

Microplastics in the Danube River Basin: A First Comprehensive Screening with a Harmonized Analytical Approach

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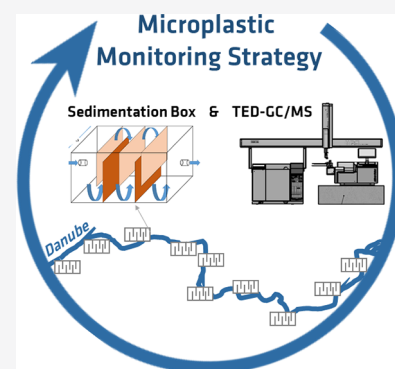
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ABSTRACT: In this study, carried out within the Joint Danube Survey 4, a comprehensive microplastic screening in the water column within a large European river basin from its source to estuary, including major tributaries, was realized. The objective was to develop principles of a systematic and practicable microplastic monitoring strategy using sedimentation boxes for collection of suspended particulate matter followed by its subsequent analysis using thermal extraction desorption-gas chromatography/mass spectrometry. In total, 18 sampling sites in the Danube River Basin were investigated. The obtained suspended particulate matter samples were subdivided into the fractions of $>100\ \mu\text{m}$ and $<100\ \mu\text{m}$ and subsequently analyzed for microplastic mass contents. The results showed that microplastics were detected in all samples, with polyethylene being the predominant polymer with maximum contents of $22.24\ \mu\text{g}/\text{mg}$, $3.23\ \mu\text{g}/\text{mg}$ for polystyrene, $1.03\ \mu\text{g}/\text{mg}$ for styrene-butadiene-rubber, and $0.45\ \mu\text{g}/\text{mg}$ for polypropylene. Further, polymers such as different sorts of polyester, polyacrylates, polylactide, and natural rubber were not detected or below the detection limit. Additional investigations on possible interference of polyethylene signals by algae-derived fatty acids were assessed. In the context of targeted monitoring, repeated measurements provide more certainty in the interpretation of the results for the individual sites. Nevertheless, it can be stated that the chosen approach using an integrative sampling and determination of total plastic content proved to be successful.

KEYWORDS: microplastics, river, suspended particulate matter (SPM), sedimentation box (SB), thermal extraction desorption-gas chromatography/mass spectrometry (TED-GC/MS), monitoring



INTRODUCTION

The Joint Danube Surveys (JDS)^{1,2} have the intention to collect reliable and comparable information on selected water quality parameters over the whole length of the Danube River, including its main tributaries. JDS is a regular monitoring activity of the International Commission for the Protection of the Danube River (ICPDR), realized each 6th year, following the regularity of the river basin management plan update at the European level. All contracting parties of the ICPDR agreed to jointly implement the EU Water Framework Directive, and therefore one of the key goals of the survey is to harmonize water monitoring practices and procedures and to strengthen efforts to protect and restore the Danube River in accordance with the EU Water legislation. Specific goals of the JDS are to collect data on parameters not normally analyzed during regular annual monitoring programs, as well as information on water characteristics and organisms living in a variety of locations along the river across various countries.

A new, controversially discussed contaminant is microplastics (MP). MP, i.e. particles mainly consisting of synthetic polymers in the dimension of $1\text{--}1000\ \mu\text{m}$ (ISO/TR 21960) can be found almost everywhere in the environment: oceans,³ soils,⁴ and air.⁵ The majority of observed MP originates from larger plastic objects that are fragmented by mechanical stress as well as aging and weathering processes. The persistence of synthetic polymers suggests that they remain in water and soil for a long time, accumulate, and cause harmful effects on flora and fauna and, maybe, ultimately on humans.⁶ Enhanced sorption of environmental pollutants⁷ or the release of toxic degradation products,⁸ which might be related to toxic effects,

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has not yet been proven in field studies. Nevertheless, based on the precautionary principle, further MP accumulation in the environment should be avoided.⁹ However, measuring MP accumulation requires representative monitoring concepts and practicable analytical methods.

