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Special Issue

Freshwater Ecosystems—Biodiversity and Protection

Edited by

Dr. Dubravka Čerba, Dr. Filip Stevic, Dr. Djuradj Milošević and Dr. Maja Raković

MDPI

https://doi.org/10.3390/w16070962

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Microplastics in the Danube River and Its Main Tributaries—Ingestion by Freshwater Macroinvertebrates

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Abstract: This study was carried out at the Danube River and its tributaries during the Joint Danube Survey 4 (JDS4) expedition. Three freshwater benthic species were used to estimate the quantity of microplastics (MPs): *Corbicula* spp., *Limnodrilus hoffmeisteri* (Claparede, 1862), and *Polypedilum nubeculosum* (Meigen, 1804). Following the kick and sweep technique, individuals were sampled using a hand net or dredge. In order to estimate the number of MP particles/individual particles/g wet body mass, the body mass and total length of all specimens were measured. Alkaline (*Corbicula* spp. and *L. hoffmaisteri*) and enzymatic (*P. nubeculosum*) protocols were performed for tissue degradation. All samples were filtered through glass microfiber filters (mesh size 0.5 µm). The particles were photographed, measured, and counted. A total of 1904, 169, and 204 MPs were isolated from *Corbicula* spp., *L. hoffmaisteri*, and *P. nubeculosum*, respectively. To confirm the chemical composition of isolated MPs, a subsample of 46 particles of the fragmented particles from 14 sampling sites was analysed via µ-ATR-FTIR spectroscopy analysis. The particles were characterised as polycarbonate (PC), polyethylene terephthalate (PET), polypropylene–polyethylene copolymer (PP-PE), nylon (polyamide-PA) and cellophane, with the domination of PET.

Keywords: microplastic; pollution; Danube River; macroinvertebrates; µFTIR spectroscopy

Citation: Stanković, J.; Milošević, D.; Paunović, M.; Jovanović, B.; Popović, N.; Tomović, J.; Atanacković, A.; Radulović, K.; Lončarević, D.; Raković, M. Microplastics in the Danube River and Its Main Tributaries—Ingestion by Freshwater Macroinvertebrates. *Water* **2024**, *16*, 962. https://doi.org/10.3390/w16070962

Academic Editors: Julie Peller and Jian Liu

Received: 7 February 2024
Revised: 15 March 2024
Accepted: 25 March 2024
Published: 27 March 2024

1. Introduction

Plastic is a synthetic organic polymer which originates from natural derivatives obtained mainly from crude oil, natural gas, or coal. Particles within the size range from 1 µm to 5 mm are referred to as microplastics (MPs) [1]. MPs are created by mechanical erosion, solar radiation, and the biodegradation of larger plastics [2] or are manufactured in extremely small sizes for special products (toothpaste, creams, and cleaning products) that can end up in freshwaters [3]. The global mishandling of synthetic organic polymer waste and low recycling rates have led to a significant increase in plastic pollution. Its ubiquitous presence and non-degradable characteristics are directly related to its persistence in the environment, on a scale range of tens to hundreds of years [4]. A rough estimate predicts that 80% of plastic litter in marine ecosystems is land-based, with rivers serving as its primary pathways [5]. On a global scale, the transport of plastic debris from rivers to the sea is estimated at 1.2 to 2.4 million tons every year [6]. Given the substantial amount of plastics transferring through estuarine systems and the relatively limited reports on microplastic

pollution in this ecosystem compared to the marine environment, priority research on MPs abundance, distribution, characteristics, and ecological impacts on estuaries is warranted.

Since the 1950s, 5 billion metric tons (MTs) of plastics have accumulated in the environment [7]. It is estimated that by the end of the century, between 2.5×10^7 and 1.3×10^8 metric tons will be in the ocean [8]. Although the number of ecotoxicological microplastic studies has increased significantly in the last decade [9], 77% of the publications reported results on marine organisms, while only 23% have been based on freshwater research [10]. The research focus of MPs has been directed toward effects on the freshwater ecosystems. The presence and occurrence of MPs were documented in urban rivers [11–13] and lakes [14–17]. Klein et al. [18] analyzed the shoreline sediments of the Rhine-Main area in Germany and Zbyszewski and Corcoran [19] in Lake Huron, Canada, to name just a few. In addition to its ubiquitous presence, MPs have a vector role, as a transport medium for invasive species [20], harmful algal bloom (HAB) [21], or opportunistic pathogens [22,23].

In the present study, the occurrence of MPs in benthic macroinvertebrates was investigated in the Danube River and its tributaries. The Danube River Basin (DRB) flows across 19 countries, occupying an area of 801,463 km^2 with a population of over 80 million inhabitants in its proximity. The DRB covers nine ecoregions, and it is classified as a special case study from the aspects of conservation and management issues [24]. As it passes through different countries, the Danube accumulates MPs, and the number of MPs in the Danube vastly outnumbers fish larvae [12]. MPs in freshwater tend to sink at a much faster rate than in marine environments since freshwater has less density. Biofouling is further increasing the mass of MPs and is aiding in their deposition and accumulation in the sediment. The filtering activities of filter feeders likely have a very significant role in MP circulation by removing MPs from the water column and depositing them in sediments through incorporation into feces and pseudofeces. Previous studies on MPs and biota within freshwater ecosystems have shown that MPs can be ingested by organisms from sediments and that the ability of macroinvertebrates to ingest MP depends on their feeding habits [25]. MPs accumulated in aquatic organisms are transferred via the food chain, and they directly affect the entire aquatic ecosystem [26]. Macroinvertebrates as widespread organisms could be suitable as bioindicators for assessing MP pollution within the different ecological niches, such as the water column and/or freshwater sediment. The reports of MPs in freshwater environments have mainly focused on the biota at higher levels in the food chain, such as fish [27,28]. Several field data have highlighted MP ingestion by freshwater macroinvertebrates [25,29–32]. More recently, Bertoli et al. [33] focused on the influence of feeding guilds and habits of macroinvertebrates on MP ingestion. Few studies have focused on the ingestion of MPs with respect to freshwater insects [34,35] and the role of freshwater benthic macroinvertebrates in MP transfers from aquatic to terrestrial ecosystems [36].

