Microplastics in Water, Sediments and Macroinvertebrates in a Small River of NW Spain

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Abstract

Microplastics (MPs) were sampled in water, sediments and macroinvertebrates in Gafos river (NW Spain) during summer 2020 in three different locations called upstream (G1), upstream of the city (G2) and downstream (G3). Different microplastic (MP) fibers and particles were found in all water samples and in G2 and G3 sediment samples. The analysis of the different types of MPs was carried out by Fourier-transform infrared spectroscopy (FTIR-ATR), Sample Technique: Attenuated Total Reflection (ATR). The majority of them were polyvinyl chloride (PVC) and polyethylene terephthalate (PET). Microplastics were found in the cases of Trichoptera families like Lepidostomatidae and Limnephilidae and inside the body in some Odonatan families such as Gomphidae.

Introduction

Plastic features (e.g., durability, lightweight) make them one of the most demanded products of our society. Since the 1950s, plastic production has exponentially increased, and nowadays, around 300 million metric tons of this material are manufactured worldwide each year (Scherer et al., 2017, Statista, 2021). Despite the multiple advantages that plastic offers and the massive economic and social benefits that we can obtain from these products, unsuitable resource management has led to litter accumulation in natural ecosystems (Barnes et al., 2009). Plastic particles with a size smaller than 5 mm are called microplastics (MPs), and can be classified into two groups (Herrera et al., 2018). On the one hand, primary MPs are particles intentionally manufactured with a size <5 mm, used in resin pellets or as ingredients of a great variety of cosmetic products (Cole et al., 2011; Chang, 2015). On the other hand, secondary MPs originate from disintegrating plastic items (Lambert & Wagner, 2018).

Similar to other persistent pollutants, microplastics are recognized globally. Their presence was first reported in the ocean in the 1970s (Carpenter & Smith, 1972). Nevertheless, some decades ago, research efforts on MP pollution have been oriented towards marine environments (Rochman, 2018). In the last years, this trend has changed, and some studies have shown the presence of these pollutants in both freshwater and terrestrial ecosystems (i.e., Wagner et al., 2014; Rillig & Lehmann, 2020).

Due to their small size, microplastics can be ingested directly or indirectly by a great variety of animals and can be incorporated and transferred through food webs (Barnes et al., 2009; Setälä et al., 2014). Different studies have tested the exposure of marine and aquatic biota such as freshwater plants, invertebrates, several fish species, and also seabirds and mammals to MPs (Rochman, 2018). In marine organisms, ingestion of large plastic items may cause choking, internal or external wounds, accelerating sores, blocked digestive tract, false sense of satiation, impaired feeding capacity, starvation, debilitation, limited predator avoidance, or death (Gall & Thompson, 2015; Unger et al., 2017). The impacts caused by MPs are less known, but they are expected to be similar to those mentioned above. For example, the ingestion of microplastics can disrupt physiological processes in marine worms, compromising their ability to store energy (Wright et al., 2013).

Moreover, MPs readily accumulate harmful chemicals such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) or heavy metals, increasing their concentration by orders of magnitude. This process is reversible, and these pollutants can be released upon ingestion, causing substantial damage in different organisms (Rochman et al., 2014). Regarding freshwater ecosystems, recent studies by Ehlers et al. (2019, 2020) found that MPs can be used by different species of caddisflies, which incorporate these particles into their cases. The respective caddisfly larvae build portable cases in which they develop during their larval stage.

The concentration of microplastics in continental waters is highly variable, to the point that only the 20 most polluted rivers in the world transport 67% of the plastic debris that reaches the oceans each year (Lebreton et al., 2017). Therefore, studies of presence and distribution of MPs in continental waters can help to understand the dynamics of these pollutants in natural ecosystems.

The Galician economy of the study area is closely related to seafood (*Salmo trutta* and *Salmo spec.*) production. Therefore, more research efforts should be focused on MP in freshwater environments and potential *Salmo* prey since MPs were found in several species of commercial interest (Barboza et al., 2018).