The occurrence of MP in the Danube River has first been reported in a study on fish larvae.¹⁰ Later, a study dedicated to addressing microplastics developed a multispot sampling tool and elaborated first transport data in the Danube River.¹¹ Various studies have documented both the presence and transport of significant amounts of plastic waste and MP to the Black Sea, which is heavily impacted by the Danube River.^{12–15} MPs were also detected in the tributary of the Danube River: 10 particles/m³ in the dimension of 100 μm–2 mm was identified in the River Tisza.¹⁶ Most critically, only particle numbers were determined so far and, according to current knowledge, these cannot be converted into particle masses, despite MP masses being widely considered essential for monitoring.

A number of previous studies investigated the presence of MP in various rivers all over the world: North America,^{17,18} South America,¹⁹ Asia,^{20,21} and Europe.^{22–24} These works used microscopic-spectroscopic equipment for unambiguous polymer identification, which reduces the misclassification of particles in relation to visual determination. The results were usually particle numbers and in some works differentiated according to particle size and shape. The use of imaging techniques, including their necessary sample preparation, is time-consuming and bears the risks of particle degradation, loss, or contamination. In relation to the number of naturally occurring particles, the number of MPs is only a small fraction.

The development and realization of a representative monitoring strategy in a large river basin is a complex challenge. In this study, a new concept used passive sampling tools²⁵ with subsequent thermoanalytical detection^{26,27} without extensive sample preparation.

In order to get a first impression on the occurrence of MP in river basin water bodies, it is initially sufficient to determine their total mass contents. At present, the determination of total contents is possible with integrals, e.g., thermoanalytical or chemical depolymerization examination methods. To determine the MP contents, the collected suspended particulate matter (SPM) samples were analyzed using thermal extraction desorption-gas chromatography/mass spectrometry (TED-GC/MS). This involves screening for a variety of different plastics, in particular those produced in large quantities such as polyethylene (PE), polypropylene (PP), polystyrene (PS), and the tire compound styrene-butadiene-rubber (SBR).

A selection of the material to be investigated is crucial in order to choose the appropriate sampling technique. Spot sampling is suitable for the assessment of short-term effects. In contrast, sampling over a longer time period offers more integral information and is less affected by random events. Various techniques are available to perform integrative sampling; however, the use of a passive sedimentation box (SB) appears to be particularly advantageous. SBs installed in the water body can be used for sampling over a period of 2 weeks and allow for the determination of MP content based on the mass of collected SPM, rather than the water volume. To include information on the water volume would require further technical equipment, such as attaching the SBs to an active pump with a flow meter. Actual running validation experiments show that the active pumping through the SB is more beneficial

regarding the control of sampling parameters. However, in the present work, we used passive SB because they are very easy to handle in praxis and do not need an electrical power supply. This is especially advantageous in less urban regions of natural surface water.

The objective of this study was to provide an initial assessment of the magnitude of MP occurrence in the Danube River Basin by using a routine analysis toolbox. Additionally, the methodological goal was to further develop a standardized way of handling a large heterogeneous real-world sample data set with focus on comparability and reproducibility of the results.

EXPERIMENTAL DETAILS

In total, 22 samples were collected and analyzed from 18 JDS4 sampling sites. NOS1 and NOS2 samples were collected additionally to the official JDS4 program. The sampling period was from late June to mid-December 2019. An overview of the sampling locations is given in Table 1 and the Supplementary Information (SI) (Figure S1).

