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Microplastics in water, sediments and macroinvertebrates in a small river of NW Spain

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ABSTRACT

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Microplastics (MPs; plastic particles < 5 mm in size) are very common nowadays and ubiquitous in the environment and can cause harm to aquatic organisms. Around 300 million metric tonnes of plastic are manufactured each year and they are regularly mismanaged. Therefore, MPs are frequently found in the environment. Anthropogenic activity in urban areas is considered one of the major sources of MPs. In view of this, we hypothesized, that MPs are present in all areas of rivers, even in riverheads. We analyzed macroinvertebrates in an urban river for MPs and discuss their potential environmental impact. We collected water samples from the centre of the river and filtered the water. Additionally, we collected sediment samples from the the bottom (S1 and S2, both samples collected specifically for sediments analysis) and from the benthic sediment (S3, sample collected for macroinvertebrates identification) from which macroinvertebrates were sampled for MP analysis and for taxonomic identification in order to estimate the water quality of the river, following the protocol for calculating the IBMWP (MAGRAMA, 2011). Sampling took place in the Gafos River (NW Spain) during summer 2020 at three different sampling sites at the head of the river called upstream (G1), in the middle part of the river and upstream of a town (G2) and in the downstream area before the river mouth in the Atlantic Ocean (G3). Different microplastic fibres and particles were found in all water samples (G1, G2 and G3) and in some of the sediment samples. Analysis of the different types of microplastics was carried out by Fourier-transform infrared spectroscopy in attenuated total reflectance mode (ATR- FTIR). The majority of microplastics in water, sediment and biota consisted of polyvinyl chloride (PVC) and polyethylene terephthalate (PET) and the abundances of MPs were very similar. We found more MP particles in biota and water (35 pieces in each compartment) than in sediments (28). Microplastics were found in the cases of Trichoptera families like Lepidostomatidae and Limnephilidae, and inside the body of some Odonata families such as Gomphidae. This confirms the presence of MPs in aquatic organisms and in habitats of an urban river in Spain. Since MPs have been found in freshwater habitats globally, future studies should analyse which macroinvertebrates could be used as MP bioindicators.

Key words: synthetic polymer, freshwater insects, microplastics, plastic pollution, Trichoptera, Odonata, sediment, urban river

RESUMEN

Microplásticos en agua, sedimentos y macroinvertebrados en un pequeño río del noroeste de España

Los microplásticos (partículas de plástico de tamaño < 5 mm, MPs) son muy comunes hoy en día y omnipresentes en el medio ambiente y pueden causar daños a los organismos acuáticos. Como cada año se fabrican unos 300 millones de toneladas métricas de plásticos y se gestionan mal, los MPs también son muy frecuentes. Nuestra hipótesis es que, debido a la alta densidad de actividad antrópica en las zonas urbanas, el medio ambiente urbano se considera como una de las principales fuentes de microplásticos (MP), estas partículas podrían ser fácilmente transferidas a las zonas ribereñas a lo largo del río y a través de *las redes alimentarias del hábitat a los organismos acuáticos. Por esta razón, se planteó la hipótesis de que los microplásticos podrían estar presentes en todas las zonas de los ríos, incluso en las cabeceras. Definimos un estudio para encontrar posibles MPs en macroinvertebrados en un río urbano y conocer su posible impacto y evaluar la presencia en la comunidad acuática. Organizamos un muestreo en la parte central del cauce del río para recoger y filtrar agua, recoger sedimentos de la orilla superficial del río y muestrear el bentos para recoger sedimentos y macroinvertebrados siguiendo el protocolo de cálculo del IBMWP (MAGRAMA, 2011) en el río Gafos (NO de España) durante el verano de 2020 en tres puntos de muestreo diferentes: en la cabecera del río llamada aguas arriba (G1), en la parte media del río y aguas arriba de una ciudad (G2) y en la zona aguas abajo antes de la desembocadura del río en el Océano Atlántico (G3). Se encontraron diferentes fibras y partículas microplásticas en todas las muestras de agua (G1, G2 y G3) y en algunas de las muestras de sedimentos. El análisis de los distintos tipos de microplásticos se llevó a cabo mediante espectroscopia infrarroja con transformada de Fourier (FTIR-ATR). La mayoría de los microplásticos encontrados en el agua, los sedimentos y la biota eran policloruro de vinilo (PVC) y tereftalato de polietileno (PET) y la cantidad de MPs era muy similar. Encontramos más partículas de MPs en la biota y el agua (35 unidades en cada compartimento) que en los sedimentos (28). Se encontraron microplásticos en los estuches de familias de Trichoptera como Lepidostomatidae y Limnephilidae, y en el interior del cuerpo de algunas familias de Odonata como Gomphidae. Esto confirma la presencia de MPs en los organismos acuáticos y en los hábitats en un río urbano, sin embargo, hay otros macroinvertebrados como bioindicadores de MPs que deben ser investigados más a fondo en diferentes ecosistemas de agua dulce en todo el mundo.*

Palabras clave: *polímero sintético, insectos de agua dulce, microplásticos, contaminación plástica, Trichoptera, Odonata, sedimento, río atlántico*

INTRODUCTION

Plastics are man-made synthetic materials comprising a wide range of polymers such as polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC) and polyamide (PA), that are moulded into shape while soft, and then set into a rigid or slightly elastic form. Their features (e.g., durability, light weight, low price) make them one of the most demanded products in our society (Herrera et al., 2018). Since the 1950s, plastic production has exponentially increased, and nowadays, around 300 million metric tonnes of this material are manufactured worldwide each year (Scherer et al., 2017; Statista, 2021). Despite the multiple advantages that plastic offers, besides the massive economic and social benefits that we can obtain from these products, unsuitable resource management has led to waste accumulation in natural ecosystems (Barnes et al., 2009). Plastic particles with a size below 5 mm are called microplastics (MPs), and can be classified into two groups (Herrera et al., 2018): MP 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), and secondary MPs arising from the fragmentation and degradation of plastic items (Lambert & Wagner, 2018).

Similar to other persistent pollutants, MPs can be found globally. Their presence was first reported in the ocean in the 1970s (Carpenter & Smith, 1972), and, research efforts on MP pollution have been oriented towards marine environments (Rochman, 2018). In recent years, this trend has changed, and some studies have shown the presence of these pollutants in freshwater, terrestrial ecosystems and other habitats (Wagner et al., 2014; Sà et al., 2018; D'Souza et al., 2020; O'Connor et al., 2022; Rillig & Lehmann, 2020).

