

Article

Assessment of Microplastic Pollution in River Ecosystems: Effect of Land Use and Biotic Indices

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Abstract: The proximity of freshwater ecosystems to anthropogenic activities makes them one of the most threatened environments by plastic pollution in the form of microplastics (MPs). Therefore, it is crucial to identify the primary drivers of MP dynamics in rivers to enhance their management. This work analyzed the concentration of MPs in water and sediments and evaluated the influence of land use and its relationship with the main biotic indices employed to assess the water quality of rivers. This research was carried out in four different catchments, with three sampling points established in each river basin. The results revealed that MPs were ubiquitous across all locations, with concentrations ranging from 0.10 to 35.22 items m⁻³ in waters and from 26 to 643 items Kg⁻¹ in sediments. The highest concentration of MPs both in water and sediments were found in the Lagares River (35.22 items m⁻³ and 643 items Kg⁻¹), while the lowest concentrations were found in the Miñor River for water (0.10 items m⁻³) and Tea River for sediments (138 items Kg⁻¹). Urbanization degree was identified as the primary driver of MP pollution in water, whereas population density correlated with sediment pollution levels. These findings explain the elevated MPs abundance in the more urbanized and populated Gafos and Lagares rivers compared to the relatively pristine Miñor and Tea rivers. Furthermore, the presence of MPs in sediments was found to negatively impact the most sensitive benthic macroinvertebrate taxa, as evidenced by lower values of the IASPT and EPT indices at sampling points with higher sediment MPs concentrations (Gafos and Lagares).

Keywords: freshwater ecosystems; land use; plastic; EPT; IASPT; IBMWP; biomonitoring



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1. Introduction

Plastic products have become one of the most demanded materials in modern society; the low manufacturing cost and the versatility of their use led to an exponential increase in their production. Around 400 million tons of plastic are produced at a global scale each year, and it is projected to double by 2050 and more than triple by 2100 [1]. Environmental Protection Agency reported that only 7% of the total produced plastic is recycled annually. Only 8% of the plastic is incinerated, and the remaining is landfilled. However, on many occasions, the high economic and energy costs involved in this process mean that waste ends up accumulating in natural ecosystems [2]. Once in nature, large plastic particles may persist and undergo various degradation processes, such as weathering, photodegradation, or biodegradation, among others, to form microplastics (MPs) [3].

Since the first time that these small particles of plastic were detected, MPs with different shapes, colors, or polymer types were considered by the scientific community as one of the most concerning emerging pollutants for the next decades [4]. The most worrying characteristic of MPs is their ubiquity since these pollutants have been found in the most remote places on earth, such as Lake Hosvölg in Mongolia [5] or in deep-sea sediments from Antarctica [6]. Most of the research efforts were focused on studying the

presence and impact of MPs in oceans and marine waters [7,8]. However, terrestrial and freshwater environments may be affected to a higher degree by MPs since all the plastic products are manufactured on land, and most of them are used and disposed of within the continent [9]. Moreover, rivers represent one of the main pathways of efficient transport of MPs from land to the oceans, and the concentration of MPs in freshwater ecosystems is expected to be at least equal to that found in the oceans, with a highly heterogeneous distribution in different areas [10,11].

These pollutants get into rivers through the wind [12], storm sewers, wastewater treatment plants, and as a consequence of direct human activity near riverbanks. The most significant sources of freshwater pollution are wastewater effluents from wastewater treatment plants and runoff from road surfaces caused by the breakdown of road markings and tire debris [13]. Previous studies [14] identified the closeness of urbanization to rivers as sources of MPs from various activities such as effluent discharge, road runoff, littering, and atmospheric deposition to aquatic ecosystems.

Once MPs are present in freshwater ecosystems, the potential threat of contamination may be greater than in the marine environment due to the closer proximity to human activities. The effect of MPs has been studied at various levels (genes, cells, tissues) and both on animals and plants [15]. Benthic macroinvertebrates represent the most abundant group of animals in freshwater ecosystems, and recent studies have evidenced their interaction with these pollutants [16]. As with many other pollutants, the effects depend on many factors, such as the habitat occupied or the MP characteristics. Hence, organisms inhabiting habitats within which MPs are likely to aggregate and be retained will be more susceptible to being affected by them [17]. This would open the possibility of using some taxa as potential indicators of MP pollution in riverine ecosystems, although no research has been conducted on it other than on marine environments where it has already been assessed [18].

Regarding humans, MPs have also entered the food web, thus becoming an emerging problem and a risk to food safety [19]. Recent studies reported the presence of MPs in food and drinking water, indicating that the exposition of MPs is also a fate that threatens human health [20,21]. MPs can cause chemical and physical damage to organisms through oxidative damage and nerve poisoning [22]. In humans, the main effects were mainly manifested in gastrointestinal toxicity and liver toxicity, involving oxidative stress, inflammation, and metabolic disorders [23]. Moreover, the release to water of additives such as bisphenol A (BPA), used as an antioxidant or stabilizing material, may cause endocrine-disrupting effects [24].