Table 1. Overview of the Sampling Locations and Sample Numbers

sampling site code	location	country	number of samples	delivery date/ sampling period
JDS4-1	Böfinger Halde	DE	1	23.08.2019
JDS4-6	Jochenstein	DE	1	27.06.2019
JDS4-9	Klosterneuburg	AT	1	11.06.2019– 25.06.2019
JDS4-10	Hainburg	AT	1	25.06.2019– 09.07.2019
JDS4-11	Pohansko	CZ	1	27.06.2019
JDS4-12	Lanzhot	CZ	2	26.07.2019
NOS-1	Brno	CZ	1	22.08.2019
JDS4-14	Bratislava	SK	2	27.06. / 18.11.2019
JDS4-23	Budapest MB	HU	1	15.07.2019
JDS4-24	Budapest MO	HU	2	26.07. / 18.11.2019
JDS4-29	Bezdan	RS	1	18.11.2019
JDS4-36	Sava	RS	1	20.12.2019
JDS4-37	Pančevo	RS	2	22.08. / 20.12.2019
JDS4-41	Timok	RS	1	26.07.2019
JDS4-46	Ruse	BG	1	27.06.2019
JDS4-47	Giurgiu	RO	1	27.06.2019
JDS4-51	Vilkove- Chilia	UA	1	15.07.2019
NOS-2	Tisza Uzhgorod	UA	1	18.11.2019

Sampling. The SPM in water bodies was sampled using SBs (Figure S2). The functional principle of an SB is based on an abrupt expansion of the cross section through which the water flow passes. Each SB has six 1" inlet and four 1" outlet openings. The cross-sectional expansion causes the flow velocity to drop significantly. As a result, particles begin to sediment inside the box. In order to additionally direct the flow inside the SB, baffle plates are attached to the bottom and the cover of the SB.

The use of SBs had two major advantages. First, it allowed the collection of large quantities of SPM. Second, a wide range of particle sizes from larger than 1000 μm down to smaller than 5 μm could be sampled, covering almost the entire size range of MPs.

The exposure time of the SBs was 14 days. They were placed directly at the sampling site so that the inflow openings were about 0.5 m below the water surface and, in some cases, attached under bridges with stainless steel cages or to buoys. Before removing from water, the inlet and outlet openings were sealed with silicone plugs. Water and solids of each SB were transferred to the 50 L stainless steel drums. The drums were then cooled to limit biological activity. The filled steel drums were stored refrigerated also during the transport to the laboratory.

After arrival to the laboratory, the contents of the steel drums were first homogenized and then filtered through stainless steel sieves with pore sizes of 1000 and 500 μm . After sufficient homogenization by vigorous stirring, 5 L aliquots were removed from the fraction <500 μm and suspended in 45 L of tap water each for a much better performance of the sieving and to prevent early clogging of the sieves. This solution was then filtered through a 100 μm stainless steel sieve, which corresponded to the fraction 500–100 μm (designated as “>100 μm ” fraction). From the fraction <100 μm , 5 L aliquots were taken and air-dried (designated as “<100 μm ” fraction) and covered with alumina foil. The drying was performed in an oven at 40 $^{\circ}\text{C}$. A schematic illustration of the filtration process can be found in the SI (Figure S3).

Further preparation and examination were performed exclusively with the samples of the fractions >100 and <100 μm . After drying, the samples were homogenized using a mortar and a pestle.

Since some samples had high inorganic matter contents, additional density separations were performed using a sodium iodide solution (NaI) with a density of 1.8 g/cm^3 . Thus, the dried sample was placed in a 500 mL Erlenmeyer flask and filled half with NaI. The sample was then mixed with a stainless steel spatula for 2 min and then treated in an ultrasonic bath. This procedure was repeated twice. The flask was then filled with NaI until it was tapered. After 24 h, the floated material was removed and the flask was cleaned with a vacuum suction device with a Whitt's bottle and a filtration unit including a 5 μm stainless steel sieve. A schematic illustration of the process is observable in the SI (Figure S4).

