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Microplastic pollution in small rivers along rural–urban gradients: Variations across catchments and between water column and sediments

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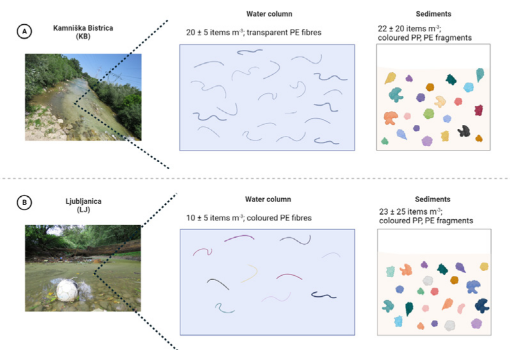
HIGHLIGHTS

- Microplastic pollution of a pre-alpine and karstic catchment was compared.
- Water and sediments of small sized catchment (< 800 km^2) were investigated.
- PE and PP were most common polymers with fibres in water and fragments in sediments.
- Sampling location, catchment characteristics and hydrogeomorphology are important.

GRAPHICAL ABSTRACT



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ABSTRACT

The aquatic ecosystems of the world are highly burdened with microplastics (MPs; particles <5 mm). There is a great need for better understanding of patterns of MP pollution across catchments and rivers of different sizes, anthropogenic pressures and hydrogeomorphological features. In this study, we investigated the MP concentrations including their characteristics (polymer type, shape, size and colour), and MP distribution in water and sediments of two hydrogeomorphologically different small-scale catchments (< 800 km^2), namely Kamniška Bistrica (KB) and Ljubljanica (LJ), Slovenia. The main objective of this study was to gain a better understanding of how WWTP effluents and catchment urbanisation together with the diversity of natural hydrogeomorphology, affect the quantity and quality of MP pollutants in the rivers with smaller catchments. Significantly different mean MP concentrations were found in the water columns (KB: 59 $\pm$ 16 items m^{-3}; LJ: 31 $\pm$ 14 items m^{-3}), but not in the sediments (KB: 22 $\pm$ 20 items kg^{-1}; LJ: 23 $\pm$ 25 items kg^{-1}). A longitudinal gradient with increasing particle concentration was observed in both water and sediment samples and in both catchments. Polyethylene (PE) and polypropylene (PP) particles dominated in all samples. Fibres were predominant in the water column samples, while fragments were more common in the sediment samples. MP particles were mostly coloured, and most of them were smaller than 2 mm in both water and sediment samples. The critical evaluation of the results and previous studies suggest that the characteristics of the catchment (anthropogenic pressures, size, climate, etc.), the hydrogeomorphology of the river (sediment type, discharge, flow velocity etc.), the sampling location along the river, the sampled compartment (water, sediment), the sampling method, and the hydrometeorological characteristics at the time of sampling, are important factors for observed MP concentrations and other characteristics.

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1. Introduction

Microplastic particles (MPs; particles <5 mm) pose an increasing threat to human health and the environment worldwide. Research on MPs and their sources, concentrations, transport pathways, environmental fates and impacts on biota, while rapidly increasing, is primarily focused on the marine environment; the origin, distribution patterns, transport pathways and impacts of MPs in the freshwater environment remain largely unexplored (Wang et al., 2021). As rivers are considered highways for plastic debris of various sizes, including MPs, an estimated 0.47–2.75 million tonnes of plastic are transported by rivers into the oceans each year (Lebreton et al., 2017; Schmidt et al., 2017). Therefore, research focusing on freshwater systems is crucial to better understand MP accumulation in the oceans and global distribution patterns.

Most studies dealing with freshwaters have investigated pollution patterns of MP in larger rivers (catchment area > 50,000 km^2 or main tributaries with catchment area > 5000 km^2), and provide information on the large-scale regional and/or global drivers of MP pollution either in the water columns or in the riverbed sediments (Klein et al., 2015; Constant et al., 2020; Eibes and Gabel, 2022). The most common polymers in freshwaters in general, and also in large rivers, are polypropylene (PP) and polyethylene (PE), with fibres and fragments being the most commonly detected shapes (Wang et al., 2021; Yang et al., 2021). Concentrations in water and sediment samples can vary considerably, from 0 to over 80 items in m^{-3} of water, depending on the sampling method (i.e. water pump, net), anthropogenic pressures and catchment size (Constant et al., 2020; Eibes and Gabel, 2022), and from 9 to over 15,000 items per kg^{-1} in river sediments, with the location of sampling playing an important role (Scherer et al., 2020).

MP are mostly produced on land and subsequently distributed throughout ecosystems, including freshwater bodies (Ziajahromi et al., 2016). Primary MPs are intentionally produced, such as industrial resins (e.g. pellets for easier transport) or personal care products (e.g. abrasive particles in toothpaste or skin care) (Waldschläger et al., 2020), while secondary MPs are products of larger displaced plastics. Under anthropogenic or environmental factors, large plastics are fragmented into numerous small pieces, becoming important sources of MP in the environment (Hidalgo-Ruz et al., 2012; Yang et al., 2021). MPs from households, industrial and other wastewater, and urban or road runoff are concentrated in wastewater treatment plants (WWTPs), which serve as a major point-source link between MP pollutants and the aquatic environment (Ziajahromi et al., 2016; Kovač Viršek et al., 2017). Although most MPs in sewage sludge are retained by WWTPs, millions of MP particles are still discharged into rivers per day due to the large amount of treated water (Prata, 2018). In addition, sewage sludge from WWTPs, when used as a fertiliser on agricultural land, can also be a source of MP pollution to freshwaters; it can be washed off by precipitation and enter surface waters (Sun et al., 2019) or be released into the atmosphere during surface treatment (Sommer et al., 2018). Atmospheric MP particles origin from a range of anthropogenic sources (WWTPs, transport, industry, agriculture, etc.) reach the land (forest, urban and agricultural) via precipitation, and flow into rivers and lakes via surface runoff (Waldschläger et al., 2020; Wang et al., 2021; Kallenbach et al., 2022).

Catchment characteristics and river hydrogeomorphology can influence the flux of MP pollution across a landscape and within water bodies. Climate, topography, hydrology and land use, for example, 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). For example, heavy rainfall causes the flux of MP particles into river systems to increase due to atmospheric fallout (Dris et al., 2015; Bergmann et al., 2019), soil erosion (Bläsing and Amelung, 2018) and stormwater runoff (Piñon-Colin et al., 2020; Treilles et al., 2021; Werbowski et al., 2021). In slow-moving stretches of water, MP are likely to settle along with sinking sediment particles, with sediment deposition further contributing to MP particles being buried (Horton and Dixon, 2018). Particles that settle on the riverbed can infiltrate the sediments into a deeper layers or be resuspended during stronger flow conditions, such as floods (Hurley et al., 2018), or even under baseflow conditions (Drummond et al., 2020). Resuspension is strongly influenced by the geomorphology of the riverbed, MP particle concentration and diameter (Nizzetto et al., 2016; Waldschläger and Schüttrumpf, 2019). In some rare cases, MP can even return to land (e.g. during floods) (de Souza Machado et al., 2018). Although the oceans act as sinks for much of the MP, freshwater bodies and soil are also involved in the plastic cycle and retain much of the MP they receive (Klein et al., 2015; Horton and Dixon, 2018; Drummond et al., 2020; Scherer et al., 2020). A study by Drummond et al. (2022) found that MPs are largely retained in headwater systems at low-flow conditions, with residence times of up to 1.7 years km^{-1}. On the other hand, the faster a river flows, the more energy it has to entrain and transport a large amount of particles. High-energy flood flows, for example, lead to the resuspension of dense MP particles together with other sediment particles (Knighton, 2014; Hoellein et al., 2017).

