Science of the Total Environment 804 (2022) 150207

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Microplastics accumulation in functional feeding guilds and functional habit groups of freshwater macrobenthic invertebrates: Novel insights in a riverine ecosystem

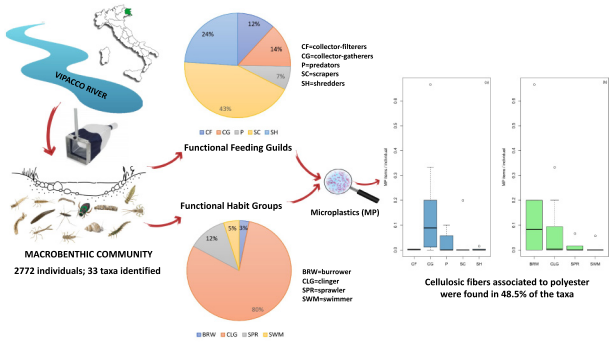
Marco Bertoli [a], Paolo Pastorino [b,*], Davide Lesa [a], Monia Renzi [a], Serena Anselmi [c], Marino Prearo [b], Elisabetta Pizzul [a]

[a] Department of Life Sciences, University of Trieste, via L. Giorgieri 10, 34127 Trieste, Italy
[b] The Veterinary Medical Research Institute for Piemonte, Liguria and Valle d'Aosta, via Bologna 148, 10154 Torino, Italy
[c] Bioscience Research Center, via Aurelia Vecchia 32, 58015 Orbetello, GR, Italy

HIGHLIGHTS

- Microplastics contamination was assessed in a macrobenthic invertebrate community.
- Cellulosic fibers associated to polyester were found in 48.5% of the taxa.
- The highest microplastics amount was detected in the collector-gatherers.
- There was no difference in microplastics amount among the functional habit groups.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 May 2021
Received in revised form 2 September 2021
Accepted 3 September 2021
Available online 8 September 2021

Editor: Henner Hollert

Keywords:
Cellulose
Collector-gatherers
Fibers
Freshwater ecosystems
Lotic ecosystems
Polyester

ABSTRACT

Microplastics pose a major threat for aquatic ecosystems, but the contamination dynamics in organisms inhabiting freshwater ecosystems is still little studied. Largely used for biomonitoring, macrobenthic invertebrates provide a pivotal trophic resource for many fish and bird species. In this study, we investigated the microplastics contamination in a macrobenthic invertebrate community (2772 individuals belonging to 33 taxa identified) in a high-plain riverine ecosystem (Vipacco River, northeast Italy) and compared the amount of microplastics accumulated in functional feeding guilds/functional habit groups. Microplastics (cellulosic fibers associated with polyester) were found in 48.5% of the taxa, with the highest amount detected in the collector-gatherers, followed by predators. The collector-gatherers showed a significantly higher microplastic accumulation than the other functional feeding guilds, whereas there was no difference among the functional habit groups. The main source of microplastics pollution was most likely urban wastewater discharge points located along the river. Our study reports a novel approach about microplastic pollution assessment in lotic environments, as it focuses into the microplastic contamination dynamics in an entire macrobenthic invertebrate community perspective and underlines the need for further study.

1. Introduction

Microplastics pose a major threat for aquatic ecosystems (Avio et al., 2017; Windsor et al., 2019), with an enormous impact on freshwater

* Corresponding author.
E-mail address: paolo.pastorino@izsto.it (P. Pastorino).

https://doi.org/10.1016/j.scitotenv.2021.150207
0048-9697/

and marine environments. Due to massive production and wrong waste management, a great number of plastics are discarded into aquatic environments: it was estimated that an amount between 1.15 and 2.41 million tons of plastics are released into the oceans via rivers each year (Lebreton et al., 2017). Microplastics can be divided into two broad categories (Singh and Sharma, 2008): a) "primary" produced voluntarily and conceived mainly as components within consumer products such as paints and cleaning products, b) "secondary", resulting from the splitting of larger plastics through mechanical abrasion, photodegradation and (micro)biological degradation processes. Conventional wastewater treatment plants may act as a microplastic source to rivers (Carr et al., 2016; Mason et al., 2016). Riverine plastic loads may both be positively correlated with inappropriate waste management (Free et al., 2014), especially when combined with a high population density (Baldwin et al., 2016). Then, plastics degrade into numerous small fragments/fibers/spheroids/granules/pellets/flakes/beads which size ranges between 1 and 5000 μm. These fragments are commonly known as microplastics (EFSA, 2016; McDevitt et al., 2017; Ding et al., 2018).

Nowadays, plastic pollution is among the topmost environmental concerns of the Anthropocene (Akindele et al., 2020) in which microplastics are ubiquitous: from high-mountain lakes to deep-sea sediments (Van Cauwenberghe et al., 2013; Free et al., 2014; Woodall et al., 2014; Pastorino et al., 2020a) and from temperate to tropical aquatic systems (Mani et al., 2015; Horton et al., 2018; Nel et al., 2018; Akindele et al., 2019). Early studies on microplastics contamination initially focused on marine ecosystems (Eerkes-Medrano et al., 2015; Li et al., 2020). The factors affecting the microplastics distribution in marine ecosystems include large-scale forces as currents driven by wind and geostrophic circulation (Law et al., 2010), turbulence and oceanographic effects (Turra et al., 2014). Also, the properties of microplastics such as density, shape and size can affect transportation and distribution patterns (Eerkes-Medrano et al., 2015). The aforementioned factors are more likely to play important roles in a large freshwater environment like riverine systems; however, they become limited on smaller isolated freshwater environments (Free et al., 2014). The mean values of microplastics abundance in freshwater systems varied greatly from almost none to several million pieces per cubic meter (Li et al., 2018). This significant difference results from some key factors as sampling locations, human activities, natural conditions, and sampling approaches (Eerkes-Medrano et al., 2015). On this path, it is well known that wastewater treatment plants are one of the dominant sources of microplastics in freshwater systems (Li et al., 2018). Although freshwater and terrestrial environments are recognized as origins and transport pathways of plastics to the oceans, the presence of plastic debris and impacts on freshwater biota represents an understudied research topic (Cera et al., 2020).

Due to their small size, microplastics are potentially available for aquatic organisms: as microplastics have the same size range of plankton species, thus they can be easily ingested by invertebrates and fish (Ding et al., 2018). In marine and freshwater habitats, several field studies have reported the occurrence of microplastics in invertebrates (Frias et al., 2014; Van Cauwenberghe and Janssen, 2014; Su et al., 2018) and in the gut contents of fish (Dantas et al., 2012; Sanchez et al., 2014; Jabeen et al., 2017). This issue raises general concerns about the ecological and human health impacts of MPs across food-chains (Duis and Coors, 2016; Miranda and de Carvalho-Souza, 2016). Moreover, microplastic ingestion can negatively affect aquatic organisms in many ways: ingestion can affect digestive system (Derraik, 2002; Tourinho et al., 2010), reproduction (Sussarellu et al., 2016), growth (Redondo-Hasselerharm et al., 2018), and can induce oxidative stress (Lu et al., 2016) and death (Teuten et al., 2009; Wright et al., 2013). Ingestion of microplastics can also lead to uptake and bioaccumulation of harmful chemicals (Betts, 2008; Teuten et al., 2009; Lavers et al., 2014). Additives in plastics such as phthalates, brominated flame retardants, nonylphenol, and antimicrobials are associated with cancer and endocrine disruption (Browne et al., 2007; Teuten et al., 2009). The high plastic sorption capacity allows the accumulation of persistent organic pollutants such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and organochlorine pesticides, with concentrations 105–106 times higher than in the surrounding water column (Mato et al., 2001; Betts, 2008). Trace metals and pathogens have also been shown to accumulate on microplastics (Nakashima et al., 2012; Lavers et al., 2014; McCormick et al., 2014).