Methodology

Study area

To carry out this study, the Gafos River was selected because it is the urban river that crosses the Pontevedra province (Galicia, Spain). This river is included in the Galicia-Costa River basin district. Moreover, it is classified as the Cantabrian -Atlantic coastal river, comprised in the typology 30 (R-T30) (MAGRAMA, 2015). Gafos river flows approximately 10 km until its mouth in the Pontevedra's sea inlet, forming a basin with 25.57 km² area that supports a population of around 69,000 inhabitants.

Sample collection

In summer 2020, a sampling campaign was conducted, and water, sediment and biota samples were collected at G1, G2 and G3 sampling points (Fig. 1). Benthic macroinvertebrate samples were taken according to the methodology established by the Water Framework Directive

(2000/60/EC) (European Communities, 2000). An aquatic entomological net was used with a mesh of 500 µm pore size to collect the macroinvertebrates. Organism samples were pooled and transported to the laboratory in glass bottles with 70% ethanol. Then, all the individuals were identified up to the family level by using a stereomicroscope and the identification keys of Tachet et al. (2002). With the obtained data, richness, IBMWP index (Iberian Biological Monitoring Working Party) (Alba-Tecedor et al., 2002), IASPT index (IBMWP value divided by the number of families) and richness were calculated to assess the ecological status of the different sampling sites.

Additionally, at each sampling point, several physical and chemical variables were measured *in situ* using a multiparameter sounding line (Hanna Instruments, Model HI 98194), including water temperature, dissolved oxygen (DO), total dissolved solids (TDS), pH and electrical conductivity (EC). Moreover, water samples were collected in glass bottles to analyse the content of nitrate, nitrite, sulphate, COD (chemical oxygen demand) and TOC (total organic carbon).

For the study of microplastics, three different samples were collected. First, to look for microplastics in the sediments, two methodologies were developed. On the one hand, the sediment from the bed surface that contained the benthic macroinvertebrates (S1) was examined. All the items visually identified as plastic were manually collected and placed into Petri dishes. On the other hand, at each sampling point, two samples of sediment were collected from the shoreline of the rivers with stainless steel rings of 100 cm³ and 5 cm depth (S2). Finally, for searching microplastics in the water column, the methodology developed by the Libera project (LIBERA, 2020) was followed. In each sampling point, 4L of water was filtered, 2L of water from the surface and 2L of water from the river's bottom. To filter water samples a manual vacuum pump and nytal-nytex filters Whatman grade 4 with 20 µm pore size were used. Immediately afterwards, filters were stored in clean glass containers and transported to the laboratory.

Contamination control

Based on Ehlers et al., (2022), besides cleaning all the field material like the hand net and sample jars, and all glassware with ultrapure water, we later cleaned the hand net with hypochlorite and then with ultrapure water between each sample site to avoid cross-contamination. We ran blanks to control for contamination of the filtered water. To prevent contamination from synthetic clothing, we wore white cotton lab coats and blue nitrile gloves during sample processing. We did not detect any fibers in the blanks for benthos, for sediments, or for water in samples. However, blue and green fibers were detected in the water column and in the benthos samples.

Water and sediments processing

In the laboratory, water and sediment samples were analyzed following and adapting the NOAA laboratory methods to analyze microplastics in water and river bed samples (Masura et al., 2015; Rodrigues et al., 2018). For water samples, filters were subjected to wet peroxide oxidation. Hydrogen peroxide (30%) was added to each filter to remove organic matter. The process was repeated until all the organic matter was removed. Then, the filter content was resuspended and filtered again. The sample was passed through vacuum filtration (0.45 µm Whatman filters), and all the suspected microplastic materials were deposited on the filter membranes.