Although several studies focused on identifying the major drivers of MP pollution in rivers, and urbanization was reported as one of the most important factors [25], this is not enough to understand the high variability of the data available in the scientific literature. These differences may concern very different magnitude orders even in the same area, indicating that there already exists a problem that could be related to the absence of a unique standardized methodology adopted by the scientific community [26].

It is, therefore, important to obtain data from new regions under different pressures and with different climatic and watershed characteristics. Thus, the main objective of this work was to evaluate the concentration of MPs both in water and in the sedimentary phase in rivers in the NW region of the Iberian Peninsula, an area where the impact of these emerging pollutants has not yet been studied. The specific objectives focused on detecting the main drivers of MP pollution within the study area and evaluating if the current biotic indexes used to assess the water quality could be indicative of MP pollution in rivers.

2. Materials and Methods

2.1. Study Area

The study area was in the province of Pontevedra (NW Spain), with a total population of 941,772 inhabitants distributed heterogeneously through a surface of 4,459 Km². The territory has a very particular climatic condition since, in the oriental area, it is considered a transition zone between the Mediterranean and Oceanic climate. According to the Koppen–

Geiger climate classification [27], the study area is characterized by a temperate climate with dry and warm summers and rainy winters (Cfb).

To carry out this work, four rivers were selected, and three sampling points were established, each of them distributed from the headwaters to the mouth in the upper (1), middle (2), and lower (3) sections (Figure 1). Three of the streams, the Gafos River (G), the Lagares River (L), and the Miñor River (M), are managed by the Galicia-Costa basin district. The Tea River is included within the Miño-Sil Hydrographic Demarcation.

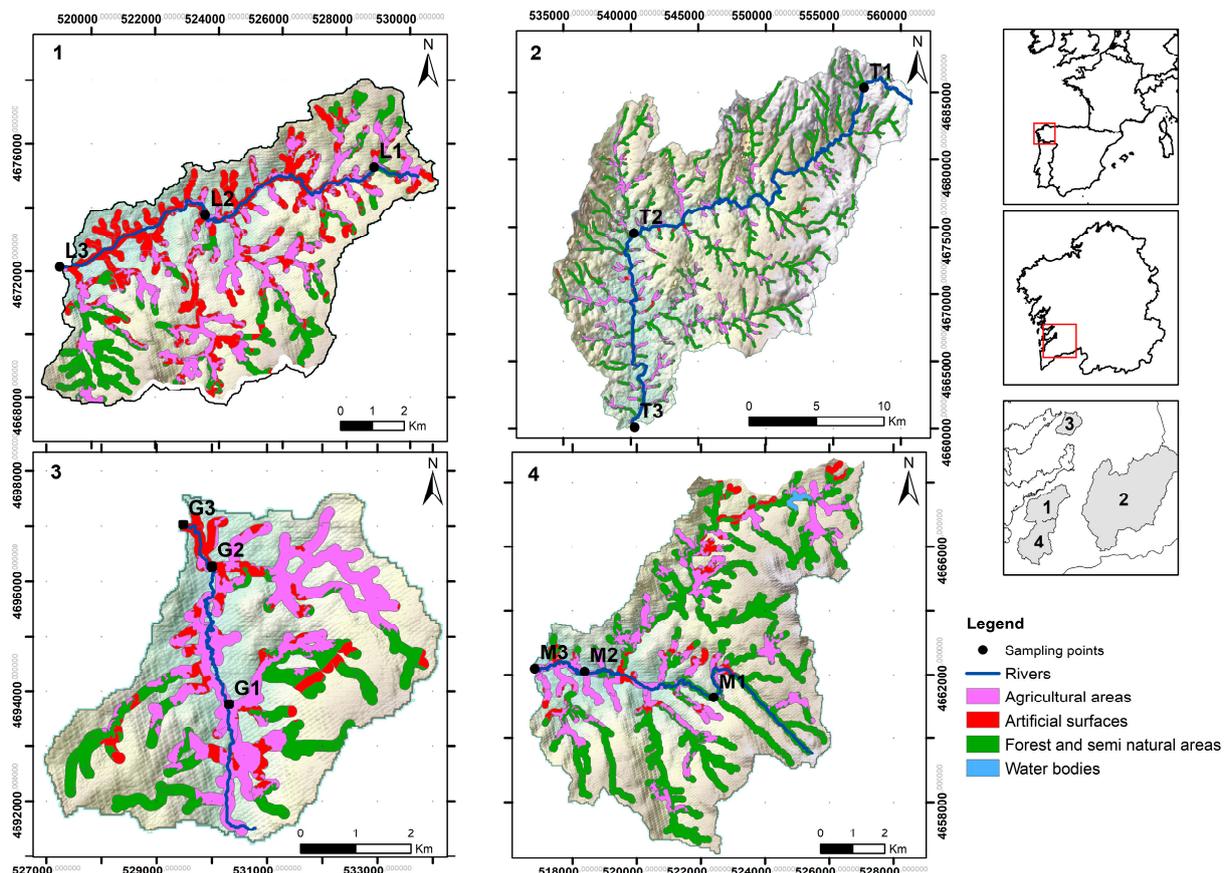


Figure 1. Study area and location of the three sampling points established throughout the course of each of the studied rivers: Lagares (1), Tea (2), Gafos (3), and Miñor (4). It represents the catchment and the land use within a buffer zone of 100 extracted from the drainage network.