Macrobenthic invertebrates occupy a central role in freshwater environments. They cover trophic functions (Cummins, 1974; Metcalfe Smith, 1994), with a wide range of feeding guilds (grazers, shredders, collector-filterers, collector-gatherers, predators) and ecological niches (Voshell, 2002) and are trophic resources for many fish and bird species (Pizzul et al., 2008; Bertoli et al., 2015). Benthic macroinvertebrates have also been largely used in biomonitoring because many taxa are sessile and their lifespan is long enough to allow assessment of site-specific ecological conditions (Rosenberg and Resh, 1993; Ghetti, 1997). By virtue of these characteristics, macrobenthic invertebrates can provide a useful tool for investigating microplastics contamination in freshwater ecosystems (Akindele et al., 2020). Recently, studies have being carried out, providing evidence of microplastic ingestion by different freshwater macroinvertebrates such as Oligochaeta (Hurley et al., 2017), Diptera Chironomidae (Nel et al., 2018), Gastropoda (Akindele et al., 2019), Ephemeroptera and Trichoptera (Windsor et al., 2019). However, these studies focused on selected target species/taxa, and they did not consider the whole community, as in classic biomonitoring protocols. It is our opinion that a "whole community perspective" could be useful, to better comprehend the microplastic pathways in freshwater environments. Within a riverine macrobenthic invertebrate community many taxa could be found (even over 20 families) covering all the feeding guilds. Usually, organisms have different size, life cycle, habits, and tolerance to environmental alterations. Moreover, different taxa live in different substrates and distribution of microplastic particles could be governed by substrate type and sediment organic matter (Nel et al., 2018). Indeed, classic riverine biomonitoring programs require the analysis of the whole macrobenthic community to maximize information. In this context, it was deemed of interest to investigate microplastic presence within a whole community, to better understand the impact at all community levels. Moreover, information about microplastic presence in many freshwater macrobenthic taxa is still lacking.

Here we measured microplastics pollution in a macrobenthic invertebrate community in a riverine ecosystem (Vipacco River; northeast Italy) and compared macrobenthic invertebrate traits (functional feeding guilds and functional habit groups) against microplastics accumulation. To do this, we examined the community as a whole without selecting target organisms. Our hypothesis was that microplastic contamination would be greater in collector-gatherers than other taxa, as previously observed by Pastorino et al. (2019, 2020b, 2020c) in riverine macrobenthic invertebrate communities for other environmental contaminants such as trace elements and rare earth elements.

2. Materials and methods

2.1. Study area

The present study was carried out in the Italian stretch of the Vipacco/Vipava River (Friuli Venezia Giulia, northeast Italy), a cross-border watercourse straddling the Italian-Slovenian border. The river is called Vipava in Slovenian and Vipacco in Italian (hereinafter referred to as "Vipacco"). This watercourse is the main left tributary of the Isonzo/Soča River (Mosetti, 1983) (Fig. 1), another cross-border watercourse flowing through Slovenian and Italian territories (Soča in Slovenian, Isonzo in Italian). The Vipacco originates from karstic springs at Mount San Lorenzo in Slovenia (1019 m a.s.l.) where it flows for 45 km before crossing the border with Italy, then runs for 4.5 km within the municipality of Savogna d'Isonzo (Friuli Venezia Giulia Region)

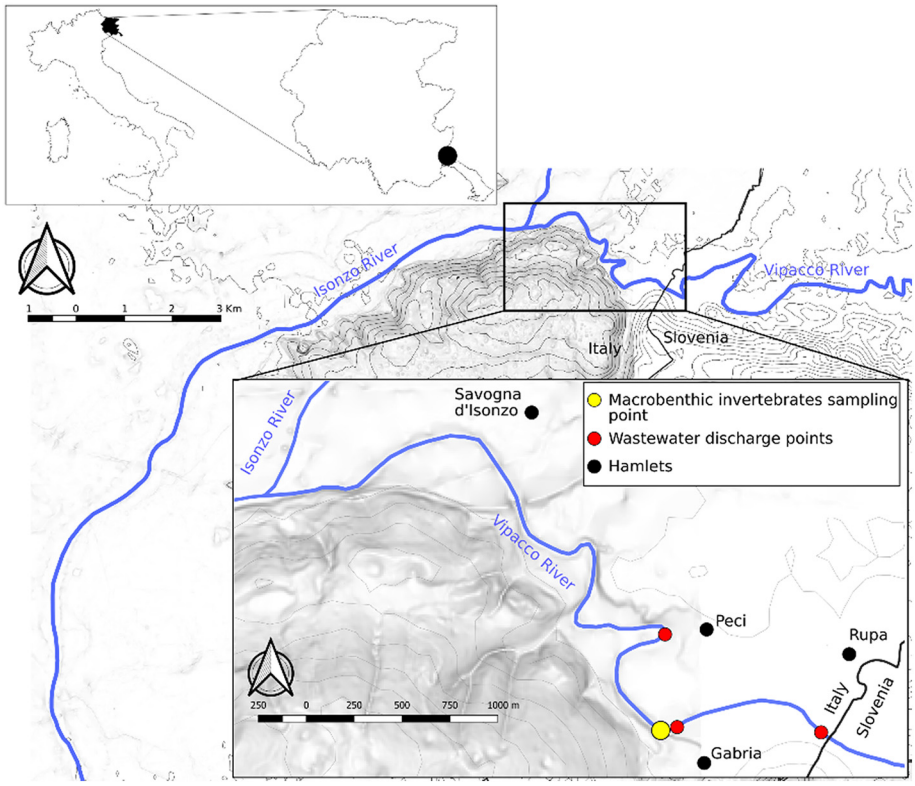


Fig. 1. Study area: geographical context and stretch of the Vipacco River in Italy (sampling site coordinates 45°53′17.670″ N, 13°34′47.800″ E).

before flowing into the Isonzo. In its Italian stretch, the Vipacco is a slightly modified high-plain watercourse set within a scarcely urbanized landscape. Land use includes mainly agriculture, while small industrial activities are more concentrated in the Slovenian territory of the low portion of the Vipacco basin: these activities include food processing factories, electronics, construction, and transport services. Three wastewater discharge points are present within the Italian stretch, collecting waters from near small urban centers (Fig. 1). Subject to anthropic pressure and potential impact from the area upstream in Slovenia, the river is included in the ecological monitoring framework of the Regional Agency for Environmental Protection of Friuli Venezia Giulia (ARPA FVG) (http://www.arpaweb.fvg.it).

For the present study, we defined a 2-km section starting from the border with Slovenia to the mid-point of the stretch in Italy. This section included mainly uniform flow areas (60%) but all comprises a variety of mesohabitats such as riffles (10%), glides (10%), and pools (20%). Cobbles, coarse and fine gravel make up the main substrates. Vegetation cover on the riverbed is low (1-20%). Three urban wastewater discharge points are located along the river (Fig. 1): one near the border with Slovenia, one in the middle of the study area, and one at the midpoint of the river in Italy. A single sampling site (45°53′17.67″ N; 13°34′47.80″ E) was identified as being representative for the river's hydrological characteristics and anthropological impacts. Mesohabitat composition and substrate distribution were considered for sampling site selection and therefore a river stretch including all the mesohabitat and substrate cited above was chosen. Accessibility was also a factor since the Vipacco is often non-wadable for most of its course in Italy due to highly variable flow rate (Fig. 2). Another factor in choosing the

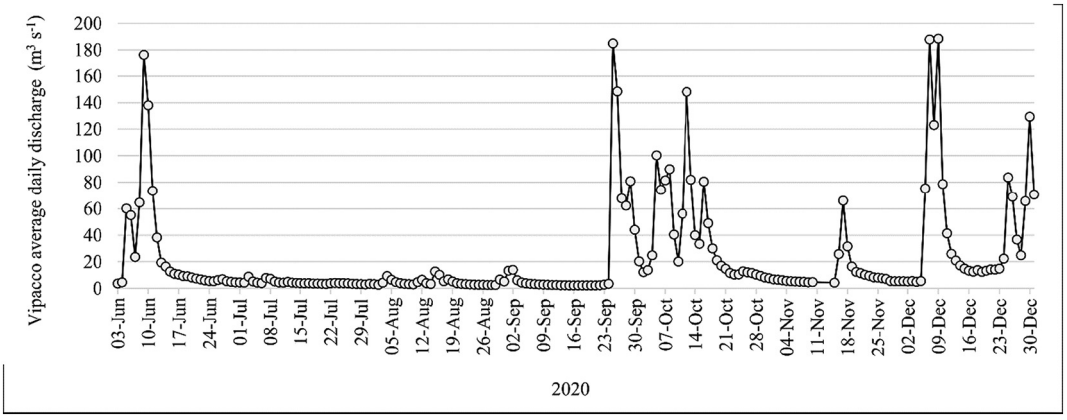


Fig. 2. Average daily flow rates ($m^3 s^{-1}$) of the Vipacco River during the period June–December 2020. Data are taken from a Slovenian Environment Agency monitoring station located near the border with Italy (https://www.arso.gov.si).

study site was that we wanted to obtain a representative picture of the impact of microplastics pollution on the macrobenthic communities. The sampling site was the midpoint of the stretch, 40 m downstream of the second discharge point, where all substrates could be sampled (Fig. 1).