Flow conditions (flow velocity, flood events) and particle properties (shape, density, type of material, microbial overgrowth) are the most important factors controlling the transport of individual MP particles in rivers (Horton and Dixon, 2018; Waldschläger et al., 2020; Winkler et al., 2022). Depending on their density, particles can either float or sink when introduced into the water (Waldschläger and Schüttrumpf, 2019). Floating particles tend to be transported to the ocean by the river current unless retained by barriers (Scherer et al., 2020), while higher density particles, such as PET or nylon, sink to the river bed unless a high-energy current transports them downstream to a low-flow area (Nizzetto et al., 2016). Furthermore, Waldschläger and Schüttrumpf (2019) reported significant differences in the sinking behaviour of pellets, fibres and fragments, indicating the importance of MP shape. Additionally, researchers have found that most fibres are of natural origin (Constant et al., 2020), in some cases as much as 90 % (Stanton et al., 2019).

Due to biofouling, i.e. the attachment of microorganisms to particles, MPs become denser and have a higher settling velocity (Miao et al., 2021). In addition, the shape and size of particles affect the retention of MP. Irregular particles such as fragments and pellets tend to sink or rise more slowly than spheres because they are slowed down by secondary movements (e.g. rotation, lateral oscillation). Fibres appear to orient themselves horizontally in the water regardless of their initial position, and rise with the same velocity, regardless of their length. However, the speed of the fibres may increase as their diameter increases (Waldschläger and Schüttrumpf, 2019). When characterising MP particles, it is also important to consider the colours of MPs, because of the interaction with the microbiota. For example, Lopes et al. (2020) analysed fish stomachs and found a prevalence of blue and black MPs, while Berglund et al. (2019) found predominantly black and transparent fibres in mussels, suggesting that mussels show a colour preference for food.

The present study focused on determining the patterns and types of MP pollution at smaller scales (500 km^2 < catchment <800 km^2, river length < 45 km) in both abiotic compartments: water columns and riverbed sediments, and along the rural-urban gradient. Since understanding MP sources and sinks is important to effectively reduce the impact of plastic pollution on receiving ecosystems (Wang et al., 2021), the main objective of the study was to understand in-depth how environmental and anthropogenic factors, such as hydrogeomorphology, urbanisation and WWTPs, impact MP pollution (i.e., concentrations, types, shapes, sizes and colour of MPs), on smaller scales. In addition, these MP characteristics were studied in both water columns and riverbed sediments to better understand the transport, retention and distribution of MPs in streams. Notably, two geologically and hydrologically different catchments (turbulent pre-alpine river and meandering lowland karst river) with similar anthropogenic pressures were compared. The hypotheses addressed in this paper are: (1) MP pollution gradually increases along the river, both in the water column and in sediments, which is due to an increase in the anthropogenic impacts, (2) MP pollution is significantly higher downstream from WWTP effluents in both water columns and sediments, (3) MP pollution in the two catchments studied differs due to differences in catchment characteristics, including anthropogenic pressures and hydrogeomorphology, and (4) MP pollution differs between water columns and sediments. Furthermore, this study calls for the standardisation of methods for estimating MP pollution in rivers by comparing and analysing the results of existing publications on European rivers MP pollution and contributes to the development of efficient mitigation methods against MP pollution in freshwater bodies by providing new field data.

2. Methods

2.1. Study catchments

The samples were obtained from two catchments in Slovenia: Kamniška Bistrica (KB) and Ljubljanica (LJ) (Fig. 1). The Ljubljanica is a typical karst river in the south-central part of Slovenia, sinking underground in several places and then reappearing back on the surface. At its last appearance on the surface, the Ljubljanica wells from numerous springs near Vrhnika, flows through the Ljubljana Moor and through the city of Ljubljana, merging with the Gradaščica and then flows as a right tributary into the Sava River. The length of the river in this section is 41 km and the catchment area of this part is about 787 km^2 (Table 1). The NW part of the Ljubljanica catchment is classified as a pre-alpine or isolated karst with steep dolomite slopes, while the background of its springs is a typical karst area. The Kamniška Bistrica, on the other hand, is a pre-alpine river with a gravel-bed in north-central Slovenia, rising in the southern Kamniško-Savinjske Alps (630 m elevation). It is 33 km long and has a catchment area of 534 km^2 (Table 1). The river flows through a narrow valley, continues through the town Kamnik, enters the Ljubljansko polje – the Ljubljana basin tectonic depression – and reaches the Sava River as its left tributary, near the outflow of the Ljubljanica. In the upper reaches, the catchment area consists of limestone and dolomite and is surrounded by forest. The catchment area around Kamnik is composed of clastic rocks (tuff, sandstone, conglomerate, clay, marl), with the lower part of the river containing alluvium.