River Basins Characterization

The hydrological basin of the rivers and each of the sub-basins for the studied points and the drainage network were extracted from the Digital Elevation Models (DEM) with a resolution of 10 m by using the specific hydrology tools of the QGIS Geographic Information System. In each sub-basin was calculated the surface percentage occupied by the land uses of level 1 of the Corine Land Cover classification in a buffer zone of 100 m along the river network up to each sampling point, following the same criteria as Gutiérrez-Rial et al. [28]. Additionally, the population density was calculated based on the population data of the IGE (Instituto Galego de Estatística) and the river basin surface. Characteristics of each of the sub-basins are reflected in Table 1.

Table 1. Characteristics of the sub-basin for each of the sampling points in the rivers Tea (T), Miñor (M), Gafos (G), and Lagares (L). CLC parameters represent the percentage of the sub-basin occupied by the Corine Land Cover Classification level 1: **CLC 1:** artificial areas; **CLC 2:** agricultural areas; **CLC 3:** forest and semi-natural areas; **CLC 5:** water bodies.

	Basin Area (Km ²)	Population Density (Hab Km ⁻²)	CLC 1 (%)	CLC 2 (%)	CLC 3 (%)	CLC 5 (%)
T1	14.03	13.79	0.00	7.80	92.20	0.00
T2	218.79	36.03	1.14	14.01	84.85	0.00
T3	406.62	85.02	2.01	18.95	79.03	0.00
M1	12.66	75.43	2.46	12.51	85.03	0.00
M2	63.74	215.38	7.70	20.28	71.62	0.41
M3	73.92	214.67	7.83	21.05	70.76	0.35
G1	11.56	173.46	11.26	29.16	59.58	0.00
G2	25.82	310.94	14.55	40.20	45.25	0.00
G3	26.94	756.02	16.94	39.68	43.38	0.00
L1	4.27	461.93	31.74	33.58	34.68	0.00
L2	47.33	1338.67	41.67	28.40	29.93	0.00
L3	69.45	1958.89	42.09	25.55	32.36	0.00

2.2. MPs Sampling Collection

Two sampling campaigns were carried out in the spring and summer of 2021 in all the mentioned locations. MPs samples from the water column were collected by filtering the water. For this purpose, a specific MP sampling net with a 220 µm mesh size was chosen to avoid the clogging of the net. The sampling net was submerged at a depth of 0–5 cm for five minutes with the open side against the river flow, forcing the MPs into the net. The amount of filtered water was calculated by measuring the flow speed with a flow meter OTT C2 considering the sampling surface of the net (50 × 12 cm/600 cm²). A clean stainless steel was employed to take 1 L of sediment from random locations from the upper 5 cm sediment layer along the river section. Both water and sediment samples were placed in correctly labeled bottles for transport to the laboratory, but first, any significant fragments of organic matter and stones were removed in situ.

2.3. MPs Samples Processing

Once in the laboratory, the samples were processed following the NOAA laboratory methods for the analysis of MPs in water and bed samples with some modifications [29]. The water processing consisted of sieving, organic matter elimination, density separation, and finally, vacuum filtration. First, samples were run through a stacked series of metal sieves (5 and 0.55 mm) and cleaned with distilled water. All the organic particles and plastic items >5 mm were discarded. Next, 30 mL of 30% hydrogen peroxide (H₂O₂) (Scharlab S.L., Sentmenat, Spain) was added to the fraction smaller than 5 mm to eliminate organic matter. The mixture was heated to 75 °C until it started to boil and left to react for 24 h at room temperature. Then, the samples underwent density separation using NaCl (density 1.2 g cm⁻³) (Labbox Labware S.L., Premià de Dalt, Spain). After 24 h, the supernatant was filtered through 0.45 µm Whatman filters (Cytiva, Global Life Sciences Solutions Operations UK Ltd., Buckinghamshire, UK) under vacuum conditions. Finally, filters were dried at 40 °C before MPs identification under a stereomicroscope.

For the sediments, the procedure was the same but with two previous steps. The wet samples were homogenized by intensive stirring. Then, three sub-samples of 50 g were taken and dried at 90 °C for 2 days. Once dried, the samples underwent the same protocol as for water samples: sieving, organic matter digestion, density separation, and vacuum filtration to retain the MPs in the 0.45 µm Whatman filters. MPs items were identified under stereomicroscope Leica S9D (Leica Microsystems, Wetzlar, Germany) with magnification from 0.6× to 5.5×. The MPs were counted and classified on fragments, spheres, fibers, and foams following the criteria of previous works [30,31].

