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Automated μFTIR Imaging Demonstrates Taxon-Specific and Selective Uptake of Microplastic by Freshwater Invertebrates

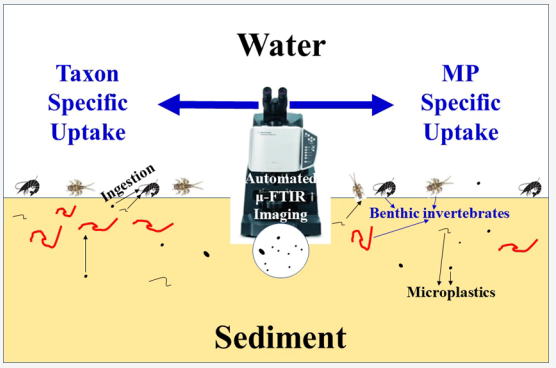
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ABSTRACT: Microplastic particles can be deposited to sediments and subsequently ingested by benthic organisms. It is unknown to what extent ingestion of microplastic is taxon-specific or whether taxa can be selective toward certain types of microplastics. Here, we used state-of-the-art automated micro-Fourier-transform infrared (μFTIR) imaging and attenuated total reflectance FTIR spectroscopy to determine small-size (20−500 μm) and large-size (500−5000 μm) microplastic particles in sediments and a range of benthic invertebrate species sampled simultaneously from the Dommel River in the Netherlands. Microplastic number concentrations differed across taxa at the same locations, demonstrating taxon-specific uptake, whereas size distributions were the same across sediments and taxa. At the site with the highest concentration, microplastic occupied up to 4.0% of the gut volume of Asellidae. Particle shape distributions were often not statistically different between sediments and taxa, except for Astacidea at one of the locations where the proportion of particles with a length to width ratio >3 (*i.e.*, fibers) was twice as high in sediments than in Astacidea. Acrylates/polyurethane/varnish was predominately found in sediments, while soft and rubbery polymers ethylene propylene diene monomer and polyethylene-chlorinated were the dominant polymers found in invertebrates. Microplastic polymer composition and thus polymer density differed significantly between invertebrates and their host sediment. Trophic transfer at the base of the food web appears to have a filter function with respect to microplastic particle types and shapes. Together with the very high ingestion rates, this has clear implications for ecological and human health risks, where uptake concerns edible species (e.g., Astacidea).



KEYWORDS: *microplastics, sediment, invertebrates, uptake, FTIR imaging*

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1. INTRODUCTION

Because of its ubiquity and potential adverse effects on organisms, pollution with microplastic (MP, particles with a size between 1 μm and 5 mm) has been recognized as an issue of global concern.[1−5] Investigation of MP has mainly focused on the qualification and quantification of MP in water, sediment, and fish samples.[6−9] Until now, few studies have investigated the occurrence of MPs in invertebrates, especially in field collected riverine invertebrates.[10−12] It has also been recognized that there are insufficient data on MP in the freshwater environment to inform the risk assessment for MP particles.[1,13]

MP can easily be ingested by a wide range of organisms, including zooplankton,[14] benthic organisms,[15−18] fish,[19] and humans.[2] Following ingestion, MP could be transferred along the food chain.[20] To date, studies on ingestion of MP by freshwater invertebrates were performed mainly under laboratory conditions and/or were limited to only a few selected species.[10,12,15,21] For example, Hurley *et al.* (2017) reported that Tubifex worms in the River Irwell (UK) ingested MP with a mean concentration of 129 particles/g wet weight (ww), with fibers being the predominant type of MP ingested.[10,22]

MP ingested by organisms can induce various adverse effects, including internal blockage, inhibition of nutrient absorption, reduction of reproduction, and mortality, as well as transfer in the food chain.[20,23−28] Consequently, studying species-specific ingestion of MP by riverine invertebrate species under field conditions is essential to understand the ecological risks of MP in freshwater systems.

Several methods have been developed to quantify MP in environmental samples. Also, protocols for quality assurance and quality control (QA/QC) have been developed.[29−32] However, there are no harmonized methods for polymer identification and the results of most reported studies are

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incomparable, which has been recognized as a significant obstacle in MP research.[33] The simplest and oldest approach used is visual inspection with the naked eye or a microscope without any further polymer confirmation.[34,35] However, this visual method has drawbacks: the misidentification rate can be high, and it does not provide information on the polymer type.[36,37] Fourier-transform infrared (FTIR) and Raman microscopy are widely used to identify MP, as the polymer identity of suspected MP can be verified. This could otherwise introduce a bias as very tiny and transparent particles are easily overlooked during the analytical process.[38] FTIR microscopy with focal plane array (FPA) detection, followed by state-of-the-art-automated image analysis, has been introduced to solve these problems.[38−40] The latter significantly decreases the time required for the interpretation of complex FTIR-imaging data and simultaneously increases the data quality. Recently, this approach has been used to quantify MP in water and sediment samples.[39−41] However, this method has not been applied to obtain such a high-resolution characterization of MP in biota samples.

The objectives of the present study were (i) to characterize the diversity and concentrations of MP in freshwater benthic invertebrates and their host sediments *in situ*, (ii) to assess the relationship between MP found in these invertebrates and that found in sediment, and (iii) explore the differences in the ingested MP across invertebrate taxonomic groups and their host sediments. To achieve these aims, invertebrates and sediments were sampled in the river Dommel (the Netherlands) and MP was extracted using strict QA/QC criteria, after which the MPs' polymer identity, size, and shape were assessed using FPA-FTIR microscopy followed by automated image analysis.

