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Occurrence and characterization of microplastics in the surface water of the Tinajillas-Río Gualaceño protected area in Ecuador

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Abstract

In recent years, plastic waste has increased exponentially. This waste enters the environment and fragments into microplastics (MPs), tiny particles < 5 mm, which are now ubiquitous in global ecosystems, particularly in aquatic environments such as rivers. In this research, we studied, for the first time, the presence, concentration, and characteristics of these pollutants in an Andean-Amazonian micro-basin within the Tinajillas-Río Gualaceño Protected Area in Ecuador. Composite samples of surface water were collected to assess the abundance, morphology, size, and polymer composition of the microplastics. An average of 25 MPs/250 mL was found, with the highest concentration recorded at the upstream site of the micro-basin (33 ± 2.10 MPs/250 mL). Fibers were the most frequent morphotype in the samples, with sizes ranging from 3.23 to 127.28 µm. The predominant polymers were PET, PP, and polyamide, materials linked to the manufacture of plastic containers, parts, and pipes, and potentially associated with local anthropogenic activities, including tourism and mining-associated activities. The lack of environmental regulations in Ecuador regarding these pollutants hinders their characterization, especially in vulnerable areas such as protected natural areas. This study provides baseline data that helps to understand the distribution of these pollutants in natural areas and elucidate the complex, poorly documented relationship between mining and microplastics, contributing to future lines of research and the strengthening of policies to combat illegal mining, one of the greatest environmental and transnational threats to developing countries. Ultimately, addressing the problem requires an approach that involves all stakeholders.

Introduction

The demand for plastic has increased exponentially in recent years (Plastics Europe, 2025), exacerbated by the use of synthetic products resulting from the COVID-19 pandemic (Fadare and Okoffo, 2020), which has led to the production of approximately 359 million tons of virgin plastic per year (Nayanathara Thathsarani Pilapitiya and Ratnayake, 2024), causing the emergence of a pollutant that poses a serious concern for the environment. These plastic materials usually degrade through physical or chemical weathering processes until they become reduced-size particles called microplastics, a term first coined in 2004 (Thompson et al., 2004), two decades ago. Microplastics (MPs) are imperceptible particles less than 5 mm in size, insoluble in water, non-degradable, and generated by the degradation of plastic. They are divided into primary and secondary microplastics based on their formation. While secondary microplastics are produced when large plastics break down into small detritus, primary microplastics are plastics designed to be microscopic in size (Lin et al., 2018). Today, these emerging contaminants, named as such due to their recent and growing detection, are present in all their forms across the planet's ecosystems (Kutralam-Muniasamy et al., 2023), especially in aquatic systems such as rivers, owing to their small size and low density (Lwanga et al., 2022; Chandra and Walsh, 2024).

Rivers are a necessary resource for the subsistence of terrestrial and aquatic life. They are vital freshwater sources that supply human communities, agriculture and wildlife. Furthermore, they act as natural corridors connecting diverse ecosystems, enabling species migration and sustaining other fragile

ecosystems such as forests and wetlands. Their importance extends beyond the ecological, as they possess great aesthetic, social-community, economic and even cultural value (Newson et al., 2024). However, their contamination, primarily by plastic waste, has generated significant environmental concern, stemming from the fact that plastics fragment and decompose until they become these MPs (Karak et al., 2025). Meanwhile, their microscopic size results in a wide variety of marine species, such as fish, absorbing them (Tanaka and Takada, 2016; Nelms et al., 2018),, entailing potential toxicological risks, including for higher trophic levels (Yeo et al., 2020); this is concerning, given that the estimated load of microplastics in the sea amounts to 448,528.84 m^3 (Zambrano-Pinto et al., 2024). Likewise, MPs are not only a ubiquitous and invisible residue but also vectors for other equally persistent contaminants (Zambrano-Pinto et al., 2024), including forever chemicals (PFAS) (Marquínez-Marquínez et al., 2023), pharmaceuticals and personal care products (Wu et al., 2016; Razanajatovo et al., 2018), antimicrobials and resistance genes (Xu et al., 2022; Zhuang and Wang, 2023), which intrinsically puts human, animal, and environmental health at risk due to constant exposure/ingestion. For this reason, the WHO (2019) considers it pertinent to study the importance of MPs due to the scarcity of data on their potential effects on health and the environment, in addition to their ease of transport to other bioregions, given their vector nature (Montecinos et al., 2022). For example, MPs have been found in the Mariana Trench at a depth of 11,000 meters (Zhao et al., 2025).

Given the concern about MP pollution in the environment in recent years, research on their abundance, occurrence, and characterization has intensified on a global scale (Hale et al., 2020; Thompson et al., 2024; Dessauvages et al., 2026), However, scientific production in Latin America, and particularly in Ecuador, the site of this study, remains notably scarce, compounded by the fact that less than 10% of plastics are recycled in the country (Jones et al., 2021). Nevertheless, evidence shows that plastic pollution and, consequently, its fragmentation into MPs, is a silent but serious danger to ecosystems, with widespread contamination found in different locations of the region, such as Andean slopes (Cabrera et al., 2022), high-altitude (Ramos et al., 2025), the Amazon region (dos Santos Silva et al., 2024) and the Galapagos Islands (Alfaro-Núñez et al., 2021; Moreira-Mendieta et al., 2023), which has been the main focus of attention. Rivers are the main indicators of microplastic pollution globally, although more than 80% of MPs in oceans come from rivers, most research focuses on oceanic dynamics, leaving limited information on riverine sources (Meijer et al., 2021). Considering that in Ecuador most drinking water comes from surface sources such as rivers and reservoirs (Vélez-Terreros et al., 2024), it is an area that also deserves attention, given that most cities do not have Wastewater Treatment Plants (WWTP) nor separate sewage systems for wastewater and rainwater, which further complicates local water treatment (Vinueza et al., 2021). This is in addition to the pressure that factors such as domestic activities, agriculture, fishing, and other terrestrial and atmospheric wastes can exert (Li et al., 2020) as well as the impact of other activities like gold mining (Arellano-Yasaca et al., 2024), all of which will ultimately affect vulnerable species and weaken the ecosystem services that sustain the local and regional economy and human well-being.