2.4. Quality Assurance and Quality Control

To avoid contamination during sample collection and laboratory processing, all the equipment was carefully washed with distilled water, and all the materials were covered with aluminum foil. During all the steps of the laboratory processing, all the samples were immediately covered, and the exposition time to air was minimized. Moreover, the personnel of the laboratory wore white laboratory coats while the samples were being processed. Whatman filters were placed near the samples exposed to the same contamination as the samples. Filters were explored under a stereomicroscope, and white fibers were found, probably from the laboratory coats, but the amount was negligible compared to the amount found in this study.

2.5. Benthic Macroinvertebrate Sampling and Processing

In each of the sampling locations, benthic macroinvertebrates were captured following the methodology established by the Water Framework Directive described in Boonsoong et al. [32]. An entomological hand net with a mesh size of 500 μm was used to collect the benthic macroinvertebrates. The samples were poled and kept in 4% formaldehyde solution to be transported to the laboratory. Once there, all the individuals were identified at the family level using a stereomicroscope and specialized identification keys [33,34], and the abundance (N) of individuals at each site was calculated. After that, several biotic indices were calculated. First, the richness (S) was calculated as the number of different taxa, and then the IBMWP (Iberian Biomonitoring Water Party) and the IASPT (Iberian Average Score Per Taxon) were used to assess the water quality following the protocol of Alba-Tercedor [35]. Additionally, the EPT and PT indexes based on the most sensitive taxa (Ephemeroptera, Plecoptera, and Trichoptera) were calculated considering the number of different taxa of these groups at each sampling station to study the effect of MPs on these individuals.

In addition, at each sampling point, the pH, water temperature, electrical conductivity (EC), redox potential, dissolved oxygen, and the total dissolved solids (TDS) were measured in situ by using the multiparametric sensor Hanna[®] HI98194 (Hanna Instruments S.L., Eibar, Spain). Moreover, water samples were taken to analyze the Total Organic Carbon (TOC) and the Chemical Oxygen Demand (COD) through the combustion method, and the main inorganic anions (SO_4^{-2} , PO_4^{-3} , NO_3^- and Cl^-) were determined by thermocatalytic decomposition method.

2.6. Data Analyses

To analyze the MP concentration in sediments and in the water column, two generalized linear models (GLM) were developed, once for each matrix. Previously, Pearson correlations were calculated to select the variables that influence the concentration of MPs. Initially, the variables considered for each sub-basin were as follows: the basin area (Basin.area), the population density (PopDen), the percentage of agricultural areas (Ag.areas), artificial areas (Art.areas), forest, and semi-natural areas (For.areas) within of the buffers of 100 m; the precipitation accumulated in the previous 15 days (Prec.15) and in the previous 30 days (Prec.30); the total dissolved solids (TDS) and electric conductivity (EC); and two factorial variables, river (Gafos, Lagares, Miñor and Tea) and season (spring or summer). An Exploratory Data Analysis (EDA) was developed in combination with a stepwise process to select the most adequate GLM until no further improvement was possible following the AIC and BIC criteria.

3. Results and Discussion

3.1. Occurrence and Distribution of Microplastics in Water

The results of this study showed that the presence and concentration of MPs in the surface waters of the studied rivers were highly variable. On the one hand, it varied between rivers and between sampling stations within the same river, and on the other hand, the results showed a marked seasonal pattern, as can be seen in Figure 2. In total, 561 MP

particles were identified, of which 81% were fibers, 20% were fragments, and, to a lesser extent, films (1.6%) and spheres (0.17%). In summer, the Lagares River had the highest concentration of MPs in the water (35.22 MPs m^{-3}) with a statistically significant difference ($p < 0.05$). The Gafos River had the second-highest concentration (11.36 MPs m^{-3}) in summer, while the lowest values were recorded in the Tea River (0.25 MPs m^{-3}) in spring and in the Miñor River (0.1 MPs m^{-3}) in summer. Despite the differences, the concentrations of MPs found in this study were similar to other European rivers, as can be seen in Table 2, much lower than in rivers from the Asian continent, considered a hot spot for plastic pollution due to rapid economic and demographic growth [36].