2. MATERIALS AND METHODS

2.1. Study Area and Sampling. The Dommel is a relatively small lowland river that originates in north-eastern Belgium and runs through the Netherlands with a base flow of 2−4 m^3/s. It receives effluents from 750,000-population equivalent wastewater treatment plants (WWTPs) (three WWTPs, Boxtel, St. Oedenrode, Eindhoven) and 200 combined sewer overflows.[42] The sampling campaign was conducted in July and August 2019 (details provided in Supporting Information). Sediment and benthic invertebrate samples were collected from seven sampling sites along the Dommel (Figure S1 and Table S1). At each site, sediments were sampled using a cylindrical polyvinylchloride (PVC) core sampler ($r \approx 3.5$ cm and depth = 10 cm) at three locations, all at a water depth of 0.8−1.0 m, with a distance of approximately 5 m from each other. The three subsamples were mixed in the laboratory in order to obtain a composite sediment sample, which resembles the spatial scale relevant to characterize the habitat of the sampled invertebrates. Subsequently, the samples were stored in clean glass bottles and covered with aluminum foil followed by polypropylene (PP) screw caps. At the same sampling sites, benthic invertebrates were collected from an area of approximately 10 m^2, using a macrofauna net with a mesh size of 0.5 mm. Once the invertebrate samples were collected, they were sieved through a stack of stainless-steel sieves with increasing mesh sizes of 0.5, 1, and 5 mm from bottom to top. Residues were placed in a stainless-steel tray filled with water from the sampling sites. All organisms were picked individually and stored in clean glass jars filled with 70% ethanol pre-filtered through a stainless-steel sieve with 20 μm mesh size to remove any potential MP present.[29] These organisms were identified to the family level under a microscope based on their morphology traits.[43] The detailed taxa classification of all sampled organisms is provided in Table S2, Supporting Information.

2.2. MP Extraction from Sediment and Invertebrate Samples. *2.2.1. Sediment.* MP was extracted from the sediment samples based on the methods reported by Coppock *et al.* (2017)[44] and Mani *et al.* (2019).[40] These methods have been reported to yield MP recoveries from sediments with 55−100% of the larger particles. Briefly, the sediment samples were dried in an oven at 60 °C and homogenized. Then, 100 g of each sediment sample was weighed and sieved using a 500 μm sieve. The <500 μm fraction was transferred into a sediment-microplastic isolation (SMI) unit and 750 mL of pre-filtered $ZnCl_2$ with a density of 1.5 g/cm^3 was added. Following Coppock *et al.* (2017),[44] the sediment was mixed for 15 min by using a glass rod and allowed to settle for 24 h. Afterward, the ball valve of the SMI unit was carefully closed and the supernatant in the headspace was poured into a filtration device which used a 20 μm metal filter. The headspace was rinsed thoroughly with ultrapure water three times to recover any remaining particles. Subsequently, the metal filter was transferred to a glass bottle filled with 50 mL of hydrogen peroxide (H_2O_2, 30%) and incubated for 3 days at 37 °C. Subsequently, the sample was filtered through a metal filter of the same size, and the residuals were transferred into a separating funnel using 100 mL of $ZnCl_2$ solution. The funnel was shaken manually for 2 min and left to enable settling of denser materials. The settled material was discarded by turning the valve of the funnel. About 20 mL of liquid was left in the funnel which were filtered again over the 20 μm metal filter. The funnel was rinsed thoroughly with ultrapure water to recover any remaining particles. The metal filter was then transferred to a glass beaker; all particles were rinsed off with ultrapure water and subsequently filtered through an Anodisc filter (diameter, 25 μm, Whatman, U.K.). The Anodisc filter was placed and dried in a half-opened glass Petri dish in an oven for 72 h at 35 °C.

2.2.2. Invertebrates. The length and wet weight of the collected organisms were measured by using a caliper and an analytical balance, respectively (Table S2). After rinsing them with ultrapure water, whole bodies per species were digested following an enzymatic purification protocol modified from a previous study.[45] For the digestion treatments, H_2O_2 and enzymes (chitinase and protease) were used in various combinations depending on the samples. The detailed protocols for the extraction of MPs from different taxa are provided as Supporting Information (Text S1 and Table S3).

2.3. MP Identification and Quantification. *2.3.1. MP > 500 μm.* MP particles >500 μm were manually separated from sediment samples with a 500 μm sieve under a stereo microscope (Olympus SZX10, Olympus). No particles larger than 500 μm were found in organisms. All putative MPs were identified using attenuated total reflectance FTIR (Varian 1000, Agilent, USA). The IR spectra were collected in the spectral range of 600−4000 cm^{-1} at a resolution of 4 cm^{-1}, which allowed the identification of the polymer type.