The Asian clam (*Corbicula* spp.) inhabits a wide range of freshwater habitats across the world, including the Danube River [37]. As benthic filter feeders with intensive activities, bivalves accumulate a considerable amount of MP particles from the environment, which is why they have been extensively used in MP studies lately [38]. Bivalves have a longer life cycle compared to other macroinvertebrates, with low mobility, which is suitable for MP studies, especially because they indicate the state in the microhabitat. Bearing in mind that their habitat is the entire Danube in high abundance [39] and other rivers, the Asian clam was selected as the target organism for this MP study. On the other hand, some aquatic oligochaetes are considered suitable for bioaccumulation studies and are included in the standard guidelines because they are easy to culture, have a high biomass yield, are tolerant to various physico–chemical properties of sediments, and are exposed to pollutants via pore water and ingested sediment [40]. *L. hoffmeisteri* (Naididae: Tubificinae) is tolerant to organic pollution and is the dominant species in almost all oligochaete assemblages along the Danube [41], together with *P. nubeculosum*, which are considered suitable bioindicators

for assessing the effects of different pollutants on freshwater biota [42]. As non-specific feeders, chironomids can ingest MPs instead of food particles [25,43].

The main aim of the present study was to quantify MPs ingested by *Corbicula* spp., *Limnodrilus hoffmeisteri*, and *Polypedilum nubeculosum* from the Danube and its tributaries. The study investigated the ingestion of MPs under real environmental conditions in different freshwater macroinvertebrates, taking into account particle size, shape and polymer type, and whether it is ingested/detected at different sites along the main course of the Danube and its tributaries.

2. Materials and Methods

2.1. Sampling Sites and Procedure

The study was conducted on the Danube River and its main tributaries. The macroinvertebrates from 23 sampling sites were analyzed, of which 15 were on the Danube, and 8 were on the tributaries (Hron, Tisza, Sava, Velika Morava, Iskar, and Jantra) (Figure 1; Table S1 in the Supplementary Materials).

