ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2022 Vol. 65, Št. 1: 80-92

Microlitter measurement in fish *Rutilus rutilus* from the Slovenian part of the Mura river basin

Meritve mikroodpadkov v ribah rdečeokah (*Rutilus rutilus*) v porečju reke Mure na območju Slovenije

Domen Bogdan[a], Tamara Kolerič[a], Marija Meznarič[a], Marko Kozjek[b], Manca Kovač Viršek[b]*

[a] Gimnazija Franca Miklošiča Ljutomer, Prešernova 34, 9240 Ljutomer, Slovenia
[b] Institute for Water of the Republic of Slovenia, Einspielerjeva 6, 1000 Ljubljana, Slovenia

*Correspondence: manca.virsek@izvrs.si

Abstract: Knowledge of the impacts of microlitter pollution on the freshwater environment is still less researched when compared to that of marine environments despite rivers being the main pathway for transport of microlitter pollution to the seas and oceans. To better understand the state of pollution with microplastics in Slovenian freshwater fish, we did the first study of microlitter in freshwater fish, in which 50 specimens of common roach (*Rutilus rutilus*) caught in the Slovenian part of the Mura river basin were examined for its presence. The gastrointestinal tract was dissected from each specimen and degraded with 10% KOH. Filtered samples were then checked for microlitter using a stereomicroscope and ATR-FTIR spectroscopy. Microlitter was separated into microplastic particles (0.3 – 5 mm) and textile fibres, which can be of synthetic or seminatural origin. Microlitter was found in 94% of specimens, with an average concentration of 5 ± 3 items/specimen. Colourful fragments and textile fibres were found. Fibres were the predominant form (96%) and indicated households as the main source of microlitter in the Mura river. A strong positive correlation between the number of microlitter and the weight of the fish was found ($R^2 = 0.70$). In the future, simultaneous monitoring of microlitter in sediments, water, and fish would be necessary to assess whether *Rutilus rutilus* is an appropriate species for biomonitoring. Given the growing evidence of the negative effects of microlitter on organisms, it will be important to carry out biomonitoring in terms of assessing environmental status and conditions for human health.

Keywords: fish, microlitter, microplastics, river ecosystem, *Rutilus rutilus*

Izvleček: Vplivi onesnaženja z mikroodpadki v celinskih vodah so še vedno zanemarljivo raziskani v primerjavi s tistimi v morskem okolju, čeprav so reke glavni vir onesnaževanja oceanov z mikroplastičnimi odpadki. Z namenom, da bi bolje razumeli stanje onesnaženosti z mikroodpadki v sladkovodnih ribah v Sloveniji, smo opravili prvo študijo mikroodpadkov v sladkovodnih ribah. Pregledali smo 50 vzorcev rib redečeok (*Rutilus rutilus*) iz slovenskega dela porečja Mure. Vsakemu osebku smo odstranili drobovje in ga razgradili v 10% KOH raztopini. Filtrirane vzorce smo nato pregledali pod stereo-mikroskopom in s pomočjo ATR-FTIR spektrometrije. Mikroodpadke smo ločili na mikroplastične delce (0,3 – 5 mm) in tekstilna vlakna, ki so lahko sintetičnega ali seminaravnega izvora. Mikroodpadke smo našli v kar 94 % vzorcev s povprečno koncentracijo 5 ± 3 delcev/vzorec. Najdeni so bili fragmenti različnih barv in tekstilna vlakna. Vlakna so močno prevladovala (96 %), kar nakazuje, da so glavni vir mikroodpadkov v reki Muri gospodinjstva. Ugotovili smo izrazito korelacijo med številom mikroodpadkov in težo rib ($R^2 = 0,70$). V prihodnosti bi bilo smiselno sočasno spremljati mikroplastiko v sedimentih, vodi in ribah, da bi ocenili, ali je *Rutilus rutilus* ustrezna ribja vrsta za izvajanje biomonitoringa mikroodpadkov. Glede na naraščajoče število dokazov o negativnih učinkih mikroplastike na žive organizme bo potrebno izvajati biomonitoring tako z vidika ocenjevanja okoljskega stanja kot tudi s stališča varne hrane in s tem vpliva na zdravje ljudi.

Ključne besede: mikroodpadki, mikroplastika, rečni ekosistem, ribe, *Rutilus rutilus*

Introduction

The production of plastic has increased from 1.5 million tons in 1950 to 367 million tons in 2020 (Plastic Europe 2021). It is becoming an ever-increasing environmental threat; however, most people are unaware of the problem hidden from our eyes, microplastic (MP) particles (plastic particles 1 µm – 5 mm in size) that have polluted the entire planet, from its deepest points to its highest summits. They have been found in the Marianas Trench (Peng et al. 2018) as well as on Mount Everest (Napper et al. 2020). In recent years, several studies have been conducted to determine the amount of microplastics in the environment, but primarily in the seas and oceans. As 80% of all plastic litter in the oceans comes from rivers (Jambeck et al. 2015), research in freshwater is equally or even more important.

Before August 2021, only 17 studies about the abundance of microplastics in freshwater fish species had been published from Europe (12 countries) and another 43 from other parts of the world (17 countries). Altogether, MPs were discovered in 199 wild freshwater fish (Galafassi et al. 2021) from 29 countries. The first evidence of microplastics presence in European freshwater fish was published in 2014 by Sanchez et al. in the species *Gobio gobio* from French rivers; MPs were documented in 12% of sampled fish. A relatively low plastic prevalence of 18.8%, with significant differences between rivers (20.6%) and lakes (16.5%) was reported from the federal state of Baden-Württemberg, Germany (Roch et al. 2019). In <10% of studies, the percentage of fish that contained MPs was <20% (Galafassi et al. 2021). In most other subsequent studies, MP abundance in fish samples was even higher; in the river Thames (UK), 33% of fish samples contained microplastics (Horton et al. 2018), in the Chi river, Thailand, 72.9% (Kasamesiri and Thaimuangphol 2020), in the Nile river, Egypt, >75% (Khan et al. 2020), and in the Widawa river, Poland, 54% (Kuśmierek and Popiolek 2020). More than 15% of studies report~~s~~ >90% of fish guts are contaminated by MPs (Galafassi et al. 2021), with some of them even up to 100%, as in Evergreen Lake and Lake Bloomington, Illinois, USA (Hurt 2020), Lake Taihu, China (Jabeen et al. 2017), and in the Rio de la Plata estuary, Argentina (Pazos et al. 2017). The average MP concentration per fish specimen was similar among studies and ranged between 0–4 items/individual, with a maximum observation from 6–30 items/individual. Most studies (80%; Galafassi et al. 2021) reported that MPs in the form of fibres dominated in >50% per sample (Pazos et al. 2017, Silva-Cavalcanti et al. 2017, Horton et al. 2018, Su et al. 2018, Kasamesiri and Thaimuangphol 2020, Khan et al. 2020, Wang et al. 2020, Yuan et al. 2019). Rare studies reported fragments as the most abundant type of MPs in their fish samples (Garcia et al. 2021, Sun et al. 2021). Overall, polyethylene terephthalate (PET), polyethylene (PE), and polypropylene (PP) were the prevalent types of MPs found in freshwater fish (Horton et al. 2018, Garcia et al. 2021, Khan et al. 2020).