2.3.2. MP < 500 μm. For each sample, the whole purified extract was filtered through an Anodisc filter. Anodisc filters with MP < 500 μm were placed on a calcium fluoride (CaF_2) crystal window and were analyzed using a FTIR microscope (Cary 620/670, Agilent) equipped with a 4× objective and an

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FPA detector. Theoretically, the lowest size limit is defined using the mesh size of the filters during the sample processing and using the pixel size during FTIR imaging (20 μm). However, MPs smaller than the mesh size can be retained when filters start clogging. Chemical imaging of these samples (100% of the total filter area) was conducted in the transmission mode. FTIR data were analyzed with the software tools siMPle and MPAPP, which have been developed and described by Primpke *et al.* (2017)[33,38,46] together with the reference database.[47] This analysis provided for all MP particles and fibers from sediment or invertebrate samples, the polymer types, the longest and shortest dimension, and their size fraction. The threshold values for the identification of individual polymer types were optimized based on manual spectral evaluation of three sediment samples and three invertebrate samples (Table S4). The masses of individual MP were estimated based on the protocol by Simon *et al.* (2018).[48] The ratio of the shortest and longest dimension of all the identified MPs was calculated; the resulting median was 0.51. For particles, it was assumed that this ratio is equal to the ratio of height and width of the MP. The weight of the particle was calculated based on the volume (assuming a prolate spheroid shape) and its corresponding polymer density. The weight of the fibers was calculated by assuming a cylindrical shape with 40% void fraction and a fixed diameter of 15 μm.[48−50] The densities of all the target polymers for mass calculation have been reported in our previous study.[41]

2.4. Quality Assurance/Quality Control. QA/QC was implemented according to Hermsen *et al.* (2018).[29] Non-plastic equipment (*i.e.,* glass and metal) was used throughout all procedures, except for PVC in the extraction units. All pieces of laboratory equipment (scalpels, forceps, glass beakers, filter manifold, *etc.*) were washed with pre-filtered (0.2 μm) deionized water and covered/wrapped in aluminum foil prior to use. The inert PVC tubes (SMI unit and sediment core sampler) were thoroughly washed and did not contain any loose particles. All samples and materials remained covered when not in use to reduce the risk of contamination from aerial sources. Natural fiber clothes and nitrile gloves were worn during the extraction process. To avoid potential MP contamination, all liquids used were filtered through 0.4 μm polycarbonate membrane filters or 20 μm metal filters before use. Moreover, the entire experimental process was conducted in a fume hood or laminar flow hood wherever possible. Triplicate laboratory procedural blanks that underwent the same treatment as field samples were conducted to detect any MP contamination. Furthermore, triplicate recovery tests were performed using sediments and invertebrates spiked with a known amount of three types of particles that differed in size and polymer type (details provided in the Supporting Information: Text S2 and Table S5). From sediments, mean MP recoveries (±standard deviation) for polyethylene (PE) (90−180 μm), polystyrene (PS) (100−500 μm), and PC (100−500 μm) were 50.0 ± 4.4, 85.3 ± 5.7, and 81.3 ± 4.2%, respectively. From invertebrates, these recoveries were 42.3 ± 4.4, 78.9 ± 6.2, and 77.6 ± 6.5% for the same polymers, respectively. The difference between the recovery rates for sediment and invertebrates was not statistically significant (independent *t*-test, $p = 0.535$). Overall, the applied method has an accumulated QA/QC score of 17 (maximum 20 for biota samples), based on Hermsen *et al.* (2018).[29]

2.5. Data Analysis. Particle number concentrations of MP were represented as the number of particles per kilogram of dry sediment (particles/kg dw), the number of particles per gram of ww organisms (particles/g ww), or the number of particles per organism/individual (particles/organism). Mass concentrations of MP in the sediments (μg/g dw) and invertebrates (μg/g ww) were also reported. In each sample, the number of MP > 500 μm and MP < 500 μm was combined for analysis. The Shapiro−Wilk test was used to assess the normality of the data. To characterize the similarities of MP polymer types in the sediment/invertebrates between sampling sites, principal components analyses (PCA) were performed using Canoco 5.

Because MP size is usually expected to show a power law distribution in the natural environment,[51,52] we fitted our data using the equation: log relative abundance = log a + b × log size. To do this, we used seven MP size bins for both sediment and biota samples to allow for meaningful data fitting. Because no or few MPs were found in several taxon samples, the fitting was only conducted on Asellidae and Chironomidae at site S1, and for all sediment data versus all organism data, pooled. Additionally, to interpret the maximum size of MP that organisms had ingested, we calculated the animal to plastic size ratio, which is defined as the length of organism divided by the corresponding maximum length of the ingested MP. For samples with hundreds of individuals, we measured the length of the shortest and longest individuals and several random individuals. Consequently, the estimated median values were used for calculation of the animal to plastic size ratio. Differences in the MP size, polymer types (densities), and shape were examined between invertebrates and the host sediment using SPSS 22.0. The χ square test was used to examine the selectivity of invertebrates with respect to MP size and density, while the Kolmogorov−Smirnov test was conducted to evaluate selectivity with respect to MP shape. The statistical significance level was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. MP Concentrations Across Sediment and Biota. None of the 26 synthetic polymer types analyzed (Table S4) were detected in the procedural blanks; therefore no blank correction was needed. Furthermore, no PVC as used in the SMI extraction devices was detected in the procedural blanks. This demonstrates the reliability of our results and the absence of MP contamination from the SMI extraction devices used or from any other source in the laboratory. MP was found in all the sediment samples, with concentrations varying between sampling sites (Table S6 and Figure S2). The mean total MP number concentrations were in the range of 2910−16740 particles/kg dw. The highest total MP concentration was observed at site S1, whereas the lowest was found at site S6. The relatively high MP concentration at site S1 might be explained by the recreational boating and swimming activities nearby the site, with particles being released from paddles, boats, clothes, and personal care products used by visitors.[53−55] This is supported by high amounts of acrylates/polyurethane/varnish (APV) detected particularly at this site because acrylates and polyurethanes are common components of antifouling paint used to protect and reduce friction on boat hulls.[56] Unlike site S1, site S6 is located in a rural area with much less anthropogenic activities, which explains the low MP level at this site. From sites S3 to S4, running through the city of Eindhoven, the total MP concentration increased from 4890 to 16,160 particles/kg, suggesting that this urban area is a significant source of MP.