In particular, one of the river systems with notable ecological and socioeconomic relevance in Ecuador is the Gualaceño River, which is located on the Amazonian slope, on the outer flank of the Eastern

Cordillera, specifically within the Santiago River system. This area covers a surface of approximately 33,080 hectares and is located within the area declared by Official Registry on June 14, 2002, as a Protective Forest and Vegetation named "Tinajillas-Río Gualaceño" (Ministerio del Ambiente del Ecuador, 2002).The area is of special interest for the conservation of a large part of Ecuadorian flora and fauna. It not only presents a high abundance of species but also maintains high rates of endemism, as most of the diversity of plants, amphibians, and reptiles is related to the Amazonian fauna or the eastern foothills of the Andes. This highlights species of high endemic value such as the lemon jambato frog (*Atelopus aff. spumarius*), species that can act as bioindicators of ecosystem health (Ortega-Andrade et al., 2021). However, they are severely threatened by local anthropogenic pressure and, therefore, by their subsequent derivation into emerging contaminants. Likewise, the area has natural forest above 3,000 m.a.s.l., constituting an enormous water potential that provides this resource to the Gualaceño River and several tributaries for drinking water supply in various communities, including the town of Indanza (Morona Santiago) and surrounding areas. Nevertheless, the pressure exerted by gold mining in the area constitutes a factor of severe predation (Gobernación del Ecuador, 2023). A massive impact is caused by the use of dredges that extract gold, removing huge quantities of material from the riverbanks and riverbeds. These dredges operate by suctioning sediments, which causes various effects on microplastics, such as mechanical resuspension, since their behavior depends on the density of the plastic polymer. This process also involves the extraction and displacement of these pollutants, even outside the original aquatic ecosystem.(Montoya et al., 2025), which, consequently, affects their accumulation in the water column. Hence the importance of this study, given the scarcity of research, as there are no methodical and systematic water studies in this basin. Furthermore, most water analyses in mining areas focus on metals, completely ignoring the measurement of plastic polymers, even though illegal mining is a major source of MPs in the environment.(Faseyi et al., 2022; Khant et al., 2024).

In accordance with the above, and with the aim of raising awareness in the future about the problems caused by the indiscriminate use of plastics and anthropogenic activities, and in line with the WHO's call for surveillance, this study aims for the first time, to evaluate the presence and characterization of microplastics in the Gualaceño River micro-basin, a protected natural environment. The abundance, shape, color, size, and type of plastic polymer (chemical composition) were determined in order to draw inferences about water quality, with a view to promoting monitoring systems with clearer, evidence-based instruments, as well as elucidating the complex interaction between mining-MPs, a poorly documented relationship.

Methods

Study area and sample collection

The study focused on the water resources of the Andean-Amazonian micro-basin within the Tinajillas-Río Gualaceño protected area in Ecuador, located in the Limón Canton, between the provinces of Morona Santiago and Azuay. The research covered an area of 1 400 hectares, from La Virgen - Gualaceo to the Interpretation Center of the Tinajillas Municipal Ecological Conservation Area, as shown in Fig. 1.

Sampling permits were reviewed and granted by the administration of the Tinajillas-Río Gualaceño Municipal Ecological Conservation Area. The environmental and biophysical characteristics of the area are shown in Table S1.To define the sampling points, location and monitoring frequency, the Ecuadorian Technical Standard on sampling NTE INEN 2226:2000 and the guidelines of Li et al. (2020), were used as references. Due to the complex Andean-Amazonian topography, the limited accessibility of the terrain and the security conditions associated with the presence of illegal and uncontrolled mining operations in the area, two strategic points were selected for this study: the upstream point (P1) as an anthropic reference point and the downstream point (P6) as the mouth of the microbasin. The location was recorded in UTM format (WGS84, Zone 17S) using ArcGIS: P1 (762451 E; 9667311 N) and P6 (768126 E; 9666274 N). Sampling was conducted during the months of April and May 2023 and samples were collected in amber glass bottles with cork stoppers, based on the recommendations of Prata et al. (2019). The technique employed consisted of collecting three water samples (1 L each) every 10 minutes, which were then homogenized to obtain a composite sample. Once the composite samples with a volumetric capacity of 1000 mL were obtained, the container was sealed, ensuring a rapid and airtight process to prevent cross-contamination. Subsequently, it was labeled according to its coding (sampling point - date and time of sampling - sample number per day). The same process was carried out for all samples every 15 days in triplicate.

Figure 1 Location map of the study area in Ecuador. Sampled sites: upstream site (P1) and downstream site (P6). Red dots indicate the sampling sites, the blue line represents the watercourse and the orange line delineates the micro-basin.

Sample treatment

Once the samples were transported to the laboratory, each 1 L sample was sieved using a steel mesh sieve (90 µm) to remove large organic matter, and with the help of a graduated cylinder, 250 mL were placed into their respective beakers, taking into consideration the coding of each replicate. After the sieving process, 25 mL of hydrogen peroxide 30% (w/v) was added to each beaker. The beakers were covered with aluminum foil and left to stand for 72 hours at room temperature in order to remove any organic matter content from the water samples, following protocols from other studies (Büngener et al., 2024). Processing was carried out in a laboratory with optimal cleanliness conditions and under a fume hood. All samples were analyzed in triplicate to ensure consistency. The entire laboratory protocol is illustrated in Fig. S1.