The average MPs concentration in fish is closely related to the state of pollution of habitat (Peters et al. 2016, Horton et al. 2018, Garcia et al. 2021), gastrointestinal tract structure (Jabeen et al. 2017), and species' feeding characteristics (McNeish et al. 2018). Fish consumed more microplastics in the urbanised sections of the river (Silva-Cavalcanti et al. 2017), where domestic sewage is the major source of MPs (Yuan et al. 2019), primarily coming from laundry. In fishing areas, fishing equipment might be the main source of microplastics (Kasamesiri and Thaimuangphol 2020). Omnivorous fish are more exposed to MPs when feeding and the percentage of MP occurrence is higher than in carnivorous species (Kasamesiri and Thaimuangphol 2020, Wang et al. 2020, Zhang et al. 2021), suggesting that particle uptake is more accidental (Roch et al. 2019) and less associated with transmission among trophic levels (Güven et al. 2017). Most particles in fish are found in the stomach and gill tissues, fewer in the edible flesh tissue (Garcia et al. 2021).

The goal of this study was to determine the amount of microlitter (ML) particles in common roach (*Rutilus rutilus*) specimens caught in the Slovenian part of the Mura river basin. This is the first study of ML abundance in freshwater fish in Slovenia. We hypothesized that common roach is common enough in the river systems and have adequate habitat and eating habits to be able to perform microplastic biomonitoring. It is a sentinel species for assessing endocrine disrupting chemicals (PCB and metals) and is widespread and ecologically important in lowland rivers throughout Eurasia (Southam et al. 2011). The results of previous research on the common roach have shown a link between microplastic abundance and anthropogenic pressures. The study was conducted as a preliminary study with a view to developing methods for conducting biomonitoring of ML in the future when ML will be considered also in the European Water Framework Directive (WFD).

Material and methods

Sampling

A sampling of the freshwater fish the common roach (*Rutilus rutilus*) was conducted in the Slovenian part of the Mura river in October 2020. The common roach was chosen because it is not endangered, is easy to catch, and is present in both standing and running inland waters. The Mura river is around 464 kilometres long and it flows through three countries in Central Europe (Austria, Slovenia, and the Croatia/Hungary border). Its basin covers an area of 13,800 km^2 (Ostroški 2015). Fish were caught at five sampling locations, with the same bait (white worm), considering the proximity of urban centres and industrial and municipal outflows. Three locations were on the Mura river (S1, Sladki Vrh; S3, Hrastje–Mota; S5, Krapje) and two were artificial lakes (S2, Lake Stara Jama in Zgornje Konjišče; S4, Lake Gajševci) (Fig. 1). This provides data on the amount of microplastics in fish from running and standing waters. Lakes are still a part of the Mura basin, as the water from Lake Gajševci flows into the Mura and Lake Stara Jama is right next to the river and has the same groundwater.

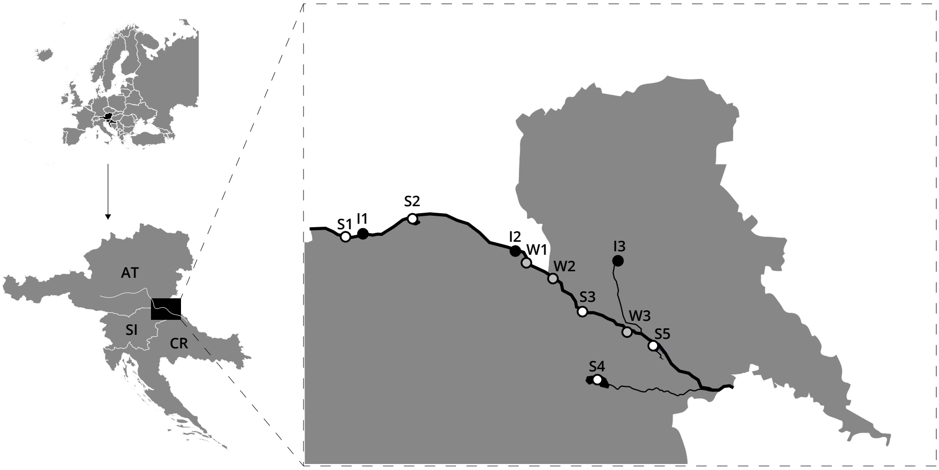


Figure 1: Sampling locations of fish samples, three on the Mura river (S1, Sladki Vrh; S3, Hrastje-Mota; S5, Krapje) and two on the lakes of the Mura basin (S2, Lake Stara Jama in Zgornje Konjišče; S4, Lake Gajševci). The map shows outflows from the WWTPs (W1 – W3) and locations of industrial centres that could have an impact on plastic pollution in the Mura basin (I1 – I3).

Slika 1: Lokacije vzorčenja rib, tri na reki Muri (S1, Sladki Vrh; S3, Hrastje-Mota; S5, Krapje) in dve na jezerih (S2, Jezero Stara Jama v Zgornjem Konjišču; S4, Jezero Gajševci) v porečju reke Mure. Zemljevid prikazuje tudi iztoke čistilnih naprav (W1 – W3) in lokacije industrijskih središč, ki imajo potencialni vpliv na onesnaževanje s plastiko v porečju reke Mure (I1 – I3).

