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Microplastic patterns in riverine waters and leaf litter: Leaf bag technique to investigate the microplastic accumulation trends in lotic ecosystems

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ABSTRACT

Microplastics (MPs) are one of the major ecological concerns of the last years and despite the increasing interest and the rise of many studies regarding freshwater habitats, many aspects about distribution patterns, transport pathways and impacts of MPs in those systems need to be investigated. The present study characterizes the temporal trends of MP concentrations in waters of a riverine stretch of the northeastern Italy, subject to flow rate variations and investigates the MP accumulations patterns in the leaf litter, simulated in situ via leaf bag technique. MP concentrations in the water were significantly and negatively correlated to the flow rate regimes, with higher concentrations observed during low discharge periods. MPs accumulation in leaf bags agreed with trends observed in the water and the presence of wastewater discharge points positively affects the levels of MP contaminations within the leaf bags. These findings seem to suggest that the maintenance of a hydrological regime at relatively high levels in the investigated system could allow to maintain the self-purifying riverine processes and the disposal of microplastics like any other polluting substance. The use of leaf bag technique for the purpose to investigate MP accumulation trends on field provided useful information, is easy to modulate in terms of time periods and allow to record the evolution of the MP patterns also in relation to high flow rate episodes. Our results suggest that the method can be employed in new a perspective, to improve the knowledge about one of the major threats of the Anthropocene.

1. Introduction

Microplastics particles (MPs), smaller than 5 mm, are unanimously recognized as one of the major environmental threats of the Anthropocene (Waters et al., 2016; Multisanti et al., 2022). Nowadays, MPs are ubiquitous and can be found in all the aquatic environments, from alpine areas to ocean floor sediments (Van Cauwenberghe et al., 2013; Free et al., 2014; Woodall et al., 2014; Pastorino et al., 2020, 2022) and to the depths of the oceanic trenches (Peng et al., 2018a; Peng et al., 2020). The research interest about MPs was initially focused on marine ecosystems, but studies regarding freshwater environments rapidly increased in the last years, including investigations about contamination in water column and sediments (Lechner et al., 2014; McCormick et al., 2014; Dris et al., 2015, 2018; Klein et al., 2015; Guerranti et al., 2017; Horton et al., 2017; Wang et al., 2017a; Sarkar et al., 2019; Valine et al.,

2020), sources, transformation, bioaccumulation in trophic levels and toxicity to aquatic organisms (Hurley et al., 2017; Nel et al., 2018; de Sá et al., 2018; Foley et al., 2018; Li et al., 2020; Windsor et al., 2019; Akindele et al., 2019, 2020; Silva et al., 2021a; Bertoli et al., 2021; Piccardo et al., 2021; Pagano et al., 2022). Among the freshwater environments, watercourses can be considered as “highways” used by plastic debris of different sizes (including MPs), and the main way for plastics to reach the seas (Matjašič et al., 2023). Modeling studies allowed to estimate that 0.47–2.75 million tons of plastics are released into the oceans via rivers each year (Lebreton et al., 2017; Schmidt et al., 2017, 2018). Recently, González-Fernández et al. (2021) calculated an amount of 1656–4997 tons per year from Europe, based on repeated direct monitoring activities of floating macrolitter. The evaluation of MP inputs to the seas is strictly related to the uncertainties in the estimations within riverine systems, which are still very limited in their spatial and

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temporal coverage (González-Fernández et al., 2021), and studies regarding different timescales are still not frequent in literature (Moore et al., 2011; Castro-Jiménez et al., 2019; González-Fernández et al., 2021). In fact, despite the increasing interest and the rise of many studies regarding freshwater habitats, many aspects about distribution patterns, transport pathways and impacts of MPs in those systems need to be explored (Wang et al., 2021; Matjašič et al., 2023).

Significant sources of MP pollution include plastic textile fibers (Browne et al., 2011) and degrading MPs whose origins are concentrated on land (Jambeck et al., 2015). From there, most of the flux of terrestrially derived plastic particles originated likely from wastewater treatments or associated overflow systems that discharge into rivers (Mani et al., 2015; Windsor et al., 2019). MPs from households, industrial and other wastewater, and urban or road runoff are collected in wastewater treatment plants (WWTPs) and discharge points/sewage waters represent a main point-source link between MPs and the aquatic ecosystems (Ziajahromi et al., 2016; Kovač Viršek et al., 2017).

Once in the water, MPs can be stored in the sediments and become available for aquatic organisms, such as fishes and invertebrates that could ingest microplastics with negative effects (Bour et al., 2018; Windsor et al., 2019; Silva et al., 2019, 2021b; Bertoli et al., 2021). Moreover, MPs can interfere with freshwater ecosystem dynamics, such as decomposition processes. Recently some works focused the effects due to MP on leaf litter, finding that MPs can negatively affect the organic matter decomposition interfering with the activities of fungal and macrobenthic invertebrate decomposers (Seena et al., 2019; Seena et al., 2022; López-Rojo et al., 2020; Du et al., 2022; Ockeden et al., 2022; Bertoli et al., 2023). MPs concentration and bioavailability in rivers is likely to be affected by many factors, including upstream land-use, urban runoff, relative volumes of discharged effluent from point wastewater sources and local hydraulics that determine entrainment or deposition (Nizzetto et al., 2016; Besseling et al., 2017; Nel et al., 2018). High MP concentrations can be found in river sediments (Hurley et al., 2018), and the significant MP removal from those sediments occurs in response to floods. However, few studies have been carried out regarding the accumulation in the leaf litter and in the organic matter debris accumulations, which could represent a retention structure for MPs. Moreover, the leaf litter represent a pivotal compartment in aquatic ecosystems, where fungi and invertebrates are involved in decomposition processes.

The present study is part of a project regarding MPs in riverine ecosystems and represents the continuation of previous investigations. In former field studies, we investigated the trends of MPs in Functional Feeding Guilds of macrobenthic invertebrate communities (Bertoli et al., 2021) and the vegetal organic matter decomposition rates via leaf bag technique (Bertoli et al., 2023). The latter study highlighted a negative contribution to decomposition rate, due to MP presence within the leaf bags. In this context, due to the importance of leaf litter in riverine environments, it was deemed of interest to investigate the trends of MP accumulation patterns within the leaf litter in the same riverine stretch characterized by high flow rate variations, trying to deepen different aspects of MP pollution in watercourses. For this purpose, the leaf bag technique (Petersen and Cummins, 1974; Basset et al., 2006) was chosen again. This method is commonly used to investigate the organic matter decomposition dynamics in aquatic ecosystems, as it simulates the natural accumulation of organic matter. Moreover, the technique allows to obtain information regarding different exposition time periods (weeks, months, seasons) directly on field, as the duration of sample submersion can be modulated in agreement with study aims and research necessities. Due to these reasons, we try to use it in this new way, as a tool for field experiment to detect MPs in leaf litter of watercourses.