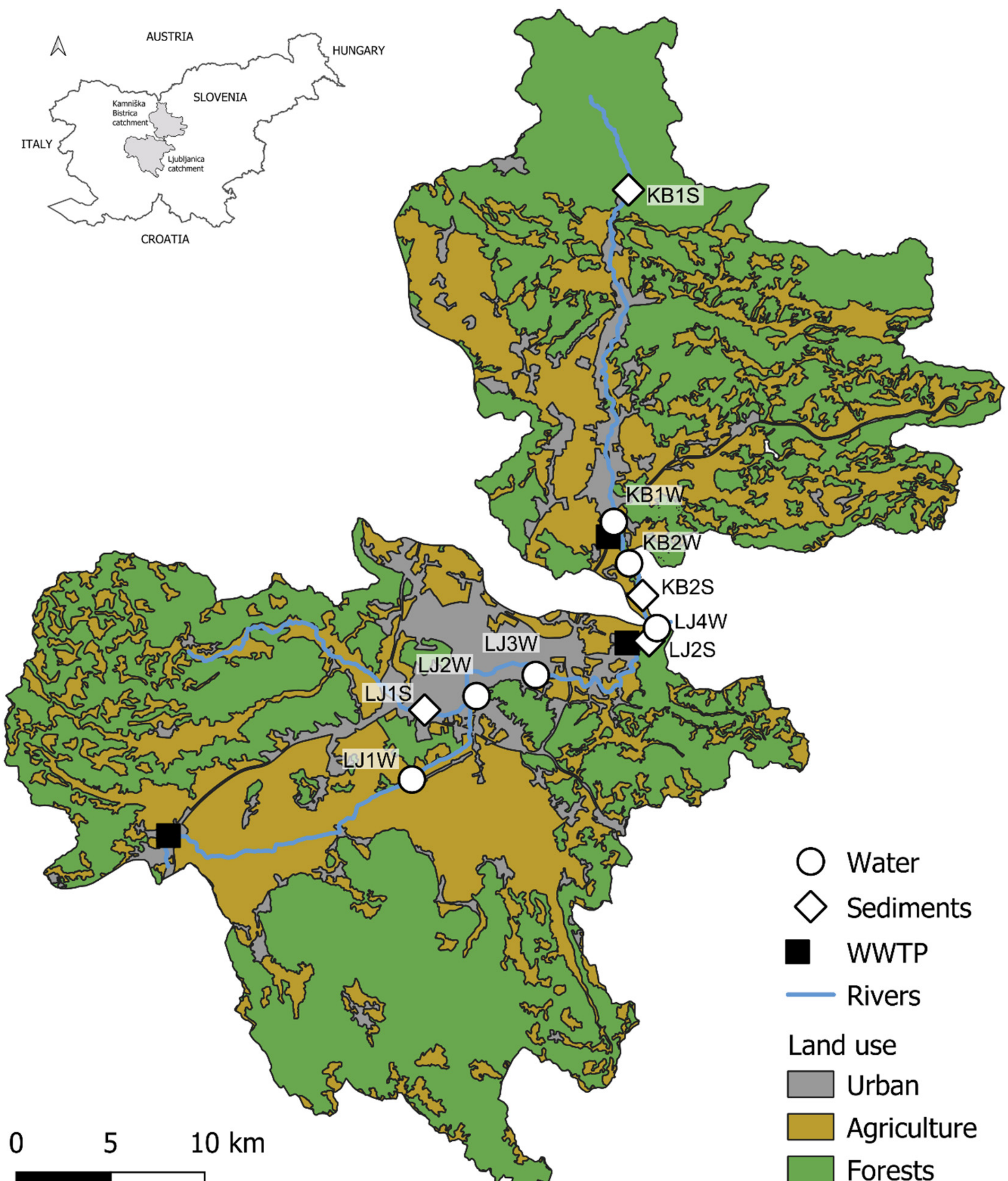

Fig. 1. The study area.

Table 1
Characteristics of study catchments. Source of data: Slovenian Environmental Agency; gauge stations: Moste I (LJ) and Vir (KB).

River	Catchment area (km^2)	River length (km)	Mean daily discharge (±SD) (2010 – 2020) (m^3/s)	Min daily discharge (2010–2020) (m^3/s)	Max daily discharge (2010–2020) (m^3/s)
Ljubljanica (LJ)	787	41	52.9 ± 53.6	4.4	343.8
Kamniška Bistrica (KB)	534	33	5 ± 8.7	0.1	156.8

2.2. Field sampling

2.2.1. Water column

Samples were obtained from two locations along the Kamniška Bistrica (upstream and downstream of the WWTP; 20 September 2019) and at four locations along the Ljubljanica (upstream, in, and downstream the city of Ljubljana; 1 October 2019) (Table 2 and Fig. 1). At each sampling location, 1 m^3 of water was pumped three times. Water samples were collected at a depth of 15–20 cm below the water level using a motor pump (Makita EW1060H) and a suction basket with a pore size of 1 × 1 cm. The water was filtered through a sieve with a pore size of 150 μm. The collected material was washed with 70 % ethanol and stored in glass containers until further analysis.

2.2.2. Sediments

Sediment samples were taken from two locations along the Kamniška Bistrica (at the pristine river reach and downstream of the WWTP; 26 August and 28 June 2019, respectively) and at two locations in the Ljubljanica catchment (in and downstream of the city of Ljubljana; 26 August 2019). Three sediment samples (500 mL) were randomly collected from the frequently flooded banks and three from the wetted channels in summer 2019 (base flow conditions). In the wetted channels, a tube (diameter = 30 cm; height = 60 cm) was inserted into the river bed. Large stones were removed and sediments (< 4 cm grain size) were carefully sieved and washed and collected in a 500 mL PP flask. 1 L of water with suspended material was also collected from the tube to obtain MPs that may have been released from the sediments during sampling.

2.3. Laboratory sample processing

2.3.1. Preprocessing of sediment samples

The sediments had to be preprocessed in order to extract MPs. In the laboratory, 300 g of sediment was thoroughly mixed with a saturated NaCl solution and left for 24 h. The supernatant was then removed and washed through a 0.063 mm steel sieve. The sediment was again mixed with NaCl solution and allowed to stand for 2 h; the supernatant was then washed through the 0.063 mm sieve and the sediment was allowed to stand in saturated NaCl solution for 2 h before being sieved again. The material was collected in glass containers and washed; $FeSO_4$ and H_2O_2 were added to break down any organic particles. The solution was heated at 75 °C for half an hour, until the chemical reaction ended. If any material remained, additional H_2O_2 was added and the solution was heated. Finally, the residues were washed again over the steel sieve and then the residues in the sieve were filtered through a glass filter (Whatman, GFF, No. 1825-047). The remains on the filter were stored in closed glass petri dishes at room temperature until they dried.

2.3.2. Characterisation of MPs from water and sediment samples

All glass containers used for sampling the water column and filters used for sediment preprocessing were thoroughly checked using a stereo microscope (20–120× magnification, StereoDiscovery V8, Zeiss, Germany). Each assumed MP particle was isolated from the samples with precise tweezers and sorted into one of five categories: fragments, fibres, foams, films and pellets/granules. The particles were then measured according to their longest length, their colours noted and their chemical composition determined using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR; SpectrumTwo, Perkin Elmer), except for fibres in the water column (see Chapter 2.3.3). Colours were categorised into one of the four obvious groups – transparent, black, white and coloured – as colour perception is subjective and can be influenced by factors such as microscope illumination/background or personal factors (Lu et al., 2021; Yang et al., 2021). MP particles were measured and sorted into size categories determined as 0–0.99 mm, 1–1.99 mm, 2–2.99 mm, 3–3.99 mm, and 4–4.99 mm, with a difference of 0.99 mm between them.

2.3.3. Characterisation of fibres

Individual textile fibres from environmental samples may be too small to be analysed by micro-FTIR. Therefore only a representative sample of fibres from the Ljubljanica River were chemically analysed using this method (Spotlight 200i, PerkinElmer). The fibre particles obtained from the water columns of the LJ were classified according to their origin – natural or anthropogenic – with the latter additionally divided into group of either semi-natural or synthetic origin. No such distinction was made for the samples from KB and they were therefore not included in the chemical analysis. From the LJ samples, a representative sample (5 %) of the fibres was selected for micro-FTIR analysis and fixed on gold-coated polyester membranes (i3-TrackPor P Membrane; i3-Membrane) to avoid interference. In Fig. 2, all fibres of both origins are included. In Fig. 3, all fibres (semi-natural and synthetic) were included in the "Shape" section, while for the presentation of material analysis, colour and size ranges, fibres found from water column samples were not included. In Fig. 4, only fibres that were visually determined to be synthetic were included.

2.3.4. Quality control

During the sampling and analytical processes, plastic was avoided where possible to minimise interference with the samples. All researchers wore non-synthetic clothes and/or cotton lab coats. Glassware was washed thoroughly with Milli-Q water before use, and the workspace was cleaned

Table 2
The sampling sites with location coordinates and location IDs, used in graphics (IWRS – Institute for Water of the Republic of Slovenia; NIB – National Institute of Biology).