Sample processing

Altogether, 50 specimens were analysed for microplastics (at each location, ten specimens were caught). At first, each fish was measured in length (cm) and weighed (g) with a precision scale (Kern 6W; d = 0.01), then the gastrointestinal tract (GIT) was removed. The fish were cut open along the abdominal region with a scalpel. The GIT was carefully removed from the oesophagus to anus and placed in pre-cleaned glass Petri dishes. GIT was weighed with a precision scale (Kern 6W; d = 0.01) and frozen (-20 °C) immediately after dissection of the fish. When we start with the degradation process, the GIT was thawed, a 10% KOH solution was added at the ratio of 1:1 of the GIT's weight and then incubated at room temperature for 72 hours. After 72 hours, the decomposed contents of the GIT samples were filtered with a Buechner funnel. Cellulose filters (pore size 12 µm) were used. The filters were washed thoroughly with distilled water to rinse off as much KOH as possible. After the filtration was completed, the filters were carefully transferred into clean Petri dishes (checked under a stereomicroscope for MPs before use) with tweezers. The Petri dishes were marked accordingly.

To prevent contamination, work surfaces were thoroughly cleaned. Gloves, face masks, and cotton lab coats were worn throughout the work in the laboratory. All utensils were cleaned and washed with distilled water before use. Other people were forbidden to enter the room during the work, windows were closed, and the air cleaner (Ideal AP30) was turned on. At the same time, procedural blanks were performed, and field blanks were collected. The data was corrected according to the contamination levels found during laboratory analysis.

Petri dishes with filters were examined under a stereomicroscope (Leica ES2; binocular; 10x and 30x magnification) for microlitter 0.3 – 5 mm in size, using a spatula and precision forceps. Microlitter particles were divided into six microplastic categories: fragments, films, foams, fibres, pellets, and granules (Kovač Viršek et al. 2016). Fibres were additionally divided into synthetic textile fibres (polyester and polyamide) and seminatural or natural-based textile fibres (cotton). Synthetic and seminatural fibres were in the first phase distinguished with microscopy (Stanton et al. 2019). In addition, the chemical composition of all fragments and 10% of fibres were identified using the Fourier transform infrared spectrometry method (Spectrum Two, Perkin Elmer), which provides information on the chemical bonds of the particles >0.3 mm in size. Carbon-based polymeric materials are easily analysed using this method, as different chemical bonds have specific vibrational characteristics in the absorption of infrared light, causing them to emit specific spectra that separate plastic from other organic and inorganic particles. By comparing the spectra of the samples with the spectra in the library of polymer spectra (Hummel spectra library), certain types of polymers were identified. To make a positive polymer identification, only matches of >70% similarity to the reference library samples were accepted, according to Frias et al. (2016). Before FTIR, each particle was photographed using a stereomicroscope with a camera (Stereo Discovery V8, software AxioVision SE64 Rel. 4.9.1) and measured lengthwise.

Statistical analysis

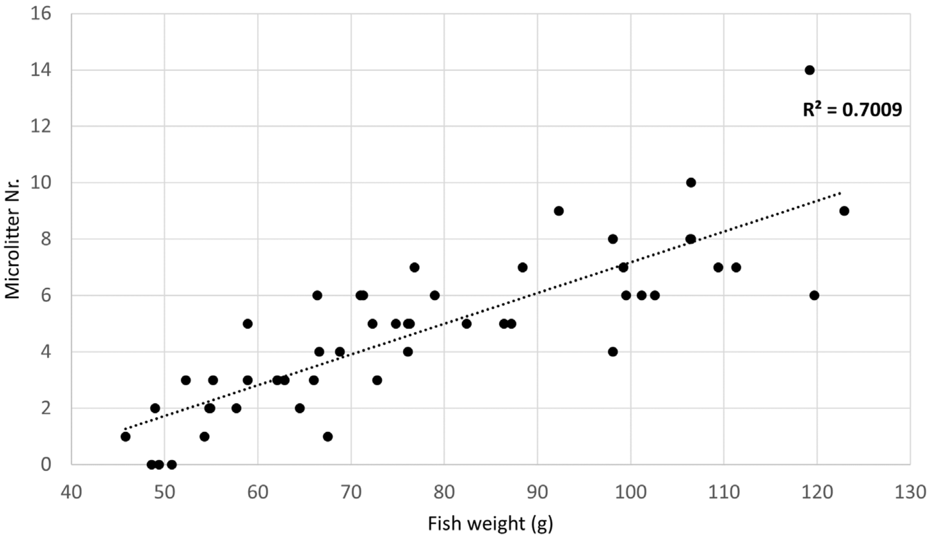
Statistical data analysis was performed using IBM SPSS Statistics 21. Kolmogorov-Smirnov test, Shapiro-Wilk test, Kruskal-Wallis test, Mann-Whitney U test, Pearson correlation and Spearman´s correlation were used.

Results and discussion

Fifty specimens of common roach (*Rutilus rutilus*) from 5 sampling locations on the Mura river were analysed for the presence of ML. The caught fish weighed between 45.8 and 122.9 g and were between 16 and 22 cm in length. ML was found in all sampling locations, in a total of 47 (94%) specimens.

A strong positive correlation was found when ML abundance (in Nr.) was compared with total fish weight ($R^2 = 0.70$; Pearson coefficient: 0.837; Spearman coefficient: 0.874) and moderate positive correlation when ML abundance was compared with fish length ($R^2 = 0.6$; Pearson coefficient: 0.781; Spearman coefficient: 0.816) (Fig. 2). Our study fully supports the results of the study by Horton et al. (2018), where the actual quantity of microplastics in the gut correlated with the size of fish. A positive linear relationship between the number of microplastic particles and the body size was found also in the fish *Neogobius melanostomus* caught in 3 major tributaries of Lake Michigan, USA (McNeish et al. 2018), while this was not found in the marine fish species (Jovanović 2017, Sun et al. 2019). Older fish are, in principle, larger, and a high number of MPs can be associated with a greater frequency of feeding and, as such, a greater chance of ingesting MPs either accidentally directly from the water or indirectly from another contaminated organism. While the whole GIT was degraded, including the wall of the digestive system, the MP particles that accumulated in the GIT tissue should be analysed as well. The particles from our study were large (>300 µm) and, as such, they could not penetrate the other tissue. It is known that fish are even more contaminated with smaller particles, which are hard to find and analyse (Roch et al. 2019).