Our aims were 1) to obtain a temporal characterization of MP contamination in waters and leaf litter, 2) to verify if accumulation of MP in the leaf bags could be positively affected by the presence of wastewater/sewage discharge points along the riverine section and 3) to investigate the variation of MP accumulation in waters and leaf bags in

relation to the discharge variation across different seasons. In this context, the leaf bag technique could assume a new role, as an experimental tool to investigate MP accumulation patterns in freshwater environments.

2. Material and methods

2.1. Study area

The study area has been already described in detail by Bertoli et al. (2021, 2023), which already analyzed the presence of MPs in waters, sediments and macrobenthic invertebrates and the relationship between MPs and leaf litter decomposition dynamics. The present work was developed in the same research context. This study was carried out in the Italian stretch of the Vipacco River (Northeast Italy), a cross-border watercourse which origins in Slovenia at 1019 m a.s.l. in the karstic area of Mount San Lorenzo. The River is 49 km in length and flows mainly in Slovenian territory, as the Italian stretch is 4.5 km long. In Italy, the Vipacco River represents the main left tributary of the Isonzo River, and it flows on an impermeable clayey or clay-sandy alluvial deposits that then change into flysch.

The catchment area is about 760 km² and the Vipacco sources are fed by a karst basin which dimensions are highly variable over time, depending on the behavior of the different drainages and rainfall regimes. Therefore, the river flow rates are highly fluctuating, with minimum below 1 m³ s⁻¹ and frequent floods with flows exceeding 300 m³ s⁻¹ (Mosetti, 1983). The average flow over the past 20 years is approximately 18 m³ s⁻¹ (www.arso.gov.si). Concerning the land use for territories contiguous to the watercourse, the Slovenian area is characterized by natural woodlands surrounded by agricultural zones (arable fields, permanent crops, fruit trees and vineyards) and scarce urbanization. Impacts can be related to small industrial spots, landfills, construction sites and railway/road networks close to the river. The Italian watercourse section is slightly modified, and it is placed in a scarcely urbanized context, with agricultural activities and small industrial spots. As reported by the Regional Agency for Environmental Protection of Friuli Venezia Giulia Region, the stretch is sensitive to anthropic impacts, and it is included in the ecological monitoring framework managed by local authorities (ARPA FVG; <http://www.arpaweb.fvg.it>).

The present study was carried out in a 2 km riverine section, from the State border to the middle of the Italian stretch. As described by Bertoli et al. (2021, 2023), the river was characterized by the presence of different habitats such as uniform flow areas (60%), pools (20%), glides (10%) and riffles (10%). Main substrates were coarse/fine gravel and cobbles. The riverbed vegetation cover was always low (absent or <20%). Microplastics were already detected in the Vipacco River both in both in sediment (300 ± 098.1 items kg⁻¹) and water (0.313 ± 0.251 items m³) (Bertoli et al., 2023).

Three waste-water discharge spots are present along the Italian stretch (Fig. 1) and three sampling sites were chosen to place the leaf bags. The first site was placed 10 m upstream to the waste-water discharge point close the border between Italy and Slovenia; the second was located 20 m downstream to the central waste-water point; the third site was placed 400 m downstream of the third water discharge point. The sampling sites number and positions were heavily affected by accessibility to the river, as the Vipacco is a non-wadable watercourse, showing very high variability in discharge and hydrometric levels. As it was not possible to add other sampling points due to hydro-morphological characteristics of the river, we considered the sites described above. Information regarding the monitored sampling sites are reported in Table 1.

2.2. Microplastic sampling in water

Water samples were collected to characterize the presence of microplastics and its temporal variability (Barrows et al., 2018).

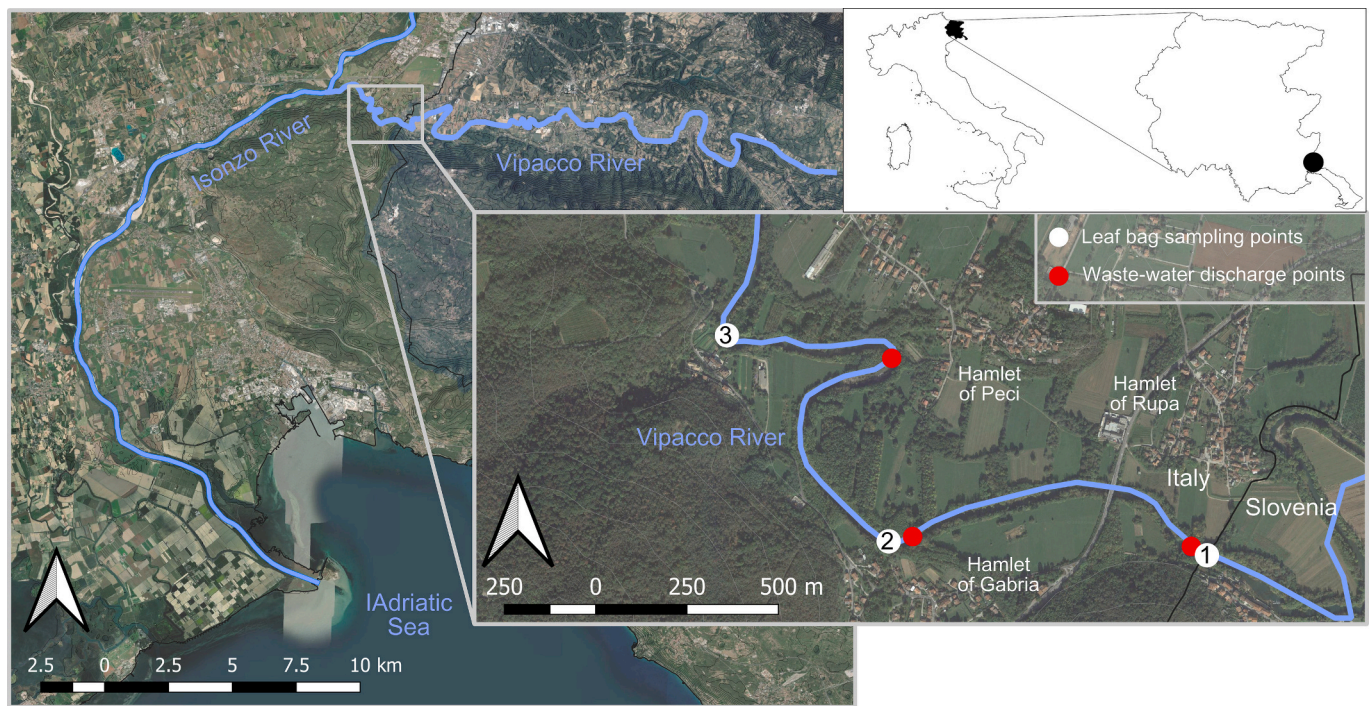


Fig. 1. Study area and sampling sites in the Vipacco River.