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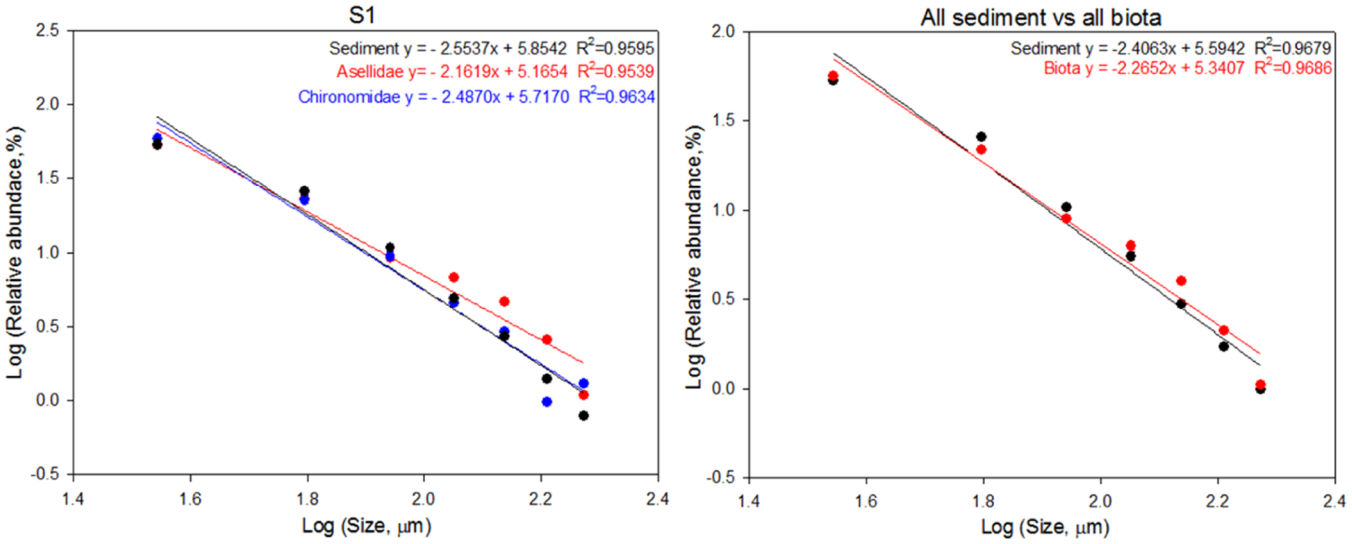


Figure 1. Size distributions for microplastic in sediment (locations S1, S2, S3, S4, S5, and S6) and various benthic invertebrate species, quantified using automated μFTIR imaging. Note: MPs <20 μm were omitted in this plot.

The mean mass-based total MP concentrations ranged between 0.49 and 6.2 μg/g dw, with a median concentration of 0.95 μg/g dw. The highest and lowest MP mass concentrations were also found at sites S1 and S6, respectively. Noteworthy, sites S1 and S4 showed 3.4−11.7 and 2.4−8.8 times higher MP mass concentrations, respectively, however only 1.3−4.8 and 0.7−4.6 times higher MP number concentrations compared to the five remaining sampling sites (S2, S3, S5, S6, and S7). This is explained by the presence of larger particles at sites S1 and S4 with concomitant increase in the particle mass. For instance, there were 17 and 3 particles >500 μm at sites S1 and S4, respectively, whereas no MP > 500 μm was observed at sites S2, S3, S6, or S7.

Results from PCA showed that the first and second ordination axes display 72 and 14% of the total variance in MP number concentrations between the sediment samples, respectively (Figure S3). Additionally, along the first axis of the ordination diagram, sediment samples from sites S1 and S4 were placed on the left side, from sites S2, S5, and S7 in the middle, and from sites S3 and S6 on the right side. This shows that sediment MP concentrations and compositions at sites S1 and S4 were different from those at sites S3 and S6.

So far, only two studies have reported MP levels in sediments using μFTIR with automated image analysis.[39,40] Mani *et al.* (2019) reported similar MP concentrations (260−11,070 particles/kg) in sediment samples from the Rhine River,[40] whereas Lorenz *et al.* (2019) reported much lower MP concentrations (3−1189 particles/kg) in marine sediments from the southern North Sea.[39] More comparisons are inapplicable due to several factors. First, previous studies reported sediment MP concentrations in different units, such as particles/m^2 or particles/(m)L.[57] Second, the reported MP size ranges are also inconsistent among studies. Finally, previous studies mainly focused on a limited number of polymers,[30] such as PE, PP, PS, polyethylene terephthalate, and PVC, whereas here, we were able to analyze 26 types of polymers simultaneously. Such a comprehensive standardized protocol is essential for better investigation and risk assessment of MP.