In the fisheries and aquaculture sector, it is considered that abandoned, lost or otherwise discarded fishing gear (ALDFG) is the main source of plastic waste in the marine environment (Lusher et al., 2017), and sources of MPs in continental waters are for example, effluents from urban wastewater treatment plants and wastewater from domestic washing machines (Waldschläger et al., 2020). Due to their small size, MPs can be ingested directly or indirectly by a variety of animals and can be incorporated and accumulated adjacent to urban centres, and transferred through food webs from the habitat to aquatic organisms (Barnes et al., 2009; Setälä et al., 2014). The impacts caused by MPs in freshwater ecosystems are not well understood, but they are expected to be similar to the effects in marine habitats. For example, the ingestion of MPs can disrupt physiological processes in marine worms, compromising their ability to store energy (Wright et al., 2013). Once ingested, MPs can affect aquatic organisms in various ways. MPs are harmful as they accumulate contaminants. Furthermore, MPs can be found in the digestive tract of a variety of aquatic organisms, such as zooplankton, mollusks, crustacea, fish, seabirds and marine mammals (Kumar et al., 2021) where they inhibit nutrient absorption and reduce the consumption of resources. As a result, MPs are limiting growth, reproduction and survival. However, the toxicological effects of ingesting nano- and micro-plastics present in sea food products are still controversial and cannot be assessed with the current level of knowledge (Barboza et al., 2018). Other xenobiotic pollutants adsorb onto the MP surface, thus providing pathways for secondary toxicity (Windsor et al., 2019). MPs may 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). There is also evidence that contaminants can be transferred throughout the food web (D'Souza et al., 2020; O'Connor et al., 2022). Regarding freshwater ecosystems, recent studies by Ehlers et al. (2019, 2020) found that MPs can be used as building materials by caddisfly larvae, which incorporate these particles into their cases. Other studies from Stanković et al., (2021) show that Mollusca, Oligochaeta, and Chironomidae like *Limnodrilus hoffmeisteri*, *Lithoglyphus naticoides* and *Chironomus acutiventris* could bioaccumulate MPs in the Danube River.

As part of the food web, humans might be directly or indirectly affected by MPs since we represent the upper ultimate predator. The uptake of plastic particles by humans occurs through terrestrial and aquatic food products, drinking water and inhalation (Carbery et al., 2018). 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 of consuming MPs is more prominent with animals eaten whole. By way of an example, Van Cauwenberghe and Janssen (2014) showed that the blue mussel *Mytilus edulis* contained, on average, 0.36 ± 0.07 MP particles g^{-1}. Globally, humans may ingest an average of 0.1–5 g/week of MPs up to 1 mm in size, or 74 000–121 000 particles per year (Cox et al., 2019). 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).

The concentration of MPs 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 examining the presence and distribution of MPs in continental waters can help to understand the dynamics of these pollutants in natural ecosystems. The aim of this study is to quantify MPs in a small urban river located in Spain, to describe their appearance and composition, and to assess the presence of MPs in macroinvertebrates.

METHODOLOGY

Study area

The Gafos River is a small urban river that crosses the town of Pontevedra (Galicia, Spain). It flows approximately 10 km down towards its mouth in the Pontevedra sea inlet, forming a basin with an area of 25.57 km^2 that supports a population of around 83 100 inhabitants (INE, 2021). The Gafos River runs through a mixture of anthropic environments, agricultural, forest and semi-natural land use areas (Fig. 1). This river belongs to the Galicia-Costa River basin district. Moreover, it is classified as a Cantabrian-Atlantic coastal river, in typology 30 (R-T30) (MAGRAMA, 2015). This typology of rivers (R-T30) includes short rivers, with steep slopes and high erosive power. The largest basins slightly exceed 1000 km^2 and 20 m^3/s of mean daily flow, with highly variable valley widths that rarely exceed 1.5 km in most of the middle and upper courses. This area has a humid oceanic temperate climate with an average annual temperature of 13.5 ºC and an average annual precipitation of 1500 mm. We chose this river because it is mostly located in the town of Pontevedra, the capital of the province. Gafos has a small basin, which is 10.6 km in length where the urban areas are surrounding almost the entire basin. Three different sampling sites were defined: one at the head of the river called upstream (G1, 42.3807625652687, -8.647587870321857), in the middle part of the river and upstream of a town (G2, 42.4172711079722, -8.635745131547248) and in the downstream area before the river mouth in the Atlantic Ocean (G3, 42.4262693927145, -8.642527190881742).

Sample collection

In summer 2020, a sampling campaign was conducted, and water (one sample for analysis of nutrients and another sample for MPs identification) and sediment (one sample subdivided into two subsamples: S1 and S2) for MPs study and biota samples (one sample of 20 kicks at available habitats in each site) for biodiversity and water quality study, were collected at upstream (G1), middle part (G2) and downstream (G3) of the Gafos River sampling sites (Fig. 1). Furthermore, the sample (S3) was collected for macroinvertebrates identification and later, those sediments were analysed for microplastics. Late spring and summer are the seasons when the biodiversity of aquatic macroinvertebrates in this type of river in the North of Spain is high, due to food availability and the presence of macrophytes, which are refuge for the fauna (Martínez et al., 2020). Benthic