Sampling site (river)	Sampler	Sample ID	Coordinates	
Črna vas (LJ)	IWRS	LJ1W	45°59′59.9"N	14°28′06.0″E
Prule – Špica (LJ)	IWRS	LJ2W	46°2′23.9"N	14°30′44.0″E
Fužine (LJ)	IWRS	LJ3W	46°3′00.8"N	14°33′09.9″E
Zalog (LJ)	IWRS	LJ4W	46°4′22.5"N	14°38′8.9″E
Above WWTP Domžale-Kamnik (KB)	IWRS	KB1W	46°07′24.6"N	14°36′21.1″E
Under WWTP Domžale-Kamnik (KB)	IWRS	KB2W	46°06′13.5"N	14°36′59.3″E
Stahovica (KB)	NIB	KB1S	46°16′55.3"N	14°36′53.3″E
Bišče (KB)	NIB	KB2S	46°05′18.4"N	14°37′33.1″E
Mali Graben (GRA)	NIB	LJ1S	46°01′59.4"N	14°28′36.1″E
Cesta v Kresnice (LJ)	NIB	LJ2S	46°04′00.6"N	14°37′49.5″E

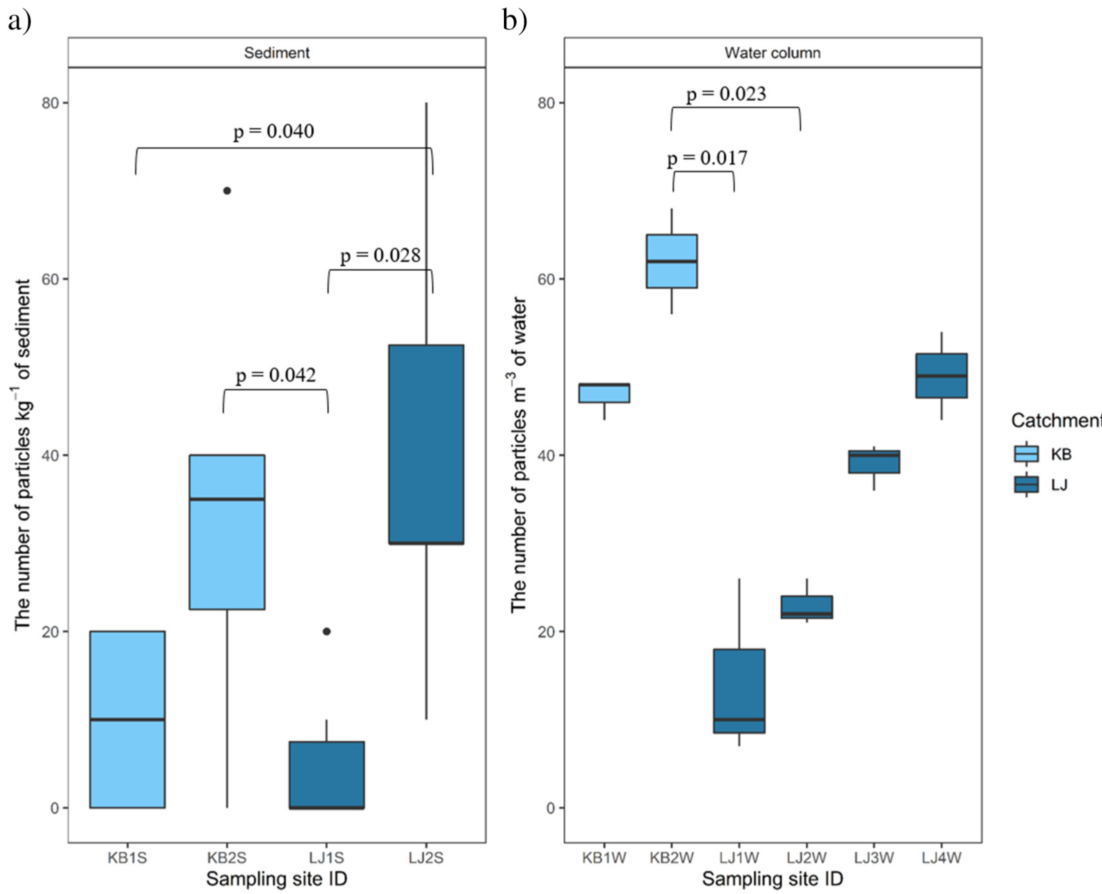

Fig. 2. Boxplots of the number of particles found in a) 1 kg of sediments and b) 1 m^3 of filtered water. Samples were taken as triplicates, and means and standard deviations were calculated as pictured in the graph. All fibres found (including of synthetic/seminatural origin) found were included in the graph. The added *p*-values are the results of the Dunn post-hoc test between the locations. In addition, the Kruskal-Wallis test revealed significant differences between the catchments ($H = 7.6575$, $df = 1$, $p = 0.006$) for the water column samples.