Table 1

Geographical coordinates and short description of the leaf bags sampling sites monitored at the Vipacco River during the present study (source of information: Bertoli et al., 2023).

Site	Latitude	Longitude	Main habitats and substrates	Wastewater discharge points
1	45°53'17.160" N,	13°35'27.798" E	uniform flow area; main substrates: coarse gravel, cobbles; no vegetation cover	Site is close to the State Border, 10 m upstream to a wastewater discharge point;
2	45°53'17.670" N	13°34'47.800" E	uniform flow areas, riffles and glides; main substrates were coarse gravel, cobbles, sometimes boulders; presence of vegetation cover during summer	The site is 20 m downstream of a wastewater discharge point
3	45°53'35.418" N	13°34'26.924" E	large pool area, low current velocity; main microhabitat: coarse and fine gravel; vegetation cover almost absent;	The site is 400 downstream of a wastewater discharge point; houses are present close to the riverbed

Collections were performed approximately every 15 days from summer 2021 to spring 2022, in agreement with flow rates that allowed the safe access to the riverbed. During the leaf bag sampling period (see below), water samplings are planned to be weekly performed. At each sampling event, parameters such as the width and depth profiles of the river section were recorded. Water samples were collected using an Apstein plankton net (opening 400 × 1000 mm; mesh size 50 µm), held stationary beneath the water surface for 5, 10, and 15 min (Valine et al.,

2020). Three replicates were collected for each time interval to examine the intra-site variability in the quantity and composition of microplastics. At the end of each sampling, the net was placed in a vertical position and thoroughly rinsed with river water to move the microplastics downward (Talbot et al., 2022). The water samples were then transferred to 500 mL glass jars, stored in a refrigerator, and brought to the laboratory until the start of analyses, which involved determining the chemical characteristics of the MPs. The water volume for each sample was calculated using the following equation:

$$Volume = A \times T \times v$$

where A is the area of the plankton net opening (m^2), T is the sampling duration (s) and v is the average water velocity ($m s^{-1}$) (Campanale et al., 2020). Based on this data, the microplastic concentrations were calculated by dividing the total concentration of each sample by the sampled water volume, thus standardizing the data (de Carvalho et al., 2021). The microplastic concentrations for each replicate were then averaged for each site during each sampling session. Furthermore, daily microplastic loads were calculated by multiplying the average microplastic concentration at each site for each sampling date by the corresponding average daily flow rate (Park et al., 2020) and then multiplying by the total number of seconds in a day (86,400).

2.3. Leaf bag samplings

To simulate the natural leaf litter accumulation within the riverbed, leaf bags were prepared following indications reported by Basset et al. (2006) and previous studies carried out in other freshwater bodies in the same Region and also in the Vipacco itself (Bertoli et al., 2016, 2020, 2022, 2023). Leaves of common reed, *Phragmites australis* (Cav.) Trin. ex Steud was used as is a widespread cosmopolitan species (Packer et al., 2017) and it grows along the Vipacco stretch, in the area where it joins the Isonzo River.

Leaves were previously collected during the senescent phase, choosing only intact ones at least 30 cm above the water surface, avoiding the apical ones. Leaves were air dried and cut into fragments about 10 cm-long, avoiding basal and apical sections. These fragments

were oven-dried to constant weight (60 °C for 72 h), for sample standardization and to get initial uniform conditions (Bärlocher, 1997). Lots of 3.0000 ± 0.0001 g (dry weight) were put inside tube-shaped bags made with cotton net (5 mm mesh). This mesh allows macrobenthic invertebrate colonization and reduces leaf material loss (Basset et al., 2006), but also allows MPs to enter the leaf bags, as they are <5 mm in size. Sampling was performed during four seasons: summer (June–August 2021), autumn (October–December 2021) winter (February–March 2022) and spring (April–May 2022). During each season, leaf bags were put gently underwater, close to the bottom and tethered with strings to stones and/or trees to avoid sample loss. Four leaf bags were collected at each site after 15, 30 and 45 days of submersion, and were used to investigate the microplastic accumulation in leaf litter, which was considered as a MPs retention tool. Samples were stored in glass jars filled with 70% ethanol and brought to the laboratory, where macrobenthic invertebrates were separated from the leaves using stainless steel tweezers and steel nets with 50 µm mesh size, to avoid microplastic loss. Leaf fragments and detritus were kept and stored in 70% ethanol. Then, samples were analyzed to investigate the MPs presence in the leaf bags material.

2.4. Microplastic analyses

Samples were treated and analyzed following the methods published by Koelmans et al. (2019) and Enders et al. (2020). Negative and positive controls were performed during the extraction and quantification procedures to avoid false positives and false negatives ($n = 5$). Laboratory analyses were conducted under a HEPA-II hood. The optimal digestion method for the matrix was selected based on Enders et al. (2020). Water samples were vacuum-filtered through circular paper filters with 10 µm pores (Whatman®, Sigma-Aldrich, St. Louis, MO, USA). The filters were placed on glass Petri dishes to prevent possible contamination during oven drying (35 °C). Once the water evaporation was complete, the filters were analyzed using a stereomicroscope, and plastic and microplastic particles were collected for chemical determination. Sediment samples were extracted three times using a prefiltered saturated NaCl solution through mechanical agitation (20 min, 100 rpm), and the resulting supernatant was filtered onto 10 µm pore size paper disks. Plastic and microplastics were analyzed using Fourier Transform Infrared Spectroscopy (FT-IR) coupled with a microscope (µFT-IR Nicolet iN 10MX®, Thermo Fischer Scientific, Waltham, MA, USA). The samples were analyzed either by reflection using a gold foil as the reflective surface or by transmission using a BaF₂ optical window (Thermo Fisher Scientific). The µFT-IR system was equipped with a liquid nitrogen-cooled MCT-A detector (spectral range 7800–650 cm⁻¹) and operated via the OMNICTM Picta user interface (Thermo Fisher Scientific). Once the chemical spectrum was obtained, it was compared to a library of known materials to determine the spectral differences. The average spectrum was calculated for each particle by plotting ten individual spectrum acquisitions together, thus obtaining a robust spectral representation of each specific fragment. The limit of detection (LOD) for chemical particle analysis was determined to be 10 µm. The limit of quantification (LOQ) for water samples was determined by the mesh size of the plankton net used during field activities, which was 50 µm. The collected items were finally classified in agreement with criteria provided by Galgani et al. (2014).

