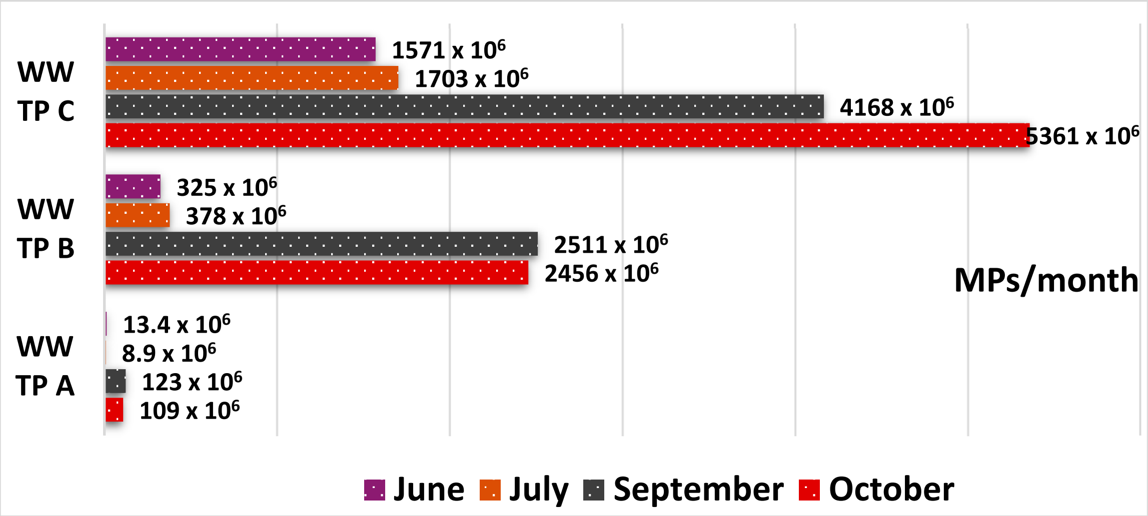


Figure 8. Monthly projections of MPs in biological sludge from the three WWTPs during June and July (dry period) and September and October (rainy period).

Annual projections (Figure 9) demonstrate that biological sludge accumulates high concentrations of MPs of various shapes and compositions. Like the findings of this study, other studies have reported annual estimates of MPs in biological sludge ranging from 3×10^5 items [91], 5.67×10^9 items [51], and 1.76×10^{1}◎items [41] up to 1.56×10^{14} items in WWTPs in China [59,62].

These projections indicate that biological sludge could represent an important source of MP pollution in agricultural soils [87]. The presence of MPs in these soils could seriously affect environmental sustainability, as synthetic polymers compromise soil integrity, biodiversity, and food security in the medium and long terms [84,87]. Therefore, given the current MP concentrations in biological sludger, they can hardly be considered suitable for agricultural reuse or for ensuring the sustainable management of wastewater treatment systems.

It is worth noting that this study considered MPs with sizes of around 300 µm, meaning that the presented projections correspond exclusively to this particle size range. Consequently, the total amount of MPs in biological sludge is likely to be significantly higher when particles of other sizes are included. Future research should therefore focus on providing a comprehensive and accurate assessment of the magnitude and implications of this emerging contaminant.

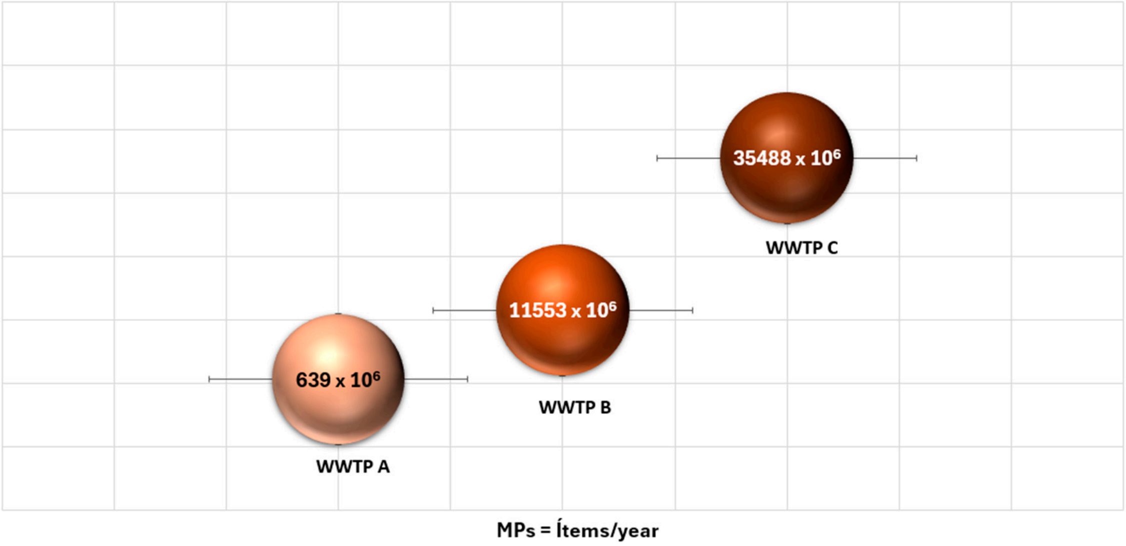


Figure 9. Annual projections of MP concentrations in biological sludge generated in the three WWTPs in relation to their generation capacities.

4. Conclusions

This study revealed significantly high concentrations of MPs in biological sludge from wastewater treatment plants, posing critical challenges for their use, sustainable agricultural reuse, and final disposal as solid waste. The high accumulation of MPs in sludge represents a serious risk to soil quality, crop safety, and terrestrial ecosystem conservation, thereby limiting their potential for sustainable reuse in agro-environmental contexts.

It is therefore essential to update regulatory protocols and technological criteria for implementing mitigation strategies aimed at reducing MP concentrations and their release into the environment. In the Mexican context, it is necessary to comprehensively revise NOM-004-SEMARNAT-2002, incorporating microplastics as emerging indicators of sludge and solid waste quality. Updating this regulation will ensure that sludge management and reuse align with sustainability principles and contribute to the conservation of terrestrial ecosystems.

Finally, it is crucial to continue strengthening research efforts to generate reliable scientific data, enabling the development of standardized protocols for MP measurement and projection in biological sludge. Such advancements will support sustainable practices, informed decision-making, and the formulation and implementation of public policies oriented toward environmental sustainability and the preservation of environmental quality.

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