Detection. Thermogravimetric analyses (TGA) of 10 mg of samples were performed to quantify the respective pyrolyzable organic matter contents (org_{pyr}) (including the microplastics fraction) to ensure an optimal SorbStar (polydimethylsiloxane adsorber, Envea GmbH, Karlsfeld, Germany) load of 1.5 mg for TED-GC/MS performance. The samples were measured in a nitrogen atmosphere (flow rate: 50 mL min^{-1} , heating rate: 10 $^{\circ}\text{C min}^{-1}$) from 25 to 600 $^{\circ}\text{C}$ using a TGA/DSC3+ (Mettler Toledo Columbus, OH, USA). Characteristic mass loss steps of contents of moisture (ML_{water} , 25–180 $^{\circ}\text{C}$), org_{pyr} (180–590 $^{\circ}\text{C}$) and unhydrolyzable mass residue (MR, 600 $^{\circ}\text{C}$) were determined. Based on the TGA results, the 44 samples were divided into three sample mass categories: $\alpha = 50$ mg ($\text{org}_{\text{pyr}} < 5\%$, 20 samples), $\beta = 20$ mg (org_{pyr} : 5%–10%, 11 samples), and $\gamma = 10$ mg ($\text{org}_{\text{pyr}} > 10\%$, 13 samples).

For the TED-GC/MS analyses, samples were measured analogously to the TGA screening using a TGA2 (Mettler Toledo) under identical thermal extraction conditions. The GC/MS measurements of the solid-phase adsorber were performed in a gas chromatograph (7890, 5977B, Agilent, Santa Clara, CA, USA), which allowed the analysis of the mass-to-charge ratio (m/z) from 35 to 350. The chromatograms

were analyzed with the Enhanced ChemStation software (version 2015, Agilent) using the database NIST17 and individual case analyses. The operating principle of the TED-GC/MS, its performance, and most important parameters are well described in the literature^{27,28} and all technical details can be found in the SI.

For MP detection, the samples were analyzed for the polymer marker compounds of polyethylene (PE), polypropylene (PP), polystyrene (PS), the tire compounds styrene butadiene rubber (SBR) and natural rubber (NR), polyethylene terephthalate (PET), polymethyl methacrylate (PMMA), biodegradable polymers polylactide (PLA), and polybutylene adipate terephthalate (PBAT). PET, PLA, and PBAT were not identified in any of the samples. The identified polymer sorts and –polymer-specific marker compounds used for MP detection with their corresponding retention times (t_{R}) and characteristic fragment ions are presented in Table 2. A detailed summary of all analyzed marker compounds can be found in Table S1.

Table 2. Overview of the Polymer Marker Compounds Used for MP Detection by TED-GC/MS with Their Corresponding Retention Times (t_{R}) and Characteristic Fragment Ions (m/z)^a

polymer	marker	compound name	t_{R} in min	characteristic fragment ions
PE	PE-3	1,12-tridecadiene	18.1	81, 55, 95, 109
	PE-4	1,13-tetradecadiene	20.8	81, 55, 95, 109
	<u>PE-5</u>	<u>1,14-pentadecadiene</u>	23.4	81, <u>55</u> , 95, 109
	PE-6	1,15-hexadecadiene	25.7	81, 55, 95, 109
PP	<u>PP-3**</u>	<u>2,4,6,8-tetramethylundec-10-ene</u>	19.2	<u>111</u> , 69, 154, 210
PS	<u>PS-2</u>	<u>2,4-diphenyl-1-butene</u>	28.7	<u>91</u> , 130, 104, 208
SBR	<u>SBR-2</u>	<u>2-phenylcyclohexene</u>	19.4	<u>104</u> , 158, 129, 115
NR	<u>NR-1</u>	<u>limonene</u>	10.9	<u>93</u> , 68
PMMA	<u>PMMA-1</u>	<u>methyl methacrylate</u>	3.7	<u>100</u> , 69, 41, 59

^aThe marker compounds used for LOD/LOQ determination are underlined. The asterisk describes diastereomer of the respective substances.