2.2. Sediment sampling

In October 2020 sediment samples were collected with a manual corer (250 cm^2 sampling surface) from the riverbed near the banks. The samples (n = 3) were placed in glass jars (1 L) to protect them from external particle contamination, frozen at −20 °C for storage, and thawed prior to extraction and determination of the chemical composition of the microplastics (Pastorino et al., 2020a).

2.3. Water sampling

Water samples (n = 3) were collected with an Apstein plankton net (opening 400 × 1000 mm; mesh 50 μm) by placing the net directly below the water surface at the same site and time as the sediment and microbenthic invertebrate samples were collected. The net was kept in place for 30 min per sample. Water volume was calculated using a manual flowmeter (fixed on the plankton net opening) (Scherer et al., 2020). The water samples were transferred into glass jars. The net was cleaned between each replicate with ultrapure water to prevent contamination of the subsequent sample. The water samples were used to determine microplastic chemical type and physicochemical features of the water (Table S1).

2.4. Macrobenthic invertebrate sampling

The sample collection was performed in autumn (November 2020), using a standardized multihabitat sampling protocol for ecological status assessment (Buffagni and Erba, 2014; Bertoli et al., 2014; Pastorino et al., 2019; Bertoli et al., 2021). The protocol conformed to the requirements of the Water Framework Directive (European Commission, 2000) and Italian law (D. Lgs 152/06, 2006; D.M. 260/2010, 2010). Samples were collected using a Surber net (mesh 500 μm; subtended area of 0.1 m^2) along a wadable stretch of the watercourse (about 50 m long), considered representative of the stream's hydrological characteristics. Ten replicates were collected two times (20 replicates in total) in diverse microhabitats proportional to their occurrences (Buffagni and Erba, 2014; Bertoli et al., 2014; Pastorino et al., 2019). Main substrates were cobbles (40%), coarse gravel (30%), fine gravel (20%), and macrophytes (10%). After collection, first sorting and initial taxonomical identification (family level) were performed in the field using glass trays and stainless-steel tweezers. The samples were frozen, brought to the laboratory, and stored until further taxonomical identification with a stereomicroscope or an optical microscope (if required). Taxonomical identification was performed to the genus level whenever possible. Glass Petri dishes and stainless-steel tweezers were used throughout to prevent contact between samples and plastic instruments. The composition of the community structure was determined including all taxa, and their densities (ind m^{-1}) estimated as described elsewhere (Buffagni and Erba, 2014). Each taxon was assigned to a functional feeding guild (FFG) and a functional habit group (FHG) according to Merritt and Cummins (2006). The samples were pooled by taxon for microplastic content determination.

2.5. Microplastic content analysis and quality assurance/quality control

The water samples were filtered by vacuum on 6-μm pore paper disk filters (Whatman®, Sigma-Aldrich, St. Louis, MO, USA). The filters were placed on a glass Petri disk to avoid pollution during oven drying (35 °C); after water evaporation, the filters were analyzed by stereomicroscopy to collect microplastic particles for chemical determination. Sediment samples were extracted three times using a prefiltered saturated NaCl solution by mechanical agitation (20 min, 100 rpm) and the supernatant was filtrated on 6-μm pore paper disks.

Invertebrate samples were pretreated by direct digestion of tissues with Creon enzyme (37 °C; TRIS-buffered pH) to quickly remove tissues without damaging plastic polymers for chemical identification (von Friesen et al., 2019) and sonicated for 1 h. Digested tissues were filtered through an apparatus fitted with a paper fiber filter disk (6-μm pore paper disks), stored in glass Petri dishes, and dried overnight at 40 °C (Ziajahromi et al., 2017). During laboratory analysis, air exposure was minimized to reduce potential airborne pollution while filtering the samples under a HEPA-filtered laminar-flow fume hood. Positive and negative controls (n = 3) were performed for each batch to ensure quality control of the analytical process. The filtered samples were sorted by stereomicroscopy at 10–80× (SMZ-800 N; software NIS-elements D, Nikon, Tokyo, Japan). Potential targets were chemically analyzed by microscopy coupled with Fourier transform infrared spectroscopy (μFT-IR; Nicolet iN10 MX, ThermoFischer Scientific, Waltham, MA, USA) equipped with an MCT-A detector (spectral range, 7.800–650 cm^{-1}) cooled with liquid nitrogen and operating in reflection mode. Identification was carried out by determining the spectral match (%) of the targeted items compared to the spectral libraries of normal and aged microplastics (OMNIC™ Picta™ software libraries, ThermoFisher Scientific) integrated with our laboratory spectral libraries and by imposing a threshold for spectra back-recognition >80% of match; the limit of detection (LOD) was a particle size of 10 μm. Recovered items were classified according to chemical type, shape, size, and color following criteria reported elsewhere (Galgani et al., 2014).

2.6. Statistical analysis

Spearman rank correlation coefficient (r_s) was used to investigate correlations between macrobenthic invertebrate size and number of microplastics per individual and between macrobenthic invertebrate size and size of the microplastics particles. As the data were not normally distributed (Shapiro-Wilk test, $p < 0.001$), and due to the small size of the sample, differences in microplastics counts between the functional feeding guilds and the functional habit groups were investigated using non-parametric Kruskal-Wallis test, then the Conover–Iman test as post hoc test (Conover and Iman, 1979; Conover, 1999). Statistical analysis was performed using RStudio version 3.5.3. Figures were produced with RStudio, except Fig. 1, which was created using QGis version 3.2.2 Bonn (QGIS.org, 2018) and processed with Inkscape version 0.92.

3. Results

3.1. Water and sediment samples

Both water and sediment were contaminated by microplastics (3.73 ± 2.11 microplastics m^{-3} per min and 3.33 ± 4.16 microplastics dm^{-3}, respectively). The chemical composition of the microplastics varied (Fig. 3): polystyrene (PS), polyethylene terephthalate (PET), polyamide (PA), polypropylene (PP), polyurethane (PU), polyethylene (PE), and polyester (P) plus cellulose (CE). The most abundant polymer in the water samples was PS (27%), followed by PET (21%), CE (12%), PE (10%), PA (8%), P (8%), PU (7%) and PP (7%). The mean size of microplastics particles was 463.2 ± 15.7 μm. The predominant shapes were fibers (46%), spherules (39%), and fragments (15%). The most frequent colors were white (80%), black (10%) and brown (10%).

In the sediment samples, the most abundant polymer was PS (26%), followed by CE (20%), PP (18%), P (12%), PA (12%), and PU (12%). The mean size was 141 ± 264 μm. The most frequent shapes were spherules (50%) followed by fibers (42%), and fragments (8%). The most frequent colors were white (75%) and black (25%).