On the other side, the wet sediments from each sampling point were first homogenized. After stirring, three 100 g sub-samples (wet weight) were created from each sampling point and dried at 40°C for 48 h to determine sediment dry weight. The microplastic debris extraction was done following the methodology described by Beibei et al. (2021). Dried sub-samples were placed in 250 mL glass beakers, and then 50 mL of hydrogen peroxide (30%) was added to remove organic matter. This step was repeated until all the organic matter was removed. The room temperature of the laboratory was 20°C. Then, sub-samples were run through a stacked series of metal sieves (5 and 0.055 mm) with Milli-Q® water, considering only particles with a size < 5mm and > 0.055 mm. Then, a zinc chloride solution (ZnCl₂; density 1.7 g cm⁻³) was added to separate the remaining high-density mineral fraction from the light fraction, in which microplastics are found. The sub-samples with MPs were again added into 250 mL beakers with 50 mL of ZnCl₂. Beaker contents were stirred with a magnetic stirrer for 15 min and then samples were kept in an ultrasonic bath for 2 min. Finally, after density separation, the subsample solution was left to settle for at least 2 hours, and the supernatant was filtered, just like with water samples. Filters were dried in an oven at 40° for 24 hours before microplastic identification.

For MP identification, a stereomicroscope was used. Following the methodology used by Rodrigues et al. (2018), microplastics were classified as fragments, fibers, spheres, sponges and films. In addition, the color of each microplastic particle was noted.

Presence of MPs in fauna

During the macroinvertebrate identification in the lab, using the stereomicroscope, we detected using the stereomicroscope that in some Odonata bodies (Gomphidae) and Trichoptera cases (Leptoceridae and Limnephilidae) there were incorporated fragments of MPs. The fragments presensent in the bodies or in the cases were of different colors and composition, we detected using the visual identification. We removed those pieces from the organisms using tweezers, labelled them and kept them in Petri dishes. Later these pieces were analyzed in the lab where we conducted FTIR analysis for obtaining more information on polymer types.

FTIR analysis

We conducted all FTIR measurements with a Nicolet 6700 ATR with a diamond crystal Smart Orbit. The spectral resolution was 4 cm⁻¹ and measurements were conducted in a wavenumber range of 4000–400 cm⁻¹.

Results

Water quality and macroinvertebrate community

Figure 2 shows the abundance of the different identified taxa. In total, 566 individuals were captured between the three sampling points. Independent of the site, insects were the most abundant group. In G1, specimens belonging to Diptera were the most abundant order, followed by Odonata and Coleoptera. Nevertheless, in the second sampling point (G2), Trichoptera were the most abundant individuals, followed by Coleoptera and, to a lesser extent, molluscs. Dipterans were again the most abundant group in the last sampling point, followed by Mollusca.

Table 1 shows the result of the Indexes calculated to assess the water quality. IBMWP index indicates that the Gafos River in sites G1 and G2 is in a good ecological state. In fact, in the high and medium course, Gafos River reached the top water quality category (Good/Moderate). Also, in these points, the water has a good chemical quality (Table 2).

Table 1
Value of the indexes calculated to evaluate the ecological status of water at the different sampling points in Gafos river.

Site	Richness	IBMWP	RSS	IASPT	Ecological Status
G1	26	146	0.65	5.6	Good/Moderate
G2	28	168	0.75	6.0	Good/Moderate
G3	14	62	0.28	4.4	Poor/Bad

Table 2
Chemical characteristics of water in each sampling points.

Site	pH	EC (µS/cm)	DO (mg/L)	Temperature (°C)	TDS (mg/L)	Chloride (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)	Sulphate (mg/L)	DQO (mgO ₂ /L)	TOC (mg/L)
G1	5.9	188	18.8	17.3	94	15.45	<0.05	4.98	<0.05	6.92	4.0	1.82
G2	6.0	224	13.4	18.8	112	15.35	<0.05	5.42	<0.05	8.32	0.8	2.16
G3	7.1	487	6.6	18.8	142	15.7	<0.05	4.99	<0.05	7.05	13.6	2.55

Nevertheless, after crossing the city of Pontevedra, the index value decreased to the point that, close to the mouth (G3), water quality was classified as poor/bad. In the same vein as the IBMWP, the richness index reached the highest value at sampling points G1 and G2. The poor water quality in G3 is also reflected by a poor diversity, shown by the low number of different taxa. Finally, IASPT also supports the results shown by the IBMWP index.