Microfiltration and staining

After this time, microplastics were extracted using vacuum filtration equipment, and all samples were filtered using cellulose nitrate membrane filters (47 mm diameter / 0.45 µm pore / sterile) PK/100. At the end of the vacuum microfiltration phase, the filters were placed in Petri dishes, covered with aluminum foil, and left to dry at room temperature for a period of 24 hours. Finally, the nitrocellulose filters were

sprayed with a Nile Red solution of 0.01 mg mL^{-1} (Sigma-Aldrich), which stains plastic microparticles (Prata et al., 2020), and then they were placed in an oven for 1 hour at 60°C.

Microplastic characterization

MPs were characterized using an Eclipse Ti2-E inverted fluorescence microscope equipped with 4x and 10x lenses, following the protocol recommended by Hidalgo-Ruz et al. (2012). The microscope allowed detailed visualization of the morphology of plastic particles, using fluorescence with an excitation wavelength of 350–359 nm and an emission wavelength of 461–463 nm (Fig.S2). Image analysis was performed with NIS-Elements AR software version 4.2 (https://www.microscope.healthcare.nikon.com/es_EU/applications/life-sciences/live-cell-imaging), providing data on particle size (length, width and diameter), shape (fibers or fragments), and quantity (concentration), which were exported in .xlsx format for further analysis.

Polymer identification

Polymer types were identified using a Nicolet™ iS™ 10 FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) accessory, utilizing the HR library of plastic polymers, that includes more than 12 000 polymer spectra. The spectroscopic data obtained in. SPA format, were processed using OMNIC™ Specta Software, allowing for the precise identification of the particle's composition according to its IR spectrum. A match of at least 70% was considered sufficient for confirming the presence of MPs in the samples (Tan et al., 2023). Finally, each MP particle was photographed beforehand and its spectra were preserved.

Prevention and contamination control

In order to prevent contamination, all materials, as well as the workspace, surfaces, and equipment, were previously cleaned with 70% v/v ethanol and ultrapure water (Milli-Q® IQ 7000), avoiding the use of plastic materials and prioritizing glass and metal materials. Throughout the experiment, pure cotton lab coats and microtextured powder-free disposable latex gloves were worn at all times; furthermore, the use of synthetic clothing was limited. The counting process was carried out by the same expert operator, always counting the samples three times in order to minimize human error. Samples were exposed for the shortest possible time throughout the process and by using the fume hood, following the guidelines of Prata et al. (2019). In addition, blank samples were used to identify plastic contamination from the laboratory environment, finding inconsequential concentrations of 0.01 MPs/L (mostly fibers), which do not significantly impact the analysis of the results.

Statistical analysis

A non-parametric Mann-Whitney U test was used, as the data did not meet the assumptions of normality, to evaluate the differences in MPs concentrations between the sampling points with a significance level

of 0.05. The data distribution was visualized using a box-and-whisker plot, which allows for observing the median and variability within each group. The abundance found at each sampling site was expressed as the number of plastic particles per 250 mL of water (MPs/250 mL), and the total abundance was expressed as the mean between the sampling sites. Finally, all statistical analyses were performed using GraphPad Prism 8.0, which was also used to generate the figures.

Results

100% of the samples exhibited the presence of plastic microparticles, and thus we decided to analyze several parameters of the particles found in each of the samples. The full characterization is presented herein:

Determination of abundance in water

The abundance of MPs varied significantly between the analyzed sampling points (Mann-Whitney U test, $p < 0.001$). The upstream site (P1) showed a mean abundance of 33 ± 2.10 MPs/250 mL, markedly higher than that recorded at the river downstream (P6), where 17 ± 1.55 MPs/250 mL were observed (Fig. 2a). This represents a decrease of 48.5%. Likewise, the total mean abundance was 25 MPs/250 mL of surface water, which is equivalent to 100 MPs/L.

Determination of particle size

Regarding the size distribution (Fig. 2c), the particles were classified into three ranges: < 50 µm, 50–150 µm, and > 150 µm, with variations observed between the sampled points. At the upstream site (P1), the smallest particles < 50 µm predominated (45.5%). Meanwhile, at the downstream (P6), the intermediate size range of 50–150 µm was the most prevalent (55.0%), and finally, the fraction of microplastics > 150 µm remained relatively similar at both points (25.0%). In addition, the mean particle sizes ranged between 3.53 and 127.28 µm for fibers and between 10.37 and 425.78 µm for fragments.

Determination of shape

Regarding morphology, two main shapes were identified: fibers (53.0%) and fragments (47.0%), with fibers being the most predominant type of plastic shape at the upstream of the micro-basin and fragments the most common shape at the downstream (Fig. 2b). The fibers were fine, elongated, and thin, similar to threads (Fig. 3a, b); therefore, they had a higher surface-to-volume ratio, whereas the fragments were more irregular, angular, or flat pieces (Fig. 3c, d).

Characterization of the microplastic type

Polymer composition analysis identified several types of plastics. Polyethylene terephthalate (PET) was the most frequent synthetic polymer, present in 70% or more of the samples in both P1 and P6 (Fig. S3), spectroscopically characterized by its intense carbonyl (C = O) stretching band between 1715 and 1725 cm^{-1} (Fig. 4).

Figure 2 Microplastics found in the Gualaceño River micro-basin. Surface water samples: abundance (a), shape (b), size (c) and polymer distribution (d).

Figure 3 Representative fluorescence microscopy images of microplastics detected in surface-water samples from the Tinajillas Río Gualaceño micro-basin: fragments (a, b) and fibers (c, d). Scale bars = 1 mm.

Figure 4 Representative ATR-FTIR spectrum of PET identified in the analyzed samples.

Discussion

Identification and characterization of MPs

This study, to the best of our knowledge, is the first evaluation of the occurrence and characterization of MPs in a micro-basin of the Tinajillas-Río Gualaceño natural area, which has had official protection records for more than two decades. This demonstrates widespread contamination in natural areas intended to preserve the water resources generated there. The total concentration was 25 MPs/250 mL, equivalent to 100 MPs/L.