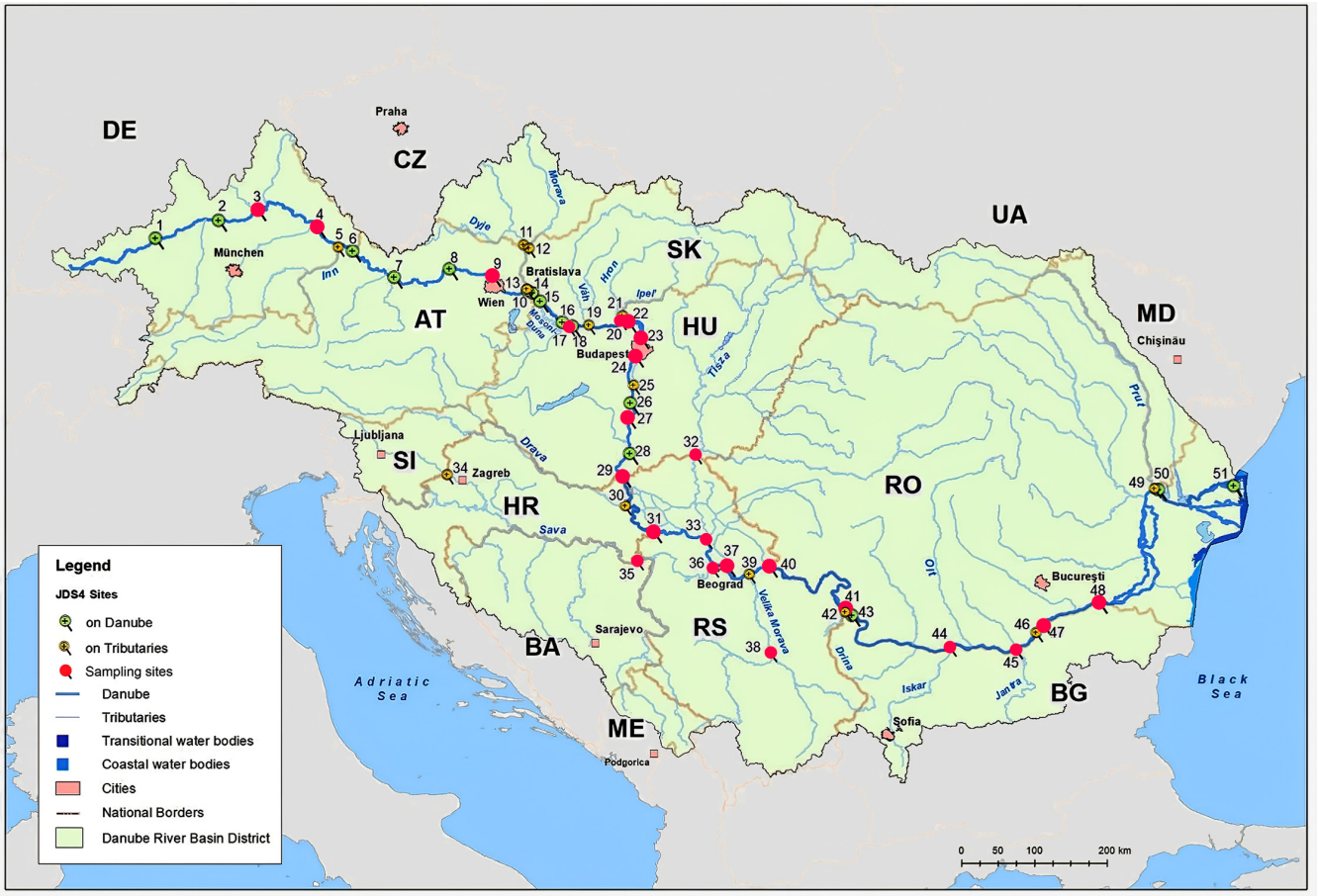

Figure 1. Area of investigation with sampling sites along the Danube River and its tributaries (adapted from https://www.danubesurvey.org/jds4/ (accessed on 2 February 2024).

Samples were collected in summer 2019 by the national Joint Danube Survey 4 (JDS4) teams. Following the multi-habitat procedure [44], individuals were sampled by the kick and sweep (K&S) sampling technique according to European Standards [45] using a hand net (ap. 25 cm × 25 cm, mesh size 500 µm). The iron-forked mouth of the triangle-shaped dredge with a collection net (mesh size 500 µm) was used for the deep water area. The dredge was pulled five times per sampling site. Each transect was considered as a separate sample. The sampling methodology is described in [46].

The samples of freshwater macroinvertebrates were counted, separated, and identified in the laboratory relative to the lowest possible taxonomic level using the following identification keys: Moller Pillot [47,48], Vallenduuk and Moller Pillot [49], Pfleger [50], and Timm [51].

2.2. Preparation of the Samples for MP Isolation

To eliminate MP contamination during the work, deionized water was filtered through 0.5 µm pore size, 47 mm GF/B glass microfibres (Whatman, Kent, United Kingdom). In order to assess potential post-sampling airborne contamination with MPs during the experimental procedure, the digestion processes without tissues ("blank") in 3 replicates per species have been checked for MP contamination.

2.3. Isolation of MPs

In total, 216 specimens of *Corbicula* spp., 130 specimens of *L. hoffmeisteri*, and 79 specimens of *P. nubeculosum* were used for MP isolation. The body mass and total length were measured for all specimens, with the addition of the total mass and total width of the shell of *Corbicula* spp. The tissue of each specimen was rinsed with pre-filtered deionized water and placed into glass beakers. Although numerous approaches have been developed for the extraction of MPs, the alkaline protocol has mostly been used for soft tissues (e.g., *Corbicula* spp. and *L. hoffmaisteri*), while only enzymatic protocols are effective for the digestion of chitinous organisms (e.g., *P. nubeculosum*). All organisms were digested in a pool of 10 specimens or less, depending on the number of individuals collected at the sampling sites.

The samples were processed using the alkaline protocol [52]—treatment with 10% (*w*/*w*) potassium hydroxide (KOH)—and incubated for 12 h at 65 °C in the water bath with a rotation speed of 80 rpm. For the enzymatic protocol, proteinase K was used as per Cole et al. [53].

The solution was filtered through 0.5 µm mesh-size glass microfiber filters. The samples were stored in a rinsed sterile glass Petri dish. The filtrated material was treated with 30% hydrogen peroxide, if needed, in order to remove the remaining organic matter. The minimum size of examined particles was 16 µm in length.

The occurrence of MPs in the organisms is expressed as item/organism and item/g wet weight (ww), where the items are divided based on fibrils and fragments as separate categories.

2.4. Particle Analysis

MPs of a size range from 16 µm to 5 mm were processed. The particles were classified into two main categories: fragments and fibres. All MPs were counted visually and photographed using a Leica MZ16A stereomicroscope (Leica microsystems, Wetzlar, Germany)(10×/21 B ocular and from 20× to 50× objective magnification) with a Leica DFC320 Digital Camera system (Leica microsystems, Wetzlar, Germany), and MPs were measured using calibrated scales in the program ImageJ (version 1.54) [54].

2.5. Micro-Fourier Transform Infrared Spectroscopy (µFTIR)

The fragment particles identified to be the most common in the samples along the Danube River and its tributaries were further chemically characterized by µFTIR. Out of 23 localities, 14 were selected for infrared measurements. Fibres were excluded from the µFTIR analysis due to technical challenges. Due to the high cost of µFTIR analyses on a large sample size, at least the 3 largest fragment particles per sample were randomly selected from the most diverse samples. In total, 46 MP fragments were analysed (see Table S2 in the Supplementary Materials). Infrared measurements were performed with individual manual readings of the particles using a Nicolet iN10 Fourier transform infrared microscope with a micro-attenuated total reflection (ATR) accessory and liquid nitrogen-cooled MCT detector in the ATR mode and carrying out 128 scans at a resolution of 4 cm^{-1}.