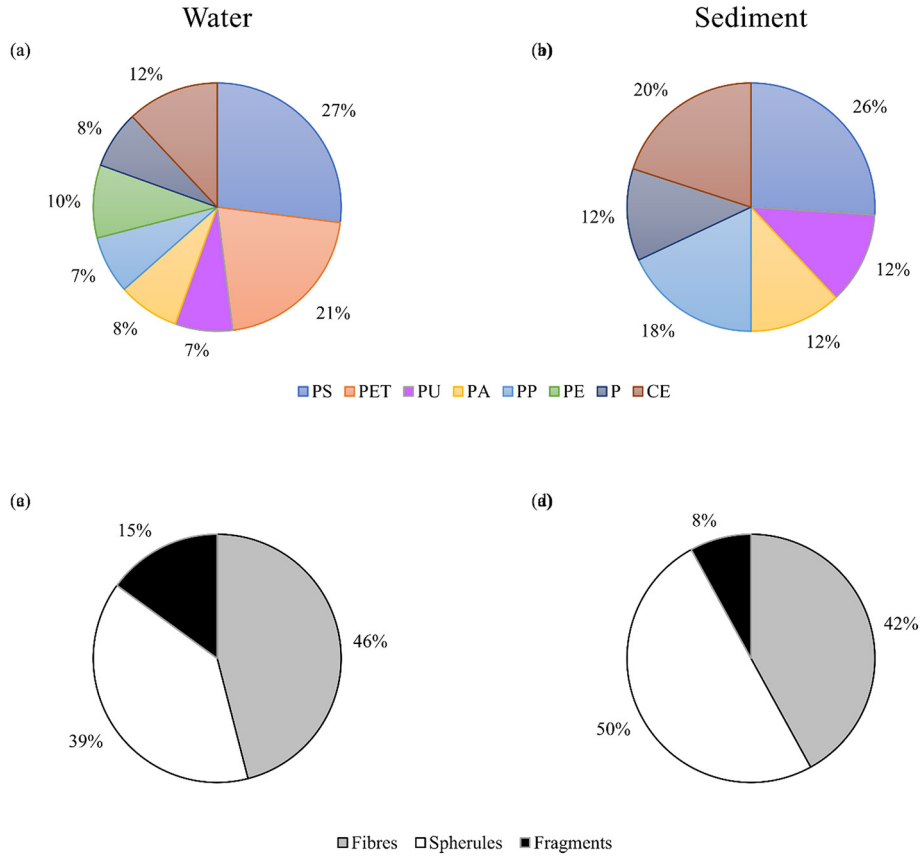


Fig. 3. Microplastic chemical composition (a, b) and microplastic shapes (c, d) observed in water and sediment samples of the Vipacco River (PS = polystyrene, PET = polyethylene terephthalate, PU = polyurethane, PA = polyamide, PP = polypropylene, PE = polyethylene, P = polyester, CE = cellulose).

3.2. Macrobenthic invertebrate samples

A total of 2772 macrobenthic invertebrate individuals belonging to 30 families were identified (Table 1). The most abundant taxon was Gastropoda (43.3% of the sample) with high densities of the genera *Theodoxus*, and *Bithynia*. The most abundant Insect orders were Trichoptera (19.6%), Plecoptera (12.4%), and Coleoptera (11.7%). The density of the trichopterans *Hydropsyche* and *Lepidostoma*, the plecopteran *Leuctra* and the coleopteran *Oulimnius* was particularly higher than the other taxa (Table 1). Among the coleopterans, the family Elmidae was very frequent with four genera (*Limnius*, *Stelnemis*, *Elmis*, *Oulimnius*). Other groups accounted for <5% of the relative abundance and low density (Table 1). Among the functional feeding guilds (FFG), scrapers were the most frequent (43%), followed by shredders (24%), collector-filterers and collector-gatherers (12% and 14%, respectively), and predators (7%) (Fig. 4a). Among the functional habit groups, the most abundant were clingers (80%), sprawlers (12%), burrowers and swimmers (5% and 3%, respectively) (Fig. 4b). All the observed taxa were previously reported by Slovenian researchers during biomonitoring sampling campaigns performed by the Slovenian Environment Agency (ARSO, https://www.arso.gov.si/en/) for the routine ecological status assessment, and our community overlaps with those collected by Slovenian Authority. Therefore, we conclude that an exhaustive picture of the whole riverine community of the Vipacco River was represented by our samples, despite we limited the sampling effort.

Microplastics were largely detected in 16 of the 33 identified taxa (Table 1). Fibers were the only observed microplastic form, ranging from 347.8 to 6994.4 μm, and cellulose (CE) was observed in association to polyester. No cellular structures were observed for CE and the fibers were variously colored (black, blue, green, grey, pink, orange, white). Therefore, we assume that CE fibers were artificial in origin. The polyester fibers were black and blue in color (Table 1).

No significant correlation was found between number of microplastics per individual and average macrobenthic invertebrate size (r_s −0.059, $p = 0.745$) or between microplastics particle size and average macrobenthic invertebrate size (r_s −0.351, $p = 0.263$). Microplastic accumulation significantly differed among functional feeding guilds (Fig. 5a; Table 2) (Kruskal-Wallis test, $H = 8.951$, $d.f. = 4$, $p < 0.05$). In particular, the number of MPs per individual was significantly higher in collector-gatherers than the other guilds (Conover-Iman test, $p < 0.02$ for all comparisons). Regarding the functional habit groups, no significant differences were highlighted by application the Kruskal-Wallis test (Kruskal-Wallis test, $H = 4.066$, $d.f. = 3$, $p = 0.25$) (Fig. 5b).

4. Discussion

With this study we analyzed microplastics contamination in an entire macrobenthic invertebrate community in a high-plain riverine system. Besides the impacts related to land use (agriculture and small industry), wastewater discharge points are located along the studied watercourse, and they represent the main microplastic pollution sources for the investigated area (Wagner et al., 2014; Windsor et al., 2019). The microplastics detected in the macrobenthic invertebrate samples were colored cellulose fibers with no cellular structure, associated to polyester (Table 1) that primarily originated from washing machine wastewater (Browne et al., 2011; Remy et al., 2015). One of the major sources of primary microplastics pollution is synthetic textiles, which release a great number of cellulosic microfibers during washing of clothes made with a blend of polyester/cellulose (De Falco et al., 2019). It is estimated that about 35% of the global release of primary

Table 1
Macrobenthic invertebrate community composition, functional feeding guilds (FFG), habit groups (HG), and size. Characteristics of microplastics (MPs) in the samples are reported for identified taxa. (FFG legend: P = predators; CG = collector-gatherers; CF = collector-filterers; SH = shredders; SC = scrapers; FGH legend = SWM = swimmers, BRW = burrower; CLG = clinger; SPR = sprawler).

Class/order	Family/genus	FFG	FHG	Size range (mm)	Densities (ind m^{-2})	Total MP Items	MP items/individual	Color	Size (μm)	Chemical
Hirudinea	*Hemiclepsis*	P	SPR	21	2	–				
Oligochaeta	Lumbriculidae	CG	BRW	12-13	3	–				
Arachnida	Hydracarina	P	SWM	2-3	87	5	0.057	Blue	1594.04	Cellulose
								Blue	1232.05	Cellulose
								Blue	1503.34	Cellulose
								Green	2899.77	Cellulose
								Blue	1073.35	Cellulose
Malacostraca	*Asellus*	SH	SPR	6-10	62	1	0.016	Blue	3954.41	Cellulose
	Gammarus	SH	SWM	7-9	6	–				
Gastropoda	*Theodoxus*	SC	CLG	6-8	816	–				
	Bithynia	SC	CLG	7-9	274	–				
	Valvata	SC	CLG	5-6	95	–				
	Gyraulus	SC	CLG	5-7	5	–				
	Lymnaea	SC	CLG	8-11	10	2	0.200	Pink	3416.01	Cellulose
								Black	1173.24	Polyester
Hexapoda										
Coleoptera	*Limnius*	CG	CLG	4	3	1	0.333	Black	4000.22	Cellulose
	Oulimnius	CG	CLG	4-7	265	3	0.011	Black	512.19	Polyester
								Black	1714.85	Cellulose
								Blue	1060.74	Cellulose
	Stenelmis	CG	CLG	4-6	6	1	0.167	Black	3313.62	Polyester
	Elmis	CG	CLG	4-7	32	3	0.094	Blue	1964.37	Cellulose
								Green	6994.36	Cellulose
								Black	353.19	Polyester
	Dryopidae	SH	CLG	5	3	–				
	Dytiscidae	P	SWM	9-11	15	–				
Ephemeroptera	*Caenis*	CG	SPR	4-6	15	1	0.067	Black	1170.48	Cellulose
	Baetis	CG	SWM	6-10	10	–				
	Heptagenidae	SC	CLG	6-7	3	–				
	Ephemera	CG	BRW	12-14	3	2	0.667	Black	462.66	Cellulose
								Black	856.21	Cellulose
	Potamanthus	CG	BRW	10	5	1	0.200	Black	507.1	Cellulose
Diptera	Chironomidae	CG	BRW	5-9	36	3	0.083	Black	483.51	Cellulose
								Blue	347.8	Cellulose
								Grey	2145.36	Cellulose
	Atherix ibis	P	SPR	9-14	7	–				
	Simulidae	CF	CLG	4-12	35	–				
	Limonidae	P	BRW	8	2	–				
Heteroptera	*Aphelocheirus*	P	SWM	8-10	15					
Odonata	Gomphidae	P	BRW	15-23	37	3	0.081	Blue	1319.84	Polyester
								Blue	566.62	Polyester
								White	1680.06	Cellulose
	Calopteryx	P	CLG	18-20	23	1	0.043	Orange	1553.42	Cellulose
	Coenagrionidae	P	CLG	20-22	10	1	0.100	Black	668.2	Cellulose
Plecoptera	*Leuctra*	SH	CLG	5-12	343	1	0.003	Black	1054.96	Polyester
Trichoptera	*Hydropsyche*	CF	CLG	5-16	290	1	0.003	Black	515.79	Cellulose
	Rhyacophila	P	CLG	10-15	5	–				
	Lepidostoma	SH	SPR	7-11	249	–				