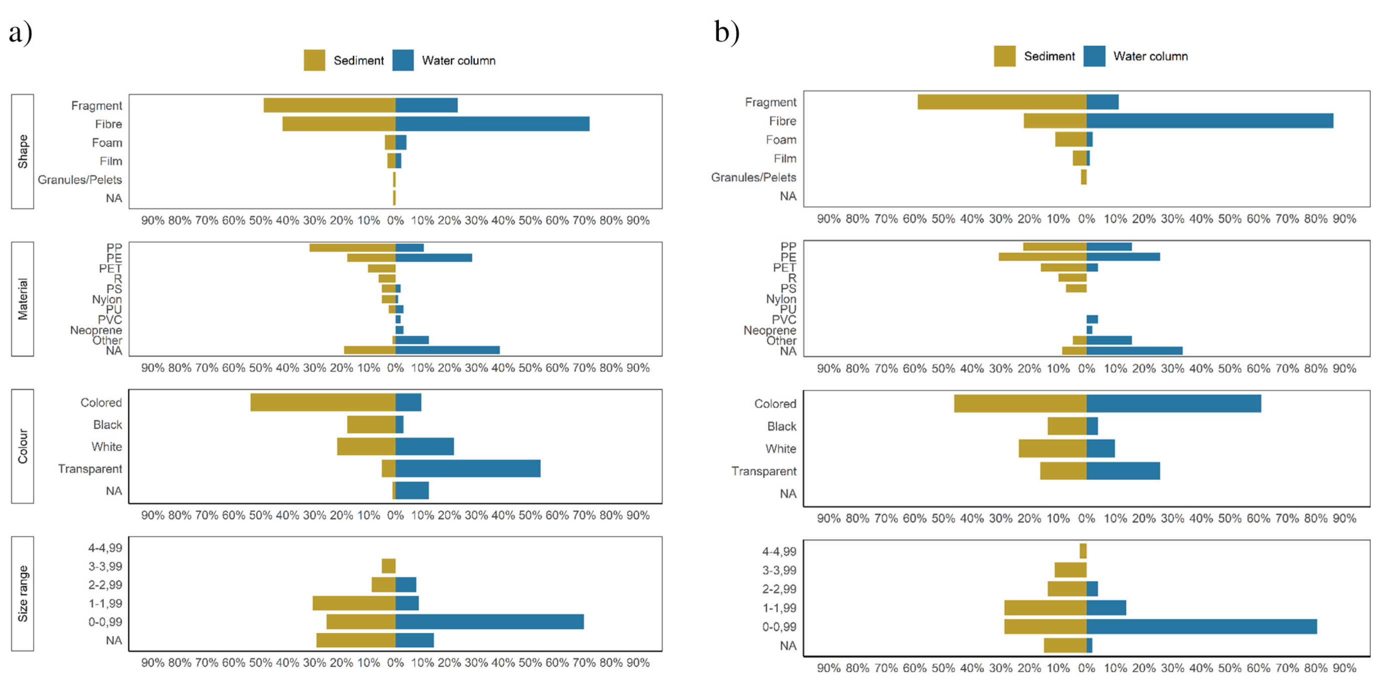

Fig. 3. Characteristics of MPs reported in the sediment and water samples as a percentage of the MPs found in the catchments a) Kamniška Bistrica and b) Ljubljanica. Shapes: fragment; fibre; foam; film; granule/pellet; NA (not defined). Materials: polypropylene (PP); polyethylene (PE); polyethylene terephthalate (PET); rubber (R); polystyrene (PS); nylon; polyurethane (PU); polyvinylchloride (PVC); neoprene; other (different single polymer materials or blends such as poly(2-hydroxyethyl acrylate); NA (not defined). Colours: coloured; black; white; transparent; NA (not defined). Size ranges (mm): 0–0.99; 1–1.99; 2–2.99; 3–3.99; 4–4.99; NA (not defined). Material, Colour and Size range do not take into account water column fibres.

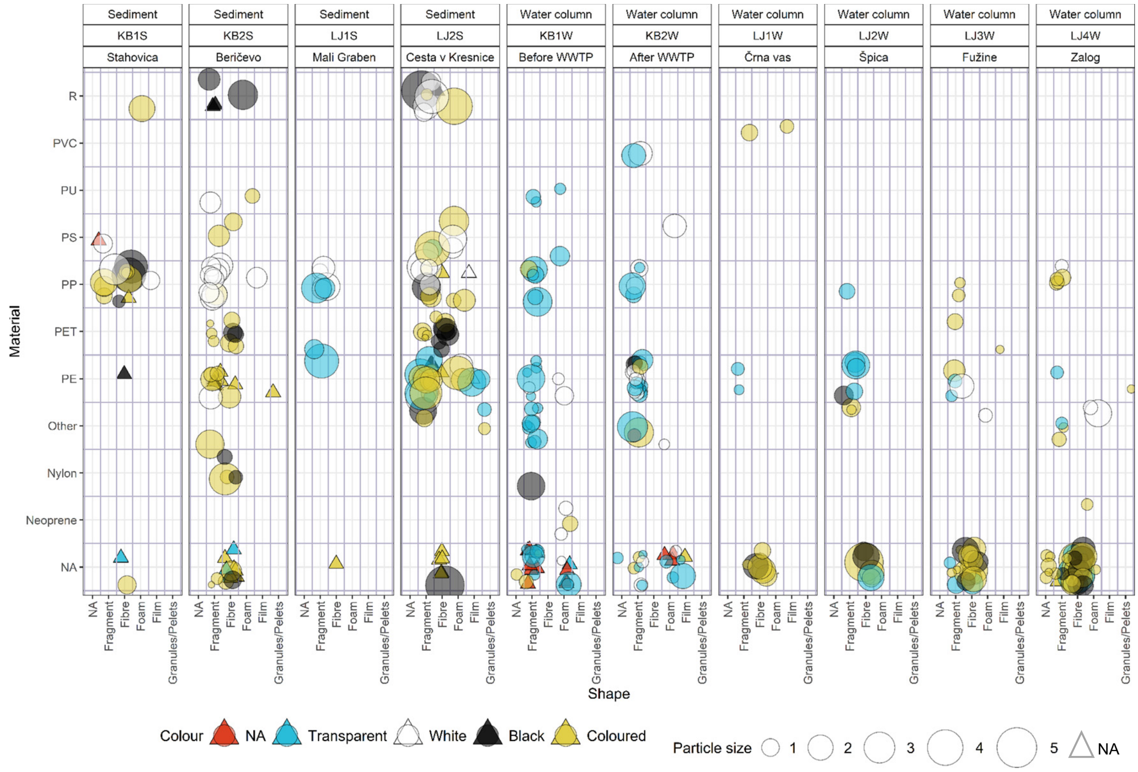

Fig. 4. Visualisation of the various materials, shapes, colours and size ranges of the MP particles collected in this study. Each circle represents one particle. The triangles represent particles whose size was not defined. For the water column samples, only fibres that were visually determined to be synthetic were included. Fibres found in KB were excluded as they were not differentiated according to their origin (semi-natural or synthetic).

with Milli-Q water before and during the research procedure. For the most part, the filters were handled in a laminar flow to prevent MP contamination from the air. When not in the laminar flow, the glass petri dishes containing the sample filters were kept closed. Microscopy was performed in a clean room with air purifier turned on. When extracting MPs from sediments, a spiked positive control was used to evaluate the MP return rate. Furthermore, distilled water was subjected to the same procedure as the environmental samples to determine blank values. The data were corrected according to the contamination levels found during the laboratory analysis.

2.4. Statistical analyses

Statistical analyses were performed using R version 4.1.2 (R Core Team, 2021) and the tidyverse package (Wickham et al., 2019) for visualisation. The level of significance for all analyses was $p < 0.05$. Due to the different methods used for sampling the water column and sediments, the data were analysed separately. As the samples were not normally distributed, the Kruskal–Wallis one-way analysis of variance was used to determine whether there was a significant difference between the mean abundance of particles in the water columns of both catchments and that in the sediments. In addition, Dunn's test was performed post hoc.