The μFTIR method for the identification of MPs provides confident chemical composition information [55].

The OMNIC Pictra Software (version 8.1.0.19) was used to identify the samples, comparing their spectra with the spectra from the Hummel-Polymer Sample Library.

2.6. Data Analysis

The morphometric parameters of the individuals and particles per organism and per g^{-1} body mass were statistically described with an average value and standard deviation (SD). The relation between morphometry-based metrics (length, height, total mass, and body mass) and the number of isolated MPs was obtained using a non-parametric Spearman's rank correlation test.

3. Results

MPs were detected in all samples of *Corbicula* spp. (1904 particles), *L. hoffmeisteri* (169 particles) and *P. nubeculosum* (204 particles). On average, the following were detected per sampling site: from 2.7 to 19.5 fibres/individual and 1.2 to 9.2 fragments/individual in *Corbicula* spp.; from 0.4 to 1.6 fibres/individual and 0.2 to 1.5 fragments/individual in *L. hoffmeisteri*; and from 0.5 to 2.4 fibres/individual and 0.5 to 2.2 fragments/individual in *P. nubeculosum*. In "blank" samples, on average, six fibres were identified, indicating airborne contamination. Therefore, fibres were excluded from μFTIR analyses. Fragments, films, or hard MPs were never present in blank samples. Selected organisms differed according to morphometric parameters (Table 1). The correlation between morphometric parameters and a number of isolated MPs was not significant (Spearman's rank correlation test; $p > 0.05$).

Table 1. Average values of morphometric parameters.

	***Corbicula* spp.**	***L. hoffmeisteri***	***P. nubeculosum***
TL ± SD	14.23 ± 3.78	8.52 ± 6.17	5.56 ± 2.07
BW ± SD	340 ± 0.21	0.76 ± 0.88	0.45 ± 0.74

Note: TL—total length (mm); BW—body weight (mg); SD—standard deviation.

The ingested particles were within the size ranges from 0.016 to 4.67 mm (Table 2). Fibres were the dominant category within *Corbicula* spp. (56.9%) and *L. hoffmeisteri* (58%), while the dominant category in *P. nubeculosum* (50.2%) comprised fragments. In *Corbicula* spp., blue-coloured fibres were dominant among fibres within all species, while transparent fragmented MPs were found to be the most abundant in *Corbicula* spp., and black-coloured particles were observed in *L. hoffmeisteri* and *P. nubeculosum*.

Table 2. Minimum and maximum length of fibres and fragments.

	***Corbicula* spp.**		***L. hoffmeisteri***		***P. nubeculosum***	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**
Fibres	0.08	4.67	0.049	4.61	0.031	4.13
Fragments	0.02	3.22	0.018	0.288	0.016	0.0241

Note: All measures are in mm.

In order to estimate the accumulation of MP particles for each species (216 specimens of *Corbicula* spp., 130 specimens of *L. hoffmeisteri*, and 79 specimens of *P. nubeculosum*), the number of isolated fibres and fragments was calculated per organism and per g^{-1} ww (Figure 2).