A



B

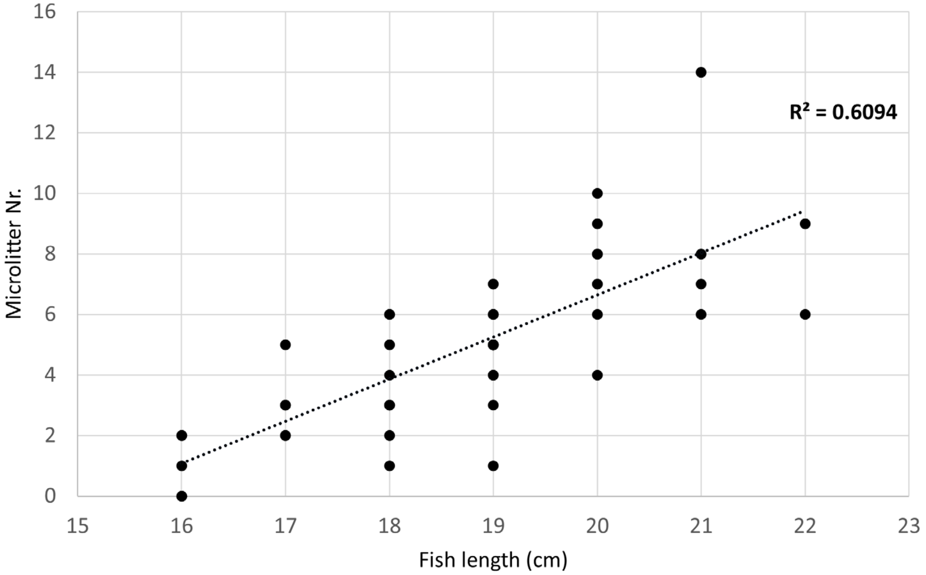


Figure 2: Microlitter correlation with fish weight (**A**) ($R^2 = 0.70$) and fish length (**B**) ($R^2 = 0.61$) for the fish common roach (*Rutilus rutilus*) caught in Mura river basin (Slovenia).

Slika 2: Korelacija mikroodpadkov z maso ribe (**A**) ($R^2 = 0{,}70$) in dolžino ribe (**B**) ($R^2 = 0{,}61$) za ribo rdečeoko (*Rutilus rutilus*), ulovljeno v porečju reke Mure (Slovenija).

The mean ML concentration among all sampling locations was 5 ± 3 items/specimen. The average ML concentration was the lowest at the first sampling location (S1: 3.8 ± 2.2 items/specimen) and the highest at the last sampling location (S5: 5.8 ± 3.5 items/specimen) (Fig. 3). The results from the riverine sampling sites show an increase in ML presence in fish downstream (total MPs S1: 38, S3: 43, S5: 58), but with no significant difference among all sampling locations (Kruskal - Wallis test, p = 0.54) and also among the first (S1) and the last (S5) sampling location (Mann - Whitney test, p = 0.28). The average ML concentration in the fish samples from the riverine system was 4 ± 2.5 items/specimen, while the average ML concentration from the two lakes (S2 and S4) was higher (5 ± 3.3 items/specimen), but with no statistically significant difference (Mann - Whitney test, P = 0.479). Statistical significance in our study was not reached most probably due to too short distances among sampling sites and small differences in land use. Otherwise, it is known that the maximum number of ingested microplastic particles for individual fish can strongly correlate to exposure (Horton et al. 2018), which is probably high in the lower reaches of the river due to agricultural and urban activities.

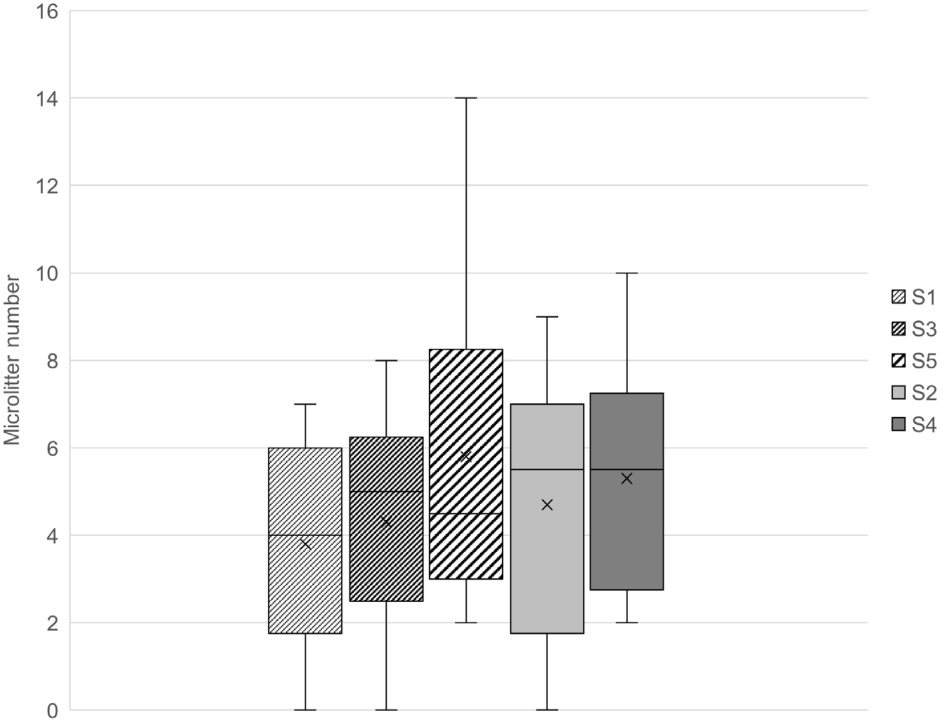


Figure 3: Boxplot for microlitter (ML) found in the fish gastrointestinal tract of common roach (*Rutilus rutilus*) from each sampling location (riverine sampling sites: S1, S3, S5; lake sampling sites: S2 and S4) in Mura river basin (Slovenia). Marks: error bars, the min and max number of ML particles found in fish; x, the average concentration of ML in fish.