3. Results

3.1. Quantification of MPs

MPs were found in all water and sediment samples. A total of 378 particles were characterised from both the water column samples (LJ, $N = 12$; KB, $N = 6$) and the sediment samples (LJ, N = 6; KB, N = 6). These and the following numbers include synthetic fibres visually determined to be of synthetic origin. For the LJ water column samples, a representative number of fibres were analysed by micro-FTIR. An average of 41 ± 20 items m^{-3} (range: 7–89 items m^{-3}) were found in the water column samples ($N = 18$), and an average of 22 ± 22 items kg^{-1} (range: 5–40 items kg^{-1}) in the sediment samples ($N = 24$) (Fig. 2). A longitudinal gradient with increasing particle concentrations was observed in the water and sediment samples from both catchments. In general, higher average concentrations of MPs were observed in the water samples from the pre-alpine river KB (Table 3). The MP concentrations in the sediments highly varied within, between the sites and the two catchments. For water columns, the lowest amount of MP particles was found in the sample obtained above the Ljubljana city in the LJ catchment (LJ1W, 14 ± 8 items m^{-3} of water), and the highest amount was found in the sample downstream of the WWTP effluent in the KB river (KB2W, 71 ± 14 items m^{-3} of water). Among the sediments, the lowest number of MP particles was detected in the city, from the samples from the regulated tributary of the LJ (LJ1S, 5 ± 7 items kg^{-1} of sediment), and the highest number was detected in the samples below the WWTP of the LJ (LJ2S, 40 ± 23 items kg^{-1} of sediment). The Kruskal–Wallis test revealed significant differences between catchments (H = 7.6575, df = 1, $p = 0.006$) and between sampling locations (H = 15.868, df = 5, $p = 0.007$) for the water columns. The Dunn post-hoc test showed that this difference was specifically between KB2W and LJ1W ($p = 0.017$) and LJ2W ($p = 0.023$). The Kruskal–Wallis test was conducted to check for differences between the sediment samples of the two catchments (KB vs LJ), but no significant difference was found. However, when considering the different sediment sampling locations (KB1S, KB2S, LJ1S and LJ2S; Fig. 1), the results of the Kruskal–Wallis test were significant (H = 11.8, df = 3 with $p = 0.0081$). The post-hoc Dunn test revealed significant differences between KB2S and LJ1S ($p = 0.042$), KB1S and LJ2S ($p = 0.040$), and LJ1S and LJ2S ($p = 0.028$), which also differed in terms of catchment land use and hydrogeomorphological characteristics.

3.2. In-depth characterisation of MP particles found in water and sediment samples

3.2.1. Water column

In terms of shape, the predominant particles in both catchments were fibres of both natural or synthetic origin (LJ 86 %, KB 72 %), followed by fragments (LJ 10 %, KB 22 %) (Fig. 3a, b). In the polymer/material analysis,

Table 3
A summary of the results of field studies investigating presence of MPs in the water column and/or sediments carried out in Europe over the last 7 years. For data on shape, size, material and colour, only the most common are given, as written by the authors. Empty cells – no data reported. EVA – Ethylene Vinyl Acetate.

		Rhine	Elbe	Rhône	Po	Dalälven	Main	Ems	Ticino	Têt	Ljubljanica	Kamniška Bistrica	Antuã
Mean river characteristics	Country	Germany	Germany	France	Italy	Sweden	Germany	Germany	Italy	France	Slovenia	Slovenia	Portugal
	Catchment area (km^2)	185,000	148,268	96,364	71,000	28,927	27,208	17,800	7228	1373	787	534	149
	Length (km)	1230	1091	783	652	520	524	371	248	116	41	33	38
Water column	Sampling method		Apstein plankton net	Manta trawl & conical plankton net	Water pump	Water pump		Driftnet	Neuston trawl	Manta trawl	Water pump	Water pump	Water pump
	Samples		n = 10	n = 13 (trawl); n = 15 (net)	n = 5	n = 10		n = 36	n = 18	n = 35	n = 12	n = 6	n = 6
	Mean density (items m^{-3})		5.57 ± 4.33	12 ± 18 (trawl); 19 ± 28 (net)	20.3 ± 13.2	4.5 ± 3		1.54 ± 1.54	33.3 ± 20.4	42 ± 18	31 ± 14	59 ± 16	
	Min – max densities (items m^{-3})		0.88–13.24	trawl: 0.3–59; net: 2.4–88	9.6–43.2	0.4–10.2		0–5.28		0.8–618	7–49	44–89	58–1265
	Shape		Fibres	Fibres	Fragments	Fibres		Fragments	Irregular particles	Fibres	Fibres	Fibres	fibres, foam
	Size (mm)		0.125–5		0.64–5	0,18–3					0.15–0.99	0.15–0.99	0.055–5
	Material		PE, PP		PE	PE		PE	LDPE, PET, PP		PE	PE	PE, PP
	Colour		Transparent		Coloured	Coloured		Coloured			Coloured	Transparent	Coloured
Sediments	Density separation	NaCl	$ZnCl_2$				NaCl			NaCl	NaCl	NaCl	$ZnCl_2$
	Samples	n = 8	n = 11				n = 2		n = 18	n = 32	n = 6	n = 6	n = 6
	Mean density (items kg^{-1})		2080 ± 4670						11 ± 7.7	258 ± 259	23 ± 25	22 ± 20	
	Min – max densities (items kg^{-1})	228–3763	9–15,962				786–1368			7.3–1029	5–40	10–33	18–629
	Shape	Fragments	Spheres, fragments				Fragments		Irregular particles	Fragments	Fragments	Fragments	Fragments
	Size (mm)	0.630–5	0.125–5				0.630–5				0.15–0.99, 1–1.99	0.15–0.99, 1–1.99	0.055–5
	Material	PE, PP, PS	PE, PP				PE, PP, PS		EVA	PE, PP	PE, PP	PP, PE	PE, PP
	Colour	Various	Transparent								Coloured	Coloured	Coloured
Reference		Klein et al., 2015	Scherer et al., 2020	Constant et al., 2020	van der Wal et al., 2015	van der Wal et al., 2015	Klein et al., 2015	Eibes and Gabel, 2022	Winkler et al., 2022	Constant et al., 2020	This study	This study	Rodrigues et al., 2018

due to methodological limitations, 63 % of the particles could be identified from the water column samples: 61 % from KB and 67 % from LJ; fibres not included. In both, most of the particles were PE (28 % and 25 % respectively; fibres not included). The chemical composition could not be determined for all particles due to their small sizes, which led to accidental losses during handling or the particles were too small to handle at all, or the instrument could not provide a signal. Of the LJ samples, a representative sample (5 %) of anthropogenic fibres was selected for chemical analysis, and 75 % of the fibres characterised were polyethylene terephthalate (PET).

Most particles in the LJ sample were coloured (60 %), while most particles in the KB sample were transparent (53 %). The majority of particles in the water samples (fibres not included) were small (0–0.99 mm), both in LJ (80 %) and KB (70 %), followed by particles of size 1–1.99 mm (LJ: 13 %; KB 9 %) and 2–2.99 mm (LJ: 4 %, KB 8 %). For some particles, the size range could not be determined (LJ 2 %; KB 14 %).

3.2.2. Sediments

Generally, the MP particles found in both the LJ (59 %) and KB (49 %) sediments were classified as fragments, followed by fibres (22 % and 42 %, respectively). The LJ sediment sample consisted primarily of PE particles (31 %), followed by PP (22 %), PET (16 %), rubber (R; 10 %) and polystyrene (PS; 7 %); the material composition of 9 % of the particles could not be determined. In contrast, the majority of the KB sediment sample consisted of PP particles (32 %), followed by PE (18 %), PET (10 %), R (6 %), PS and nylon (both 5 %) and PU (3 %); 19 % of the particles could not be determined. Materials classified as “other” in both LJ (5 %) and KB (1 %) included polymethyl methacrylate (PMMA), PE-PP copolymers and acrylonitrile butadiene styrene (ABS), among others. The sediment particles were mostly coloured (LJ 46 %; KB 54 %) or white particles (LJ 23 %; KB 22 %). The majority of MP particles in the sediment samples fell into the smallest size categories: 0–0.99 mm (LJ 28 %; KB 26 %), 1–1.99 mm (LJ 28 %; KB 31 %), and 2–2.99 (LJ 14 %; KB 9 %). The size ranges of some particles could not be determined (LJ 15 %; KB 30 %).