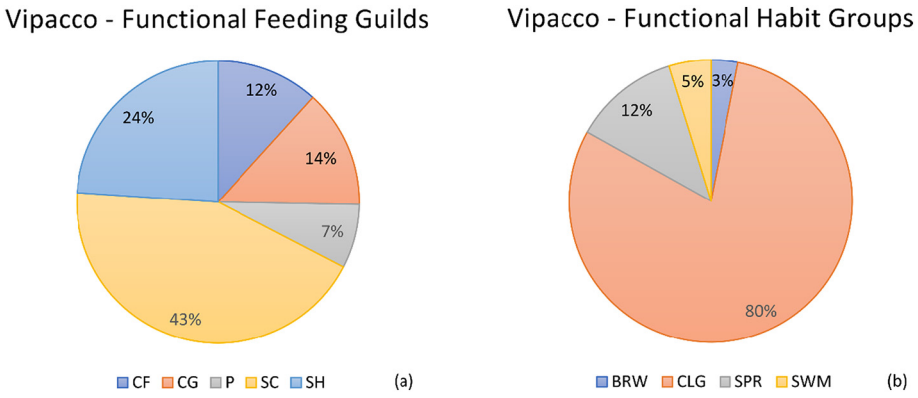


Fig. 4. Macrobenthic invertebrate community in the Vipacco River according to functional feeding guild (FFG) and functional habit group (FHG) (FFG legend: P = predators; CG = collector-gatherers; CF = collector-filterers; SH = shredders; SC = scrapers; FGH legend = SWM = swimmers, BRW = burrower; CLG = clinger; SPR = sprawler).

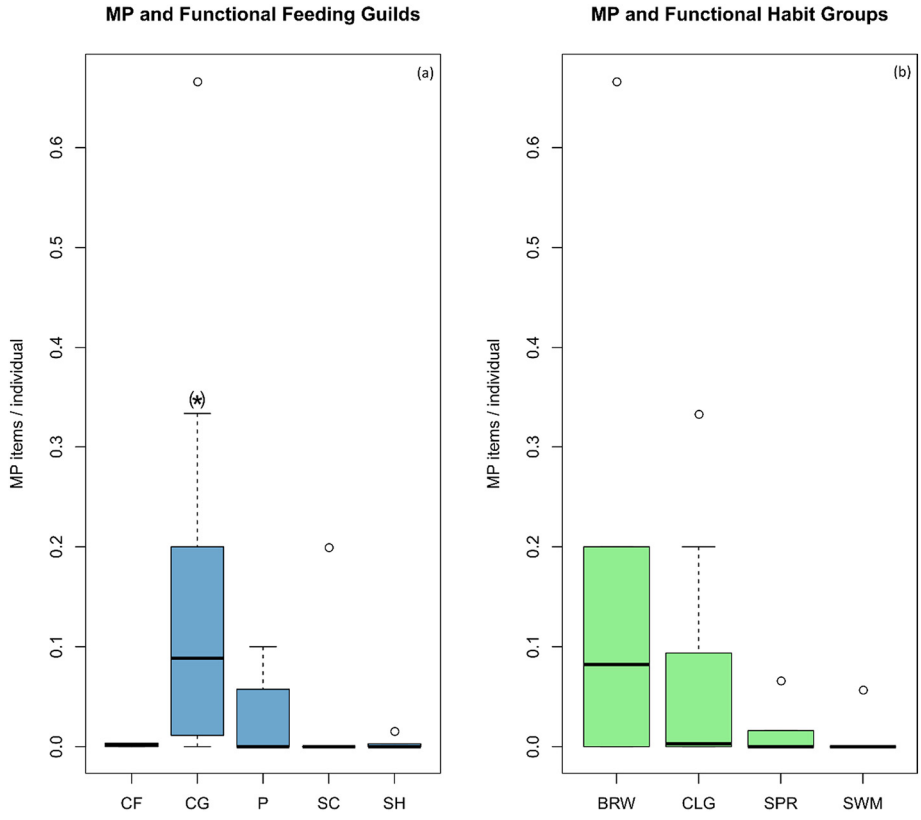


Fig. 5. Microplastics accumulation per individual in relation to functional feeding guild and functional habit group (FFG legend: P = predators; CG = collector-gatherers; CF = collector-filterers; SH = shredders; SC = scrapers; FGH legend = SWM = swimmers, BRW = burrower; CLG = clinger; SPR = sprawler). Asterisks indicate significant differences with other groups/guilds.

microplastics into the world's oceans is from synthetic fabric garments (Boucher and Friot, 2017; De Falco et al., 2019).

In the Vipacco River, fibers accounted for the main type of microplastics detected in the water and the sediment samples (46% and 42%, respectively). This finding is shared by previous studies (Nel and Froneman, 2015, Nel et al., 2018; Hurley et al., 2017; Horton et al., 2018; Akindele et al., 2019, 2020) that reported that fibers were the most abundant microplastics in abiotic and biotic samples. The fibers settle on the riverbed when flow velocity is too slow to keep them suspended (Voshell, 2002), making them available to ingestion by aquatic animals (Rosenkranz et al., 2009; Akindele et al., 2019, 2020; Windsor et al., 2019). Ingestion depends also on the characteristics of the microplastics (size, density, polymer shape and type), biological factors, and life history traits (Sidney et al., 2016).

We noted widespread microplastics contamination in the macrobenthic invertebrate community: at least one type of microplastics fiber was detected in 16 of the 33 identified taxa (48.5%). This finding recalls the observations by Windsor et al. (2019), who reported microplastics contamination in 50% of invertebrate specimens monitored in Wales. In Vipacco River, we found microplastic fibers within all feeding guilds, suggesting that different taxonomic categories could be predisposed to MP accumulation. This result agrees with observations provided by Akindele et al. (2020) in freshwater tributaries of the Guinea Gulf (Africa). In the present study, the highest level of contamination was observed in collector-gatherers, also termed deposit-feeders, which feed on material sedimented or deposited on submerged substrata (Berg, 1995). Sediment-feeding taxa may randomly ingest microplastics which are embedded in the substrate, while filtering taxa may select microplastics by size (Windsor et al., 2019) and/or shape. Collector-gatherers in our study are mainly represented by Coleoptera Elmidae, Ephemeroptera and Diptera Chironomidae. Elmidae, or riffle beetles, inhabits moss covered stones or gravel substratum, feeding mainly on algae and detritus (Elliott, 2008). It is reasonable to state that they can easily ingest microplastics attached to algae or embedded in the sediment. The present work represents the first report of microplastic accumulation for Coleoptera Elmidae. As other ephemeropterans larvae of *Caenis*, *Ephemera* and *Potamanthus* feed on algae or detritus (Sansoni, 1988) and they could easily ingest microplastics retained in the substratum. Recently, Akindele et al. (2020) reported that collector-gatherer taxa (Ephemeroptera of the family Siphlonuridae and Diptera of the genus *Chironomus*) seemed to accumulate more diverse polymers than predatory insects and suggested that these organisms could be best employed as microplastics bioindicators in freshwater ecosystems. Microplastics ingestion by deposit feeders (e.g., ephemeropterans and dipterans) has recently been reported (Nel et al., 2018; Windsor et al., 2019), and the use of invertebrates (e.g., *Chironomus* sp.) as microplastics bioindicators in freshwater systems have been strongly recommended (Nel et al., 2018; Scherer et al., 2018).