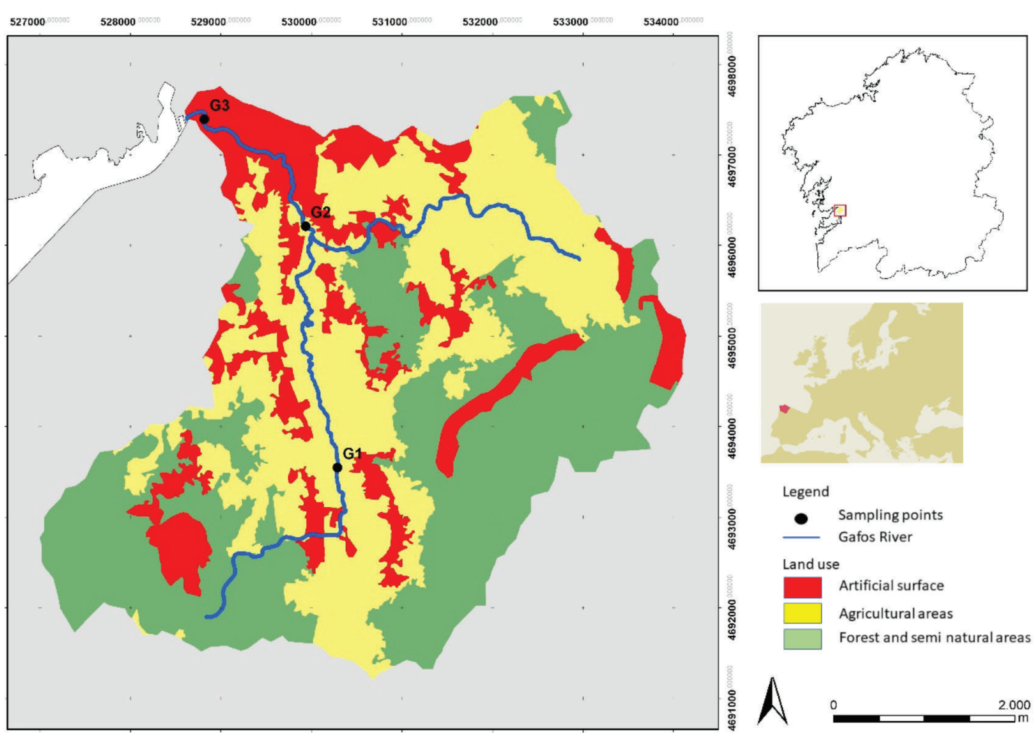

Figure 1. The Gafos River and sampling sites (G1, G2 and G3), showing the different land uses in the basin. In red the artificial surfaces, in yellow the agricultural areas and in green the forest and semi natural areas. *El río Gafos y los puntos de muestreo (G1, G2 y G3), mostrando los diferentes usos del suelo en la cuenca. En rojo las superficies artificiales, en amarillo las zonas agrícolas y en verde las zonas forestales y seminaturales.*

macroinvertebrates were sampled according to the methodology established by the Water Framework Directive (2000/60/EC) (Council of the European Communities, 2000) using a kick net with a mesh size of 500 µm in different habitats as running freshwater, macrophytes, mosses, stones in current, lotic stones, roots, woody debris, etc. 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 using a stereomicroscope and the identification key of Tachet et al. (2002). With the data obtained, the IBMWP index (Iberian Biological Monitoring Working Party) (Alba-Tecedor et al., 2002), the 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 site, several physical and chemical variables were measured *in situ* using a multiparameter probe (Hanna Instruments, Model HI 98194), including water temperature, dissolved oxygen (DO), total dissolved solids (TDS), pH and Electrical Conductivity (EC). Moreover, water samples (4L) were collected in glass bottles for analysing the content of nitrates, nitrites, sulphates, COD (Chemical Oxygen Demand) and TOC (Total Organic Carbon). The sediments were sampled in the selected sampling area.

For the study of MPs, water, sediments and macroinvertebrates were analysed. Sediments were collected using the methodologies of Rodrigues et al. (2018) and Masura et al. (2015). On the one hand, two subsamples of 100 g each (S1 and S2) of the sediment from the riverbed surface (benthic substrate) were taken specifically for the MPs analysis. On the other hand, we also checked for the possible presence of MPs in the sediments from the benthic substrate of the benthic macroinvertebrates sample (S3). For the macroinvertebrates, we sampled the area of the 20 kicks (as indicated in the sampling protocol for macroinvertebrates) that included different microhabitats throughout the stretch of the river (100 m), which covers an area of 2.5 m^2 (the area of the net for each kick is 0.125 m^2.) All the particles for polymer type analysis from the sediments (S3) from the macroinvertebrates' samples were manually collected and placed onto Petri dishes. Furthermore, the particles that resembled microplastics in the sediment samples (S1 and S2) were sorted out at the lab and polymer types were analysed. For assessing MPs in water, the methodology developed by the Libera project (LIBERA, 2020) was followed. At each sampling site, 4L of water were collected from the central area of the river basin using glass jars and all the volume was filtered, 2L of water from the surface and 2L of water from the riverbed. To filter water samples, a manual vacuum pump and Whatman grade 4 nytal-nytex filters with 20 µm pore size were used. Immediately afterwards, filters were stored in clean glass containers and transported to the laboratory to check for the presence of MPs.

Contamination control

The prevention of cross-contamination is essential in MP research. Cross-contamination can lead to overestimation of microplastic concentrations (Bogdanowicz et al., 2021). Cross-contamination was controlled using the sampling procedure described by Ehlers et al. (2022). Besides cleaning all the field material like the hand net and sample jars, and all glassware with ultrapure water, the hand net was cleaned with hypochlorite and then with ultrapure water between sites to avoid cross-contamination. Blanks to control for contamination of the filtered water were run. Distilled water was filtered in the lab in the same filters and compare to the filter with the water filtered from the river. To prevent contamination from synthetic clothing, white cotton lab coats and blue nitrile gloves were worn during sample processing. No fibres were detected in the blanks for benthos, sediments or for water samples.

Water and sediment processing

At the laboratory, we analysed the water and sediment samples for MPs following and adapting the NOAA laboratory methods (Masura et al., 2015; Rodrigues et al., 2018).

The sediment samples S1 and S2 were first homogenized. After stirring, three 100 g sub-samples (wet weight) were created from each sampling site and dried at 40 ºC for 48 h to determine sediment dry weight. MP extraction was conducted following the methodology described by He 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 ºC. 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. Subsequently, 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 to 250 mL beakers with 50 mL of $ZnCl_2$. 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, as with water samples. Filters (0.45 µm Whatman filters) were dried in an oven at 40 ºC for 24 hours before MP identification.

For water samples, filters were subjected to wet peroxide oxidation. Hydrogen peroxide (30 %) was added to each filter to remove organic matter, with a room temperature of 20 ºC. The process was repeated until all the organic matter was removed. Then, the filter content was resuspended and filtered again to ensure that there were no other organic particles present. The sample was passed through vacuum filtration (0.45 µm Whatman filters), and all the possible MP materials were deposited on the filter membranes.