2.5. Statistical analysis

Correlations between MP concentration in the waters of the Vipacco River and the flow rates were investigated with Spearman's non-parametric coefficient ρ . Data relating to the hydrometric level and flow rate of Vipacco were obtained from the site <https://www.arso.gov.si/> by consulting the hydrometric station “Miren I”, located about a hundred meters upstream from the state border between Italy and Slovenia.

Differences in seasonal mean MP concentrations within the leaf bags were investigated using the nonparametric Kruskal-Wallis test and the Conover–Iman post-hoc test (Conover, 1999; Conover and Iman, 1979). The same test was used to compare MP concentrations between sampling sites in the context of the same season.

All analyses were performed using RStudio version 2021.9.0.351 (R Core Team, 2021; RStudio, 2021). Figures were produced with RStudio and processed with software Inkscape version 0.92.

3. Results

3.1. Microplastics in the water

MPs were always detected in all the water samples and different polymer types were observed, such as polypropylene (PP), polyurethane (PU), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyethylene (PE), polyamide (PA) and polystyrene (PS). PET was the most frequent polymer in summer (46%) while its levels remain fairly constant in the Vipacco water during the other seasons (13–19%). PP represented the 20% of MPs found in summer samples, showing higher levels in the other monitoring periods (35–47%). PP and PET were most of the MPs collected in water samples, representing together at least 48% of the collected polymers in Vipacco waters (Fig. 2). Among the other MP types, PA and PE were also frequently found, though their frequencies were lower than PP and PET (Fig. 2): PA was 10% of the samples in summer and autumn, increasing to 20% in winter and spring, while PE showed lower percentages in summer and spring (9% and 8%, respectively) than in autumn and winter (15% and 13%, respectively). PS ranged between 2% and 10%, with the maximum frequency observed in autumn, while it was absent in spring. PU was found only in summer (12%) and winter (5%) while PVC was absent in summer and showed reduced variability in frequencies, ranging between 6% and 12% (Fig. 2).

The MP density in the Vipacco waters ranged between 0.041 and 0.600 items m⁻³ (Fig. 3a). The mean size of the collected items was 460.2 ± 10.5 µm. A decreasing density trend was observed in relation to the increase of flow rate values and vice-versa (Fig. 3a). Seasonally, MP were significantly more abundant in summer and spring (0.224 ± 0.134 items m⁻³ and 0.342 ± 0.172 items m⁻³ respectively) than in the autumn and winter (0.152 ± 0.073 items m⁻³ and 0.139 ± 0.100 items m⁻³ respectively), which did not differ significantly. The application of Spearman's non-parametric correlation coefficient allowed to highlight a significant negative correlation between MP concentrations in the waters and the flow rate ($\rho = -0.670$, $n = 28$) (Fig. 3a, b).

3.2. Microplastics in leaf bags

MP items were collected via leaf bag technique during each monitoring season and at each site and the same polymer types recorded in water samples were observed. The most abundant MPs were PP, PET and PE, which were collected almost at each sampling site (Fig. 4). PP and PET were generally the most of MPs in leaf bags (42–76%), reflecting the trends recorded for water samples and showing fairly constant seasonal trends. Exceptions were observed in site 3, where PP was absent in summer and showed lowest frequency in winter (7%). Low PP frequencies were also observed in autumn in site 1, while in winter the highest value (54%) was recorded. PET showed slightly constant trends in all seasons and was absent only in site 1 in winter. PE was the third polymer in terms of frequencies observed within the leaf bags, and showed similar trends in all seasons and sites, except in site 1 in summer, when it was not found, and in site 4 in autumn, when the highest value was recorded (37%). PS showed also slightly decreasing trend through seasons, showing frequencies between 12 and 17% in summer, 6–17% in autumn, 8–13% in winter and 7–8% in spring, it was not recorded at site 3. PVC (6.7–20.0%) wasn't found during summer and autumn, while PU was the less abundant MP in leaf bags and was found only in two sites in