Table 2
Results of the application of the Kruskal Wallis nonparametric test and of the Conover-Iman test to the data regarding accumulation per individual in relation to functional feeding guild (FFG legend: P = predators; CG = collector-gatherers; CF = collector-filterers; SH = shredders; SC = scrapers). Significant results are highlighted in bold.

Kruskal-Wallis test, $H = 8.951$, $d.f. = 4$, $p < 0.05$				
	CF	CG	P	SC
CG	-0.604			
	0.275			
P	0.419	2.399		
	0.339	**0.012**		
SC	0.697	2.685	0.607	
	0.246	**0.006**	0.274	
SH	0.630	2.417	0.458	-0.104
	0.267	**0.011**	0.325	0.459

As Shredders feed on larger fragments, it is reasonable that deposit feeders represented by collector-gatherers are the most affected guild for MP accumulation. Predators feed on other consumers and higher content in MP accumulation, despite not significant. The trend observed for collector-gatherers FFG agrees with our hypothesis and with observation provided by Pastorino et al. (2019), Pastorino et al. (2020b, 2020c) about accumulation of other contaminants (trace elements and rare earth elements) in macrobenthic riverine communities of the same geographical area, suggesting that this FFG plays a pivotal role in contaminant accumulation. Collector-gatherers have a key role in collecting fine particulate organic matter (FPOM) into larger particles after ingesting them from the sediment (Merritt and Cummins, 2006). As observed by Akindele et al. (2020) the guild could provide a useful proxy to assess pollutant accumulation, as deposit feeders may therefore be suitable as MP bioindicators in lotic freshwater systems since they are not only site-specific, but they can also indicate impacts over a period of time.

The scrapers (mainly represented by Gastropoda) were the most abundant in the Vipacco samples, but microplastics accumulation was lowest for this functional feeding guild. Scrapers feed on algae and associated materials and could ingest microplastics attached to algae (Gutow et al., 2016). Microplastics accumulation was reported in three Gastropoda species in two Nigerian streams (Akindele et al., 2019). Reduced presence of microplastics in Vipacco gastropods could be maybe explained by the reduced size of the observed taxa: in fact, only two fibers were found in *Lymnaea* specimens, which was the bigger Gastropoda observed during the present study (Table 1). As reported by Akindele et al. (2019), larger gastropod species show a much higher MP load per individual and body size can influence the rate of microplastic uptake for different gastropod species.

However, in general we found no correlation between size of the invertebrate and size or number of ingested microplastics particles. In addition, we found no correlation between functional habit groups and microplastics accumulation in macrobenthic organisms. These findings suggest that microplastic contamination in macrobenthic invertebrates depends mainly on feeding behavior of the organisms. However, Microplastics accumulation in the biotic components of freshwater food webs likely results from a combination of biotic and abiotic factors (Windsor et al., 2019).

A future area of focus could be the study of various macrobenthic invertebrate taxa to gain a better picture of the contamination dynamics of microplastics in riverine ecosystems and freshwater environments and to add information regarding new taxa, as knowledge is still lacking form many organisms. Moreover, seasonal analysis of the whole macrobenthic community, as in classic biomonitoring programs, could help to better understand temporal variation patterns of microplastic accumulation in riverine ecosystems. Macrobenthic invertebrates are largely used in biomonitoring and monitoring programs, and they could provide a key tool to investigate the microplastics dynamics in the trophic webs of freshwater ecosystems.

5. Conclusion

The present study is the first to analyze the contamination dynamics of microplastics in a riverine ecosystem from a "whole community perspective", while previous studies focused on microplastic accumulation on few selected taxa. Our findings agree with previous observations regarding presence of microplastics in different taxa and in different functional feeding guilds, adding information regarding presence of MP in new organisms (such as Coleoptera Elmidae). Collector-gatherer FFG is the most affected by pollutant accumulation. Further studies of the seasonal effects on variation in community composition, life history stages, and watercourse discharge are desirable. Watercourse discharge rate can considerably alter microplastics dynamics in freshwater riverine ecosystems (Windsor et al., 2019), particularly in watercourses with highly variable hydrometrics, such as the Vipacco River. Research is needed to inform remediation efforts based on a more complete biological risk assessment than is currently available for many freshwater ecosystems.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.150207.

CRediT authorship contribution statement

Marco Bertoli: Conceptualization; Investigation; Data curation; Methodology; Writing - Original draft. **Paolo Pastorino**: Data curation; Methodology; Investigation; Writing - Reviewing and editing. **Davide Lesa**: Investigation; Methodology; Writing - Reviewing and editing. **Monia Renzi**: Investigation; Methodology; Writing - Reviewing and editing. **Serena Anselmi**: Investigation; Methodology; Writing - Reviewing and editing. **Marino Prearo**: Methodology; Writing - Reviewing and editing. **Elisabetta Pizzul**: Conceptualization; Investigation; Supervision; Writing - Reviewing and editing.

All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

English Language editing service was supplied by Avicenna di Kenneth Adolf Britsch & C. snc.

References

Akindele, E.O., Ehlers, S.M., Koop, J.H.E., 2019. First empirical study of freshwater microplastics in West Africa using gastropods from Nigeria as bioindicators. Limnologica 78, 125708. https://doi.org/10.1016/j.limno.2019.125708.

Akindele, E.O., Ehlers, S.M., Koop, J.H.E., 2020. Freshwater insects of different feeding guilds ingest microplastics in two gulf of Guinea tributaries in Nigeria. Environ. Sci. Pollut. Res. 27, 33373–33379. https://doi.org/10.1007/s11356-020-08763-8.

Avio, C.G., Gorbi, S., Regoli, F., 2017. Plastics and microplastics in the oceans: from emerging pollutants to emerged threat. Mar. Environ. Res. 128, 2–11. https://doi.org/10.1016/j.marenvres.2016.05.012.

Baldwin, A.K., Corsi, S.R., Mason, S.A., 2016. Plastic debris in 2 Great Lakes tributaries: relations to watershed attributes and hydrology. Environ. Sci. Technol. 50 (19), 10377–10385. https://doi.org/10.1021/acs.est.6b02917.

Berg, M.B., 1995. Larval food and feeding behaviour. In: Armitage, P.D., Pinder, L.C., Cranston, Peter (Eds.), The Chironomidae, Biology and Ecology of Non-Biting Midges. Springer, Dordrecht, pp. 136–168 https://doi.org/10.1007/978-94-011-0715-0.

Bertoli, M., Azzoni, M., Pizzul, E., 2014. A comparison between biomonitoring methods for the analysis of macrobenthic invertebrate communities in different river types of friuli venezia Giulia. Ann. Ser. Hist. Nat. 24, 139–146.

Bertoli, M., Brichese, G., Michielin, D., Ruzic, M., Vignes, F., Basset, A., Pizzul, E., 2015. Seasonal dynamics of macrobenthic community in the wetland of the natural regional Reserve of the Isonzo River Mouth, Northeast Italy: a three-years analysis. Ann. Ser. Hist. Nat. 25, 55–66.

Bertoli, M., Piazza, G., Pastorino, P., Prearo, M., Cozzoli, F., Vignes, F., Basset, A., Pizzul, E., 2021. Macrobenthic invertebrate energy densities and ecological status in freshwater watercourses (Friuli venezia-Giulia, Northeast Italy). Aquat. Ecol. 55 (2), 501–518.

Betts, K., 2008. Why small plastic particles may pose a big problem in the oceans. Environ. Sci. Technol. 42 (24), 8995. https://doi.org/10.1021/es802970v.

Boucher, J., Friot, D., 2017. Primary Microplastics in the Oceans: A Global Evaluation of Sources. IUCN, Gland, Switzerland https://doi.org/10.2305/IUCN.CH.2017.01. en.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. Environ. Sci. Technol. 45, 9175–9179. https://doi.org/10.1021/es201811s.