Microplastics in the sediment from the bed surface sediments

During the processing of the macroinvertebrate samples, 34 particles were visually identified as plastic debris. Figure 3 shows the abundance of the different polymers found in the Gafos River. Polyester (PEST) was the most abundant polymer independent of the sampling site, followed by Polyethylene (PE) and Polyethylenerephthalate (PET; which is a polyester) in G1 and G2. For differentiating PET from other PEST we observed peaks at the wavenumbers 1722, 1597, 1251, 1115, 1066, and specifically the peaks among 738 and 704 cm⁻¹(Pandey et al, 2016). For PET the wave number range found was 1714, 1241, 1095, 873 and 724 cm⁻¹ (Pereira et al, 2017). Also, the wavenumbers 1712, 1409, 1340,1244, 1095, 1017, 872, 847 and 722 cm⁻¹ show the presence of polyethylene terephthalate (PET) (Jung et al. 2018).

At the G3 sampling point, Polyvinyl chloride (PVC) and Polypropylene (PP) were more abundant than at the other points. For characterizing PVC, the wave number range that it is analyzed is the rank between 600-750 cm⁻¹ (D’Amelia et al, 2016). For the G3 sampling the results showed that the values were in that rank.

Microplastics in the water column and S2 sediments

Figure 4 shows the MPs extracted from sediments (S2) and water samples. On the one hand, in sediment samples 28 MPs were found: 19 in G2 point and 9 in G3. No microplastic was found at point G1. On the other hand, in water samples, MPs were detected at all three studied points. G2 was the point with the highest abundance of MPs (19), followed by G1 (12) and G3 (2) to a lesser extent. All the microplastics collected in this study were fibers and fragments of 4 different colors (Fig. 5).

Fibers were more abundant than fragments in all studied samples, with the exception of sediments in G2. Moreover, the most frequent color was blue, followed by black and red.

Microplastics in the Gomphidae body and Trichoptera cases

The polymers found in Gomphus were ethylene-vinyl- acetate and polyesterurethane particles with the shape of balls. In the Trichoptera cases the polyethylene was found as a small piece of 50 mm.

Figure 6 shows the graphic with the wavenumbers of the FTIR spectra representing the presence of PEST in the Odonata sample. The red arrows show the wavenumbers related to the polymeric read. For complementing this, the figure 7 shows the body of a *Gomphus* with the presence of ethylene-vinyl- acetate and polyesterurethane particles inside. In addition, figure 8 shows the pieces of MPs present in the Trichoptera cases that were identified as polyethylene.

Discussion

The results that were obtained related to the water quality indicate that the Gafos river is in an excellent ecological state in G1 and G2 points. After crossing the city of Pontevedra and the industrial area located in the suburbs (G3 point), the river suffers a degradation process that reduces the water quality, assessed by the IBMWP and the IASPT indexes. This fact is also supported by the richness values calculated for each point. The highest richness values were reached in G1 and G2 (Table 1), while in G3, the diversity of taxa was much smaller than in the sites located upstream. The biodiversity in rivers is one of the most commonly used indicators for different disturbances (Strayer & Dudgeon, 2010). In this study, as can be seen in Fig. 1, the Gafos river flows mostly through agricultural areas until it reaches the city of Pontevedra. The impact of agriculture and the increase in organic pollution might explain the high abundance of chironomids found in G3 (Fig. 2), which could be a consequence of an increase in the nutrient content from these crop zones, or by the higher levels of organic matter found in G3 (Marziali et al., 2010).

The MP concentration found was similar to the findings reported by other authors at different sites worldwide (Table 3). Regarding polymer types, PE and PP are the most abundant polymers in river sediments. Previous studies support our findings (Cole et al., 2011; Dris et al., 2016; Rodrigues et al., 2018), explaining that these low-density polymers are likely to be transported longer distances, while plastics with a high density like PET or PSA would likely accumulate close to the source points (Beibei He et al., 2021).

Table 3
The microplastic concentration found in sediments and the water column in different studies including this one.