Slika 3: Boxplot diagram za mikroodpadke, ki smo jih našli v prebavnem traktu rdečeok (*Rutilus rutilus*) z vsake vzorčne lokacije (rečna vzorčna mesta: S1, S3, S5; jezerska vzorčna mesta: S2 in S4) v porečju reke Mure (Slovenija). Oznake: odkloni, najmanjše in največje število delcev mikroodpadkov, ki smo jih našli v ribah; x, povprečna koncentracija mikroodpadkov v ribah.

Microplastic concentration in the fish species *Rutilus rutilus* was also investigated in the river Thames (UK) (Horton et al. 2018), rivers and lakes in the federal state of Baden-Württemberg (Germany) (Roch et al. 2019), and Widawa river, Poland (Kuśmierek and Popiolek 2020). The mean ML concentration found in *Rutilus rutilus* in our study was 4.2 and 7.2 times higher than the mean concentration from the Widawa and Thames, respectively. As the methodology for sample analysis differs among studies, this comparison does not necessarily reflect the true state. Detection of ML under a stereomicroscope is subjective and dependent on the experience of the expert. The most numerous mistakes are made in the determination of fibres, which are not addressed in all studies and where it is hard to distinguish whether they are of natural or synthetic origin. To some extent, it is possible to distinguish between them with a stereomicroscope, however, chemical analysis by FTIR is crucial to get proper results. Altogether around 50% (Galafassi et al. 2021) of studies did not use any chemical analysis of microparticles, which can lead to over- or underestimation of the concentration of ML._

So far, no study on the presence of ML/MPs in the Mura river (water or sediments) has been reported. Thus, the obtained results cannot be directly correlated to ML pollution in the river. A few studies reported that MP concentrations in river water/sediment and fish seemed not to be strictly dependent (McNeish et al. 2018, Galafassi et al. 2021), nevertheless, our results indicate high ML pollution of the Mura river at the time of sampling. The incidence of ML in the water rises with resuspension of sediments and in them accumulated ML, thus pelagic fish are more exposed to incidentally ingest ML. *Rutilus rutilus* is a pelagic omnivorous fish and the incidence of MPs in pelagic fish should be higher in more polluted rivers and near sewage discharges (Pazos et al. 2017). The concentration of MPs in fish is also closely related to feeding habits. Omnivorous fish are more susceptible to MP ingestion than carnivorous ones (Wang et al. 2020). But regardless of the eating habits of individual fish species and their habitat, the uptake of microplastics by fish is inadvertent rather than intentional (Li et al. 2021).

Only two categories of microparticles were found: fibres and fragments, of which most were fibres (96%), as in many previous studies that addressed microplastic quantity in freshwater fish (Pazos et al. 2017, Silva-Cavalcanti et al. 2017, Horton et al. 2018, Su et al. 2018, Yuan et al. 2019, Kasamesiri and Thaimuangphol 2020, Khan et al. 2020, Wang et al. 2020). Most of them do not distinguish between synthetic and seminatural textile fibres with any method (microscopy or spectroscopy). It follows that, in such cases, the term microplastic can be misleading, as it is not necessary that all the fibres were of synthetic polymer origin. Therefore, in our study, the term ML was used as in the document Guidance of Monitoring of Marine Litter in European Seas (MSFD Technical Subgroup on Marine Litter 2013).

In this study, altogether 230 fibre particles were isolated from the 50 specimens of the common roach. The fibres were different in colour, shape, length, and thickness. Based on these characteristics and ATR-FTIR analysis (10% of fibres), we distinguished between anthropogenic fibres of seminatural (cellulose-cotton) and artificial origin (polymer material). Fibres of natural origin are flat, twisted, and uneven in diameter, while fibres of artificial origin are equal in diameter along their length (Stanton et al. 2019). Based on ATR-FTIR analysis, 86% of all fibres were of anthropogenic seminatural origin, similar to the studies of Garcia et al. 2021 and Jabeen et al. 2017. While these kinds of fibres are of anthropogenic origin with potential risk to nature due to their dye content and other chemical used in processing material (Sharma et al. 2007, Khan and Malik 2018), is crucial to include them into microlitter studies, although they are not of polymer origin.

Among fibres of artificial origin, PET (1x), PE (1x), and ethylene-vinyl acetate (EVA) (1x) were also detected (Fig. 4). PET is most widely used (60%; Li-Na, 2013) in the production of synthetic fibres (polyester fibres). Polyester fibres are often spun together with natural fibres such as cotton and wool to produce a fabric with blended properties. For this reason, both contribute to ML environmental pollution. Fish are exposed to fibres via feeding and breathing, but not all the fibres accumulate in the fish gills or GIT since they can be spontaneously excreted with mucus (Li et al. 2021). The abundance of fibres in the GIT increases in the presence of food (Li et al. 2021). Fibres were most likely to be intertwined in other organic matter (natural cellulose fibres) in water environments, thus, with feeding, omnivorous fish also accidentally eat textile fibres.

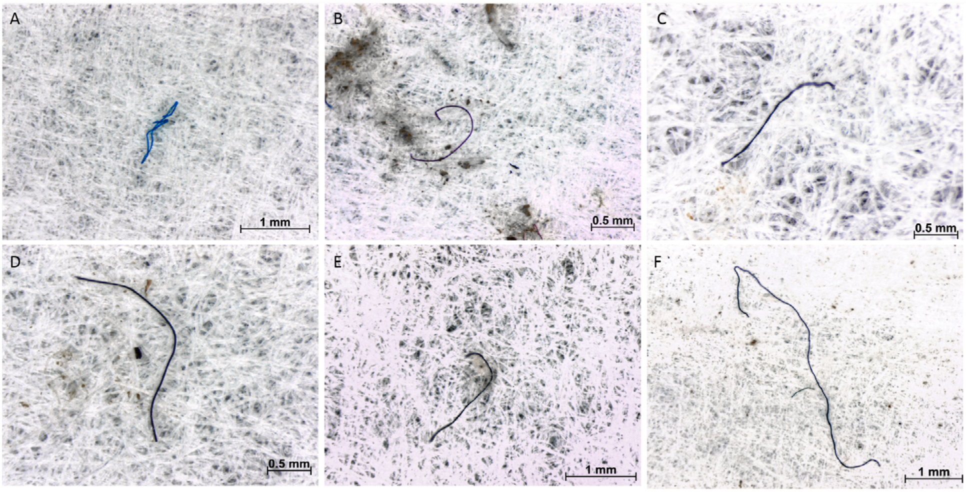


Figure 4: Examples of fibres of anthropogenic origin from the fish samples (*Rutilus rutilus*) caught in the Mura river basin. Figures **A, B, C** show cellulose fibres of various colours, **D** was identified as ethylene vinyl acetate, **E** as polyethylene, and **F** as polyethylene terephthalate.