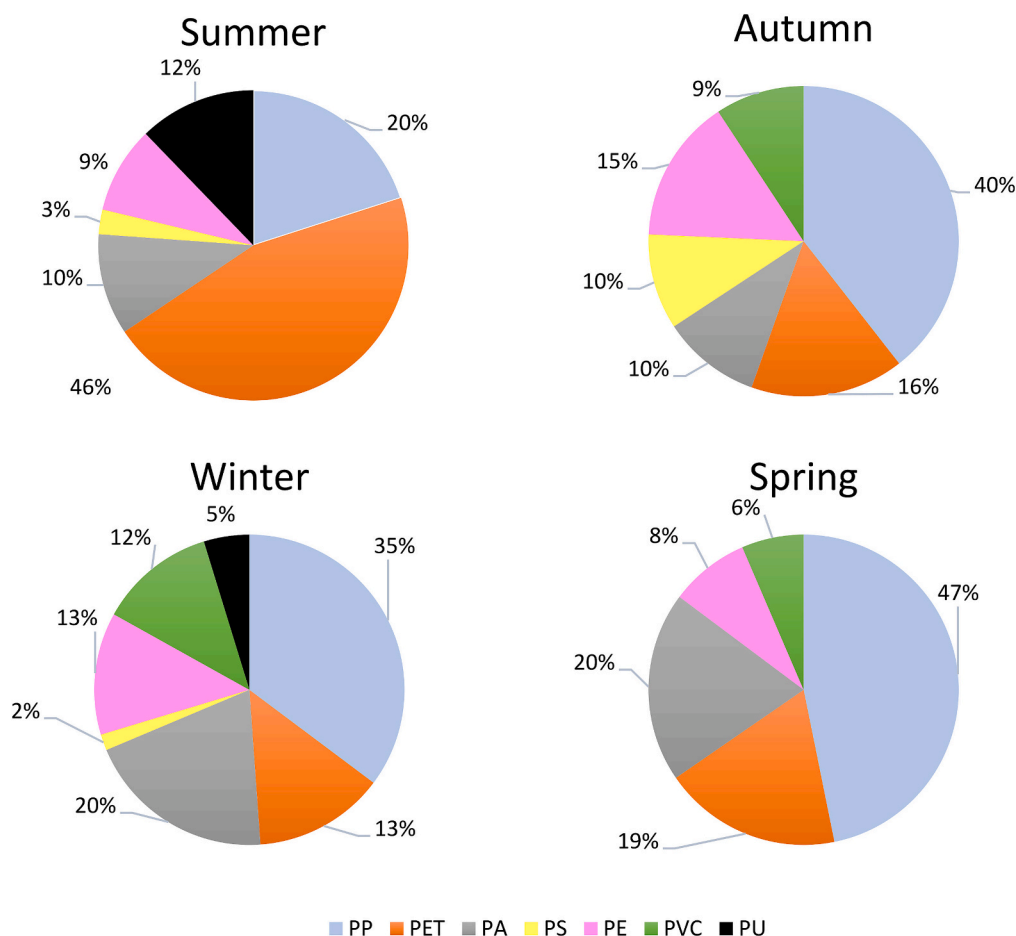


Fig. 2. Microplastic chemical composition observed in Vipacco water samples (PP = polypropylene; PU = polyurethane; PET = polyethylene terephthalate; PVC = Polyvinyl chloride; PE = polyethylene, PA = polyamide; PS = polystyrene).

summer (Fig. 4).

MPs in leaf bags showed high abundances with low flow rate values, especially in site 2 (Fig. 5a). During summer, MP concentrations in leaf bags were higher in site 2 than in sites 1 and 3, while the latter showed the lowest MP densities (Kruskal Wallis test: $H = 6.713$, $d.f. = 2$, $p < 0.05$; Conover – Iman test: $p < 0.05$). A similar trend was observed in autumn, despite the Kruskal Wallis test was not significant, with site 2 showing values slightly higher than the others (Kruskal Wallis test: $H = 4.545$, $d.f. = 2$, $p = 0.10$). In winter, concentrations did not differ between sampling points, despite site 3 showed slightly higher levels (Kruskal Wallis test: $H = 0.963$, $d.f. = 2$, $p = 0.62$). Finally, the spring trend was similar to those observed in autumn, with the lowest concentrations observed in site 3 and slightly higher values in site 2 (Kruskal Wallis test: $H = 1.384$, $d.f. = 2$, $p = 0.50$) (Fig. 5b).

Concerning seasonal differences for each sampling point, MPs abundances in site 1 did not differ among seasons (Kruskal Wallis test: $H = 1.170$, $d.f. = 3$, $p = 0.76$), while significant variations were highlighted for site 2 (Kruskal Wallis test: $H = 4.408$, $d.f. = 3$, $p < 0.05$; Conover – Iman test: $p < 0.05$), where concentrations of MPs were dramatically higher in summer, decreased significantly in autumn and then remained at the same levels in winter and spring, when a higher variability was observed and the levels seem to increase again. In site 3, levels of MPs in the leaf bags were comparable in summer and autumn, significantly increased in winter and then decreased in spring (Kruskal Wallis test: $H = 5.554$, $d.f. = 3$, $p < 0.05$; Conover – Iman test: $p < 0.05$).

4. Discussion

The present study investigates the presence of microplastics (MPs) in water and leaf litter within a riverine stretch subjected to high flow rate variability and some anthropogenic impacts. In this context, the leaf bag technique is a useful tool for these investigations.

4.1. Microplastics in water samples

The waters of the Vipacco River are known to be affected by MP pollution, with main MPs belonging to several chemical types such as PET, PP, PA and PE (Bertoli et al., 2022, 2023). These polymers were the most abundant in water samples, and are commonly employed for packaging, containers fishing gears, agricultural films, household tools and other uses. The presence of these MPs has been related to the land use in the Vipacco River area, as small industrial spots are present both in Italian and Slovenian territory. Agricultural activities could also be a source, as arable land, permanent crops of fruit trees and vineyards are present in the area. However, how observed in other studies, Eventually, the wastewater discharge points can be considered as one of the main MP sources for the investigated river. Among the observed polymers, PP is used for the production of synthetic garments (Mishra et al., 2022) and PET is an indicator of fibers dispersion from clothes through washing (Wang et al., 2019), because it is commonly used for clothes and represent the highest number of artificial fibers produced worldwide (Alvarez Troncoso et al., 2022). Moreover, PET is one of the most abundant MP types recovered in sewers (Talvitie et al., 2015) and across urban and industrial areas (Su et al., 2016; Wang et al., 2017a). A large