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

Presence of MPs in fauna

We identified the macroinvertebrates in the lab using the stereomicroscope, and we found MPs in some Odonata bodies (Gomphidae) and Trichoptera cases (Leptoceridae and Limnephilidae). These fragments had different colours, and they were detected using visual identification. The MPs were removed from the organisms using tweezers, labeled, and kept in Petri dishes. Afterwards, these pieces were analyzed in the lab where FTIR analysis was conducted to obtain information on polymer types. The small particles from the water and sediments, which were similar to MPs (both fibres and fragments), were not analysed with FTIR because of their size.

FTIR analysis

All FTIR measurements were conducted 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}. Plastic polymer types were identified based on the wavenumbers mentioned by Jung et al. (2018) and Käppler et al. (2015).

RESULTS

In total, 566 individuals were captured at the three sampling sites. Figure 2 shows the abundance of the different identified taxa where Diptera was the most abundant taxon in G1 (33 %) and G3 (53 %), and in G2 Trichoptera (27 %) and Coleoptera (22 %). Odonata was also very abundant in G1 (19 %) and Coleoptera in G2 (22 %). Independent of the site, insects were the most abundant

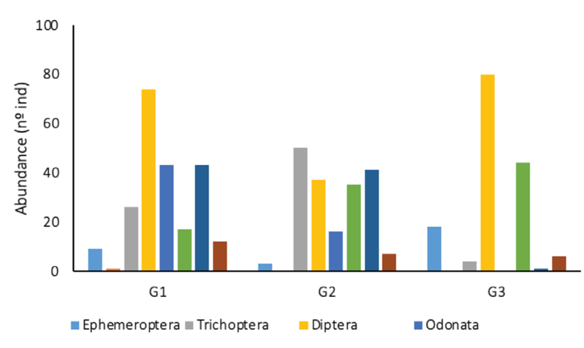

Figure 2. Abundance of the different macroinvertebrate taxa captured at each sampling site (G1, G2 and G3). *Abundancia de los diferentes taxones de macroinvertebrados capturados en cada punto de muestreo (G1, G2 y G3).*

Table 1. Chemical characteristics of water at each sampling site and ecological status assessment based on IBMWP calculation. *Características químicas del agua en cada punto de muestreo y evaluación del estado ecológico basada en el cálculo de IBMWP.*

Site	pH	EC (µS/cm)	DO (mg/L)	Temperature (ºC)	TDS (mg/L)	Chloride (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)	Sulphate (mg/L)	CDO (mgO_2/L)	TOC (mg/L)	Richness	IBMWP	RSS	IASPT	Ecological Status
G1	5.9	188	18.8	17.3	94	15.45	<0.05	4.98	<0.05	6.92	4.0	1.82	26	146	0.65	5.6	Good/Moderate
G2	6.0	224	13.4	18.8	112	15.35	<0.05	5.42	<0.05	8.32	0.8	2.16	28	168	0.75	6.0	Good/Moderate
G3	7.1	487	6.6	18.8	142	15.7	<0.05	4.99	<0.05	7.05	13.6	2.55	14	62	0.28	4.4	Poor/Bad

group. In G1, specimens belonging to Diptera were the most abundant order, followed by Odonata and Coleoptera. In G2, Trichoptera were the most abundant individuals, followed by Coleoptera and, to a lesser extent, Mollusca. Diptera was again the most abundant group in G3, followed by Mollusca. In G3 Chironomidae, Physidae, Bythinidae and Baetidae were the most common families in the sample.

The IBMWP index indicates that the Gafos River in sites G1 and G2 has a good ecological state (Table 1). In fact, in the high and medium course, the Gafos River reached the top water quality category (Good/Moderate). In addition, at these sites, the water is in good condition according to the parameters defined in the Directive 2000/60/EC of the European Parliament and of the Council (Table 1). Nevertheless, after crossing the town of Pontevedra, the index value decreased so that, close to the mouth (G3), water quality was classified as Poor/Bad. Similar to the IBMWP, the richness index reached the highest value at sampling sites G1 and G2. The poor water quality in G3 is also reflected by a poor diversity, shown by the low number of different taxa.

During the processing of the macroinvertebrate samples from G1, G2 and G3, 34 particles were visually identified as plastic debris and the abundances of the different polymers in the Gafos River sediment from the locations where the macroinvertebrates were sampled are shown in Figure 3. Polyester (PEST) was the most abundant polymer independent of the sampling site, followed by Polyethylene (PE) and Polyethylene terephthalate (PET; which is a polyester) in G1 and G2.

On the one hand, in sediment samples 28 MPs were found (Fig. 4): 19 in G2 (14.96 MPs/m^2) site and 9 in G3 (7.08 MPs/m^2). No microplastic was found at site G1 in the two subsamples of sediments (S1 or S2). However, there are MPs in the S3, the sediments coming from the biota sample. We found 9 fragments in G1, 16 fragments

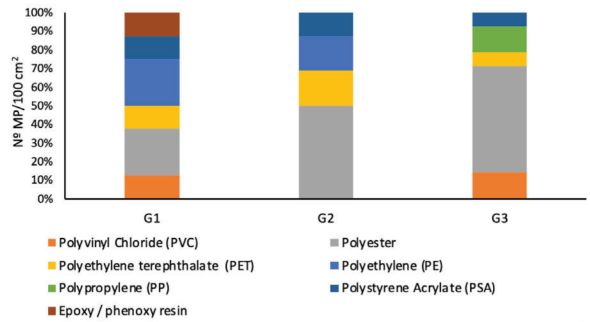

Figure 3. Relative abundance (%) of the different polymers found in sediments from bed surface (S3) in each sampling site (G1, G2 and G3). *Abundancia relativa (%) de los diferentes polímeros encontrados en los sedimentos de la superficie del lecho (S3) en cada punto de muestreo (G1, G2 y G3).*

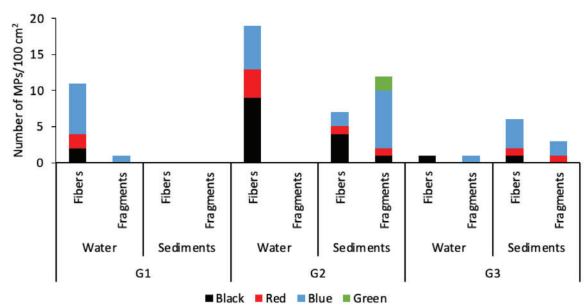

Figure 4. Abundance of microplastics (MPs) according to morphology and colour found in water and sediments from the samples S1 and S2 in each sampling site (G1, G2 and G3). *Abundancia de microplásticos (MPs) según morfología y color encontrados en agua y sedimentos de las dos submuestras S1 y S2 en cada punto de muestreo (G1, G2 y G3).*