Slika 4: Primeri vlaken antropogenega izvora iz vzorcev rib (*Rutilus rutilus*), ujetih v porečju reke Mure. Slike **A, B, C** prikazujejo celulozna vlakna različnih barv, **D** je vlakno etilen vinil acetata, **E** polietilen in **F** polietilen tereftalat.

The finding that most of the ML were fibres suggests that the main sources of pollution in the Mura river are wastewater treatment plants. Fibres enter the freshwater systems mainly via wastewater treatment plant outflows (Cesa et al. 2017). Just one load of polyester laundry can release hundreds to thousands of fibres per gram of fabric and the values depend on the kind of fabric tested and on the washing conditions/laundry products (de Falco et al. 2018). The majority accumulate in wastewater sludge and the percentage that is released from WWTPs depends on the WWTP technology. Secondary treatment plants have been found to retain approximately 92% of microplastics, tertiary treatment plants up to 96%, and membrane filtration plants more than 99% of microplastics (Blair et al. 2019). The presence of fibres rises with levels of urbanisation (Huang et al. 2020). Fibres can also be transported into the atmosphere and enter freshwater systems via precipitation. Atmospheric transport allows them to reach even very distant areas. Rain and storm events are key for microplastic contamination and microplastic cycling in the environment. One storm event can multiply microplastic contamination in rivers over 40-fold (Hitchcock 2020).

Altogether nine colourful fragments were found (white, transparent, pink, green, and blue) (Fig. 5), 0.37 mm - 0.89 mm in length (S1, 1 fr.; S2, 1 fr.; S3, 2 fr.; S4, 2 fr.; S5, 3 fr.). ATR-FTIR analysis proved diverse chemical compositions – PE (3x), PP (2x), PET (2x), polystyrene (PS) (1x), and styrene copolymer (1x). Similar chemical composition of MPs from the freshwater fish was also reported by Horton et al. (2018), Khan et al. (2020) and Yuan et al. (2019). The polymer composition of plastic fragments was expected, while the largest groups in total non-fibre plastic production are PE (36%), PP (21%), and polyvinyl chloride (PVC) (12%), followed by PET, polyurethane (PUR), and PS (<10% each). The majority (42%) of the PE, PP, and PVC is used only for packaging (Geyer et al. 2017). This relationship is reflected in environmental studies on MPs. The main polymer constituents of microplastics found in freshwaters, identified as PE, PP, PS, and PET, account for nearly three-quarters of the pollution in freshwater systems (Li et al. 2020).

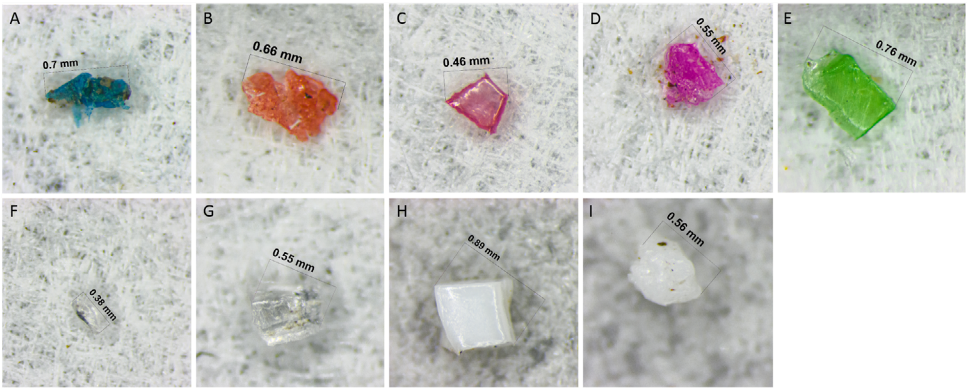


Figure 5: Microplastic fragments from the fish samples (*Rutilus rutilus*) caught in the Mura river and its tributaries: **A**, styrene copolymer; **B**, polyethylene; **C**, polystyrene; **D**, polypropylene; **E**, polyethylene terephthalate; **F**, polyethylene terephthalate; **G**, polyethylene; **H**, polyethylene; **I**, polypropylene.

Slika 5: Mikroplastični fragmenti iz vzorcev rib (*Rutilus rutilus*), ujetih v porečju reke Mure; **A**, stiren kopolimer; **B**, polietilen; **C**, polistiren; **D**, polipropilen; **E**, polietilen tereftalat; **F**, polietilen tereftalat; **G**, polietilen; **H**, polietilen; **I**, polipropilen.

Conclusions

This is the first study on the presence of microlitter in freshwater fish in Slovenia, which will contribute to a better understanding of ML in fish. We demonstrated relatively high concentrations of ML in the fish species *Rutilus rutilus* from the Mura river basin in comparison with other European studies. Fibres as the main ML type indicate households and WWTPs effluents as the main source of ML pollution. The study also clearly demonstrates a positive correlation between the amount of microplastics and the weight/length of the fish. These results indicate the potential suitability of fish *Rutilus rutilus* for the microlitter biomonitoring.

A significant amount of research is being carried out on microplastics in the marine environment, which in Europe is directly linked to the implementation of the Marine Strategy Framework Directive that treats marine litter as one of the key indicators of the health status of marine environments. The fact that rivers are the main route of microplastics to the sea, makes the research on freshwater ecosystems even more important. As a start, identification of sources of pollution through regular monitoring in freshwater ecosystems should be necessary in the frame of the European Water Framework Directive. Only by taking measures to reduce pollution in freshwater, will the measures to improve ecological status regarding litter in the marine environment be effective.