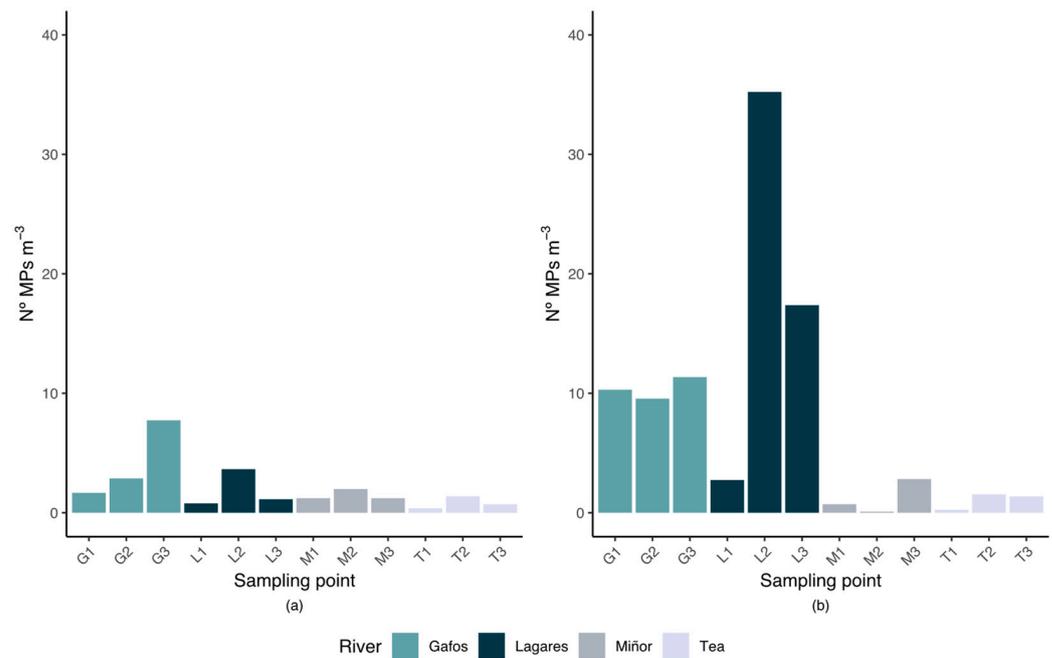


Figure 2. Microplastic concentration in water in all the sampling stations in both spring (a) and summer (b).

Previous works [37] suggested that the difference in MP concentration in rivers depended on different characteristics of the river catchments, such as land use, population density, or the socioeconomic conditions of the area. In this study, the concentration of MPs in rivers varied significantly depending on the artificial surface area in the sub-basin ($p < 0.05$). Previous studies demonstrated that the abundance of MPs increases in urbanized areas with respect to sub-urban [38,39], which would explain the higher concentration of MPs found in the Gafos and Lagares rivers compared to the rest of the rivers. These two rivers, among those studied, have the highest proportion of their catchment area urbanized, as can be seen in Figure 1 and in Table 1. These characteristic conditions were reflected in the higher abundance of MPs found in the most urbanized areas (G2, G3, and L2 and L3). The basin of rivers such as the Miñor or the Tea are characterized by lower population density, and its catchments are much more dominated by forested/natural areas (Table 1). So, anthropogenic activities are less intensive within their basins, and therefore, the potential sources of MPs are less abundant. This is reflected in the lower concentration of MPs found in these rivers.

Another observed trend was the increase in the concentration of MPs from upstream to downstream, something found before by Zhao et al. [40]. Generally, rivers close to their source tend to have steeper gradients and faster flow rates, while downstream sections are flatter. This means that MPs can migrate from upstream to downstream, resulting in higher amounts of MPs as the river flows [41]. However, the dilution capacity of the river may result in a lower concentration of MPs in the lower section of the rivers, where the river flow is higher, so the concentration may be low, as found by Tan et al. [4]. But, in these

cases, the total amount of MPs transported by the river continues to be higher than in the upper and middle zones when considering the total volume of water carried by the river. Moreover, human settlements and industrial areas are usually concentrated in downstream sections; in these sites, the high degree of urbanization results in the presence of a high proportion of less permeable soil that facilitates the transport of MPs by runoff to different environmental compartments, including rivers [25].

Table 2. Compilation of the abundance of MPs in the water column from different locations available in the recent scientific literature. The list is ordered from the lowest to the highest maximum concentration of MPs.

Study Area	Continent	MPs Water (MPs m ⁻³)	Reference
Paraíba do Sul and Pomba rivers	South America	0.52	da Costa et al. [42]
Paraíba do Sul and Dois rivers	South America	0.65	da Costa et al. [42]
Paraíba do Sul and Muriaé rivers	South America	0.96	da Costa et al. [42]
Tea River. Spain	Europe	1.54	This study
Miñor River. Spain	Europe	2.82	This study
Pearl River	Asia	4.21	Li et al. [43]
Liane River	Europe	4.52	Pasquier et al. [44]
Ebro River. Spain	Europe	4.9	Simón-Sánchez et al. [45]
Milwaukee Rivers (Milwaukee)	North America	5.67	Lenaker et al. [46]
Gafos River. Spain	Europe	11.36	This study
Elbe River	Europe	13.24	Scherer et al. [47]
Lagares River. Spain	Europe	35.22	This study
Ljubljana	Europe	45	Matjasic et al. [48]
Kamniška Bistrica Basin	Europe	75	Matjasic et al. [48]
North Saskatchewan River	America	88.3	Ross et al. [49]
Neuse River Basin	North America	131	Kurki-Fox et al. [50]
Neuse River Basin	North America	221	Kurki-Fox et al. [50]
River Ganga	Asia	237.9	Rajan et al. [51]
Yangtze River. China	Asia	258	Yuan et al. [52]
Swat River	Asia	594	Bilal et al. [53]
Ergene River	Europe	1206	Akdogan et al. [54]
Yangtze River	Asia	35,986	Huang et al. [55]

The concentration of MPs was also affected by a seasonal pattern; as shown in Figure 2, the number of MPs by cubic meter was higher in all the sampling points in the dry period (summer) rather than in the wet period (spring). During the wet season, with the first rains and floods, surface runoff from the areas surrounding the river increases, resulting in an input of MPs that would increase the abundance and concentration of MPs in the water [25]. However, after a few days of precipitation, the effect would be the opposite; the rainfall would cause a dilution effect of the concentration in the water, and the increased flow would reduce the retention time of the MPs transporting them along the river [56]. Like other types of pollutants, microplastics in dynamic ecosystems such as rivers experience fluctuations over short periods due to the short residence time that water has [47].