The concentration observed in the Tinajillas–Río Gualaceño micro-basin should be interpreted within the broad variability reported for freshwater environments (Table 1). Available studies span several orders of magnitude, from values below 0.1 MPs L^{-1} in some river systems to concentrations exceeding 200 MPs L^{-1} in mining-impacted and highly anthropized watersheds. The present value of 100 MPs L^{-1} is therefore comparable to the upper range reported for several freshwater environments, including protected and mining-affected systems. Nevertheless, direct quantitative comparisons require caution because reported concentrations are strongly influenced by differences in sampling volume, particle-size thresholds, sample processing, and analytical identification procedures (Li et al., 2020; Uddin et al., 2020). Importantly, the predominance of fibers and PET in the present study is also consistent with patterns reported across several Andean, Amazonian, and other freshwater systems (Table 1), suggesting that these characteristics are not uniquely diagnostic of a particular source. Thus, although mining-associated activities constitute a plausible local input and may also promote sediment resuspension, the current evidence does not allow the observed MPs to be attributed specifically to mining.

Location	Water body type	MP Concentration (MPs L^{-1})	Dominant morphology	Dominant polymer(s)	References
Gualaceño River, Ecuador	Protected micro-basin (Andean-Amazonian)	100	Fiber (53%)	PET (73.4%)	This study
Huaibei, China	River (coal mining)	60–405 (avg. 216.7)	N/R	N/R	(Ling et al., 2026)
Cañete River, Lima, Peru	River	0.055–0.170[1]	Fiber (61.72%)	N/R	(Jacay et al., 2026)
Hengshi and Wengjiang rivers, South China	River (metal mining)	0.2–6.2	N/R	N/R	(Cai et al., 2025)
Taff Bargoed, Wales, UK	River (rural, mining)	0.0003–0.029 (avg. 0.015) [1]	Fiber	Acrylic/polyester	(Lofty et al., 2025)
High-altitude Andean Lakes, Ecuador	Lake	N/R	N/R	PET/PP	(Ramos et al., 2025)
Sierra Gorda Biosphere Reserve, Mexico	River (protected area)	≤ 110.87	Fiber	PET/PP/HDPE/PA	(Granados-Sánchez et al., 2024)
Lacramarca River, Ancash, Peru	River	280.7[1]	Fragment (87.5%)	N/R	(Melgarejo-Velásquez and Reyes-Avalos, 2024)
Choqueyapu River, La Paz, Bolivia	River	> 44	Fibers	PET	(Maupas et al., 2024)
Amazon Basin	Rivers	0.005–74.5[1]	Fiber and fragment	PA/PET/PP	(dos Santos Silva et al., 2024)
Guapimirim, Macacu, Maracanã rivers, Brazil	River	0.004–51.17 (mean 3.65)	Fiber (63.3%)	PE (22%)	(Drabinski et al., 2023)
Yangtze River, Chongqing, China	River	N/R	Fiber	N/R	(Fan et al., 2021)
Chibunga River,	River (urban)	6.4–12.0[2]	N/R	SBR/BR/CR	(Paredes et al., 2021)

Location	Water body type	MP Concentration (MPs L^{-1})	Dominant morphology	Dominant polymer(s)	References
Riobamba, Ecuador					
Guayllabamba River, Ecuador	River (tropical Andean)	N/R	Fiber	N/R	(Donoso and Rios-Touma, 2020)

[1]Converted from MPs/m³ (÷ 1000); [2]Converted from MPs/250 mL (×4); **Abbreviations**: MP, microplastic; PET, polyethylene terephthalate; PP, polypropylene; PE, polyethylene; PA, polyamide; HDPE, high-density polyethylene; SBR, styrene-butadiene rubber; BR, butadiene rubber; CR, chloroprene rubber; N/R, not reported.

The highest concentration was recorded at the upstream point (P1) (33 ± 2.10 MPs/250 mL) compared to the downstream point (P6), which may reflect differences in proximity to anthropogenic inputs and/or downstream transport and retention processes (Li et al., 2020). For example, the high-density plastic polymers identified in this study, such as PET, also tend to deposit in river sediments, and fiber shapes are susceptible to accumulating in riparian vegetation (Fan et al., 2021; Bošković et al., 2022). However, dredging of the riverbed can cause resuspension of these contaminants, which could increase their bioavailability/abundance in the water (Ling et al., 2026).

In this research, several factors can explain the distribution and occurrence of MPs: Unlike other natural parks in Ecuador (such as Cajas or Cotopaxi National Park) that have main ranger stations and interpretation centers with automated daily visitor logs, the Tinajillas-Río Gualaceño area has scattered and open access points. This suggests that uncontrolled local anthropogenic activities, such as tourism and the expansion of the agricultural frontier, can be considered a likely source of contamination, could be considered a likely source of contamination and contribute to higher concentrations of MPs (Ossa-Yepes et al., 2026). Indeed, this fragmented access facilitates the establishment of mining operations without environmental regulations or controls, which are affected by the actions of Organized Crime Groups (OCGs), a problem that is becoming increasingly prevalent in Ecuador's natural areas, with more than 796 reports of these illegal activities recorded within the National System of Protected Areas (Asamblea Nacional del Ecuador, 2025), including the Tinajillas-Río Gualaceño protected area, the focus of this research, where camps engaged in illegal gold mining have been found, along with heavy machinery and dredges operating on the riverbeds (Gobernación del Ecuador, 2023). The organization Amazon Underworld (2023), in its report "In the World's Largest Rainforest," identifies illegal gold mining as a key pillar of criminal activity in the region. Specifically, in the Ecuadorian Amazon, these activities have multiplied, consequently generating large volumes of plastic waste (Khant et al., 2024), including the intensive use of plastic hoses and pipes, the use of tarps, worn-out tires and heavy machinery parts, and bottles, among other items, which quickly break down into small fragments due to the conditions of the activity.