Povzetek

Študije mikroodpadkov v prebavilih sladkovodnih rib so še dokaj redke, čeprav so reke zelo izpostavljene onesnaževanju s plastičnimi delci in glavna transportna pot odpadkov do morja. Opisana študija predstavlja prve rezultate o prisotnosti mikroodpadkov v prebavilih rib rdečeok (*Rutilus rutilus*), ki so bile ulovljene v reki Muri jeseni 2020 na petih lokacijah (S1, Sladki Vrh; S2, jezero Stara Jama / Zgornje Konjišče; S3, Hrastje–Mota; S4, jezero Gajševci; S5, Krapje). Iz vsake lokacije so bila analizirana prebavila desetih rib. Vzorci prebavil so bili razgrajeni v 10 % KOH in filtrirani. Tako pripravljeni vzorci so bili pregledani s streomikroskopom za prisotnost mikroodpadkov, katerim je bila določena tudi kemijska sestava s ATR-FTIR spektrofotometrom. Mikroodpadke smo ločili na mikroplastične delce, kamor uvrščamo fragmente, pene, filme, pelete in granule ter na tekstilna vlakna, katere ločimo glede na izvor na sintetična in naravna (bombaž).

Izmed 50 pregledanih rib, jih je 94 % vsebovalo mikroodpadke v povprečni koncentraciji 5 ± 3 delcev/osebek, med katerimi so prevladovala tekstilna vlakna (96 %). Dokazana je bila korelacija med koncentracijo mikroodpadkov in težo rib ($R^2 = 0{,}70$). Skupno je bilo najdenih 9 mikroplastičnih fragmentov različnih barv in kemijskih struktur (polietilen - 3x, polipropilen - 2x, polietilentereftalat - 2x, polistiren - 1x, stiren kopolimer - 1x). Med tekstilnimi vlakni so prevladovala vlakna naravnega izvora (barvan bombaž; 86 %), med sintetičnimi vlakni so bili identificirani primerki iz etilen vinil acetata, polietilena in polietilentereftalata. Rezultati, s prevladujočimi mikroodpadki v obliki tekstilnih vlaken, kažejo na gospodinjstva in z njimi povezane odpadne komunalne vode, kot največji vir onesnaževanja reke Mure z mikroodpadki. Rezultati se po strukturi mikroodpadkov ne razlikujejo od študij opravljenih v morskem okolju v Sloveniji. Okvirna direktiva o morski strategiji že obravnava morske odpadke in predvideva izvajanje monitoringa morskih odpadkov. Poleg tega se znotraj izvajanja direktive sprejemajo ukrepi za zmanjševanje onesnaženosti morskega okolja s plastičnimi odpadki in doseganje dobrega stanja morskega okolja, medtem ko Okvirna vodna direktiva te problematike še ne obravnava, čeprav se glavna pot odpadkov do morskega okolja prične na rekah. Tako so študije mikroodpadkov v celinskih vodah izrednega pomena, tako z vidika ugotavljanja stanja okolja in vpliva na organizme kot tudi vpliva na zdravje ljudi.

Acknowledgement

This study was made possible by local fishermen who caught the fish sampled. The laboratory work was performed with the help of Mrs. Sonja Koroša.

References

Blair, R.M., Waldron, S., Phoenix, V.R., Gauchotte-Lindsay, C., 2019. Microscopy and elemental analysis characterisation of microplastics in sediment of a freshwater urban river in Scotland, UK. Environmental Science and Pollution Research, 12491–12504.

Cesa, F.S., Turra, A., Baruque-Ramos, J., 2017. Synthetic fibers as microplastics in the marine environment: a review from textile perspective with a focus on domestic washings. Science of the Total Environment, 598, 1116-1129.

De Falco, F., Gullo, M. P., Gentile, G., Di Pace, E., Cocca, M., Gelabert, L., Brouta-Agnésa, M., Rovira, A., Escudero, R., Villalba, R., Mossotti, R., Montarsolo, A., Gavignano, S., Tonin, C., Avella, M., 2018. Evaluation of microplastic release caused by textile washing processes of synthetic fabrics. Environmental Pollution, 236, 916-925.

Frias, J. P., Gago, J., Otero, V., Sobral, P., 2016. Microplastics in coastal sediments from Southern Portuguese shelf waters. Marine Environmental Research, 114, 24–30.

Galafassi, S., Campanale, C., Massarelli, C., Uricchio, V.F., Volta, P., 2021. Do freshwater fish eat microplastics? A review with a focus on effects on fish health and predictive traits of mps ingestion. Water, 13(16), 2214.

Garcia, A.G., Suárez, D.C., Li, J., Rotchell, J.M., 2021. A comparison of microplastic contamination in freshwater fish from natural and farmed sources. Environmental Science and Pollution Research, 28, 14488–14497.

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Science Advances, 3, 25–29.

Güven, O., Gökdağ, K., Jovanović, B., Kıdeyş A.E., 2017. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. Environmental Pollution, 223, 286–294.

Hitchcock, J.N., 2020. Storm events as key moments of microplastic contamination in aquatic ecosystems. Science of the Total Environment, 734, 139436.

Horton, A.A., Jürgens, M.D., Lahive, E., van Bodegom, P.M., Vijver, M.G., 2018. The influence of exposure and physiology on microplastic ingestion by the freshwater fish Rutilus rutilus (roach) in the River Thames, UK. Environmental Pollution, 236, 188–194.

Huang, Y., Tian, M., Jin, F., Chen, M., Liu, Z., He, S., Li, F., Yang, L., Fang, C., Mu, J., 2020. Coupled effects of urbanization level and dam on microplastics in surface waters in a coastal watershed of Southeast China. Marine Pollution Bulletin, 154, 111089.

Hurt, R., O'Reilly, C.M., Perry, W.L., 2020. Microplastic prevalence in two fish species in two U.S. reservoirs. Limnol. Oceanographic Letters, 5, 147–153.

Jabeen, K., Su, L., Li, J., Yang, D., Tong, C., Mu, J., Shi, H., 2017. Microplastics and mesoplastics in fish from coastal and fresh waters of China. Environmental Pollution, 221, 141–149.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347. 6223, 768–771.

Jovanović, B., 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integrated Environmental Assessment and Management, 13(3), 510-515.

Kasamesiri, P., Thaimuangpho, W., 2020. Microplastics ingestion by freshwater fish in the Chi River, Thailand. International Journal of GEOMATE, 18, 114–119.