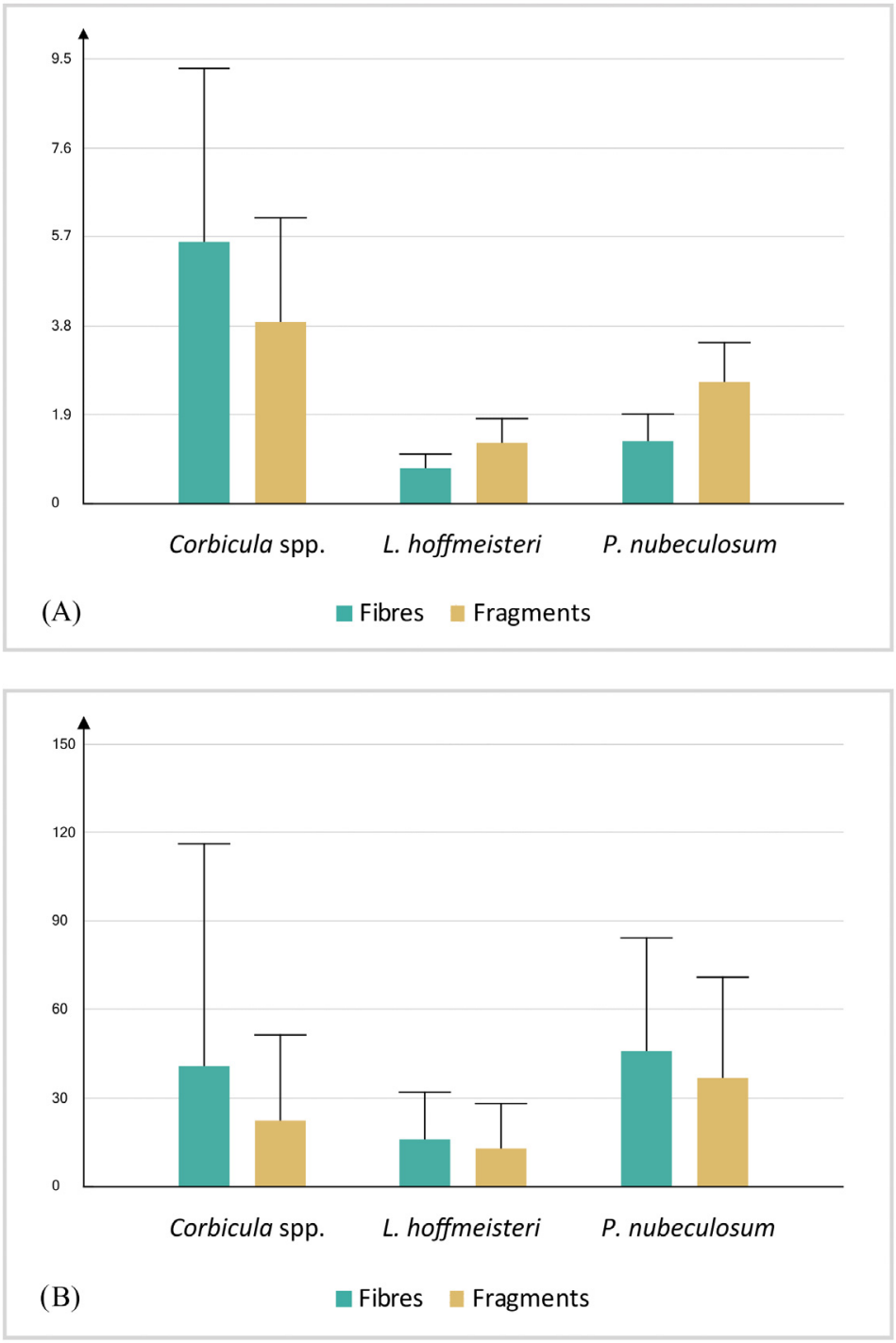

Figure 2. An average number of fibres and fragments (bars) per (**A**) organism and (**B**) g^{-1} ww for each species. Lines represent the variance in the number of particles.

The data show a higher abundance of MPs at sampling sites JDS4–3, JDS4–23, JDS4–24, JDS4–40, and JDS4–41 in *Corbicula* spp.; JDS4–37 in *L. hoffmeisteri*; and JDS4–31 in *P. nubeculosum* along the Danube. The abundance of MPs was higher at sampling sites JDS4–20, JDS4–35, JDS4–36, and JDS4–38 in *Corbicula* spp.; JDS4–38 in *L. hoffmeisteri*; and JDS4–33 and JDS4–35 in *P. nubeculosum* on tributaries (Figure 3A).