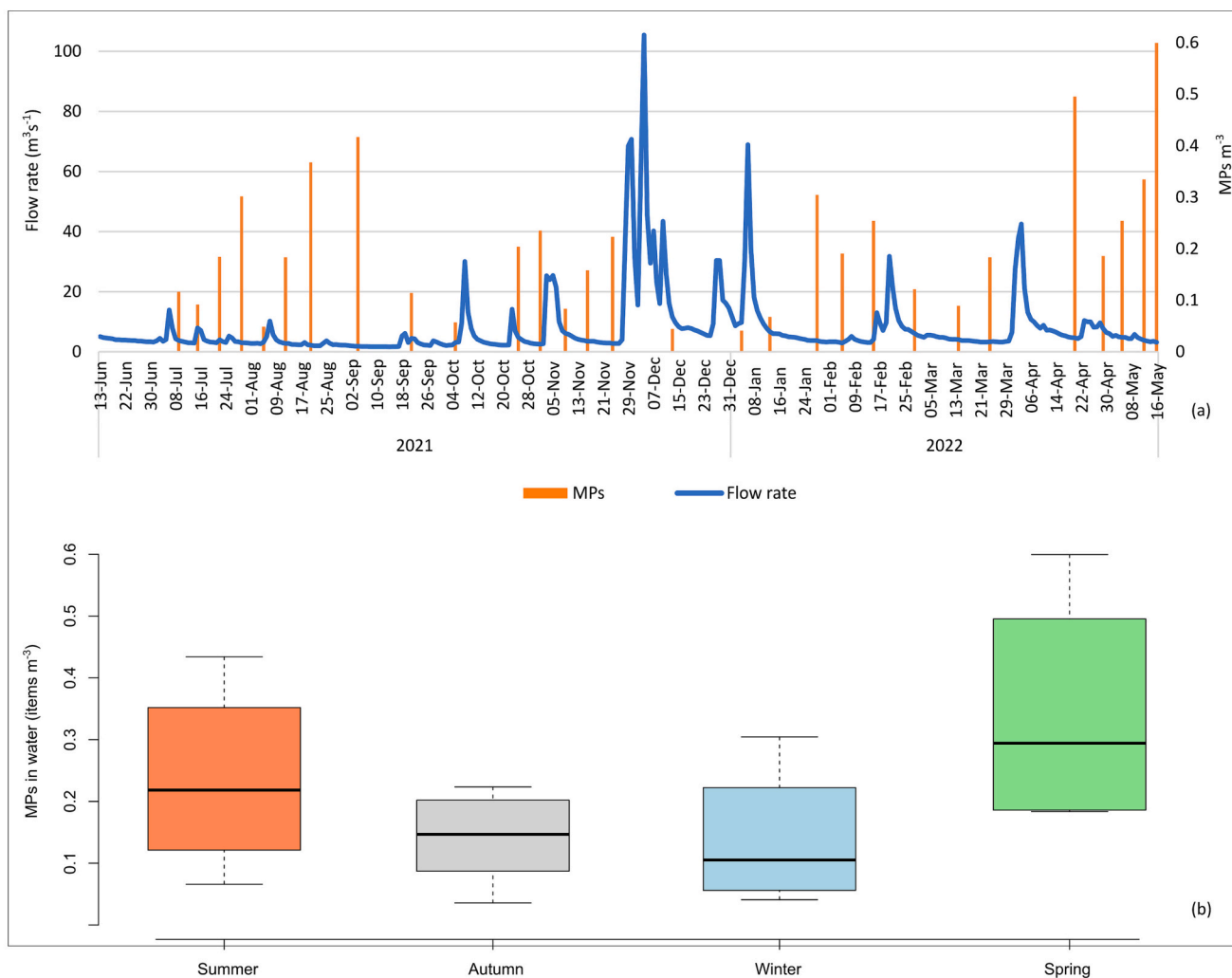


Fig. 3. MP concentrations in the Vipacco River waters (items m^{-3}) in relation to the flow rate (a) and seasonal variations observed during the study period (b).

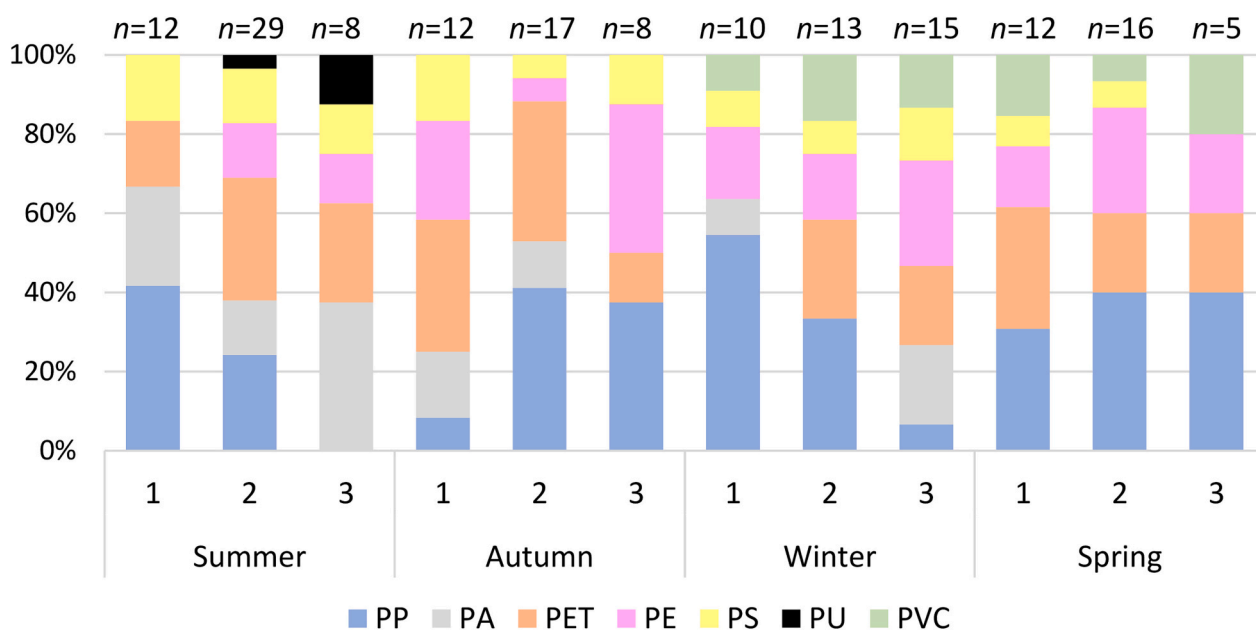


Fig. 4. Seasonal and spatial composition of MPs observed in leaf bags in the monitored sampling points (PP = polypropylene; PA = polyamide; PET = polyethylene terephthalate; PE = polyethylene; PS = polystyrene; PU = polyurethane; PVC = Polyvinyl chloride).