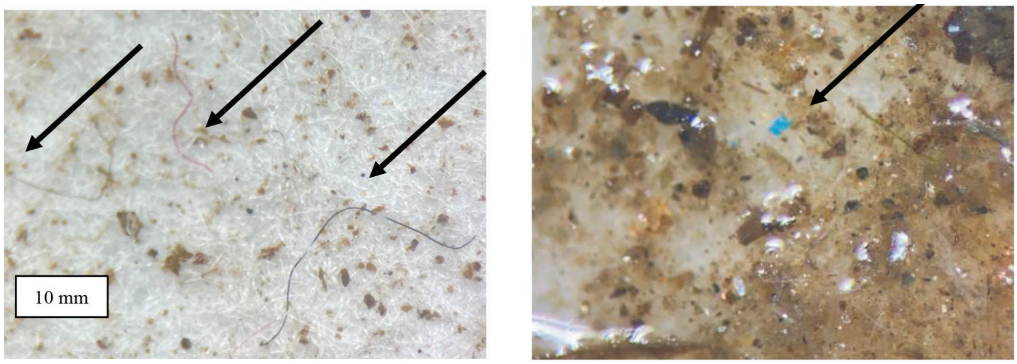

Figure 5. Different microplastic typologies in Gafos River were identified under a stereoscopic microscope. The black arrows indicate plastic items: fibre on the left and blue fragment on the right. The scale is 10 mm. *Se identificaron diferentes tipologías de microplásticos en el río Gafos bajo un microscopio estereoscópico. Las flechas negras muestran las partículas de plástico: fibra a la izquierda y fragmento azul a la derecha. La escala es de 10 mm.*

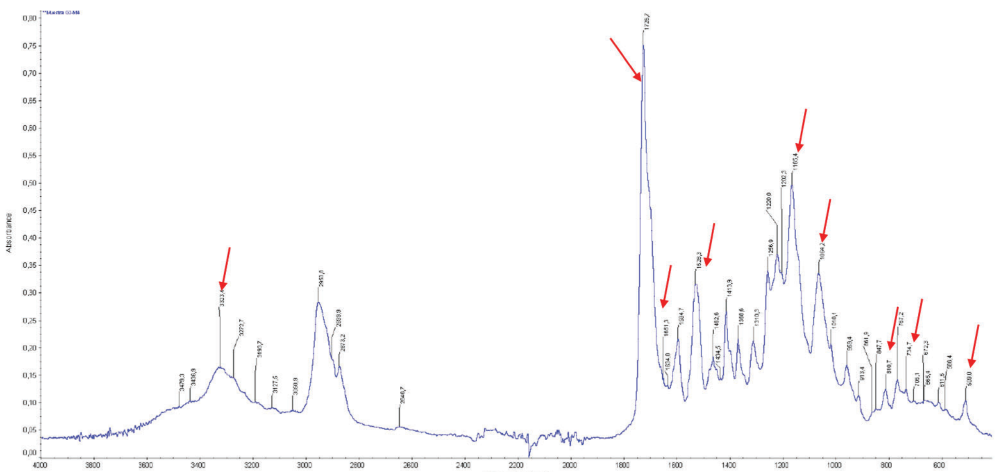

Figure 6. FTIR spectra representation of the presence of Polyester (PEST) in the Odonata sample. The red arrows show the wavenumbers related with the polymeric read. *Representación de espectros FTIR de la presencia de Poliéster (PEST) en la muestra de Odonata. Las flechas rojas muestran los números longitud de onda relacionados con la lectura polimérica.*

in G2 and also in G3. On the other hand, in water samples, MPs were detected at all three study sites. G2 was the site 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 fibres and fragments with 4 different colours (Fig. 5).

Regarding the colours of the different particles detected in water and sediments, blue MPs were the most frequent, followed by black and red (Fig. 4).

The polymers found in the samples were around 1-5 mm and had a weight of 0.012-< 0.0001 mg. We found ethylene-vinyl-acetate and polyester urethane particles in the shape of spheres in *Gomphus*. In the Trichoptera cases polyethylene was found as a small fragment of ca. 5 mm.

Figure 6 shows a FTIR spectrum of a PET MP, which was found in the Odonata sample from Aeshnidae. The red arrows show the wavenumbers related to the polymeric read. To comple-

Figure 7. Gomphus, from the Gomphidae family. It has spheres (red arrow) of ethylene-vinyl- acetate and polyester urethane incorporated inside its body. The microplastics were extracted using tweezers. The scale is 10 mm. *Gomphus, de la familia Gomphidae. Lleva incorporadas en el interior de su cuerpo esferas (marcadas con la flecha roja) de etileno-vinil-acetato y poliésteruretano. Los microplásticos se extrajeron con pinzas. La escala es de 10 mm.*

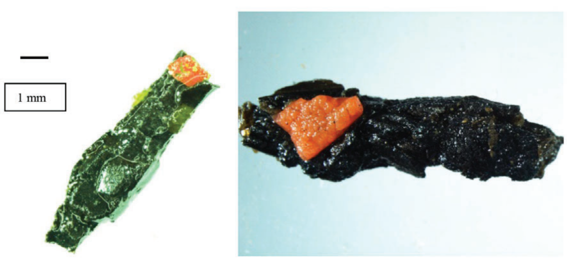

Figure 8. Two Trichoptera (Lepidostomatidae on the left and Limnephilidae on the right) cases with incorporated fragments of polyethylene. The scale is 1 mm for both figures. *Dos estuches de Trichoptera (Lepidostomatidae a la izquierda y Limnephilidae a la derecha) que han incorporado fragmentos de polietileno durante su construcción. La escala es de 1 mm para ambas imágenes.*

ment this, Fig. 7 shows a *Gomphus* (Odonata) body with particles of ethylene-vinyl-acetate and polyester urethane inside. In addition, Fig. 8 shows polyethylene MPs in the Trichoptera cases.