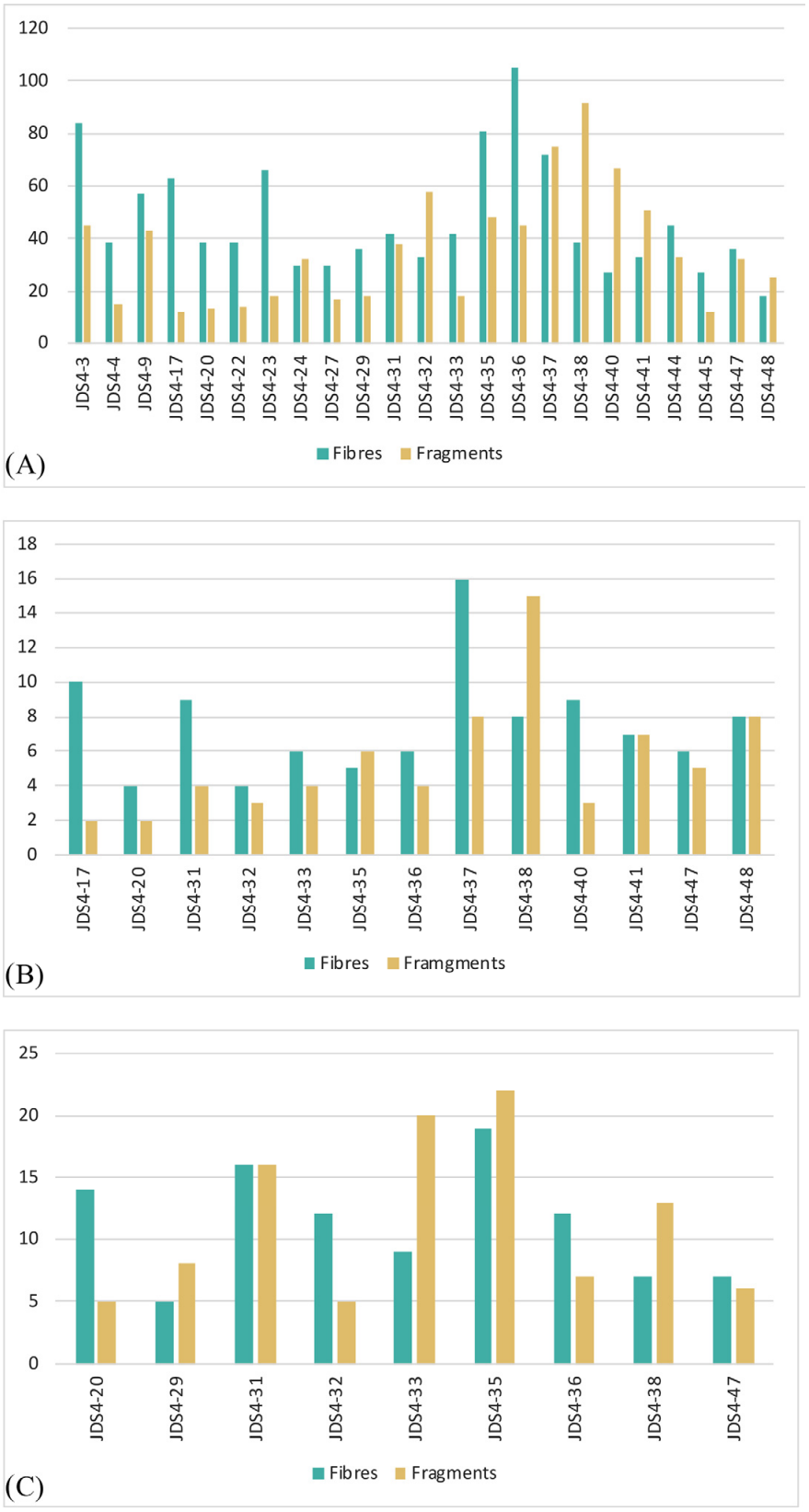

Figure 3. The quantities of fibres and fragments in (**A**) *Corbicula* spp., (**B**) *L. hoffmeisteri*, and (**C**) *P. nubeculosum* at sampling sites along the Danube and its tributaries.

In order to confirm that extracted particles were indeed MPs and to determine the polymer type of the isolated MPs, *Corbicula* spp. samples were selected for the μ–ATR–

FTIR analysis. From the total of 46 particles, 40 were confirmed as plastic polymers via μ-ATR–FTIR. Analyzed MPs were identified as polycarbonate (12/40), polypropylene–polyethylene co-polymer (3/40), nylon (Polyamide) (1/40), cellophane (2/40), and PET, which was the most dominant with 21 particles out of 40 (Figure 4; see Tables S2 and S3 in the Supplementary Materials).

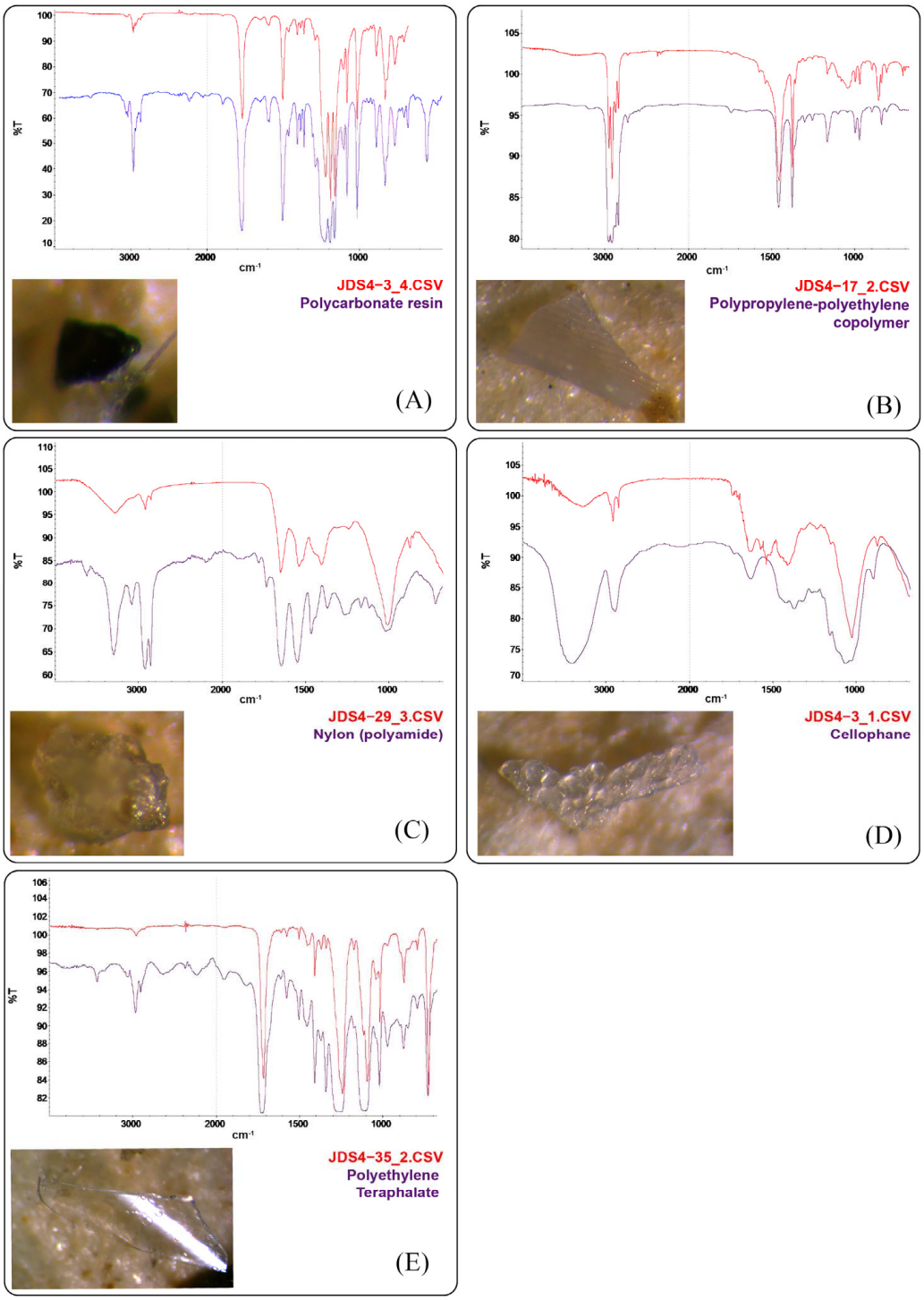

Figure 4. Results of μ–ATR–FTIR analyses of the MP sample (red line); the chemical substance standard database (blue/purple line): (**A**) polycarbonate—JDS4–3_4; (**B**) polypropylene–polyethylene copolymer—JDS4–17_2; (**C**) nylon (polyamide)—JDS4–29_3; (**D**) cellophane—JDS4–3_1; (**E**) polyethylene terephthalate—JDS4–35_2.