Quality Control. To minimize the risks of cross contamination, all sampling and laboratory work was carried out under maximally plastic-free conditions with plastic-free equipment and minimal exposure times. The materials with sample contact during preparation in the laboratory were limited to stainless steel, glass, and silicone. Also, the clothing and the gowns were made of plastic-free materials.

The results of the duplicate TGA determinations served as a quality control and showed a high homogeneity (deviation $\leq 1\%$, Table S2) for the two crucial parameters org_{pyr} and ML_{water} in all analyzed samples. The stated deviation in the values refers to the mean value of two measurements per sample. Consequently, the analysis samples were homogeneous.

For quality assurance and to prevent possible carry-over and contamination, blanks were measured and implemented into the analyses. For TED-GC/MS analysis, the procedural blank is equivalent to the analytical blank measurement. The addition of the internal standard fivefold deuterated polystyrene could not be used within this sample set because H/D

exchanges were observed in samples with high inorganic matter contents.

For MP quantification, the matrix-dependent response factors of the detected polymers PE, PP, PS, SBR, NR, and PMMA were determined by standard addition for each of the three sample mass categories α , β , and γ . The limit of detection (LOD) and of quantification (LOQ) values were determined using their quintuple or decuple signal-to-noise ratio (LOD: S/N 5, LOQ: S/N 10). This was done in triplicate for each polymer and averaged for the calculated noise ratio. The respective marker compounds with their corresponding m/z and t_R are underlined in Table 2. The resulting LOD values are presented as mean values with their standard deviations in Table 3. Since PET, PLA, and PBAT were not detected in any

Table 3. Matrix-Dependent Limit of Detection Values of the Sample Mass Categories of α (50 mg), β (20 mg), and γ (10 mg)

polymer	limit of detection in μg		
	α ($\text{org}_{\text{pyr}} < 5\%$)	β ($\text{org}_{\text{pyr}}: 5\% - 10\%$)	γ ($\text{org}_{\text{pyr}} > 10\%$)
PE	5.05 ± 0.32	3.75 ± 0.41	6.72 ± 1.14
PP	0.21 ± 0.01	0.21 ± 0.01	0.23 ± 0.02
PS	0.15 ± 0.02	0.12 ± 0.01	0.16 ± 0.02
SBR	0.14 ± 0.01	0.15 ± 0.01	0.18 ± 0.02
NR	0.09 ± 0.00	0.07 ± 0.00	0.09 ± 0.01
PMMA	0.07 ± 0.01	0.08 ± 0.02	0.08 ± 0.01

sample, no LOD and LOQ determinations were performed. However, from validation experiments using other matrices, we know that the LOD of PET is in the range of $1.1 \pm 0.2 \mu\text{g}$ for PBAT and for PLA even higher.

The LOD values of PP, PS, SBR, NR, and PMMA were quite homogeneous with very low standard deviations ($\leq 0.2 \mu\text{g}$) in all analyzed mass categories. PE was the only exception with varying LOD values from $3.75 \pm 0.41 \mu\text{g}$ in category β to $6.72 \pm 1.14 \mu\text{g}$ in category γ . Thus, the matrix compositions only had a significant effect on the LOD determinations of PE but not on PP, PS, SBR, NR, and PMMA.

Since the goal of this survey was a first screening and not a comprehensive study, all MP contents found above their respective LOD values are discussed and presented in Table 3. The results of the TED-GC/MS analyses showed that various

polymers could be detected in different mass contents in all analyzed samples. Concretely, a minimum of two different decomposition products of PE, PS, SBR, and PP were unambiguously identified by TED-GC/MS.