Khan, S., Malik, A., 2018. Toxicity evaluation of textile effluents and role of native soil bacterium in biodegradation of a textile dye. Environmental Science and Pollution Research, 25(5), 4446-4458.

Khan, F.R., Shashoua, Y., Crawford, A., Drury, A., Sheppard, K., Stewart, K., Sculthorp, T., 2020. "The plastic nile": First evidence of microplastic contamination in fish from the Nile river (Cairo, Egypt). Toxics, 8(2), 22.

Kovač Viršek, M., Palatinus, A., Koren, Š., Peterlin, M., Horvat, P., Kržan, A., 2016. Protocol for Microplastics Sampling on the Sea Surface and Sample Analysis. Journal of Visualized Experiments 1–9.

Kuśmierek, N., Popiołek, M., 2020. Microplastics in freshwater fish from Central European lowland river (Widawa R., SW Poland). Environmental Science Pollution Research,. 27, 11438–11442.

Li, C., Busquets, R., Campos, L.C., 2020. Assessment of microplastics in freshwater systems: A review. Science of the Total Environment, 707, 135578.

Li, B., Liang, W., Liu, Q. X., Fu, S., Ma, C., Chen, Q., Su, L., Craig, N. J., Shi, H., 2021. Fish Ingest Microplastics Unintentionally. Environmental Science & Technology, 55(15), 10471-10479.

Li-Na, J., 2013. Study on preparation process and properties of polyethylene terephthalate (PET). Applied Mechanics and Materials, 312, 406–410.

McNeish, R.E., Kim, L.H., Barrett, H.A., Mason, S.A., Kelly, J.J., Hoellein, T.J., 2018. Microplastic in riverine fish is connected to species traits. Scientific Reports, 8, 1–12.

MSFD Technical Group on Marine Litter, 2013. Guidance on monitoring of marine litter in European seas. Publications Office of the European Union.

Napper, I.E., Davies, B.F.R., Clifford, H., Elvin, S., Koldewey, H.J., Mayewski, P.A., Miner, K.R., Potocki, M., Elmore, A.C., Gajurel, A.P., Thompson, R.C., 2020. Reaching New Heights in Plastic Pollution—Preliminary Findings of Microplastics on Mount Everest. One Earth 3, 621–630.

Ostroški, L., December 2015. Statistical Yearbook of the Republic of Croatia 2015. Zagreb: Croatian Bureau of Statistics. p. 49. ISSN 1333-3305. Retrieved 27 December 2015.

Pazos, R.S., Maiztegui, T., Colautti, D.C., Paracampo, A.H., Gómez, N., 2017. Microplastics in gut contents of coastal freshwater fish from Río de la Plata estuary. Marine Pollution Bulletin, 122, 85–90.

Peters, C.A., Bratton, S.P., 2016. Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos River Basin, Central Texas, USA. Environmental Pollution, 210, 380–387.

Peng, X., Chen, M., Chen, S., Dasgupta, S., Xu, H., Ta, K., Du, M., Li, J., Guo, Z., Bai, S., 2018. Microplastics contaminate the deepest part of the world's ocean. Geochemical Perspectives Letters, 9, 1–5.

Plastic Europe-Association of Plastics Manufactures, 2020. Plastics – the Facts 2020. PlasticEurope 1–64.

Roch, S., Walter, T., Ittner, L.D., Friedrich, C., Brinker, A., 2019. A systematic study of the microplastic burden in freshwater fishes of south-western Germany – Are we searching at the right scale? Science of the Total Environment, 689, 1001–1011.

Sanchez, W., Bender, C., Porcher, J.M., 2014. Wild gudgeons (Gobio gobio) from French rivers are contaminated by microplastics: Preliminary study and first evidence. Environmental Research, 128, 98–100.

Sharma, S., Suryavathi, V., Singh, P.K., Sharma, K.P., 2007. Toxicity assessment of textile dye wastewater using swiss albino rats. Australasian Journal of Ecotoxicology, 13(2), 35-39.

Silva-Cavalcanti, J.S., Silva, J.D.B., França, E.J. de, Araújo, M.C.B. de, Gusmão, F., 2017. Microplastics ingestion by a common tropical freshwater fishing resource. Environmental Pollution, 221, 218–226.

Southam, A.D., Lange, A., Hines, A., Hill, E.M., Katsu, Y., Iguchi, T., Tyler, C.R., Viant, M.R., 2011. Metabolomics reveals target and off-target toxicities of a model organophosphate pesticide to roach (Rutilus rutilus): implications for biomonitoring. Environmental Science and Technology, 45(8), 3759-3767.

Stanton, T., Johnson, M., Nathanail, P., MacNaughtan, W., Gomes, R.L., 2019. Freshwater and airborne textile fiber populations are dominated by ‘natural’, not microplastic, fibers. Science of the Total Environment, 666, 377–389.

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

Sun, X., Li, Q., Shi, Y., Zhao, Y., Zheng, S., Liang, J., Liu, T., Tian, Z., 2019. Characteristics and retention of microplastics in the digestive tracts of fish from the Yellow Sea. Environmental Pollution, 249, 878-885.

Sun, D., Wang, J., Xie, S., Tang, H., Zhang, C., Xu, G., Zou, J., Zhou, A., 2021. Characterization and spatial distribution of microplastics in two wild captured economic freshwater fish from north and west rivers of Guangdong province. Ecotoxicology and Environmental Safety, 207, 111555.

Wang, S., Zhang, C., Pan, Z., Sun, D., Zhou, A., Xie, S., Wang, J., Zou, J., 2020. Microplastics in wild freshwater fish of different feeding habits from Beijiang and Pearl River Delta regions, south China. Chemosphere 258, 127345.

Yuan, W., Liu, X., Wang, W., Di, M., Wang, J., 2019. Microplastic abundance, distribution and composition in water, sediments, and wild fish from Poyang Lake, China. Ecotoxicolology and Environmental Safety, 170, 180–187.

Zhang, C., Wang, J., Zhou, A., Ye, Q., Feng, Y., Wang, Z., Wang, S., Xu, G., Zou, J., 2021. Species-specific effect of microplastics on fish embryos and observation of toxicity kinetics in larvae. Journal of Hazardous Materials, 403, 123948.