3.2. Occurrence and Distribution of Microplastic in Sediments

In total, 460 MPs were identified, and, as in the water column, fibers were the most abundant type (70.6%), followed by fragments (26.9%) and, to a lesser extent, spheres (2.4%). The results obtained for the sediments differed markedly from those shown for the water column. Higher concentrations of fragments in sediments rather than in the water column were also found by Lin et al. [57]. Fragments appear more frequently in sediments because of their morphological characteristics; these particles have a higher surface-to-volume ratio and tend to have higher density, which increases the sedimentation of these particles rather than fibers. [58].

Although the concentration of MPs was highly variable between rivers and between sampling points in the same rivers, no clear pattern was observed; nevertheless, the highest

concentrations of MPs in the sediments were reported in the same locations as those for the water column as indicated in Figure 3 but differed by a few orders of magnitude between rivers. The highest concentration was found in L3 in summer (643 MPs Kg^{-1}) and the lowest in T2 in spring (26 MPs Kg^{-1}).

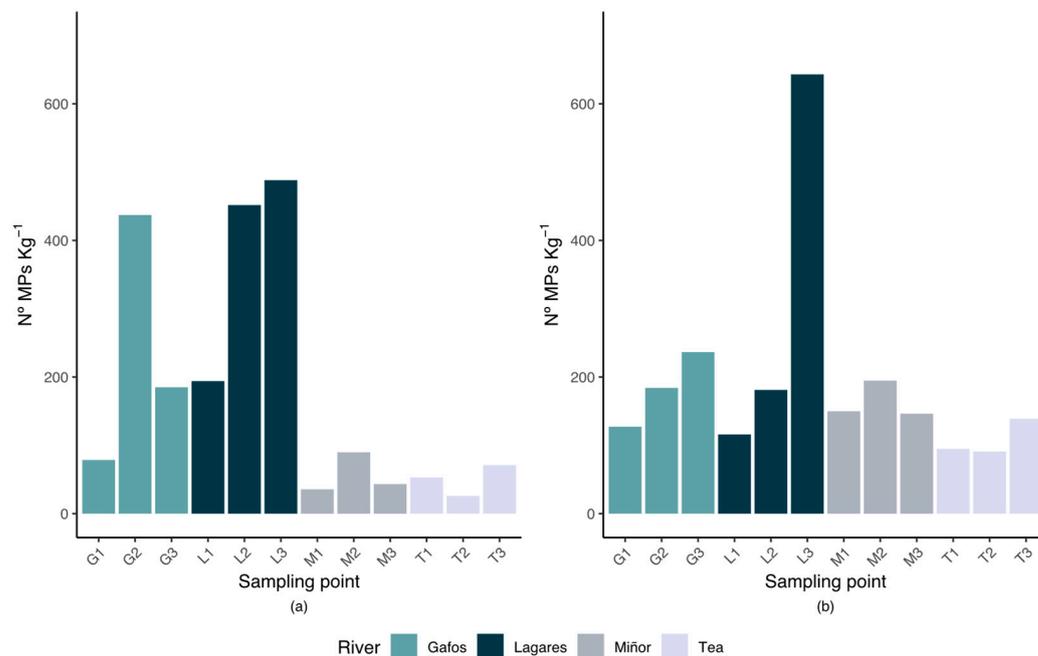


Figure 3. Microplastic concentration in sediments in all the sampling stations in both spring (a) and summer (b).

In contrast to the results obtained for the water column, the concentration of MPs in sediments did not show a spatial or temporal distribution pattern, something common among previous studies [59]. In the case of surface waters, there was a pronounced pattern resulting in Asian rivers being the most affected by MP pollution; the concentration in sediments shows significant spatial variability, as indicated in Table 3. It is expected to be higher in the same areas as for surface waters. The absence of this pattern could be indicative of a lack of consistency in the methodology, previously announced by Lu et al. [56]. Once the differences caused by the methods have been eliminated or at least minimized, the effect of the environmental variables can be quantified with certainty. Logically, river hydrodynamic conditions, as well as river characteristics, should be one of the main drivers of MPs dynamics in rivers.

The results obtained in this study indicated that the concentration of MPs increased from the source of the rivers to the river mouth. This pattern was especially marked in the Gafos and Lagares rivers in summer and in the Lagares River in spring. Other marked pattern was observed in the Miñor River in both seasons and in the Gafos River in spring. In this case, the highest MP concentration in sediments was found in the middle section, similar results to those found by Scherer et al. [47] in the Elbe River. The sampling points in these rivers in the low section are very close to the mouth of the Atlantic Ocean (Figure 1). Thus, these locations are affected by tidal effects, which move marine sediments from the Atlantic Ocean into the rivers, leading to sediment mixture and a further dilution of MP levels [60] compared to that found in the middle section (Figure 3).