A total of 11 benthic invertebrate taxa, including two Clitellata (Tubificidae and Erpobdellidae), six Insecta (Calopterygidae, Chironomidae, Sialidae, Coenagrionidae, Ephemeroptera, and Sigara), and three Malacostraca (Asellidae, Gammaridae, and Astacidea), were sampled in the present study, with Gammaridae, Asellidae, Tubificidae, and Chironomidae being the dominant benthic invertebrates (Table S2). MP was found in 88.6% of invertebrate samples, with concentrations varying between taxa and between sampling sites (Table S6). Generally, the MP number concentration was higher in Tubificidae, Chironomidae, and Asellidae compared to the remaining taxa. For instance, Asellidae at site S1 had the highest MP number concentration (19,023 particles/g ww), followed by Chironomidae at site S1 (5509 particles/g ww). In contrast, Gammaridae had very low levels of MP at all sampling sites (0−34.8 particles/g ww). Regarding MP mass concentrations, higher levels were observed again in Asellidae, Chironomidae, and Tubificidae compared to the other taxa. The highest MP mass concentration of 404 μg/g ww was found for Asellidae at site S1. We calculated the total MP volume ingested by Asellidae at S1 (N = 10), which was 0.04 mm^3. For this species, a gut volume of 0.1 mm^3 has been reported,[58] which would imply that an average of 4.0% of the gut volume was occupied by MP. Note that as this is an average for 10 individuals, the gut volume occupancy could theoretically be as high as 40% if all the particles would have resided in a single individual. This high percentage further illustrates the potential risk for Asellidae and other invertebrate taxa and supports food dilution as a very relevant mechanism of the effect for invertebrate taxa.[28] Generally, samples with high number-based MP concentrations also had high mass-based concentrations, which is consistent with the results found for the sediment samples (Table S6). This result is quite obvious as here we calculated the number and mass MP concentrations based on the whole sample rather than on an individual basis, which is a limitation of the methodology. Nonetheless, the MP mass concentration in Chironomidae sampled from site S6 was relatively low with a value of 1.9 μg/g, despite the high MP number concentration of 2442 particles/g ww. This can be explained by the predominance of MP < 50 μm ingested by Chironomidae (>99%; Figure S4) or by particle fragmentation occurring inside the Chironomidae body.[59] Density (*i.e.,* polymer identity) showed a

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significant difference between Asellidae and Chironomidae at site S1 ($p < 0.05$, Figure 2). This significant difference in characteristics of MP ingested by different taxa on the same location demonstrates that uptake is taxon-specific.

Results from PCA showed that the first and second ordination axes display 56% and 17% of the total variance in MP number concentrations between invertebrate samples, respectively (Figure S5). MP number concentrations varied between invertebrate taxa and between sampling sites. For instance, PE, PE oxidized, PP, ethylene propylene diene monomer (EPDM), and polyamide had higher concentrations in Asellidae collected from site S1 than the remaining invertebrate samples, again demonstrating taxa-specific ingestion of MP.

3.2. MP Size Distributions Across Sediment and Biota. The size distributions of MP detected in the sediments and organisms at each sampling site demonstrate that number concentrations increased with decreasing size (Figures 1 and S4). The percentages of MP smaller than 75 μm were 88 and 92% in the sediments and organisms, respectively (Figure S6). Mani *et al.* (2019) used the same size cutoff and calculated a percentage of 96% in sediments from the Rhine River,[40] which thus is very similar to our results, confirming the generic similarity of MP size distributions in the environment. Even though we used 20 μm filters during the extraction process, MP < 20 μm was still detected due to clogging. For example, 43.5% of the particles detected in sediment at site S1 were around or smaller than 20 μm. Consequently, the concentration or relative percentage of MP < 20 μm is likely to be underestimated in the current study.

The MP relative abundance showed a log-linear relationship with MP size for both sediment and biota samples (Figure 1; Table S7). All fitted linear regressions showed similar negative slopes with values ranging from (minus) 2.16 to 2.55 (mean: 2.37). Due to recoveries being lower for smaller particles, the actual slopes are estimated to be around 0.3 higher. The slopes are within the range of 0.62−2.81 reported for different sediments.[52] Theoretically, a slope of 3 represents 3D fragmentation with full conservation of mass.[60,61] The value of 2.7, obtained by correcting for the incomplete recovery of small particles, suggests that fragmentation of the plastics has partly been two-dimensional (e.g., for flakes and sheets).[61] Given the MP number concentrations found, meaningful size distributions could be constructed for all taxa grouped together and for Asellidae and Chironomidae at the site with the highest concentrations (site S1). There was no significant difference in the MP size distribution between the sediments and invertebrates (Asellidae and Chironomidae) at site S1 or between all sediments and all biota (Figure 1). These results suggest limited selectivity in MP size by the taxa analyzed in the present study.