RESULTS AND DISCUSSION

Determination of Organic Content. Figure 1 illustrates the organic matter content of TED-GC/MS analyses along the Danube River, which ranged between 0.9% (JDS4-9) and 16.1% (JDS4-1). JDS4-6, JDS4-9, JDS4-10, and JDS4-47 showed the smallest values. The highest values were observed for JDS4-1, JDS4-11, JDS4-36, and JDS4-37 for the fraction $>100 \mu\text{m}$. The results showed a general tendency for higher organic contents in the fraction $>100 \mu\text{m}$. However, a few exceptions were also found, e.g., JDS4-47, where the highest organic content was in the fraction $<100 \mu\text{m}$. All detailed TGA data, including ML_{water} and MR values, can be found in the Table S2.

Determination of MP Contents. The results of the MP determination are illustrated in Figure 2. All detailed MP content data, can be found in the Table S3.

In general, PE was by far the most predominant polymer with the highest detected mass contents of all polymers analyzed. PE was detectable in 43 of 44 samples with a maximum mass content of $10.63 \mu\text{g}/\text{mg}$. The second most dominant polymer was PS, which was identified in 30 samples with a maximum content of $3.32 \mu\text{g}/\text{mg}$. Further, the tire compound SBR was found in 17 samples (max. $1.03 \mu\text{g}/\text{mg}$) and PP in seven samples (max. $0.32 \mu\text{g}/\text{mg}$). In addition, a decomposition product of PMMA was detected in two samples and the tire compound NR in 16 samples. PET, PLA, and PBAT were not identified in any of the samples. Especially the absence of PET is surprising because PET signals were expected from contamination of clothing fibers in environment transported by air or particle release by abrasion of PET bottles. A possible reason is that the nonpolar solid phase adsorber in the TED-GC/MS suppressed the detection of polar markers such as the markers from PET, PLA, or PBAT, especially when they are only present in low concentrations in the sample. In contrast, nonpolar markers, such as the markers of PE, PP, PS, and SBR, might be overrepresented.

To date, and in our best knowledge, only particle count-based MP results from microscopic-spectroscopic methods are

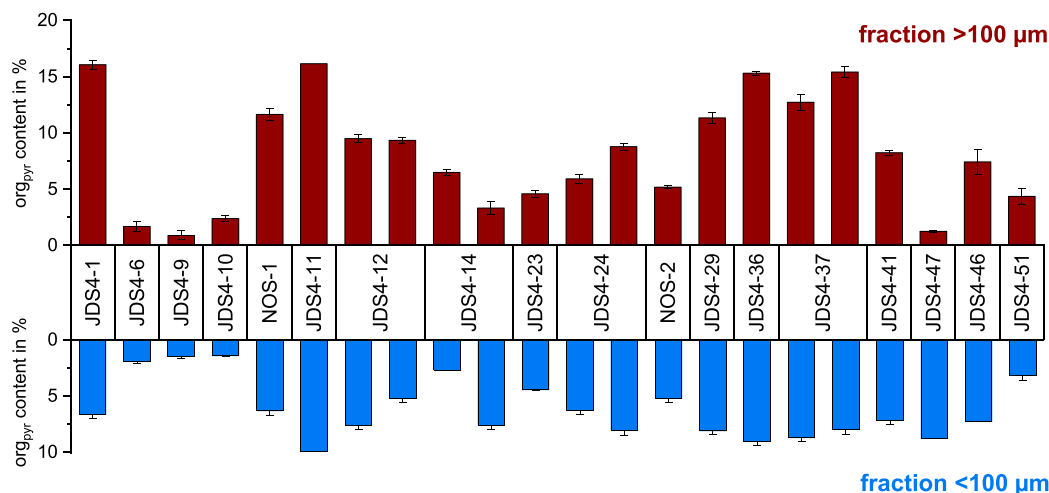


Figure 1. Illustration of organic content of the pyrolyzable organic matter of the samples along the Danube River.