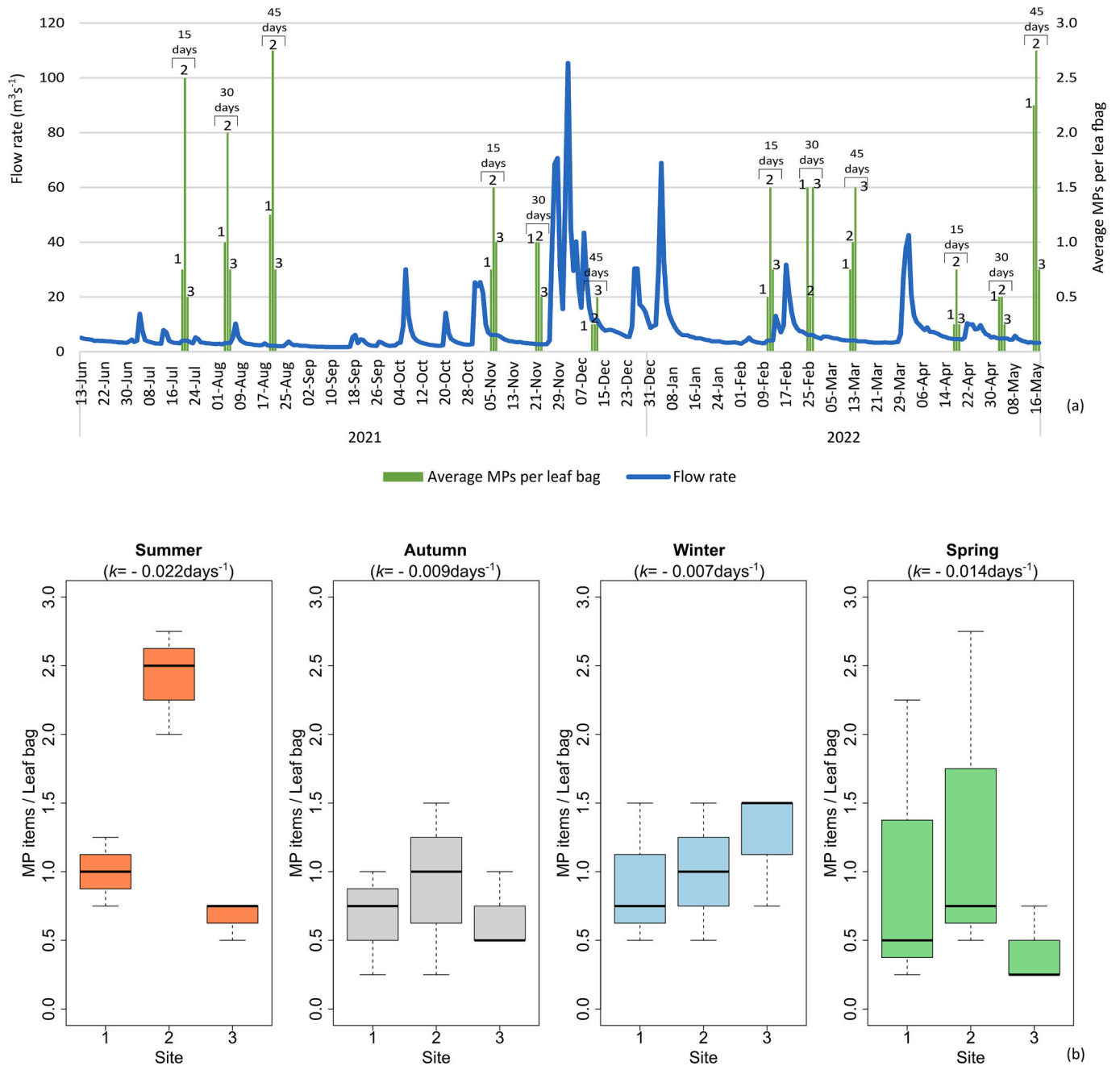


Fig. 5. Seasonal average number of MP items observed in leaf bags at each sampling site (1,2,3) and each sampling time (15, 30 and 45 days of submersion) (a), and box-plots for seasonal density (average MP items per leaf bag) trends observed at each site (b). At the top of the seasonal boxplots, values of decomposition rates k reported in Bertoli et al. (2023) are reported.

presence of fibers was already detected in Vipacco macrobenthic invertebrates Feeding Guilds during a previous study in the watercourse stretch (Bertoli et al., 2022).

The MP concentrations observed in water samples analyzed herein are in line with those reported in literature; in particular, they are similar to those recorded in Danube in Austria ($0.32 \text{ items m}^{-3}$) (Lechner et al., 2014), and for the hydrological network of the Oregon State ($0\text{--}0.4 \text{ items m}^{-3}$) (Valine et al., 2020). Our values seem to be lower than those recorded by Matjašič et al. (2023) for some river catchments in Slovenian territories, where the Vipacco River springs are located. The Authors reported concentrations about $31 \pm 14 \text{ items m}^{-3}$ and $59 \pm 16 \text{ items m}^{-3}$, consistently higher than those observed in the Vipacco. However, comparisons could be very difficult due to many factors affecting MPs quantification, such as sampling methods and expressions

of the results (Kumar et al., 2021; Razeghi et al., 2021). Moreover, catchment characteristics and river hydrogeomorphology can heavily affect the flux of MP pollution across a landscape and within water bodies. Climate, topography, hydrology, catchment dimension, human activities and land use alter the mass balance of MP within a catchment by affecting the diversity and volume of MP emitted from different sources, the nature and extent of transport processes and the likelihood of temporary storage in ecosystems (Windsor et al., 2019; Schirinz et al., 2020; Matjašič et al., 2023).

Regarding the temporal pattern observed in the waters of the Vipacco River, higher MP concentrations were related to lower flow rates, especially in summer and spring. This is in contrast with results reported by Rodrigues et al. (2018), which highlighted seasonal trends with higher concentrations in autumn in comparison to spring, in

relation to precipitation regimes. Similar results were also obtained in Poland by Pol et al. (2022). Some Authors highlighted that MP concentrations in rivers increase with rainfall and flow speed (Gündoğdu et al., 2018; Schirizzi et al., 2020; Winkler et al., 2022). It was also observed that after rainfall MPs can come from moved bottom sediment and from the ground (Zbyszewski and Corcoran, 2011; Pol et al., 2022). On the other hand, other authors highlighted a negative correlation between MP densities in lotic waters and the flow rate levels (Watkins et al., 2019; Windsor et al., 2019; de Carvalho et al., 2021; Talbot et al., 2022), as observed in our data for the Vipacco. As observed by Pol et al. (2022), it is difficult to compare results of studies carried out in different watercourses, due to differences in land use, geological/hydrological conditions and population density (Bordos et al., 2019; Kataoka et al., 2019), human activities and impacts (such as tourism, agriculture, industrialization) (Driedger et al., 2015; Magnuson et al., 2016; Tibbetts et al., 2018), density/position of wastewater treatment plants (Kay et al., 2018) and sampling seasons (Nel et al., 2018; Rodrigues et al., 2018). In the case of the Vipacco River, the geology and the hydromorphological characteristics may have a pivotal role in moving/accumulation dynamics of MPs. This River is characterized by highly fluctuating flow rates, with frequent floods exceeding $300 \text{ m}^3 \text{ s}^{-1}$ (Mosetti, 1983), due to the karstic nature of the spring area. Moreover, the bottom of the analyzed watercourse stretch is made by coarse sediment, mainly coarse/fine gravel and cobbles instead of sand and mud. This could reduce the accumulation of MPs, in the bottom, despite they have been recorded in previous studies carried out in the same stretch (Bertoli et al., 2023).