The particles in the sediments were generally larger than in the water column samples (Fig. 4). The largest particle from the sediment samples was a black, 4.73-mm fibre in LJ2S, while the largest particle from the water column samples was a pink, 4.69-mm fibre in LJ2W. All identified particles in the samples from KB were smaller than 4 mm. No rubber particles were found in either of the water column samples and no neoprene or PVC particles were found in the sediments. In addition, no nylon or PU was found in the LJ sediment or water column samples. The most common particles in the water column samples were transparent PE fragments with a size between 0 and 0.99 mm, while the most common particles in the sediments were white PP fragments with a size of 1–1.99 mm. Almost no white particles were found in the LJ water column sample; most were coloured. Most particles from the KB water column were transparent fragments.

4. Discussion

4.1. Potential drivers of MP concentrations in river water

In this study MP sampling in the water column was carried out using the water pump method, which proved to be a very suitable method, as also shown by the linear trend of increasing concentrations downstream of the Ljubljanica River. The average concentrations of MP particles in the water were significantly lower in the Ljubljanica catchment (31 ± 14 items m^{-3}) compared to Kamniška Bistrica (59 ± 16 items m^{-3}), although KB is shorter and has a smaller catchment area. The main reason for this is most likely the selection of sampling sites, which in the case of KB was in the immediate vicinity of the WWTP. The comparison between Po and Kamniška Bistrica Rivers (Table 3) is also interesting, as the sampling methods are the same, but the MP concentrations much lower in the Po River, although the Po catchment is many times more densely populated (17 million inhabitants). The study in the Po reports on increased discharge (floods) during the sampling which could cause the dilution of MP pollution. In general, comparison of the results of other studies does not show any straight forward linkages between MP concentration, the size of catchment area and sampling method. Most likely, the main factors affecting the MP concentration in the riverine water column is the selection of sampling site, where the local anthropogenic pressures and the hydrological features of the river at the time of sampling play the most important role. This is in accordance with the study on the Ems River, where higher amounts of MP items m^{-3} have been found in the Ems downstream of WWTP (Eibes and Gabel, 2022). Interestingly, Eibes and Gabel (2022) also reported a significantly lower concentration of MP downstream of cities, thus identifying cities as potential sinks of MPs. A possible explanation for this phenomenon was the presence of obstacles (e.g. weirs) that reduced flow velocities upstream to the weir, which in turn increased sedimentation of floating MPs. A similar observation regarding obstacles altering flow velocities was made in the Elbe study (Scherer et al., 2020), where the MP concentration tended to decrease along the river course (higher in Middle Elbe than in the Lower and Outer Elbe). In the case of the Antuã River (Portugal), there was no significant upstream-to-downstream gradient in MP concentration (Rodrigues et al., 2018), as observed in our study. However, some seasonal differences were observed with higher concentrations in autumn in comparison to spring, while the opposite was true for sediment samples (Rodrigues et al., 2018). This is most likely due to precipitation. It is known, that the MP concentration in rivers increases with the amount of precipitation and flow velocity (Gündoğdu et al., 2018; Winkler et al., 2022), which is due to the resuspension of plastic particles from river sediments and river banks. This is also supported by the observation that the sampling sites with the lowest MP concentrations in the surface water samples had the highest concentrations in the sediment samples (Winkler et al., 2022). The findings of our and previous studies suggest that the hydrogeomorphology of the river studied is important for the MP distribution, indicating the need for further studies that take into account both spatial and temporal variability and carefully designing the sampling campaign.

4.2. Potential drivers and main characteristics of MPs in river sediments

Compared to previous studies (Table 3), the amounts of the MPs in the sediments of the two catchments studied were much lower. It seems that in the case of sediments, the intensity of MP pollution is related to the size and length of the catchment, followed by the intensity of anthropogenic pressures. For example, similar MP concentrations were found in samples from the Antuã, Têt and Ticino rivers, which have a comparable length and catchment area, while the larger Elbe, Rhine and Main rivers had much higher concentrations of MP in the sediments (Table 3). Furthermore, a strong gradient of higher MP concentrations along the river was observed in both study catchments, which is consistent with the pattern in the water samples.

Most of the particles found in the samples of previous studies were PE and PP in the form of fragments, which is consistent with the present study (Table 3). These results are in agreement with the review paper by Yang et al. (2021): they reported that PE was the main polymer type identified, followed by PP and PS, but also emphasised the diversity of MPs found in different regions of the world. For example, ethylene-*co*-vinyl acetate (EVA) was the main polymer found in the Ticino River (Winkler et al., 2022). On the contrary, Yang et al. (2021) reported that fibres were the most commonly occurring particles in freshwater sediments; however, it is important to note that the results of many studies may have been erroneous, as fibres were usually of natural origin (semi-natural fibres, most likely cotton) (Stanton et al., 2019).

Most of the MP particles were coloured in the present study, similar to the Antuã River, while Klein et al. (2015) found different colours and Scherer et al. (2020) found mostly transparent particles (Table 3). The latter was probably due to the different consideration of coloured MPs: Scherer et al., 2020 considered each individual colour in their calculations, while coloured MPs were grouped under the category “coloured” in the present study, highlighting the importance of standardised methods and reporting procedures.

Similar to water samples, the sampling season and hydrological events prior to sampling can have an important influence on MP concentrations in sediments. In the Têt River, samples taken just after a rain event in October 2015 had the highest concentration (13 mm, 1029 items kg^{-1}), while the lowest mean concentration was found in samples collected a few days after a major rain event in December 2016 (59 mm, 24 ± 19 items kg^{-1}), which caused peaks in the river flow rate (87 m^3/s); this suggests that precipitation and river flow rate influence the MP concentrations in sediments (Constant et al., 2020).

4.3. Differences in MPs' concentrations and characteristics between water and sediments

Due to differences in the field sampling, sample processing, characteristics of water and sediments and the units used in this study, a direct comparison of the number of MPs between water and sediments is not meaningful, and only a qualitative comparison could be made. The qualitative analysis of the particles shows the difference in the shape of the particles – water samples are dominated by fibres, while sediment samples are dominated by fragments. The shape is one of the crucial factors (besides the material structure – density and size) that influence the buoyancy of the particles. Hoellein et al. (2017) have documented that fibres can float longer and have a longer transport length compared to fragments. In addition to shape, the surface-to-volume ratio also influences the state of aggregation and sinking behaviour of the particles. Additionally, Hoellein et al. (2017) observed that of the differently shaped plastics, for instance, fragments have the shortest transport length in the streams due to the surface-to-volume ratio. The results showed that the majority of particles in both types of samples (water and sediments) fit in the size range 0–0.99 mm and are coloured PE or PP.

The particles found in the water column samples were on average smaller than those found in the sediments. In the case of the Ticino River, for example, the sediment samples contained a significantly larger proportion of smaller MP than the water column (Winkler et al., 2022). However, comparison between different studies in terms of MP concentration by size can be difficult, as noted in recent reviews (Hartmann et al., 2019; Lu et al., 2021; Yang et al., 2021). MP studies consider pieces smaller than 5 mm and are limited by the sampling mesh or filter used. In addition, studies use a different size binning between their size classes (Klein et al., 2015; Constant et al., 2020; Yang et al., 2021). Lu et al. (2021) found that 73 different size classes were reported in the reviewed studies, while some of the reviewed studies did not mention particle size at all. Studies should report on MP size with standardised range categories to avoid overestimation.