DISCUSSION

The water quality of the Gafos River is excellent considering its ecological status (according to EU Water Framework Directive 2000 (WFD)) both in G1 and G2 sites. The selection of sampling area for checking the presence of MPs was done by taking the heterogeneity of the river into consideration in order to avoid a possible bias. The impact of agriculture and the increase in organic pollution might explain the high abundance of chironomids found in G3. This site is located in the middle of the city and downstream of the whole river basin area (Fig. 2). This area is surrounded by crop zones that might produce the higher levels of organic matter found in G3 as other researchers found out in other rivers (Marziali et al., 2010).

Regarding the MP concentration, the value found was similar to the findings reported by other authors at different sites worldwide, especially close areas like Portugal (Table 2). As far as polymer types are concerned, PE and PP are the most abundant polymers in river sediments. However, other authors in Portugal such as Sá et al. (2022) found that polyethylene terephthalate (PET, 29 %) and polyacrylate (PAcr) (23 %) were the most common polymer types in sediments. In the study area, there are many industrial sites and companies and different settlements around the town of Pontevedra. Previous studies support our findings regarding the presence of PE and PP throughout the rivers and their distribution (Cole et al., 2011; Rodrigues et al., 2018), explaining that low-density polymers are likely to be transported longer distances, while plastics with a high density like PET or polyvinylidene fluoride (PVDF) would likely accumulate in sediments close to the source sites, which can be primarily attributed to industrial effluent or storm water runoff from the surrounding industrial areas (Nizzetto et al., 2016, He 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, fibres were the most abundant type, similar to the findings by Rodrigues et al. (2018) and Hübner et al. (2020). Our findings linked MPs composition and distribution with the accumulation of MP from various inputs throughout the course of the river, and they are very similar to the findings in a closer region in Portugal where a similar proportion of MPs was found (Sá et al., 2022).

The main entry route for MPs into Gafos River might be the runoff produced in riverbanks,

Table 2. The microplastic concentration found in sediments and in the water column in different studies including this one. *La concentración de microplásticos encontrada en sedimentos y en la columna de agua en diferentes estudios, incluido éste.*

Nº	Location	Concentration	Reference
1	Hudson River, USA	0.98 particles/litre 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 litre	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	Rhine, Koblenz	0.26 ± 0.01 to 11.07 ± 0.6 × 103 MP particles kg^{-1} in the 11–500 µm size range	Mani et al. (2019)
9	Lis River Basin	fibres 0.02 to 1111.11 $items.m^3$ in water and 10.66 to 1609.64 $items.kg^{-1}$ in sediment	Sá et al. (2022)
10	Lis River Basin	fragments 0.02 to 2311.11 $items.m^3$ in water and 10.66 to 501.01 $items.kg^{-1}$ in sediment	Sá et al. (2022)
11	Gafos River, Spain	0-83.27 particles per kg of dry sediment	This study
12	Gafos River, Spain	0-4.75 particles per litre	This study

which accumulates waste products in the banks that will later be carried into the river. For instance, Rodrigues et al. (2018) found higher MP levels in the Antigua River's sediments after rainfall and wind periods due to the intense surface runoff. It is crucial to analyse if there are differences in MP levels between dry seasons and wet seasons in future studies, as other authors suggested and presented (Sá et al., 2022) for the Lis River Basin.

Nevertheless, there is evidence that MP 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 colours. In our study, fibres were more abundant than fragments in all studied samples, with the exception of sediments in G2. However, only fragments and no fibres were incorporated into the cases or inside the bodies of *Gomphus*.

In this study, MPs were detected in the cases of different trichopterans and in the bodies of odonatan species (Fig. 6-8) for the first time in this area and this river type in Spain. Although MPs were previously detected in mineral caddisfly cases (Ehlers et al., 2019), this finding is one of the first that shows MPs in biological constructions of freshwater organisms that are made of wood and leaves. MPs can act as a vector for persistent organic pollutants and toxic leachates (Campanale et al., 2020). 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 made of stone and inorganic particles. This change in the stability of the cases probably 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).

MPs in freshwater fauna, either as part of biological structures or inside the digestive tract, are 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. (2020) 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 supplied from parents to nest-bound offspring. Our study can contribute to finding a bioindicator for detecting MP pollution in European rivers (Vitecek et al., 2021).

CONCLUSIONS

MPs were detected in water, sediments and macroinvertebrates of a small river in northwestern Spain, and our results suggest that their concentration increased as human activity and presence increased. The examined caddisflies can incorporate microplastics into their organic cases during the larval stage. Hence, Trichoptera could be used as bioindicators for microplastic pollution.

On the other hand, the ecological quality indices of rivers do not provide information on the presence of MPs. WFD indexes do not cover emerging or multiple stressors including pollution by MPs. Therefore, future research should consider the possible inclusion of these emerging pollutants into the indexes of freshwater quality and the water quality protocol for ecological assessment of waterbodies.

We conclude that there is a need to conduct studies on standardized MP analysis in aquatic fauna. Our analysis confirmed that microplastics of different sizes, polymer types and shapes are present in freshwater aquatic invertebrates, and so they might be used as MP bioindicators in freshwater ecosystems. 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. Furthermore, it should be investigated if macroinvertebrates could be used as bioindicators for microplastic pollution.

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All authors agreed with the content, and all gave explicit consent to submit and they obtained consent from the responsible authorities at the University of Vigo (Spain) where the work has been carried out, prior to being presented.

Romina Álvarez-Troncoso, David Gutiérrez, Iria Villar, Benedicto Soto, Salustiano Mato and Josefina Garrido contributed to the study conception and design. Material preparation, data collection and analysis were performed by Romina Álvarez-Troncoso, David Gutiérrez, Iria Villar, Benedicto Soto, Salustiano Mato and Josefina Garrido. Sonja M. Ehlers assessed the microplastics extraction techniques and assisted with the preparation of this paper.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENTS

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

REFERENCES

Alba-Tercedor, J., Jáimez-Cuéllar, P., Álvarez, M., Avilés, J., Bonada, N., Casas, J, et al. 2002. Caracterización del estado ecológico de ríos mediterráneos ibéricos mediante el índice IBMWP (antes BMWP'). *Limnetica*, 21(2), 175–185.

Barboza, L.G.A.; Dick Vethaak, A.; Lavorante, B.R.B.O.; Lundebye, A.-K.; Guilhermino, L. 2018. Marine microplastic debris: An emerging issue for food security, food safety and human health. *Marine Pollution Bulletin*, 133, 336–348.