Furthermore, the dredges operating in riverbeds exert a great impact on ecosystems, both in the short and long term, because they seriously pollute the water by removing huge quantities of sediments; they severely alter the riverbed and riverbanks (including the biota and riparian vegetation) (Arellano-Yasaca et al., 2024). For instance, one of the most concerning harmful effects that mining extraction can cause on water resources is acid drainage, as it has the potential to produce devastation (Mestanza-Ramón et al., 2023). Recent studies have identified mining operations as a critical source of MP pollution in remote rivers (N. Khant et al., 2024; Ling et al., 2026) and in particular, high bioavailable concentrations of heavy metals such as Cd, Hg, and Pb have been found in Amazonian rivers (Montoya et al., 2025), the region of this study.

In light of this, the relationship between illegal mining and microplastics, which has been very little studied, represents one of the most destructive and least visible combined environmental crises for river ecosystems. Although they appear to be independent problems, MPs enhance the toxicity of heavy metals in the water, derived from mining activity, by acting as vectors for these persistent contaminants due to their surface-to-volume ratio and hydrophobicity. For example, polymers such as polypropylene (PP) and polyethylene (PE) are capable of trapping heavy metals in high concentrations on their surface (N. Khant et al., 2024) and it has been found that mining river sediments accumulate large amounts of fibers and fragments that change the density and stability of the riverbed (Cai et al., 2025). Consequently, the MPs deposited there accidentally become food for aquatic fauna (Santonicola et al., 2024), which are subsequently consumed by higher organisms in the food web and riverine human communities that depend on fishing. The detection of MPs in human feces provides evidence of human exposure and ingestion, although it does not identify the specific exposure pathway (Refosco et al., 2025).

Another evaluated parameter was size distribution, which was categorized into three classes: < 50 µm, 50–150 µm, and > 150 µm. At the Upstream(P1), particles < 50 µm (45.5%) were the most common. At the downstream site (P6), the intermediate size range of 50–150 µm was the most common with 55.0%, while the > 150 µm fraction remained relatively consistent at both points with 25.0%, observing a trend similar to Cai et al. (2025), who found MPs < 100 µm in several rivers in China. On the other hand, Malyuskin (2020) mentions that using microwave spectroscopy and optical microscopy, MPs were found with a length varying from 50 µm to 5 mm, and finally, in other natural environments, the size range was between 50 and 150 µm (Vélez-Terreros et al., 2024), as large particles can fragment into smaller particles. However, it must be acknowledged that size usually varies significantly depending on its origin and that in natural conditions, plastics can accelerate the fragmentation of much smaller particles (Zambrano-Pinto et al., 2024).

Fiber was the most common shape recorded in this study (53.0%), followed by fragments (47.0%). A pattern consistent with that reported in other rivers in Ecuador (Villegas et al., 2021) and other environments with similar pollution. For example, in tropical Andean rivers such as the Guayllabamba (Esmeraldas, Ecuador), fibers were the predominant shape, originating from synthetic textiles. For its part, in the Magdalena River (Neiva, Colombia), a high prevalence of this plastic shape was found along the river, as products of wastewater discharges (Donoso and Rios-Touma, 2020). In fact, fibers (the

predominant particles in this research) possess low density, which facilitates their mobility over long distances (Rebelein et al., 2021). Furthermore, once settled, they can accumulate in the aquatic ecosystem and become incorporated throughout the trophic web.

Additionally, in this research, the highest incidence of color was blue > red > black when observed under brightfield light, and considering that these are fluorescent under a green fluorescence field, the presence of MPs is confirmed. A study analyzing the color distribution of MPs in rivers and lakes worldwide mentions that blue MPs (47.1%) are the most frequent color pattern in South American rivers, including the Amazon basin. In addition, blue and black MPs collectively represent more than 60% in lakes in North and South America (Jiang et al., 2025), originating from activities such as industrial inputs, agricultural runoff, and anthropogenic sources. Bright pigments such as red and blue absorb different solar energy, which accelerates the photoaging and fragmentation of the plastic into smaller particles (Zhao et al., 2022), and which could attract the attention of aquatic species and be confused with food sources (Du et al., 2025), potentially affecting those that consume them.

Regarding chemical identification by FTIR, polyethylene terephthalate (PET, 73.40%) and polypropylene (PP, 24.09%) were the most common polymer types in this study. These plastic materials have a high annual demand, and several studies have already reported their high prevalence in aquatic environments worldwide (Sánchez-Paz et al., 2025). PET is a plastic component used in the production of containers such as water, soft drink, and oil bottles, food trays, egg cartons, jars for cosmetic and hygiene products, and durable clothing and carpet textiles (through recycling), and it is even used as an engineering plastic in combination with fiberglass (FRP) (Muringayil Joseph et al., 2024). Meanwhile, PP is a heat-resistant material that does not let moisture and grease pass through, which is why it is widely used in the manufacture of yogurt and butter containers, as well as for straws, bottle caps, sacks, awnings, textiles, hoses, and equipment parts (Hossain et al., 2024). According to Ramos et al. (2025), these two types of polymers are sensitive to breakage under environmental conditions, which facilitates their mobility and accumulation in water systems such as rivers Finally, polymers such as nylon (polyamide, 0.7%) and polytetrafluoroethylene (PTFE, 0.6%) were found; these are high-performance technical plastics, also reported in other rivers in Ecuador (Vélez-Terreros et al., 2024), because they are very frequently used in fishing nets, textile fibers, and the manufacturing industry (Mejías et al., 2023). Lastly, 1.26% represented other polymers such as polyethylene (PE), polyester (PES), polystyrene (PS), high-density polyethylene (HDPE), and polyvinyl chloride (PVC), which are common synthetic materials in the plastics world, due to their low cost and characteristics of easy use and resistance, in addition to being frequently reported in freshwater systems (Hale et al., 2020; Bexeitova et al., 2024).