Browne, M.A., Galloway, T., Thompson, R., 2007. Microplastic–an emerging contaminant of potential concern? Integr. Environ. Assess. Manag. 3, 559–561. https://doi.org/10.1002/ieam.5630030412.

Buffagni, A., Erba, S., 2014. Linee guida per la valutazione della componente macrobentonica fluviale ai sensi del DM 260/2010. ISPRA, Manuali e Linee Guida 116/2014, Rome.

Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res. 91, 174–182. https://doi.org/10.1016/j.watres.2016.01.002.

Cera, A., Cesarini, G., Scalici, M., 2020. Microplastics in freshwater: what is the news from the world? Diversity 12, 276. https://doi.org/10.3390/d12070276.
Conover, W.J., 1999. Practical Nonparametric Statistics. 3rd edition. Wiley, Hoboken.
Conover, W.J., Iman, R.L., 1979. On Multiple-comparisons Procedures. Technical Report LA-7677-MS. Los Alamos Scientific Laboratory, Los Alamos.
Cummins, K.W., 1974. Structure and function of stream ecosystems. Bioscience 24, 631–641.
Dantas, D.V., Barletta, M., Da Costa, M.F., 2012. The seasonal and spatial patterns of ingestion of polyfilament nylon fragments by estuarine drums (Sciaenidae). Environ. Sci. Pollut. Res. 19 (2), 600–606.
D. Lgs 152/06, 2006. Norme in materia ambientale. Decreto Legislativo N.152. Gazzetta Ufficiale N. 88(96). http://www.parlamento.it/leggi/deleghe/06152dl.htm.
D.M. 260/2010, 2010. Regolamento recante i criteri tecnici per la classificazione dello stato dei corpi idrici superficiali, per la modifica delle norme tecniche del decreto legislativo 3 aprile 2006, n. 152, recante norme in mate- ria ambientale, predisposto ai sensi dell'articolo 75, comma 3, del medesimo decreto legislativo. http://www.gazzettaufficiale.it/eli/id/2011/02/07/011G0035/sg.
De Falco, F., Di Pace, E., Cocca, M., Avella, M., 2019. The contribution of washing processes of synthetic clothes to microplastic pollution. Sci. Rep. 9, 6633. https://doi.org/10.1038/s41598-019-43023-x.
Derraik, J.G., 2002. The pollution of the marine environment by plastic debris: a review. Mar. Pollut. Bull. 44 (9), 842–852. https://doi.org/10.1016/S0025-326X(02)00220-5.
Ding, J., Zhang, S., Mamitiana Razanajatovo, R., Zou, H., Zhu, W., 2018. Accumulation, tissue distribution, and biochemical effects of polystyrene microplastics in the freshwater fish red tilapia (Oreochromis niloticus). Environ. Pollut. 238, 1–9. https://doi.org/10.1016/j.envpol.2018.03.001.
Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ. Sci. Eur. 28, 2. https://doi.org/10.1186/s12302-015-0069-y.
Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 75, 63–82. https://doi.org/10.1016/j.watres.2015.02.012.
EFSA (European Food Safety Agency), 2016. Presence of microplastics and nanoplastics in food, with particular focus on seafood. EFSA panel on contaminants in the food chain (CONTAM). https://doi.org/10.2903/j.efsa.2016.4501.
Elliott, J.M., 2008. The ecology of riffle beetles (Coleoptera: Elmidae). Freshw. Rev. 1 (2), 189–203. https://doi.org/10.1608/FRJ-1.2.4.
European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32000L006.
Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., Boldgiv, B., 2014. High levels of microplastic pollution in a large, remote, mountain lake. Mar. Pollut. Bull. 85 (1), 156–163. https://doi.org/10.1016/j.marpolbul.2014.06.001.
Frias, J.P.G.L., Otero, V., Sobral, P., 2014. Evidence of microplastics in samples of zooplankton from portuguese coastal waters. Mar. Environ. Res. 95, 89–95. https://doi.org/10.1016/j.marenvres.2014.01.001.
Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R.C., van Franeker, J., Vlachogianni, T., Scoullos, M., Veiga, J.M., Palatinus, A., Matiddi, M., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J., 2014. Guidance on Monitoring of Marine Litter in European Seas. Publications Office of the European Union, Luxembourg, pp. 1–128 https://doi.org/10.2788/99475.
Ghetti, P.F., 1997. Indice Biotico Esteso (I.B.E.) - I macroinvertebrati nel controllo della qualità degli ambienti di acque correnti - Manuale di applicazione. Provincia Autonoma di Trento, APPA, Trento.
Gutow, L., Eckerlebe, A., Giménez, L., Saborowski, R., 2016. Experimental evaluation of seaweeds as a vector for microplastics into marine food webs. Environ. Sci. Technol. 50, 915–923. https://doi.org/10.1021/acs.est.5b02431.
Horton, A.A., Jürgens, M.D., Lahive, E., van Bodegom, P.M., Vijver, M.G., 2018. The influence of exposure and physiology on microplastic ingestion by the freshwater fish Rutilus rutilus (roach) in the river Thames, UK. Environ. Pollut. 236, 188–194. https://doi.org/10.1016/j.envpol.2018.01.044.
Hurley, R.R., Woodward, J.C., Rothwell, J.J., 2017. Ingestion of microplastics by freshwater tubifex worms. Environ. Sci. Technol. 51, 12844–12851. https://doi.org/10.1021/acs.est.7b03567.
Jabeen, K., Su, L., Li, J., Yang, D., Tong, C., Mu, J., Shi, H., 2017. Microplastics and mesoplastics in fish from coastal and fresh waters of China. Environ. Pollut. 221, 141–149.
Lavers, J.L., Bond, A.L., Hutton, I., 2014. Plastic ingestion by fleshfooted shearwaters (Puffinus carneipes): implications for fledgling body condition and the accumulation of plastic-derived chemicals. Environ. Pollut. 187, 124–129. https://doi.org/10.1016/j.envpol.2013.12.020.
Law, K.L., Moret-Ferguson, S., Maximenko, N.A., Proskurowski, G., Peacock, E.E., Hafner, J., Reddy, C.M., 2010. Plastic accumulation in the North Atlantic subtropical gyre. Science 329.
Lebreton, L.C.M., van der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the World's oceans. Nat. Commun. 8, 15611. https://doi.org/10.1038/ncomms15611.
Li, C., Busquets, R., Campos, L.C., 2020. Assessment of microplastics in freshwater systems: a review. Sci. Total Environ. 707, 135578. https://doi.org/10.1016/j.scitotenv.2019.135578.
Li, J., Liu, H., Chen, J.P., 2018. Microplastics in freshwater systems: a review on occurrence, environmental effects, and methods for microplastics detection. Water Res. 137, 362–374.
Lu, Y., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (Danio rerio) and toxic effects in liver. Environ. Sci. Technol. 50, 4054–4060. https://doi.org/10.1021/acs.est.6b00183.
Mani, T., Hauk, A., Walter, U., Burkhardt-Holm, P., 2015. Microplastics profile along the Rhine River. Sci. Rep. 5, 17988. https://doi.org/10.1038/srep17988.
Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D., Rogers, D.L., 2016. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. Environ. Pollut. 218, 1045–1054. https://doi.org/10.1016/j.envpol.2016.08.056.
Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ. Sci. Technol. 35, 318–324. https://doi.org/10.1021/es0010498.
McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., Kelly, J.J., 2014. Microplastic is an abundant and distinct microbial habitat in an urban river. Environ. Sci. Technol. 48 (20), 11863–11871. https://doi.org/10.1021/es503610r.
McDevitt, J.P., Criddle, C.S., Morse, M., Hale, R.C., Bott, C.B., Rochman, C.M., 2017. Addressing the issue of microplastics in the wake of the microbead-freewaters actda new standard can facilitate improved policy. Environ. Sci. Technol. 51, 6611e6617. https://doi.org/10.1021/acs.est.6b05812.
Merritt, R.W., Cummins, K.W., 2006. Trophic relationships of macroinvertebrates. In: Hauer, F.R., Lamberti, G.A. (Eds.), Methods in Stream Ecology. Academic Press, San Diego, pp. 585–610.
Metcalfe Smith, J.L., 1994. Biological water quality assessment of rivers: use of macroinvertebrate communities. In: Calow, P., Petts, G.E. (Eds.), The Rivers Handbook. Vol. 2. Blackwell Science, London, pp. 144–170.
Miranda, D.D.A., de Carvalho-Souza, G.F., 2016. Are we eating plastic-ingesting fish? Mar. Pollut. Bull. 103 (1-2), 109–114.
Mosetti, F., 1983. In: Quaderni, E.T.P. (Ed.), Sintesi sull'idrologia del Friuli-Venezia Giulia. Rivista di Limnologia, Udine.
Nakashima, E., Isobe, A., Kako, S., Itai, T., Takahashi, S., 2012. Quantification of toxic metals derived from macroplastic litter on Ookushi Beach, Japan. Environ. Sci. Technol. 46 (18), 10099–10105. https://doi.org/10.1021/es301362g.
Nel, H.A., Dalu, T., Wasserman, R.J., 2018. Sinks and sources: assessing microplastic abundance in river sediment and deposit feeders in an austral temperate urban river system. Sci. Total Environ. 612, 950–956. https://doi.org/10.1016/j.scitotenv.2017.08.298.
Nel, H.A., Froneman, P.W., 2015. A quantitative analysis of microplastic pollution along the south-eastern coastline of South Africa. Mar. Pollut. Bull. 101, 274–279. https://doi.org/10.1016/j.marpolbul.2015.09.043.
Pastorino, P., Bertoli, M., Squadrone, S., Brizio, P., Piazza, G., Oss Noser, A.G., Prearo, M., Abete, M.C., Pizzul, E., 2019. Detection of trace elements in freshwater macrobenthic invertebrates of different functional feeding guilds: a case study in Northeast Italy. Ecohydrol. Hydrobiol. 19 (3), 428–440.
Pastorino, P., Pizzul, E., Bertoli, M., Anselmi, S., Kušce, M., Menconi, V., Prearo, M., Renzi, M., 2020a. First insights into plastic and microplastic occurrence in biotic and abiotic compartments, and snow from a high-mountain lake (Carnic Alps). Chemosphere 265, 129121. https://doi.org/10.1016/j.chemosphere.2020.129121.
Pastorino, P., Zaccaroni, A., Doretto, A., Falasco, E., Silvi, M., Dondo, A., Elia, A.C., Prearo, M., Bona, F., 2020b. Functional feeding groups of aquatic insects influence trace element accumulation: findings for filterers, scrapers and predators from the Po Basin. Biology 9 (9), 288.
Pastorino, P., et al., 2020c. Macrobenthic invertebrates as tracers of rare earth elements in freshwater watercourses. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2019.134282.
Pizzul, E., Guiotto, S., Moro, G.A., 2008. Osservazioni sulle comunità macrozoobentoniche dell'Isola della cona (Friuli-Venezia Giulia, nordest Italia). Ann. Ser. Hist. Nat. 18, 79–90.
QGIS.org, 2018. QGIS geographic information system. Opensource geospatial foundation project. http://qgis.org.
Redondo-Hasselerharm, P.E., Falahudin, D., Peeters, E.T.H.M., Koelmans, A.A., 2018. Microplastic effect thresholds for freshwater benthic macroinvertebrates. Environ. Sci. Technol. 52, 2278–2286. https://doi.org/10.1021/acs.est.7b05367.
Remy, F., Collard, F., Gilbert, B., Compère, P., Eppe, G., Lepoint, G., 2015. When microplastic is not plastic: the ingestion of artificial cellulose fibers by macrofauna living in seagrass macrophytodetritus. Environ. Sci. Technol. 49 (18), 11158–11166. https://doi.org/10.1021/acs.est.5b02005.
Rosenberg, D., Resh, V., 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, New York.
Rosenkranz, P., Chaudhry, Q., Stone, V., Fernandes, T.F., 2009. A comparison of nanoparticle and fine particle uptake by Daphnia magna. Environ. Toxicol. Chem. 28, 2142–2149. https://doi.org/10.1897/08-559.1.
Sanchez, W., Bender, C., Porcher, J.M., 2014. Wild gudgeons (Gobio gobio) from french rivers are contaminated by microplastics: preliminary study and first evidence. Environ. Res. 128, 98–100. https://doi.org/10.1016/j.envres.2013.11.004.
Sansoni, G., 1988. Atlante per il riconoscimento dei macroinvertebrati dei corsi d'acqua italiani. Provincia Autonoma di Trento. Stazione Sperimentale Agraria Forestale. Servizio Protezione Ambientale. APR & B Editrice, Trento.
Scherer, C., Brennholt, N., Reifferscheid, G., Wagner, M., 2018. Feeding type and development drive the ingestion of microplastics by freshwater invertebrates. Sci. Rep. 5 (2017), 17006. https://doi.org/10.1038/s41598-017-17191-7.
Scherer, C., Weber, A., Stock, F., Vurusic, S., Egerci, H., Kochleus, C., Reifferscheid, G., 2020. Comparative assessment of microplastics in water and sediment of a large European river. Sci. Total Environ. 738, 139866.