Besides the evaluation of complete size distributions, specific size fractions can be evaluated, which may constitute more sensitive indicators of ingestion. As discussed above, there was no significant difference in MP size distributions among sediments and organisms. However, the relative abundance of MP < 50 μm was significantly higher in organisms than in their corresponding host sediments (paired t-test, $p < 0.05$). This suggests that invertebrates prefer to ingest small-size MP in the sediment and that the bioavailability of MP and its potential to accumulate in invertebrates increased with decreasing MP size. This is likely because small MPs can be ingested along with similarly fine-grained sediments and organic matter whereas large MPs may be too large to be ingested by these small organisms.[10] Such selectivity of particles has also been demonstrated for *Gammarus pulex*, who selected particles between 20 and 165 μm from a range of 20−500 μm.[15] In addition, it has also been reported that Tubifex worms preferred to ingest sediments <63 μm.[62]

Recently, Jâms *et al.* (2020) established an empirical relationship between the size of an organism and the maximum MP size it could ingest.[63] To examine the effect of size (length) of invertebrates on ingestion of MP, we categorized Asellidae and Gammaridae (the most abundant taxa) into two groups based on their size/length in the present study. However, there was no difference in the level or size of MP ingested by these two taxa between groups (Table S6). This result is in line with Hurley *et al.* (2017), who observed no significant correlation between the size (length or weight) of worms and the number or size of MP ingested.[10] Following Jâms *et al.* (2020),[63] we calculated ratios between the animal length and maximum ingested MP size found. These ratios ranged from 29 for Asellidae to 340 for Chironomidae, with site S1 showing the lowest ratios (29−110) (Table 1). Even for the same species, the ratios varied greatly among sampling sites. For example, the ratios of Asellidae were 29 and 170 at sites S1 and S4, respectively. These results suggest that the animal to MP size ratio was taxon- and site-specific. The lowest ratios of 29 and 31 both occurred at site S1 where MP showed the highest abundance, which suggests that the MP levels and size in the sediment would influence the size of MP in organisms, especially for organisms with very small living areas. The average ratio of 160 observed here is approximately an order of magnitude higher than that of 20 reported by Jâms *et al.* (2020) who summarized this ratio based on 2000 gut content analyses from animals ranging over three orders of magnitude in body size (length from 9 mm to 10 m).[63] This difference could be caused by biological variability and MP aspects other than size.[12,64−67] In addition, this difference suggests that the benthic invertebrates analyzed in the present study prefer to ingest small particles.

Table 1. Ratio of Animal Size to the Ingested Maximum Length of MP in the Invertebrates Sampled[a]

taxa	S1	S2	S3	S4	S5	S6	S7
Asellidae	29	--	--	170	140	--	--
Calopterygidae	102	--	--	--	--	--	--
Chironomidae	31	--	--	--	340	--	--
Sialidae	110	--	--	--	--	--	--
Tubificidae	127	--	--	84	91	--	--
Astacidea	--	133	--	--	--	--	--
Ephemeroptera	--	--	80	--	--	--	--
Gammaridae	--	--	--	133	--	300	200
Coenagrionidae	--	--	--	--	320	--	240
Sigara	--	--	--	--	--	93	--

[a]-- no MP or invertebrates were found at the sampling site.

3.3. MP Polymer/Density Distributions Across Sediment and Biota. APV and EPDM were the dominant polymers found in sediments and invertebrates, which accounted for 64.9 and 58.6% of the total number of MP particles, respectively (Figure S7). The high abundance of EPDM in invertebrates might be related to its special characteristics and the release of odorants and infochemicals.[64,65] There were significant differences in polymer

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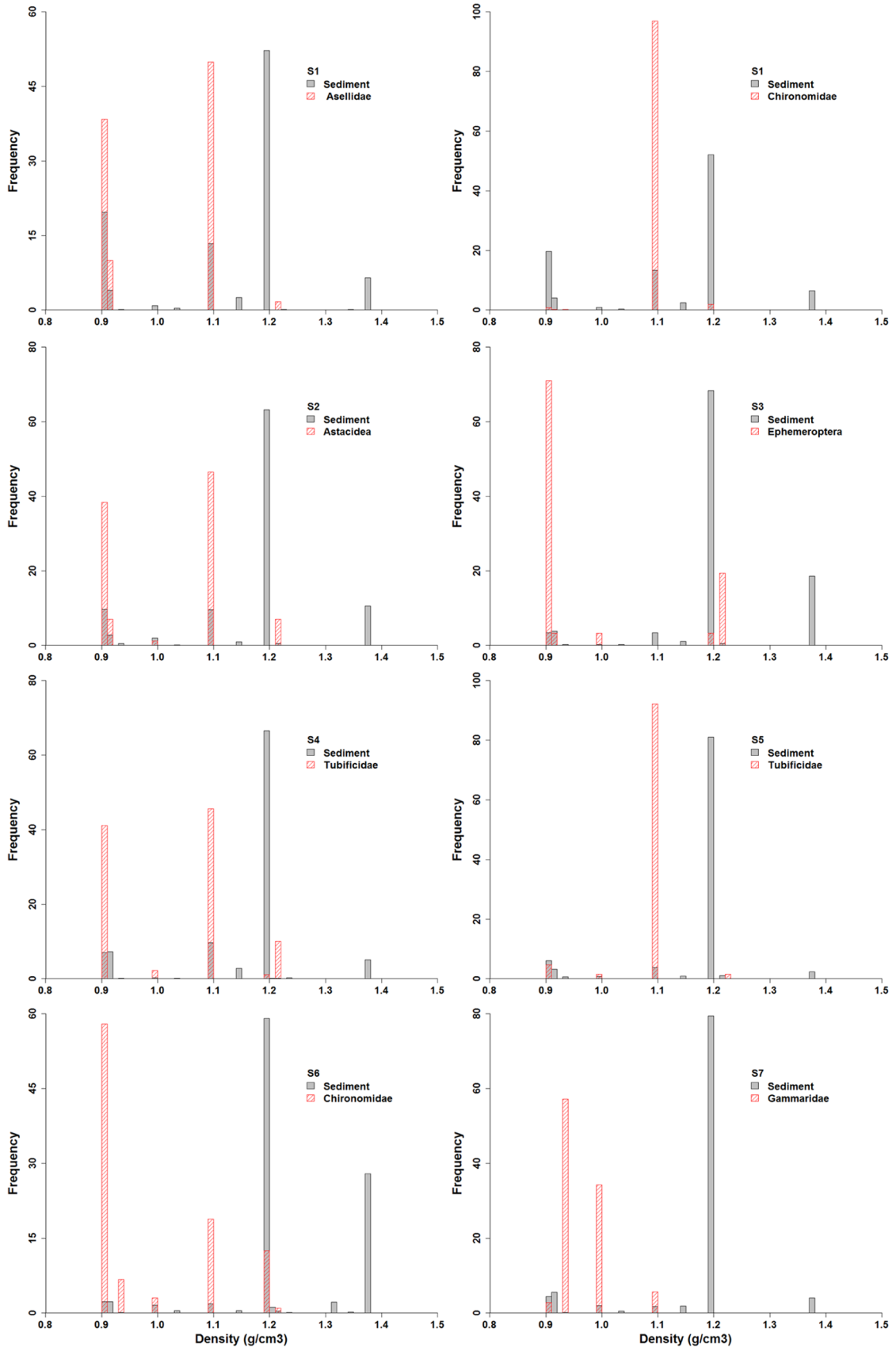