Our findings suggest that the maintenance of a hydrological regime at relatively high levels could allow to maintain the self-purifying processes and the disposal of microplastics like any other polluting substance. The results for water analyses reported in the present study seem to support this hypothesis, at least for the flow rate range in which it was possible to perform the sampling operations ($1.67\text{--}9.7 \text{ m}^3 \text{ s}^{-1}$). High but not too intense flow regimes can contribute to the mobilization and removal of microplastics from the river environment. It was also observed how big rivers in China (Yangtze, Hangjiang) large amount of water are associated to reduced levels of microplastic pollution in comparison to rivers with lower discharge levels (Zhao et al., 2014; Wang et al., 2017b; Peng et al., 2018b; Pol et al., 2022). On the other hand, flood events could promote the aggregation and mobilization of microplastics (Roebroek et al., 2021). Due to the impossibility to access to the river in safe conditions during periods when levels are too high (for Vipava, flow rates above $10 \text{ m}^3 \text{ s}^{-1}$), it was not possible to measure MP quantity in the water in such conditions. However, the increase in transport and the consequent accumulation could have been recorded by the leaf bags, which remained in place during the period of river level increase.

4.2. Microplastics in leaf bags

The same polymers found in water samples were collected in the leaf bags, suggesting that the technique can accurately represent the MP trends in the investigated ecosystem. MP frequencies within the bags were generally higher in site 2, which is located only 20 m downstream to the middle waste-water discharge point in the investigated section. This result agrees with other studies that report higher amounts of MPs downstream to wastewater treatment plants, sewers and discharge points (Wagner et al., 2014; Windsor et al., 2019; Eibes and Gabel, 2022; Matjašič et al., 2023). Wastewater treatment points are, in fact, one of the main sources of MPs for freshwater ecosystems (Estahbanati and Fahrenfeld, 2016; Kay et al., 2018; Edo et al., 2020), especially in relation to plastic fibers originated by clothes washing (Boucher and Friot, 2017; De Falco et al., 2019). In some cases, site 3 showed high MP abundance, especially after periods when flow rates increased, such as at 45 days in autumn and during the winter sampling campaign (Fig. 5a). This fact could be explained by the hydromorphological characteristics

of the site, which is placed by a large pool area, with low current, where the river curves (Fig. 1) and the flow velocity decreases. This could lead to a local reduction of increased sedimentation of floating MPs (Scherer et al., 2020; Matjašič et al., 2023). Capturing MPs, leaf bags could likely take a record of these events, even the access to the sampling sites is forbidden due to high hydrometric levels.

Except for these cases, MPs inside the leaf bags showed generally higher concentrations at low flow rates, in agreement with results of water analysis. This finding supports the hypothesis that certain flow regimes can favor the dynamics of self-purification in the watercourse also for microplastics. This could be likely observed in autumn, when concentrations of MPs were reduced also in site 2 and they progressively decreased in all the monitored sites from 15 to 45 days of submersion (Fig. 5a, b). Flow rate is one of the fundamental factors in determining the presence, transport and accumulation of microplastics in watercourses (Sarkar et al., 2019) and level variations are implicated in the mechanisms of mobilization and accumulation of microplastic debris on the aquatic vegetation and river sediments (Wolanski and Elliott, 2015; Scherer et al., 2020). The observed dynamic should be carefully considered in a context of reduced rainfall regimes and consequent decreasing of flow rates, that can lead to a significant reduction in the self-purification power of watercourses, likely enhancing the effects of MP pollution, such those of other contaminants. Regarding leaf litter, accumulated MPs can interfere with system dynamics, such as decomposition processes (Seena et al., 2019; Seena et al., 2022; López-Rojó et al., 2020; Du et al., 2022; Ockeden et al., 2022; Bertoli et al., 2023).

5. Conclusions

The present study provides temporal patterns of MP concentrations in the waters and of a riverine ecosystem subject to high flow rate. Those new data provide useful information regarding levels and composition of MPs in the studied river that will eventually hit the Adriatic Sea, increasing the MP levels in the Mediterranean sea area. One of the most valuable aspects of the present study is that data presented herein were obtained via direct field experiments and field monitoring activities, while many studies were not carried out measuring MPs in rivers or the sea, because they are based on modeling activities. Despite the high importance of modeling studies, direct detection of MPs in rivers is still very relevant to know how much MP is reaching the coastal sea waters, just to improve the knowledge and provide useful data to sustain and improve also the modeling works.

The observed trend is negatively affected by flow rates regimes, which seem to act via self-depuration dynamics as for common pollutants. Temporal patterns observed via leaf bag techniques are in line with those observed in water samples, but this technique appears to be a useful tool to analyze accumulation of MPs in riverine ecosystems: it simulates the natural leaf litter accumulation in freshwater environments, and it allow to obtain information regarding different time periods (weeks, months, seasons) as the times of submersion can be modulated in agreement with study aims and research necessities. On the other hand, water samplings provide an instant picture of the MP pollution, recorded at the sampling time. Other containers could be probably used to study temporal trends of MP accumulations, but for the purposes of the present study, the leaf bag technique was surely a good choice. Different meshes and/or different materials must be tested in the future, as different organic matter accumulation in the riverine stretches (such as different leaf types, woody debris) can probably work differently and keep different amounts and types of MPs. Further investigations are needed to deepen the dynamics of MP accumulation in leaf litter, especially in relation to heavy droughts events like those that occurred in the last years in the analyzed watercourse. These events are caused both by variations in rainfall regimes and/or by the use of the water resource (increasing amount of water diversion) and can alter the system dynamics in watercourses, enhancing the effects of MP accumulation due to reduced self-depuration power in lotic environments. In

this context, more experimental studies measuring the levels of MPs in rivers are needed, especially considering different timescales (seasonal, annual or multiannual) to better understand the variability of MP fluxes which can change in time.

CRediT authorship contribution statement

Marco Bertoli: Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Davide Lesa:** Methodology, Investigation. **Paolo Pastorino:** Writing - review & editing, Methodology. **Antonella Mele:** Data curation, Investigation. **Serena Anselmi:** Investigation. **Damià Barceló:** Writing - review & editing. **Marino Prearo:** Supervision, Writing - review & editing, Methodology. **Monia Renzi:** Supervision, Investigation, Methodology. **Elisabetta Pizzul:** Supervision, Writing - review & editing, Methodology, Investigation.

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.

Data availability

Data will be made available on request.

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