4. Discussion

The JDS4 MP study with respect to biota provided comparable information on the MPs in the biota along 2040 rkm of the Danube, contributing to the general knowledge of their distribution in biological systems. To the best of our knowledge, besides the study of Su et al. [56], this is the second study on MPs in benthic macroinvertebrates in large-spatial-scale rivers.

Our results confirmed that there is no correlation between the morphometric parameters of the organisms and the quantity of ingested MPs. Similar findings are documented for fish [57] and shellfish [58]. In the case of selected species, the size of the organism does not affect the quantity of ingested MPs.

Hohenblum et al. [59] reported MPs in the Danube's Austrian stretch in water samples with a concentration range of 0.039–0.205 mg/m^3 and 0.029–0.516 mg/m^3, specifically in the entry and exit points, respectively. Accordingly, the annual average range of transport of MPs is estimated from 6 to 66 kg per day in the Austrian Danube River. Isolated particles in their study are categorized as fragments (over 50%), pellets (4–10%), and green lenticular flakes (2.1–2.8%), while in this study, the category of fibres was the most dominant.

Scherer et al. [25] demonstrated the ingestion of polystyrene among different freshwater invertebrates: *Physella acuta* (Draparnaud, 1805) (Mollusca), *Lumbriculus variegatus* (Müller, 1774) (Oligochaeta), and *Chironomus riparius* (Meigen, 1804) (Diptera: Chironomidae). Hurley et al. [60] isolated particles from the tissue of *Tubifex tubifex* (Müller, 1774) from the Salford Quays basin (Manchester City, UK), where the majority are fibres (87%), while the rest of the particles are fragments. This is similar to our findings where fibres were dominant in the samples of *Corbicula* spp. (56.9%) and *L. hoffmeisteri* (58%). Lin et al. [61] detected microgranules (0–28%), microfilms (0–16%), microfragments (3–47%), and microfibres (40–64%) within the midge larvae (Diptera: Chironomidae) at five sampling sites in the Wu River basin, Taiwan, while in this study, fragments were the dominant category of particles in *P. nubeculosum* (50.2%). As the dominant category in most studies, the origin of fibres has become questionable. Some authors suggested that the majority of fibres have natural origins, as they are cotton fibres [62]. On the other hand, the extent of plastic use in the textile industry is dominant, as it is estimated to be the third largest industry for plastics worldwide [7]. Synthetic textile fibres dominate, with a share of over 60% of total global textile fibre production in 2019, with 52% being polyester fibres (58 million tons), followed by polyamides (nylon) with 5% (5.6 million tons) [63].

The negative effects of MPs on freshwater biota have been documented previously. Exposure to polystyrene MPs caused a decrease in the weight of *L. hoffmeisteri* and induced inflammatory responses and sediment-avoidance behaviour [64]. When exposed to MPs, Asian clams not only showed statistically significant fitness reduction and an increase in lipid oxidative damage, but neurotoxicity was also detected [65]. In association with polychlorinated biphenyls (PCBs), MP exposure leads to tubular dilation [66]. No data have been published for MP ingestion by *Polypedilum nubeculosum* species, but various studies have demonstrated negative effects on chironomids [43,67,68].

Su et al. [56] provided the results of MP analyses in *C. fluminea* in the Middle and Lower Yangtze River Basin. Their results show that Asian clams are a good bioindicator for describing MP pollution, especially for sediments, as in 61 out of 63 samples of Asian clams, MPs are detected. Su et al. [56] reported abundance ranges from 0.3 to 4.9 items/g wet body mass and from 0.4 to 5.0 items/individual per site. MP pollution in *C. fluminea* from Taihu Lake (China) resulted within the range of 0.2–12.5 items/g wet mass [69]. Baldwin et al. [70] have isolated 18 to 105 MP particles per individual, with a mean value of 51.7 items/organism. Our results show a higher abundance of MP particles in the *Corbicula* spp. of DRB in comparison to the mentioned studies in China, indicating the pressure caused by plastic pollution in the Danube Basin. The size range of all MPs in this study was from 0.016 to 4.67 mm, which is a very similar size range of particles ingested by Asian Clams from the middle–lower Yangtze River Basin, from 0.021 to

4.02 mm [56], and the Taihu Lake, from 0.05 to 5 mm [69]. In addition, all previous studies [56,69,70] documented the dominance of fibres ingested by *C. fluminea*.

Cellophane was dominant in *C. fluminea* from the Taihu Lake, followed by PET, polyester, and polypropylene [69]. Our study showed that PET particles (used for the production of plastic bottles) were dominant in the *Corbicula* spp. samples from the Danube, while cellophane was present with lower abundances. It should be noted that cellophane particles can be difficult to identify via infrared spectroscopy in the presence of cellulose because of their similar molecular structure [71]. For the infrared identification of cellophane, we selected larger irregular-shaped particles that are not characteristic of natural regularly ordered structures like fibres and compared them with spectra from the library. These particles achieve a spectral matching score of 60% for cellophane and 37% for cellulose. The domination of PET fragments in the samples, besides potentially high quantities in the Danube, could be due to the clam's preference for ingestion. Li et al. [52] demonstrated a higher intake of PET fibres (4.1 items/g) than five other polymer types (1 item/g or less). In addition, natural colour particles (brown and white) are identified as calcium carbonate. Thus, these subcategories should be analysed with much more scrutiny in future analysis in order to avoid the misidentification of MPs with inorganic materials.