Barnes, D.K., Galgani, F., Thompson, R.C., Barlaz, M. Accumulation and fragmentation of plastic debris in global environments. 2009.

Philosophical Transactions of the Royal Society Biological, 364, 1985–1998. DOI: 10.1098/rstb.2008.0205

Bogdanowicz, A.; Zubrowska-Sudol, M.; Krasinski, A.; Sudol, M. 2021. Cross-Contamination as a Problem in Collection and Analysis of Environmental Samples Containing Microplastics—A Review. *Sustainability*, 13, 12123. DOI: 10.3390/su132112123

Campanale, C., Dierkes, G., Massarelli, C., Bagnuolo, G., & Uricchio, V. F. 2020. A relevant screening of organic contaminants present on freshwater and pre-production microplastics. *Toxics*, 8(4), 100.

Carpenter, E.J., Smith, K.L., 1972. Plastics on the Sargasso Sea surface. *Science*, 175, 1240–1241.

Carbery, M., O'Connor, W., and Palanisami, T. 2018. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. *Environment International*, 115, 400–409. DOI: 10.1016/j.envint.2018.03.007.PMID:29653694

Chang, M. 2015. Reducing microplastics from facial exfoliating cleansers in wastewater through treatment versus consumer product decisions. *Marine pollution bulletin*, 101(1), 330-333.

Cole, M., Lindeque, P., Halsband, C., & Galloway, T. 2011. Microplastics as contaminants in the marine environment: A review. *Marine pollution bulletin*, 62. 2588-97. DOI: 10.1016/j.marpolbul.2011.09.025

Cox KD, Covernton GA, Davies HL, Dower JF, Juanes F, Dudas SE. 2019. Human Consumption of Microplastics. *Environmental Science & Technology*, 53 (12), 7068–74. DOI: 10.1021/acs.est.9b01517

Council of the European Communities, 2000. Directive 2000/60/EC of the European Parliament and Council of Benetti, 20 October 2000 establishing a framework for community 19 action in the field of water policy. *Official Journal of the European Communities*, L327. 72 pp.

De Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T. L., Futter, M. N. 2018. Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? *Science of The Total Environment*, 645, 1029-1039.

D'Souza, J. M., Windsor, F. M., Santillo, D., & Ormerod, S. J. 2020. Food web transfer of plastics to an apex riverine predator. *Global change biology*, 26(7), 3846-3857.

Ehlers, S., Manz, W. & J.H.E. Koop. 2019. Microplastics of different characteristics are incorporated into the larval cases of the freshwater caddisfly *Lepidostoma basale*. *Aquatic Biology*, 28, 67–77. DOI: 10.3354/ab00711

Ehlers, S. M., T. Al Najjar, T. Taupp & J. H. E. Koop. 2020. PVC and PET microplastics in caddisfly (*Lepidostoma basale*) cases reduce case stability. *Environmental Science and Pollution Research*, 27, 22380–22389. DOI: 10.1007/s11356-020-08790-5

Ehlers, Sonja M., Julius A. Ellrich, Jochen H.E. Koop. 2022. Microplastic load and polymer type composition in European rocky intertidal snails: Consistency across locations, wave exposure and years. *Environmental Pollution*, Volume 292, Part A, 118280, ISSN 0269-7491. DOI: 10.1016/j.envpol.2021.118280

Jung, M. R., Horgen, F. D., Orski, S. V., Rodriguez, V., Beers, K. L., Balazs, G. H., & Lynch, J. M. 2018. Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. *Marine pollution bulletin*, 127, 704-716.

He, B., Wijesiri, B., Ayoko, G. A., Egodawatta, P., Rintoul, L., & Goonetilleke, A. 2020. Influential factors on microplastics occurrence in river sediments. *Science of The Total Environment*, 738, 139901.

He, B., Smith, M., Egodawatta, P., Ayoko, G. A., Rintoul, L., & Goonetilleke, A. 2021. Dispersal and transport of microplastics in river sediments. *Environmental pollution*, 279, 116884.

Herrera A., P. Garrido-Amador, I. Martínez, M.D. Samper, J. López-Martínez. 2018. Novel methodology to isolate microplastics from vegetal-rich samples. *Marine pollution bulletin*, 129 (1), 61-69.

Horn, D., M. Miller, S. Anderson, And C. Steele. 2019. Microplastics are ubiquitous on California beaches and enter the coastal food web through consumption by Pacific mole crabs. Marine *Pollution Bulletin*, 139, 231–237. DOI: 10.1016/j.marpolbul.2018.12.039

Hübner, M.K., Michler-Kozma, D.N., Gabel, F. 2020. Microplastic concentrations at the water surface are reduced by decreasing flow velocities caused by a reservoir. *Fundaments of Applied Limnology*, 194/1,49–56.

Hussain, N., Jaitley, V., & Florence, A. T. 2001. Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. *Advanced Drug Delivery Reviews*, 50, 107–142.

National Statistics Institute, 2021 https://www.ine.es/en/index.htm.

Käppler, A., Windrich, F., Löder, M. G., Malanin, M., Fischer, D., Labrenz, M., & Voit, B. 2015. Identification of microplastics by FTIR and Raman microscopy: a novel silicon filter substrate opens the important spectral range below 1300 cm^{-1} for FTIR transmission measurements. *Analytical and bioanalytical chemistry*, 407, 6791-6801.

Kolandhasamy, P., Su, L., Li, J., Qu, X., Jabeen, K., & Shi, H. 2018. Adherence of microplastics to soft tissue of mussels: a novel way to uptake microplastics beyond ingestion. *Science of the total environment*, 610, 635-640.

Kumar, R., Sharma, P., Manna, C., & Jain, M. 2021. Abundance, interaction, ingestion, ecological concerns, and mitigation policies of microplastic pollution in riverine ecosystem: A review. *Science of The Total Environment*, 782, 146695.