Figure 2. Polymer density distribution of microplastics in sediments (locations: S1, S2, S3, S4, S5, and S6) and various benthic invertebrate species, quantified using automated μFTIR imaging. Panels relate to the different locations.

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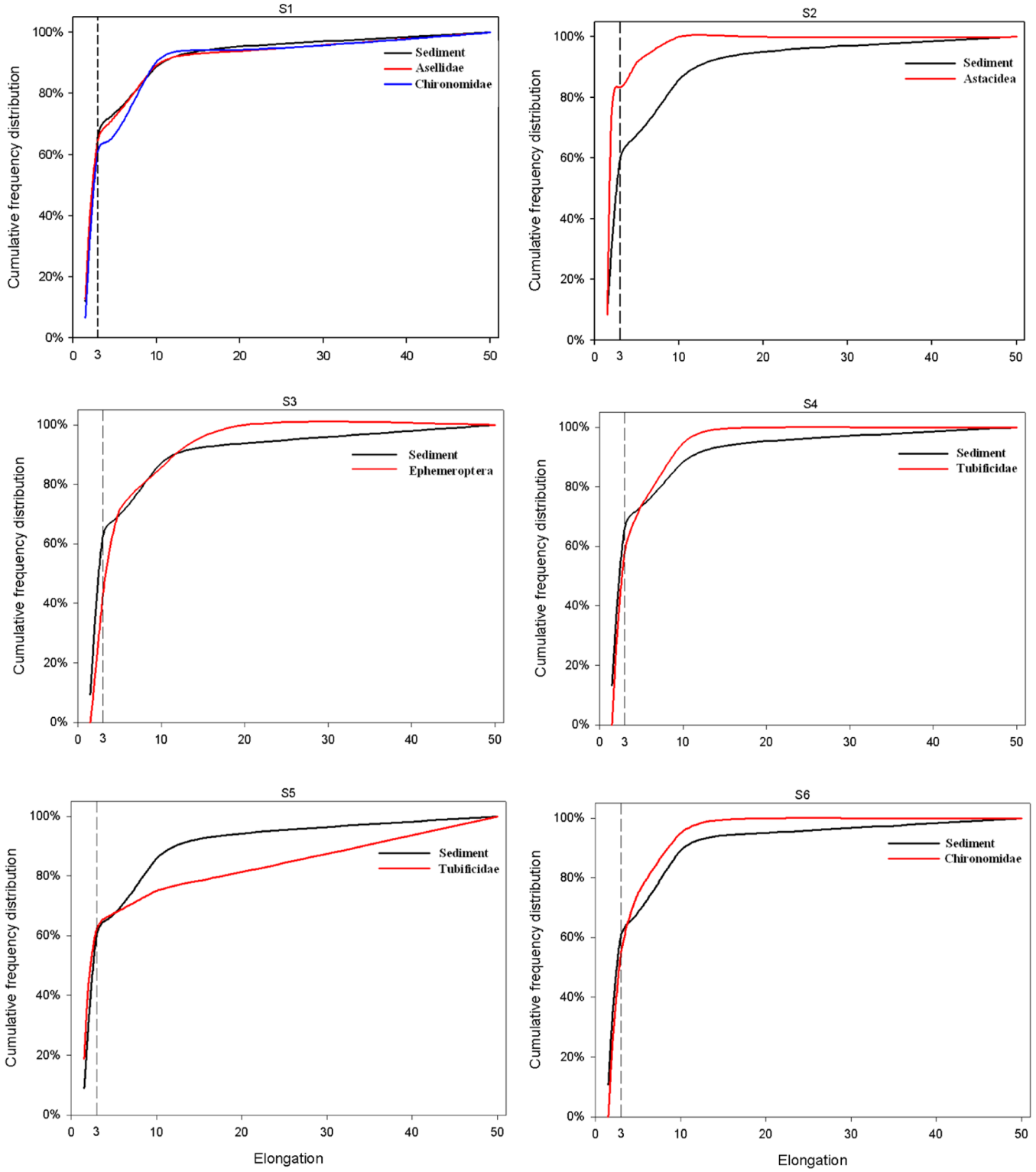