Nº	Location	Concentration	Reference
1	Hudson River, USA	0.98 particles/liter surface water	Miller et al. (2017)
2	River Thames, UK	66 particles per 100 g of dry sediment	Horton et al. (2017)
3	Shangai River, China	80.2 ± 59.4 particles per 100 g of dry sediments	Peng et al. (2018)
4	Changjiang Estuary, China	12.1 ± 0.9 particles per 100 g of dry sediment	Peng et al. (2017)
5	Ciwalengke River, Indonesia	5.85 ± 3.28 particles per liter	Chaya Alam et al. (2019)
6	Ciwalengke River, Indonesia	3.03 ± 1.59 particles per 100 g of dry sediment	Chaya Alam et al. (2019)
7	Antigua River, Portugal	18-629 particles per kg of dry sediment	Rodrigues et al. (2018)
8	Gafos river, Spain	0-83.27 particles per kg of dry sediment	This study
9	Gafos river, Spain	0-4.75 particles per liter	This study

Polyester and, more specifically, PET fibers represent the most significant volume of synthetic fibers produced worldwide. These materials are produced in higher quantities than any other natural or synthetic fiber (Jaffe et al., 2020). Moreover, PET is one of the most frequently produced thermoplastics, and their primary use is for manufacturing single-use products like beverage bottles, plastic food packaging films, or containers. Although waste-derived materials can be recycled, less than half of the total products are recovered, and the remaining accumulate in natural environments (Kawai et al., 2020). The more urbanized area around Gafos would explain that the most abundant polymer in the Gafos river was PEST, and more specifically PET (Fig. 3).

Regarding the morphology of the particles, independent from the sampling point, fibers were the most abundant type, similar to the findings by Rodrigues et al. (2018) and Hübner et al. (2020). These pollutants are released during the washing process. If domestic water treatment is not sufficient in wastewater treatment plants, fibers can reach rivers, where they then end up in the water column (Fig. 4) (Browne et al., 2011). Another main entry route of microplastics into freshwater ecosystems is the runoff produced in riverbanks, which accumulates the garbage in the banks that later will be carried into the river. For instance, Rodrigues et al. (2018) found higher microplastic levels in the Antigua river's sediments

after rainfall and wind periods due to the intense surface runoff. It is crucial to analyze if there are differences in microplastic levels among dry seasons and wet seasons in future studies.

Regarding the colors of the different particles, blue microplastics were the most frequent, followed by black and red. These results are in line with the findings of other studies like Zhang et al. (2019) and Yan et al. (2019). Nevertheless, there is evidence that the ingestion is not the only problem associated with the presence of MPs in aquatic environments (Ehlers et al. (2019)) found MP particles in caddisfly cases, and their results showed that the microplastic particles incorporated into the caddisfly cases also showed a wide spectrum of colors.

In this study, MPs were also detected in the cases of different trichopteran and in the bodies of odonatan species (Fig. 6-8). This is one of the first times that the presence of microplastics in the biological construction of freshwater organisms has been shown (Ehlers et al. (2019); Gallitelli et al. (2020) and Gallitelli et al. (2021)). Microplastics can act as a vector for persistent organic pollutants and toxic leachates. When these plastic particles are incorporated into the cases, the proximity to the larval body may be harmful for caddisfly larvae (Ehlers et al., 2019). Another recent study showed that PVC and PET particle content in the cases might threaten caddisflies by destabilizing these cases. This change in the stability of the cases reduces the protective function of the cases and presumably increases the animals' predation risk (Ehlers et al., 2020). Moreover, as plastic is lighter than sand, larvae may be positively buoyant and could be carried away by currents. Both effects could limit the survival of caddisflies, which are critical primary consumers in aquatic ecosystems (Ehlers et al., 2020).