Lambert S. & Wagner M. 2018. Microplastics Are Contaminants of Emerging Concern in Freshwater Environments: An Overview. In: Wagner M., Lambert S. (eds) Freshwater Microplastics. *The Handbook of Environmental Chemistry*, vol 58. Springer, Cham. DOI: 10.1007/978-3-319-61615-5_1

Lebreton, L., Van Der Zwet, J., Damsteeg, Jw, Slat B., Andrady A. & Reisser, J. 2017. River plastic emissions to the world's oceans. *Nature Communications*, 8, 15611. DOI: 10.1038/ncomms15611

Libera. 2019. Protocolo de muestreo e identificación de microplásticos en ríos (HyT – LIBERA). 68 pp.

Lusher, A., Hollman, P., & Mendoza-Hill, J. 2017. *Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety.* FAO.

MAGRAMA 2011. Sampling protocol and laboratory of benthic fauna of wadeable invertebrates. Code: ML-RvI-2011. 23 pages Ministry of Agriculture and Environment. Spanish Government.

MAGRAMA 2015. Spanish Royal Decree 817/2015, of September 11, which establishes the criteria for monitoring and evaluating the state of surface waters and environmental quality standards. *Official State Gazette*, September 11, 2015, number 219.

Martínez, Y., Gutiérrez, D., Álvarez-Troncoso, R. & Garrido, J. 2020. Impact of small-scale hydropower stations on macroinvertebrate communities for regulated rivers. *Limnetica*, 39 (1), 317–334. DOI: 10.23818/limn.39.21

Marziali, L., Armanini, D. G., Cazzola, M., Erba, S., Toppi, E., Buffagni, A., & Rossaro, B. 2010. Responses of Chironomid larvae (Insecta, Diptera) to ecological quality in Mediterranean river mesohabitats (South Italy). *River Research and Applications*, 26(8), 1036-1051.

Mani, T., Primpke, S., Lorenz, C., Gerdts, G., & Burkhardt-Holm, P. 2019. Microplastic pollution in benthic midstream sediments of the Rhine River. *Environmental science & technology*, 53 (10), 6053-6062.

Masura, J., Baker, J., Foster, G., & Arthur, C. 2015. Laboratory Methods for the Analysis of Microplastics in the Marine Environment: *Recommendations for quantifying synthetic particles in waters and sediments*.

Miller, M. E., Kroon, F. J., & Motti, C. A. 2017. Recovering microplastics from marine samples: a review of current practices. *Marine Pollution Bulletin*, 123 (1-2), 6-18.

Nizzetto, L., Bussi, G., Futter, M. N., Butterfield, D., & Whitehead, P. G. 2016. A theoretical assessment of microplastic transport in river catchments and their retention by soils and river sediments. *Environmental Science: Processes & Impacts*, 18 (8), 1050-1059.

O'Connor, J. D., Lally, H. T., Koelmans, A. A., Mahon, A. M., O'Connor, I., Nash, R., & Murphy, S. 2022. Modelling the transfer and accumulation of microplastics in a riverine freshwater food web. *Environmental Advances*, 8, 100192.

Rillig, M. C., & Lehmann, A. 2020. Microplastic in terrestrial ecosystems. *Science*, 368(6498), 1430-1431.

Rochman, C. M., Kurobe, T., Flores, I., & Teh, S. J. 2014. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. *Science of the total environment*, 493, 656-661.

Rochman, C. M. 2018. Microplastics research-from sink to source. *Science*, 360 (6384), 28−29.

Rodrigues Mo, Abrantes N, Gonçalves Fjm, Nogueira H, Marques JC, Gonçalves Amm. 2018. Spatial and temporal distribution of microplastics in water and sediments of a freshwater system (Antuã River, Portugal). *Science of The Total Environment*, Aug 15, 633,1549-1559. DOI: 10.1016/j.scitotenv.2018.03.233. Epub 2018 Apr 4. PMID: 29758905.

Sá, B.; Pais, J.; Antunes, J.; Pequeno, J.; Pires, A.; Sobral, P. Seasonal abundance and distribution patterns of microplastics in the Lis River, Portugal. 2022. *Sustainability*, 14, 2255. DOI: 10.3390/su14042255

Scherer, C., Brennholt, N., Reifferscheid, G., & Wagner, M. 2017. Feeding type and development drive the ingestion of microplastics by freshwater invertebrates. *Scientific reports*, 7(1), 1-9.

Schmid, C., Cozzarini, L., & Zambello, E. 2021. Microplastic's story. *Marine pollution bulletin*, 162, 111820.

Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M. 2014. Ingestion and transfer of microplastics in the planktonic food web. *Environmental Pollution*, 185, 77–83. DOI: 10.11016/j.envpol.2013.10.013

Stanković, J., Raković, M., Paunović, M., Atanacković, A., Tomović, J., & Milošević, Đ. 2021. Isolation of microplastics from freshwater macroinvertebrates in the Danube River. *Facta Universitatis. Series: Medicine and Biology*, 23(2), 21-27.

Tachet, H., P. Richoux, M. Bournaud & P. Usseglio-Polatera. 2002. *Invertebrés d'Eau Douce* (2nd corrected impression). CNRS editions. Paris. France. 588 pp.

Van Cauwenberghe, L. & Janssen, C. 2014. Microplastics in bivalves cultured for human consumption. *Environmental pollution* (Barking, Essex: 1987), 193C. 65-70. DOI: 10.1016/j.envpol.2014.06.010

Vitecek, S. & Johnson, R. & Poikane, S. 2021. Assessing the Ecological Status of European Rivers and Lakes Using Benthic Invertebrate Communities: A Practical Catalogue of Metrics and Methods. *Water*, 13, 346. DOI: 10.3390/w13030346

Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, D., Winther-Nielsen, M., & Reifferscheid, G. 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. *Environmental Sciences Europe*, 26, 12. DOI: 10.1186/s12302-014-0012-7

Waldschläger, K., Lechthaler, S., Stauch, G., & Schüttrumpf, H. 2020. The way of microplastic through the environment-Application of the source-pathway-receptor model. *Science of the Total Environment*, 713, 136584.

Windsor, Fredric M., Rosie M. Tilley, Charles R. Tyler, Steve J. Ormerod. 2019. Microplastic ingestion by riverine macroinvertebrates, *Science of The Total Environment*, Volume 646, 68-74, ISSN 0048-9697. DOI: 10.1016/j.scitotenv.2018.07.271

Wright, S.L., D. Rowe, R.C. Thompson, T.S. Galloway. 2013. Microplastic ingestion decreases energy reserves in marine worms. *Current Biology*, 23 (23), pp. 1031-1033.