Sidney, L.A., Diepens, N.J., Guo, X., Koelmans, A.A., 2016. Trait-based modelling of bioaccumulation by freshwater benthic invertebrates. Aquat. Toxicol. 176, 88–96. https://doi.org/10.1016/j.aquatox.2016.04.017.

Singh, B., Sharma, N., 2008. Mechanistic implications of plastic degradation. Polym. Degrad. Stab. 93 (3), 561–584.

Su, L., Cai, H., Kolandhasamy, P., Wu, C., Rochman, C.M., Shi, H., 2018. Using the Asian clam as an indicator of microplastic pollution in freshwater ecosystems. Environ. Pollut. 234, 347–355.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. U. S. A. 113, 2430–2435. https://doi.org/10.1073/pnas.1519019113.

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Hung Viet, P., Seang Tana, T., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. B 364 (1526), 2027–2045. https://doi.org/10.1098/rstb.2008.0284.

Tourinho, P.S., Ivar do Sul, J.A., Fillmann, G., 2010. Is marine debris ingestion still a problem for the coastal marine biota of southern Brazil? Mar. Pollut. Bull. 60 (3), 396–401. https://doi.org/10.1016/j.marpolbul.2009.10.013.

Turra, A., Manzano, A.B., Dias, R.J.S., Mahiques, M.M., Barbosa, L., Balthazar-Silva, D., Moreira, F.T., 2014. Three-dimensional distribution of plastic pellets in sandy beaches: shifting paradigms. Sci. Rep. 4, 4435.

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70. https://doi.org/10.1016/j.envpol.2014.06.010.

Van Cauwenberghe, L., Vanreusel, A., Mees, J., Janssen, C.R., 2013. Microplastic pollution in deep-sea sediments. Environ. Pollut. 182, 495–499. https://doi.org/10.1016/j.envpol.2013.08.013.

von Friesen, L.W., Granberg, M.E., Hassell, M., Gabrielsen, G.W., Magnusson, K., 2019. An efficient and gentle enzymatic digestion protocol for the extraction of microplastics from bivalve tissue. Mar. Pollut. Bull. 142, 129–134. https://doi.org/10.1016/j.marpolbul.2019.03.016.

Voshell, J.R., 2002. A Guide to Common Freshwater Invertebrates of North America. The McDonald and Woodward Publishing Company, Granville.

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, A.D., Winther-Nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. Environ. Sci. Eur. 26, 12. https://doi.org/10.1186/s12302-014-0012-7.

Windsor, F.M., Tilley, R.M., Tyler, C.R., Ormerod, S.J., 2019. Microplastic ingestion by riverine macroinvertebrates. Sci. Total Environ. 646, 68–74. https://doi.org/10.1016/j.scitotenv.2018.07.271.

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1, 140317. https://doi.org/10.1098/rsos.140317.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031.

Ziajahromi, S., Neale, P.A., Rintoul, L., Leusch, F.D.L., 2017. Wastewater treatment plants as a pathway for microplastics: development of a new approach to sample wastewater-based microplastics. Water Res. 112, 93–99. https://doi.org/10.1016/j.watres.2017.01.042.