Although the number of sampling points was limited by the complex topography of the area and the serious safety risks associated with uncontrolled mining activities, this study provides a valuable baseline on the potential effect of these anthropogenic stressors on Andean-Amazonian protected ecosystems, which are of high interest due to their high levels of biodiversity and the generation of water resources for human supply (Koo et al., 2024).With all the aforementioned, the diverse characteristics of MPs (size, shape, color) reflect different origins and distinct properties, which alter the rate at which they

accumulate, fragment, and disperse in the aquatic environment (Hidalgo-Ruz et al., 2012; Hu et al., 2022).

Exploring sustainable strategies to mitigate microplastic pollution

Plastic pollution has generated deep environmental concern, including the plastisphere (Li et al., 2021), due to its widespread reach that also disrupts human and animal health, associated with their constant ingestion/exposure. Addressing the pollution problem requires a comprehensive and multidisciplinary approach, as it is known that multiple sectors contribute to its contamination and that the pace of the production chain globally has far exceeded the responsible way to manage them. The Zero Draft that exists on the global treaty against plastics, signed in 2022 by 175 countries and proposed by the UN, is consolidated as one of the first key conventions that addresses the full life cycle of plastics, highlighting the goal of changing the form of production and consumption through actions such as banning or gradually eliminating the use of single-use plastics, monitoring hazardous substances (plasticizers) that must be eliminated and/or reduced, requiring products to comply with circular design (promoting recycling), and improving the handling and infrastructure for plastic waste management (Zhao and You, 2025). Furthermore, it proposes financing to help developing countries with economic and technological items. However, these guidelines must be properly structured in order to avoid contamination by the substitution of inappropriate materials. Also, for example, in several countries, more than 100 signatory countries limit the use of microbeads (plastic spheres < 5 mm used in cosmetics) through strict regulations or mandate the installation of microfiber filters in washing machines. In addition, several countries promote the use of biodegradable plastics that decompose naturally, and although it is a promising material, there are increasing reports of the danger of the release of oligomer compounds, which are highly persistent and whose effects are still poorly understood (Wang et al., 2023; Zhao et al., 2025). On the other hand, Extended Producer Responsibility (EPR) obliges companies that manufacture or import plastics to bear the associated costs of the full life cycle of the products, with special attention to when they become waste. Also, financing sources and incentives, public-private sector investments, and tax rates on the production of single-use plastic have been used, which complement control and regulatory measures, and which ultimately favor the adoption of greener and alternative practices.

In Latin America, and specifically in Ecuador, there is no specific law for microplastics or nanoplastics (Orona-Návar et al., 2022). However, pollution by these contaminants is addressed indirectly through regulations governing the plastic cycle and its waste, such as the National Plan for the Reduction of Plastic Waste (PNRRP) and the Organic Law for the Rationalization, Reuse, and Reduction of Single-Use Plastics, with a view to reducing plastic waste by 19% by the year 2040 (Asamblea Nacional del Ecuador, 2024). In addition, since 2024, it has joined the National Plastic Action Partnership (NPAP), setting a roadmap for the first time with the goal of reducing plastic generation at the source, improving waste management (collection, sorting, and processing), fostering recycling plans, promoting the circular economy, and strengthening the research and monitoring of these plastic wastes. Nevertheless, despite significant efforts and the development of consistent legal frameworks, their execution presents

important structural and investment limitations, which include infrastructure deficiencies, as most landfills lack mechanized sorting technology (Ochoa-Herrera et al., 2025), and the country's main cities also lack efficient wastewater treatment systems (Vélez-Terreros et al., 2024), Although national laws require the reduction of plastic waste, municipalities do not have suitable management systems for handling these residues, which hinders compliance with regulations at the local level and paradoxically contributes to the generation of bottlenecks, because the demand from national industry exceeds the supply of recycled plastic. Likewise, although statistics show that 6 out of 10 households attempt to separate their waste (INEC, 2025), sorting remains defective, and ultimately, the burden of recycling management falls almost exclusively on grassroots recyclers, most of whom are informal. Therefore, work must continue on strengthening the systems for the collection and treatment of plastic waste, reducing plastic waste in national protection areas, as well as improving recycling programs, important challenges, that the circular economy model, driven by law, will have to face.

Conclusions

The excessive increase in plastic consumption has become a major environmental concern due to its serious implications for river ecosystems. To date, research in Ecuador on the presence and distribution of MPs in environmental matrices, especially in national protected areas, remains scarce, as coastal zones have received the most attention. In this study, we used a combination of techniques for a detailed characterization of MPs in the Gualaceño River micro-basin, located within the Tinajillas-Río Gualaceño Natural Protection Area, being the first documented evidence of the presence of these pollutants in the area, with an average of 25 MPs/250 mL. Fibers were the most frequent form in the samples, with sizes ranging from 3.23 to 127.28 µm, highlighting the predominance of polymers such as PET, PTFE, PP, and polyamide, which are used in the manufacture of containers, bottles, plastic parts, and pipes. The observed predominance of fibers and PET/PP, together with the higher abundance recorded at the upstream site (P1), indicates substantial spatial heterogeneity in MP contamination within this protected watershed. Although nearby mining, tourism, agriculture, and local waste inputs represent plausible anthropogenic sources, the present data do not permit source-specific attribution. Rather, this study establishes a baseline for future source-resolved and hydrologically informed monitoring of MP pollution in Andean-Amazonian protected freshwater systems.

The lack of current environmental regulations in Ecuador limits the comparison of the presence of these emerging pollutants, which are ubiquitous in virtually all global ecosystems. However, this study provides a valuable methodological reference that can improve the understanding of the distribution of plastic particles in protected natural areas and contribute to future lines of research in these vulnerable ecosystems, as well as to strengthening policies in the fight against illegal mining, given that the relationship between mining and microplastic pollution is an emerging field of study that has historically been ignored. Furthermore, more long-term research is needed to study the potential ecotoxicological impacts on riparian biota and river biodiversity in order to better understand the distribution of these pollutants and develop appropriate mitigation strategies. It is also recommended to assess the influence of the physical characteristics of rivers, such as water flow and substrate type, on the accumulation of

microplastics. In summary, addressing the problem urgently requires a comprehensive approach involving governments, legislators, industries, and consumers.