Table 3. Compilation of the abundance of MPs in river sediments from different locations available in the scientific literature. The list is ordered from the lowest to the highest maximum concentration.

Study Area	Continent	MPs Concentration (MPs Kg ⁻¹)	Reference
Nanhuizui tidal flat	Asia	6	Peng et al. [57]
Ljubljana	Europe	44	Matjasic et al. [48]
Kamniška Bistrica Basin	Europe	48	Matjasic et al. [48]
Yujiabang River	Asia	53	Peng et al. [57]
Huangpu River branch	Asia	102	Peng et al. [57]
Shajinggang River	Asia	104	Peng et al. [57]
Jiangjiagang River	Asia	117	Peng et al. [57]
Tea River. Spain	Europe	138	This study
Beishagang River	Asia	179	Peng et al. [57]
Miñor River. Spain	Europe	194	This study
Tibet Plateau	Asia	195	Jiang et al. [61]
Caohejing River	Asia	230	Peng et al. [57]
Gafos River. Spain	Europe	437	This study
Lagares River. Spain	Europe	643	This study
Tisza River	Europe	5147	Kiss et al. [62]
Milwaukee Rivers	America	6229	Lenaker et al. [46]
Elbe River	Europe	6750	Scherer et al. [47]
St. Lawrence Rive	America	7562	Crew et al. [63]
Amazon River	America	8178	Gerolin et al. [64]
West River	Asia	10,240	Huang et al. [65]

In this study, the concentration of MPs in sediments varied significantly depending on the population density of each sub-basin ($p < 0.05$), something previously found by Matjasic et al. [48], who also reported the size and length of the catchment as one of the major driver of MPs pollution in sediments from freshwater systems. Our results contrast with those found by Klein et al. [66], who did not find any relationship between the MP concentration in the sediments and the population density. But, as for many other previous studies [11,67,68], our model showed that the population density may be a good indicator of MP pollution in the riverbank. This would explain the higher concentration of MPs found in the Lagares and Gafos rivers, whose basins show a higher population density than the rest of the rivers, even in the headwater points (Table 1). Generally, in these highly populated areas, it is expected that high pollution levels may be observed in sediments [69,70]. These rivers, which are much more urbanized, have lower water velocities and less turbulent hydrodynamic conditions. In addition, potential sources of MPs are more abundant, and migration velocity is lower [4]. These conditions facilitate a higher abundance of MPs by increasing sedimentation and retention of MPs in the sediments, compared to much more natural rivers such as the Miñor or Tea, where hydrodynamic conditions increase the transport of MPs along the river course.

Finally, there were no statistically significant differences ($p < 0.05$) between the concentration of MPs found in spring compared to summer. Although some authors consider that seasonal variation is a key factor affecting MP distribution in rivers [71], it should not be applicable in the region of this study, where rainfall is frequent throughout the year. However, in areas where dry and wet periods are much more differentiated, these differences may be more marked. In these cases, rainfall events promote the wet deposition of MPs suspended in the air [72], and the storm events lead to faster river flows that can wash the river sediments and transport them downstream, leading to a lower abundance of MPs in headwater sediments [73]. In contrast, during the dry season, the lower flow velocity may facilitate the accumulation and sedimentation of MPs, which may result in a higher abundance of MPs in the sediments [74].

3.3. Biotic Indices and MPs Pollution

The use of benthic invertebrates as indicators to assess different pollution sources has been extended since the last century to guarantee the sustainability of biodiversity and ecosystem services [75]. For this reason, it has been recognized as one of the most useful tools to develop monitoring programs in rivers [76]. Nevertheless, when the biotic indices were developed, threats such as microplastics were not considered, so the potential use to assess the presence of these emerging pollutants remains unknown.

In this study, 21,726 individuals were identified as belonging to 92 different taxa between spring and summer, with insects being the most abundant group (77%). The maximum abundance was found in G2 in spring ($N = 3579$) and the minimum in L3 in summer ($N = 97$), while the highest and the lowest values for richness were reported in T1 in spring ($S = 40$) and M3 ($S = 8$) and L3 ($S = 8$) in summer, respectively (Table 4). As shown in Table 5, the correlation test did not show a significant ($p > 0.05$) correlation between the abundance of macroinvertebrates and the concentration of MPs. Although some authors previously found that long-term expositions to these pollutants can result in a decrease in the total abundance of macroinvertebrates [77], this may not happen under natural conditions in rivers. The concentration of MPs in sediments used in experimental conditions usually are much higher than realistic [78], and the presence of MPs in the water column in a dynamic system as a river is not enough to affect these individuals as a consequence of the less prevalence time caused by the water flow. Considering the richness, we found a significant ($p < 0.05$) negative correlation ($\rho = -0.427$) with the concentration of MPs in sediments (Table 5). So, the presence of high levels of MPs in the sediments could affect the structure of the benthic macroinvertebrate communities by reducing the diversity, as found by Silva et al. [79].