Microplastics in freshwater fauna, either as part of biological structures or inside the digestive tract upon ingestion, represent a risk for global freshwater ecosystems (Kumar et al, 2021). There are many studies focusing on MP transport through the food web in marine environments (Horn et al., 2019; Schmid et al., 2021). However, such studies in freshwater ecosystems are mostly lacking. Nevertheless, some authors have studied the transport of microplastics through the aquatic food web. D'Souza et al. (2019) found high microplastic concentrations in the fecal and regurgitated pellets from Eurasian dippers (*Cinclus cinclus*). This study found that plastics were present in both adult and nestling diets, showing trophic transfer of plastics from invertebrate prey to apex predators. Furthermore, this study demonstrated the intergenerational transport of plastics in prey provisioned from parents to nest-bound offspring.

The bioaccumulation and biomagnification processes of microplastics through the food web are well known (Miller et al, 2020; Carbery et al., 2018). As part of the food web, humans are also affected by these pollutants since we represent the upper apical predator. The uptake of plastic particles by humans occurs through terrestrial and aquatic food products, drinking water, and inhalation (Carbery et al., 2018; Vethaak and Lestie, 2016). For example, seafood may be contaminated with microplastics through ingestion of natural prey or adherence to the organism's surface (Kolandhasamy et al. 2018). The risk is more prominent with animals eaten in whole. For instance, Van Cauwenberghe and Janssen (2014) showed that the blue mussel *Mytilus edulis* contained on average 0.36 ± 0.07 MP particles g^{-1} . Some studies have demonstrated the translocation of polystyrene and PVC particles from the gut cavity to the lymph and circulatory system of the marine mussel, *Mytilus edulis* (L.). Tiny particles (<30 nm) have the potential to cross cell membranes, the blood-brain barrier, and the placenta, with adverse effects like oxidative stress, cell damage, inflammation, and impairment of energy allocation (Hussain et al, 2001).

Conclusions

Microplastics were detected in water, sediments and organisms of a small river in northwestern Spain and their concentration increased as human activity increased.

The examined caddisflies can incorporate microplastics into their cases during the larval stage. Hence, these animals could be used as bioindicators for microplastic pollution (Ehlers et al., 2019).

On the other hand, the ecological quality indices of rivers do not provide information on the presence of microplastics. Therefore, future research should consider the possible inclusion of these emerging pollutants in the indexes of freshwater quality.

More studies are needed to establish a standardized methodology for studying microplastics in freshwater ecosystems. We conclude that based on the limited number of high-quality studies identified, standardization of microplastic analysis in aquatic fauna is needed, as we found in caddisfly larval cases and other groups (Odonata), these groups might have the potential to be used as bioindicators. Despite the quality limitations, our analysis confirmed that microplastic is frequently present in freshwaters aquatic invertebrates. More studies are needed to better understand selection, occurrence, shape, polymer types, and particle sizes, particularly for the small plastic particles inside the macroinvertebrates and as part of the benthos.

Declarations

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All authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the University of Vigo (Spain) and University of Koblenz-Landau, Koblenz, Germany, in where the work has been carried out, before the work is submitted.

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Romina Álvarez-Troncoso, David Gutiérrez, Iria Villar, Sonja. M. Ehlers, Benedicto Soto, Salustiano Mato and Josefina Garrido. The first draft of the manuscript was written by Romina Álvarez-Troncoso and David Gutiérrez and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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Figures

Figure 1
Watershed of Gafos river and sampling points (G1, G2 and G3).

Figure 2
Abundance of the different macroinvertebrate taxa captured in each sampling point.

Figure 3

Abundance of the different polymers found in sediments from bed surface (S1) in G1, G2 and G3 sampling points.

Figure 4

Abundance of microplastics (MPs) according to morphology and color found in water and sediments from each sampling point.

Figure 5

Different microplastic typologies in Gafos river were identified under a stereoscopic microscope: fiber on the left and blue fragment on the right.

Figure 6

FTIR spectra representation of the presence of polyester (PEST) in the Odonata sample. The red arrows show the wavenumbers related with the polymeric read.

Figure 7

Gomphus, from the Gomphidae family. It has fragments of ethylene-vinyl- acetate and polyesterurethane incorporated inside its body. The microplastics were extracted using tweezers.

Figure 8

Two Trichoptera (Lepidostomatidae on the left and Limnephilidae on the right) cases that have incorporated fragments of polyethylene for their construction.