Declarations

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

M.N.C. :Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. P.A. : Conceptualization and Funding acquisition. L.P.G. : Writing – review & editing. V.A. : Writing – review & editing. U.L. : Supervision, Writing – review & editing, Conceptualization. G.O. : Supervision, Writing – review & editing, Conceptualization.

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Data Availability

The data that support the findings of this study are available in the article and in its supplementary information. The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

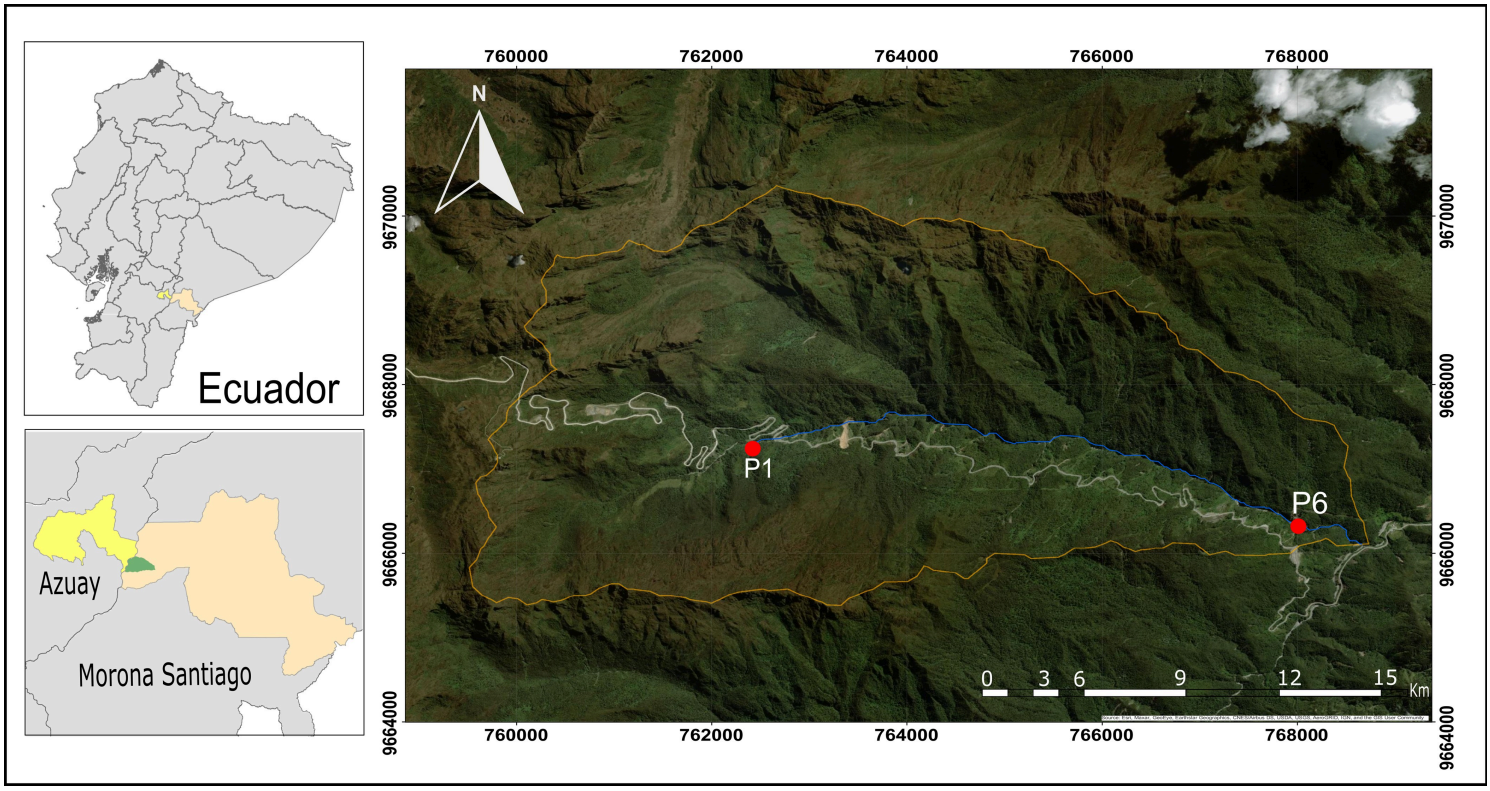

Figure 1

Location map of the study area in Ecuador. Sampled sites: upstream site (P1) and downstream site (P6). Red dots indicate the sampling sites, the blue line represents the watercourse and the orange line delineates the micro-basin.