The results are reasonable because PET is used in manufacturing plastic bottles, which could be found floating in rivers. Over time, mechanical forces, sunlight, and biological processes altogether break down macroscopic plastic bottles into smaller pieces, which became available to smaller organisms. The specific density of PET is 1.38 g cm^{-3}, which is already dense by itself and sufficient for sinking to the sediment. In addition, after the microbial or algal inhabitancy of the plastic surface, MPs became even denser and can sink into the sediments easier. Their increased presence in the downstream region of the Danube could not only be explained by local pollution but also by the floating plastic debris from upper stream regions, which are more developed. The data from this study could indicate that tributaries greatly contribute to MP loads in the Danube. Furthermore, the higher presence of MP debris on sites JDS4-37, JDS4-40 and JDS4-41 could indicate the influence of Belgrade, as well as the tributaries Sava and Velika Morava.

Lechner et al. [12] estimated an average of 7.5 g of plastic litter per 1000 m^3 s^{-1} with respect to the mean flow (4.2 t per day or 1533 t per year), transported via the Danube to the Black Sea.

Some of the bivalves are widely used in the human diet. At the time of consumption, commercially grown bivalves *Mytilus edulis* and *Crassostrea gigas* contain on average 0.36 ± 0.07 and 0.47 ± 0.16 particles g^{-1} ww (wet weight), respectively [72]. The same study concluded that 250 g ww of mussels or 100 g ww of oysters results in the ingestion of 90 or 50 particles of MPs, respectively. When estimated per year, humans ingest 11,000 MP particles just through a diet of bivalves [72]. The presence of marine MPs in seafood could potentially be a threat to food safety because of the additives in plastics, mainly endocrine disruptors phthalates and bisphenol A [73] or the adsorption of POPs, PCBs, PAHs, organo-halogenated pesticides, nonylphenol, and dioxins [74–76] on the MPs' surface.

Freshwater Asian clams are invasive species [77] and are useful bioindicators of emerging contaminants [78,79], including MPs [69]. Due to increasing synthetic pollution in aquatic environment, there is a need to include MPs in the standard procedures of water analysis in order to gather more data on this problem.

5. Conclusions

The present study is the first field study to investigate 2040 km of the Danube River, and to the best of our knowledge, the second study on MPs in macroinvertebrates in the river. MPs isolated from *Corbicula* spp. showed the presence and bioavailability of five types of polymers—cellophane, polyamide, polypropylene–polyethylene copolymer, polycarbonate, and PET, which was the most dominant polymer (58%).

The analyzed parameters (number of MPs per site, mean number of MPs per individual per site, and mean number of MPs per body mass—g/wet weight) indicated a higher MP load for tributaries, as well as an important influence of tributaries and settlements on the presence of MP debris in the Danube.

The results of the JDS4 MPs study confirmed that bivalves are suitable test organisms for the assessment of MP loads in the aquatic environment.

As benthic macroinvertebrates are an important component of food chains, as well as the basis of many services and functions of freshwater ecosystems, such as nutrient cycling and water quality, assessing the ecological risk of MPs in freshwater ecosystems is crucial for the successful implementation of strategies to ensure clean water supplies and to prevent the loss of biodiversity in freshwater. Therefore, further standardised studies providing comparable data on MPs in biota within the Danube River Basin using Asian clams are not only needed but other test organisms are also needed in order to assess the MP load and possible consequences more accurately. In addition, the uptake of particles, pathways, and quantities and their relation to particular size need to be further studied.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w16070962/s1; Table S1: List of sampling sites with codes and information for each site; Table S2. Number of *C. fluminea* specimens per sample and analysed particles using micro-ATR-FTIR spectroscopy; Table S3. Description of the analyzed particles.

Author Contributions: Conceptualization, M.P.; methodology, M.R., D.M. and J.S.; formal analysis, J.S., B.J., N.P., K.R. and D.L.; investigation, J.S., N.P., J.T. and A.A.; resources, M.P.; data curation, J.S.; writing—original draft preparation, J.S., D.M. and M.R.; writing—review and editing, D.M., M.P., B.J., N.P., J.T., A.A. and M.R.; visualization, J.S., B.J., N.P., K.R. and D.L.; supervision, D.M., M.P. and M.R.; project administration, M.P.; funding acquisition, M.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-47/2023-01/200007).

Data Availability Statement: Data is contained within the article (and Supplementary Materials).

Acknowledgments: We are grateful to the ICPDR for familiarizing us with the physiognomy of the River Danube during several Joint Danube Surveys. Special thanks should be given to Igor Liška (ICPDR Secretariat), Jaroslav Slobodník (logistic officer of the four JDS missions), and Franz Wagner (chairman of the Monitoring and Assessment Working Group of ICPDR), who all provided essential help for the successful actions of the Core Team during all Danubian activities of our group.

Conflicts of Interest: The authors declare no conflicts of interest.

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