In order to confirm and better understand the sources of the identified MP items, the visual placements of these items were confirmed using FTIR analysis. In addition, due to the difficulties in handling smaller pieces with FTIR, visual inspection remains important. Fibres of natural origin are flat, twisted and of uneven diameter, whereas fibres of synthetic origin are of uniform diameter along their entire length. Facilitating the digestion of organic matter in MP samples, along with visual separation of MPs, is effective for MP separation and is used in most studies (Stanton et al., 2019). Sediment samples were found to contain mostly PP followed by PE particles, while conversely most water column samples contained mostly PE particles followed by PP (Fig. 3). In most rivers, the predominant polymer was PE (Table 3) and these results are also consistent with the report by Lu et al. (2021) that PE, PET and PP were the predominant MP types in the water samples of previous studies, while PE and PP dominated in the sediment samples, which is consistent with the present study. PE is in high demand in Europe (17.4 % and 12.9 % for low and high density, respectively) and is mainly used for creating reusable bags, food packaging, shampoo bottles and agricultural film (PlasticsEurope, 2021).

In the present study, half of the items found in the sediments were coloured, followed by white, black and transparent items. In the water column samples, coloured and transparent items were common, followed by white and black items, and some were non-defined (6 %) (Fig. 3). Previous studies have reported similar results, different rivers were polluted with particles of different colours (Rodrigues et al., 2018; Scherer et al., 2020). Coloured MPs are usually associated with products with long shelf life (Prata et al., 2019). Analysing the colour of MP can also identify possible sources of MP or contaminants during sample preparation (Prata, 2018; Fahrenfeld et al., 2019; Stanton et al., 2019), which in turn can also facilitate the identification of shapes (Lu et al., 2021). In this study, the most common shapes were fibres and fragments. Coloured fibres may be associated with effluent from a nearby WWTP, while transparent (or discoloured) particles could be due to long-term environmental exposure to UV light or chafing against sediments. In contrast, coloured particles could be indicate fragments of recently discarded (and brightly coloured) larger plastic particles, usually from products with a long shelf life.

4.4. Methodological constraints and recommendations for improving MP characterisation

Due to differences in sampling methods and laboratory analyses, it is difficult to compare the results of MP concentrations in riverine systems between studies. Table 3 compares studies on European rivers of different sizes and under high anthropogenic pressure, using different sampling methods.

Water sampling with a water pump is a rather rare method in MP research. Before commencing the present study, the epineuston net and pump-based sampling methods were tested and it was found that the MP concentrations obtained by sampling with the pump were 10 times higher at smaller volumes of individual samples. Sampling with a net was deemed unsuitable for rivers, because a lot of organic (leaves, branches, aquatic organisms) and inorganic (sand, fine sediment) material gets trapped in the net, making further separation of sample particles difficult. Techniques such as organic material degradation and density separation are not suitable because organic particles such as leaves, cannot be completely degraded by techniques that do not simultaneously affect plastic particles. Furthermore, density separation would not work because the organic particles float on the surface together with the MPs. MP particles are therefore hidden under the other materials. In contrast, the samples obtained with the pump sample were extremely clean because the suction basket did not allow particles larger than 1 cm to pass through. Therefore, the samples did not need to be processed further before MP separation. This method also allowed sampling at different depths. In rivers, MPs tend to be more evenly distributed in the water column due to the river flow, and their concentrations are not necessarily highest at the water surface, as is typical for seawater, so sampling with a water pump in the upper half of the water column made even more sense.

An important consideration when investigating MP concentrations is the choice of an appropriate sample size. Sufficiently large water and sediment samples should be taken to reduce the error in the results, as extrapolating results from, say, 100 g sample to one kilogramme is not realistic as the samples are not homogeneous, and can lead to an error.

In MP research, laboratory analysis of MP particles is often based on separating particles using tweezers. However, this is limited by particle size and may result in a small proportion of particles whose chemical composition can be determined. We therefore propose to filter the washed samples from the filter on an inorganic membrane in the laboratory and examine them with an FTIR microscope. This avoids manual separation of the particles, which saves time and reduces the risk of sample contamination. The use of a glass fibre filter should be avoided, as such filters consist of several layers between which MP particles can hide, affecting the final result.

A current debate among researchers revolves around which fibres can be counted as MP. In particular, studies have differed on the counting of cotton fibres, which are chemically cellulose but are often dyed (Stanton et al., 2019). In the present study, anthropogenic fibres of synthetic and natural origin were distinguished by visual recognition using a stereomicroscope. Anthropogenic fibres of natural origin were classified as microlitter – a term also used in the implementation of marine litter monitoring in the context of the Marine Strategy Framework Directive (Hanke et al., 2013). Therefore, the term microlitter was deemed prudent to describe microplastics as well as particles of other materials (e.g. rubber, paints). Accordingly, all particles from anthropogenic materials in the natural samples were classified to be microlitter, and those particles originating from polymer were categorised as microplastics.

5. Conclusion

The present study offers one of the rare in-depth insights into the quantities, distribution and main features (polymer type, shape, size and colour) of MPs in rivers with small catchments (< 800 km^2) along rural-urban gradient, differing in the intensity of anthropogenic pressures and hydrogeomorphology and addressing MP pollution in both the water column and sediments.

In both the water and sediment samples and in the two rivers studied, the MP concentrations increased further downstream, i.e. with increasing distance from the river spring. PE and PP particles were most frequent and abundant in the water and sediment samples, with smaller particles dominating in the water columns. Fragments prevailed in the sediments, and fibres were predominant in the water columns; this can be attributed to differences in the horizontal movement of the particles in the water. The colouration of the particles was not consistent across catchments, indicating that the sources of MP pollution are highly variable. While pollution sources of MP and pollution intensity are key factors determining the types and quantities of MP present in rivers, patterns of downstream distribution, retention and transport depend on the hydrogeomorphological characteristics of the river.

In past studies on MP pollution, different field sampling and processing methods have been used, making a comparison of different field studies difficult. Therefore, standardisation of sampling methods, laboratory procedures and reporting on results is crucial. In addition, more data needs to be collected in order to make a comprehensive risk assessment of MP in the environment. In sum, further research, with standardised methodologies and reporting procedures, is needed for a better understanding of MP distribution and its influencing factors including catchments with different anthropogenic pressures and of different sizes and natural characteristics.

CRediT authorship contribution statement

TM participated in conceptualization and development of methodology, in field and laboratory work, analysed and organized the data and wrote the first draft of MS; NM lead the conceptualization, participated in field sampling, data analysis and writing of MS; IH participated in conceptualization and development of methodology, laboratory work and writing of MS; OB participated in development of methodology, as an expert in FTIR and writing; MKV lead the development of methodology, participated in field sampling, data analysis and writing of MS.

Data availability

Data will be made available on request.

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.

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