Table 4. Diversity and water quality indices calculated based on the benthic macroinvertebrate communities of each point for the rivers Tea (T), Miñor (M), Gafos (G), and Lagares (L) in spring and summer. N: abundance; S: richness; EP: Ephemeroptera and Plecoptera; EPT: Ephemeroptera, Plecoptera, and Trichoptera; IBMWP: Iberian Monitoring Water Party; IASPT: Iberian Average Score Per Taxa.

	N		S		EP		EPT		IBMWP		IASPT	
	Spring	Summer										
T1	1556	269	40	29	8	6	20	14	279	185	6.8	6.8
T2	417	640	23	32	5	7	13	17	161	210	7	6.56
T3	391	429	14	28	5	4	7	13	91	169	6.5	6.26
M1	1211	1660	35	18	11	0	19	7	296	112	7.05	5.89
M2	2007	464	32	15	4	2	15	2	203	75	6.64	5
M3	792	881	13	8	2	0	5	0	82	32	6.31	4.57
G1	956	569	31	27	3	4	8	11	151	170	5.2	6.3
G2	3579	2676	25	22	4	3	7	7	141	126	5.84	5.73
G3	153	792	14	18	1	1	3	3	81	78	4.5	4.33
L1	482	370	29	30	3	4	9	12	142	182	5.68	6.07
L2	304	454	25	28	3	4	6	13	128	170	5.33	6.3
L3	577	97	14	8	2	0	3	1	67	41	4.78	5.12

The EP index shows the number of different taxa belonging to the orders Ephemeroptera and Plecoptera and, as can be seen in Table 5, also presented a significant ($p < 0.05$) negative correlation ($\rho = -0.427$) with the concentration of MPs in sediments. When the number of different trichopteran taxa was added to the previous index (EPT index), the correlation was significant ($p < 0.01$) and much higher ($\rho = -0.427$) (Table 5). These indexes only reflect the most sensitive taxa but are considered good indicators of water quality, especially in altered and urbanized rivers [28]. So, according to the results of this study, the most sensitive taxa may be susceptible to being affected by the presence of MPs in sediments, and as it was shown in this study, the effect can be measured with more severity when considering the trichopterans. Ehlers et al. [80] found that caddisflies can incorporate MPs of different characteristics into the larval cases where they live. This increases the exposition time to

MPs and the risk of being affected by some of the additives, but it also reduces the stability of the cases and the protective function, making the larvae more prone to predation [81,82]. The impact of MPs on some benthic macroinvertebrate groups could affect ecosystem functions by causing changes in community structure, as benthic macroinvertebrates are crucial for ecological parameters such as primary production or leaf decomposition [83,84]. These effects may also affect the energy transfer across trophic levels [85], and the uptake of MPs by different benthic macroinvertebrates represents the pathway for these pollutants to enter food webs [16]. Previous studies evidenced the transfer of MPs from basal to upper levels [86], indicating that further bioaccumulation and biomagnification processes are likely to be produced through food webs [87].

Table 5. The Pearson correlation coefficient (ρ) obtained for the concentration of MPs in water and sediments with each of the biotic indices. N: abundance; S: richness; EP: Ephemeroptera and Plecoptera; EPT: Ephemeroptera, Plecoptera, and Trichoptera; IBMWP: Iberian Monitoring Water Party; IASPT: Iberian Average Score Per Taxa.

	MPs Water	MPs Sediments
N	−0.125	0.024
S	−0.112	−0.427 *
EP	−0.201	−0.468 *
EPT	−0.101	−0.558 **
IBMWP	−0.130	−0.500 *
IASPT	−0.132	−0.551 **

Note(s): ** 0.01; * 0.05.

The IASPT also showed a significant ($p < 0.01$) negative correlation ($\rho = -0.551$) with the MP concentration in sediments, supporting the findings mentioned for EP and EPT indices. The IASPT index informs about the mean average degree of tolerance of the taxa found in the benthic macroinvertebrate community, so according to our results, the higher the concentration of MPs in sediments, the lower the IASPT as a consequence of the replacement of the most sensitive by more tolerant taxa [88]. This would explain the lower values for these indexes reported in the rivers Gafos and Lagares with respect to the more pristine rivers (Tea and, to a lesser extent, Miñor rivers), as can be seen in Table 3. Finally, a significant ($p < 0.05$) negative correlation ($\rho = -0.551$) was found with the IBMWP that indicates that the water quality decreases while the concentration of MPs in sediments increases. It would be expected since the main threats to water quality are caused by anthropogenic activities as equal to the source of MP pollution. However, the use of this index has some disadvantages, as previously shown by several authors [89,90], and as for many other pollutant types, its use is not recommended when considering MPs.

4. Conclusions

This study confirmed the ubiquitous presence of MPs in the study area, as these contaminants were detected in all the sampled points, both in water and sediment samples. The study found that urbanization was the primary driver of microplastic pollution in water, while in sediments, the most important factor was the density of the population within the catchment. Finally, the results demonstrated that biotic indices based on the most sensitive taxa, such as EPT, PT, or IASPT, may be suitable for assessing MP pollution in sediments from rivers, although further research is necessary.

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