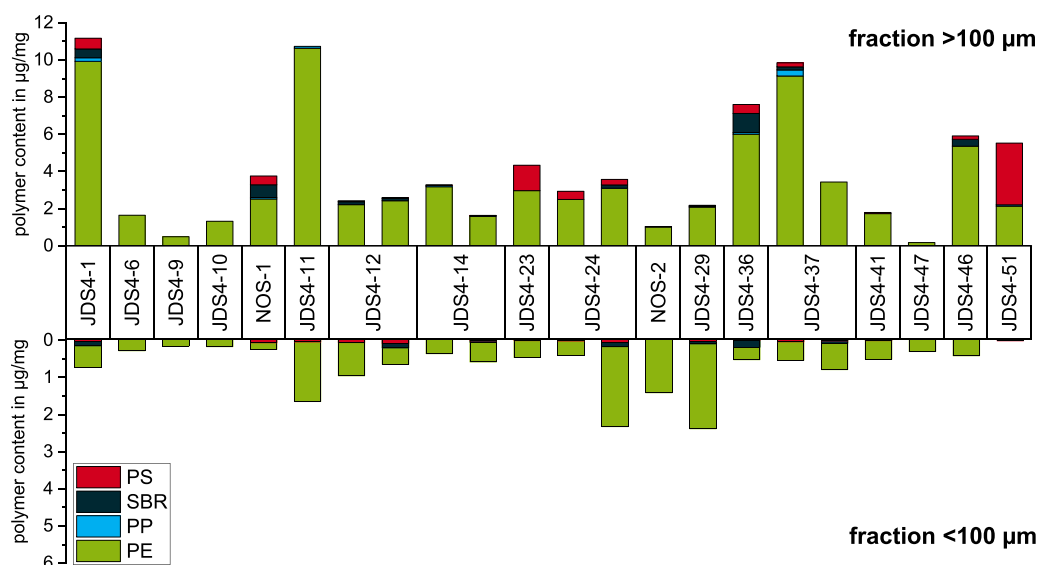


Figure 2. Detected MP contents in the samples whose contents were above the corresponding LOD values. For a comparable representation of both fractions, the scaling of the y axis of the fraction $<100 \mu\text{m}$ was halved due to the significantly lower polymer contents.

available in river water, which do not allow for conclusions on assessing the polymer mass content results and therefore they are not quantitatively comparable with this study. However, the observation of a predominance of PE or PS in European river waters is in accordance with the results of other studies.^{22,23}

A separate analysis showed two differences between both fractions. First, the polymer mass contents in the fraction $>100 \mu\text{m}$ were significantly higher than the MP contents detected in the smaller fraction $<100 \mu\text{m}$ with only three exceptions (JDS4-29, JDS4-47, NOS-2, see Figure 2). As a result, the maximum mass contents in the fraction $<100 \mu\text{m}$ were about a magnitude smaller for PE (max. $2.38 \mu\text{g}/\text{mg}$), PS (max. $0.09 \mu\text{g}/\text{mg}$), and SBR (max. $0.21 \mu\text{g}/\text{mg}$). Secondly, there was a greater polymer variety in the fraction $>100 \mu\text{m}$ than in the fraction $<100 \mu\text{m}$, where no PP was detected. One explanation for these findings could be the generally lower weight of smaller particles compared to the weight of larger particles. Therefore, it needs many more smaller MP particles to correspond to the high mass content of one larger MP particle and to be measurable above the LOD. Furthermore, some micro- and nanoplastic particles have been found to form plastic agglomerates. This has previously been demonstrated for PS in marine water.²⁹

The MP levels determined do not allow any conclusions to be drawn as to the extent to which the results are influenced by, for example, tributaries, sewage treatment plants, large cities, or roads. Also, no increase of the MP contents in the water column can be observed along the Danube River.

Underestimation: Influence of Inorganics on MP Contents. For MP monitoring, the detected MP levels must not only be above their LOD values as previously discussed, but also above their respective LOQ values. For a large-scale investigation of this comprehensive data set, the use of LOQ instead of LOD had only a minor impact on the results due to the high sensitivity of the TED-GC/MS system and the comparatively high mass contents found.