Figure 3. Cumulative frequency distribution of MP length-to-width ratios (elongation) in sediments (locations: S1, S2, S3, S4, S5, and S6) and various benthic invertebrate species, quantified using automated μFTIR imaging. Fibers are defined as particles with an elongation >3 (vertical dashed line). Panels relate to the different locations.

composition between the sediment and invertebrate samples at each sampling site ($p < 0.05$, Figure S8). This indicates that ingestion of MP by benthic invertebrates to some extent is polymer or density specific. Generally, the relative abundance of EPDM and polyethylene-chlorinated (PEC) was much higher in invertebrates than in their host sediment. For instance, at site S1, EPDM represented 49.9 and 96.8% of the total MP found in Asellidae and Chirononidae, respectively, whereas it only accounted for 13.3% in the host sediment. Similarly, at site S4, PEC constituted 41.1 and 2.6% of the total MP found in Tubificidae and its host sediment, respectively. Benthic invertebrates thus seemed to prefer EPDM and PEC

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compared to the remaining polymers found. Unlike the current study, Hurley *et al.* (2017) found that MP polymer composition of ingested MP by Tubifex worms only differed slightly from the polymers in the host sediments.[10]

Concerning density, most of the ingested MP corresponded to polymer types with densities lower than 1.1 g/cm^3, whereas in the sediment, MPs with density >1.1 g/cm^3 accounted for more than 60% of the total MPs (Figure 2). This suggests that invertebrates prefer to ingest low-density particles (*i.e.*, selectivity in polymer density), which is further statistically confirmed (χ^2 test; $p < 0.05$).

3.4. MP Shape Distributions Across Sediment and Biota. The shape (particle and fiber) of individual MP was evaluated based on the length-to-width ratio (elongation) of individual MP. Primpke *et al.* (2019) applied an elongation of 3.0 for distinguishing a particle from fiber.[46] Because the fiber width is not reported, we assumed a width of 15 μm for all fibers[50] and used this value to compute the corresponding elongation. The cumulative frequency distribution for elongation shows that particles were more prominent than fibers in sediment as well as in organisms (Figure 3). Specifically, in the sediment, particles and fibers represented 72.5 and 27.5% of the total MP, respectively. Unlike our study, most previous studies reported that MP fibers were more prominent than particles both in the sediments and organisms.[8,22] Such differences could be attributed to the different methods applied to evaluate MP shape among studies, as here we used MPAPP software to automatically determine MP shape (based on elongation) while most previous studies ascertained MP shape with the naked eye or under a microscope, which is more prone to bias and to overseeing, especially the small and transparent MPs.[22,68−70] The difference may also be related to the taxon-specific feeding behaviors and/or difference in MP composition between habitats. Astacidea (site S2) showed a significantly different MP shape distribution compared to its host sediment ($D = 0.495$ and $p = 0.006$). For other invertebrates and their host sediments, no significant difference in the MP shape distribution could be detected, suggesting either absence of shape selectivity for these other organisms or lack of statistical rigor given the poorer data availability. However, it should be noted that at site S4, the contribution of fibers to MP in Tubificidae was more than 20% higher than that in its host sediment (Figure S9), suggesting that Tubificidae prefer fibers to particles. Similar results have been reported in previous studies.[10,62] For instance, Hurley *et al.* (2017) reported that 87% of MP ingested by Tubifex was fibers.[10]

The selectivity of MP ingestion by benthic organisms, as detected in the present paper, is probably affected by many factors, including the characteristics of MP, species traits and environmental conditions/ecological traits. For instance, it has been demonstrated that MP characteristics (*e.g.*, shape, polymer/density, and size) influence MP ingestion by organisms, whereas urban runoff, effluents of WWTPs, and river hydraulics affected MP transport and thus bioavailability.[66,71−73] Species traits have also been shown to influence MP ingestion and retention, including individual size, mouthpart morphology, feeding modes, and gut recharge rate.[12,21] Considering the potential adverse effects of MP, more attention should be paid to species-specific ecological risks of MP in the freshwater aquatic environment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c03119.

MP extraction for invertebrate samples; recovery tests, sampling sites; MP number concentrations; principal component loadings for MP polymers; relative contribution of individual size fractions; PCA ordination diagram; mean percentage of each size fraction; mean percentage of each polymer type; percentage contributions of particles and fibers; sampling locations and corresponding coordinates; detailed information about the sampled invertebrates; summary of digestion treatments and conditions applied; overview of manmade polymers and natural material types; recoveries of different MPs; total MP levels in the sediment and invertebrate samples; and regression statistics (PDF)

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Notes

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