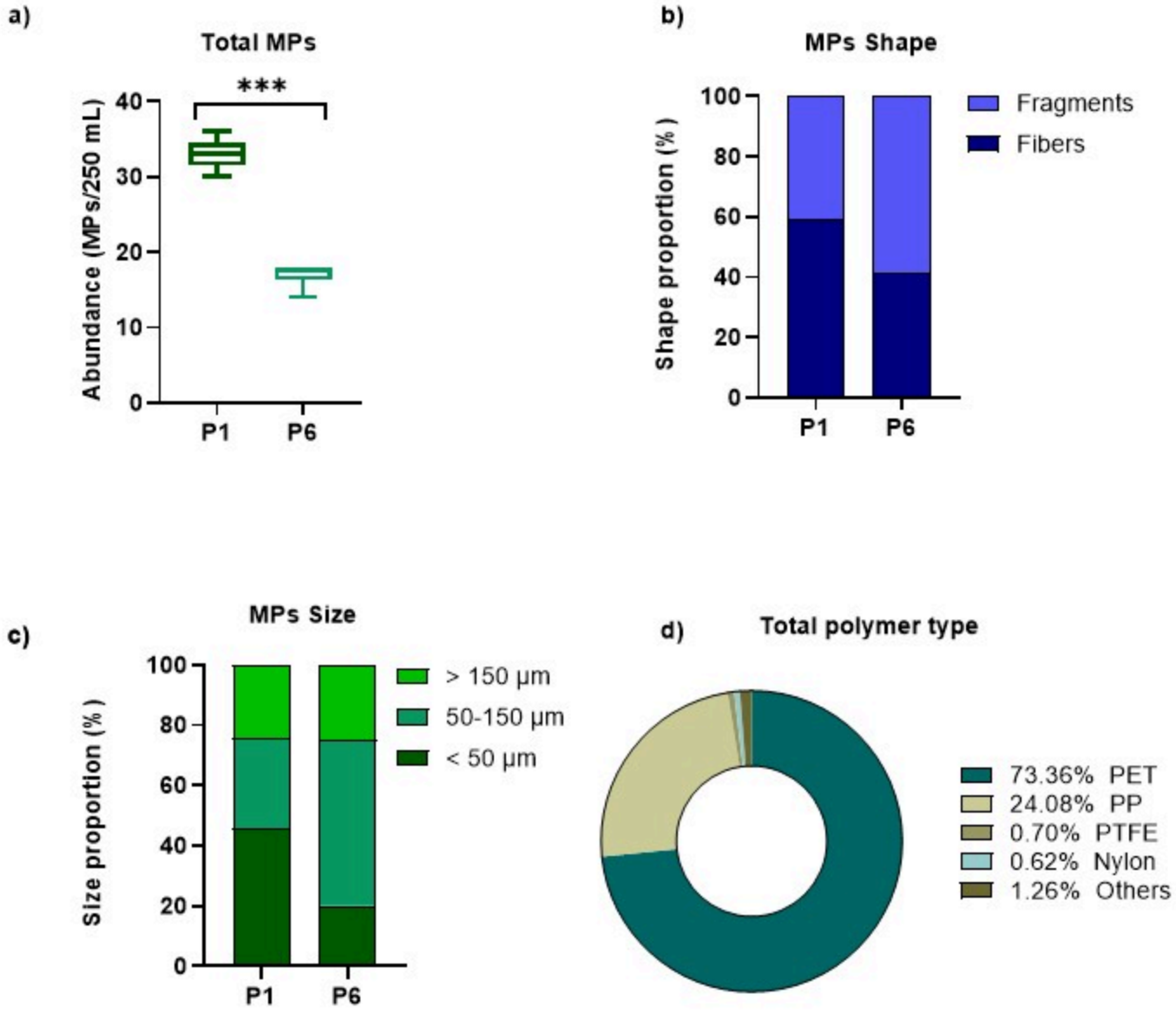

Figure 2

Microplastics found in the Gualaceño River micro-basin. Surface water samples: abundance (a), shape (b), size (c) and polymer distribution (d).

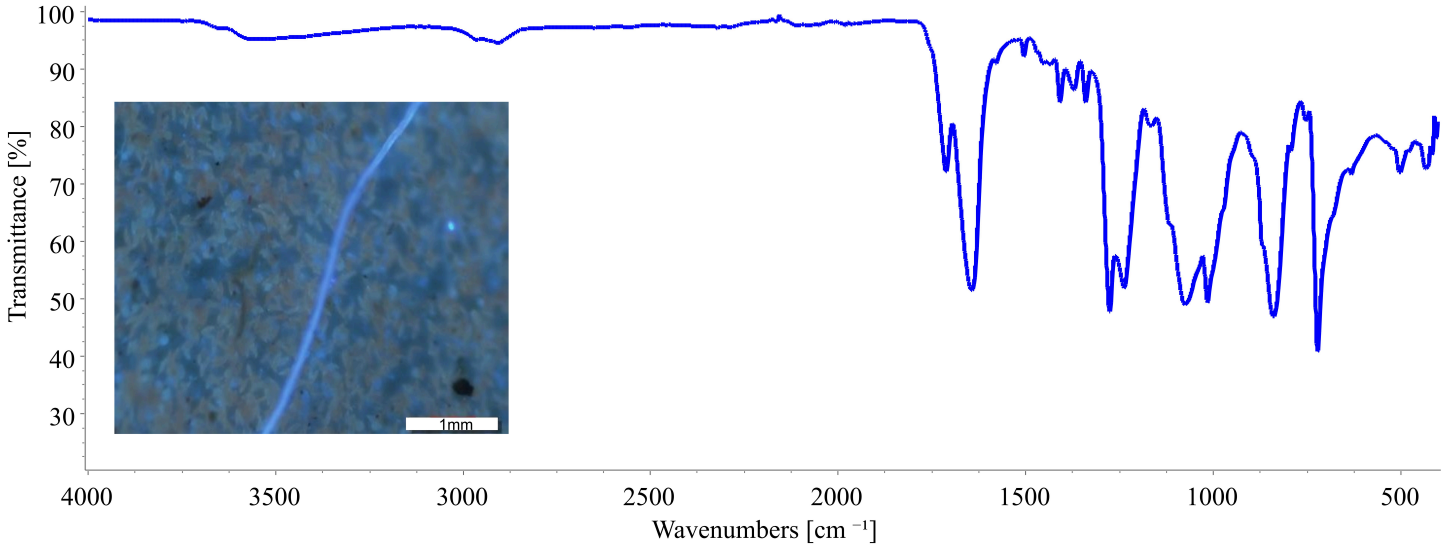

Figure 4

Representative ATR-FTIR spectrum of PET identified in the analyzed samples.

Supplementary Files

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- SupplementaryMaterialCuencaCastillo2026.docx