However, for a detailed investigation, all MP contents should be adequately assessed and quantified above their LOQ. One possible solution is to concentrate the organic matter by

density separation. This was carried out exemplarily for both fractions of two particularly low organic samples JDS4-6 and JDS4-9 ($\text{org}_{\text{pyr}} < 2\%$). Subsamples of these selected samples were taken and homogenized by quartering and coning. The sample masses for the density separations were between 54 and 74 g (Table S4).

The results of the fraction $>100 \mu\text{m}$ showed that density separation increased the organic matter content by a factor of about 10. Fittingly, this enrichment by a factor of 10 was also reflected in the total MP contents of both samples. Similar but stronger enrichment effects of the density separations were seen in the fractions $<100 \mu\text{m}$. Here, the organic matter contents increased roughly by a factor of 20. Concerning the MP enrichment, even factors of 73 and 142 were observed, respectively. This effectiveness of density separations for inorganic-rich media has already been proven in the literature.³⁰

In summary, the density separation not only changed the quantity of the results but also provided more comprehensive qualitative MP information of both analyzed samples, allowing the detection and quantification of the contained MP above LOQ.

Overestimation: Influence of Algae on PE Content.

Since PE was the predominant polymer found in almost all samples, further investigations were carried out to assess the MP results. For PE quantification, the mean of the marker compounds PE-3, PE-4, PE-5, and PE-6 (see Table 2) was used to equalize possible matrix-induced signal intensity differences. It has been found in practice that naturally occurring hydrocarbons, e.g., spruce needles or specific lipids, can also form thermal decomposition products similar to those of PE. Thus, the possible origination of PE signals by algae was investigated.

The Danube River Basin, such as all larger rivers, contains biomass of algae aka autotrophic phytoplankton. First qualitative studies in 1898 and 1899 indicated a similar phytoplankton species composition in the Danube River as nowadays,^{31,32} with diatom species (*Bacillariophyceae*) dominating and green algae (*Chlorophyceae*) co-dominating the assemblage, together contributing to about 90% of the total

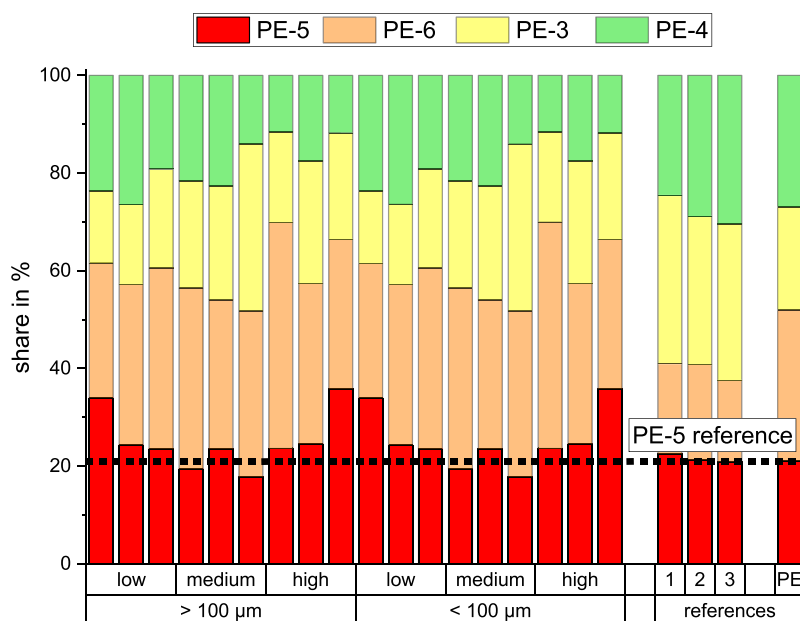


Figure 3. Comparison of the relative proportions of PE marker compounds in three samples with different PE contents (low, medium, high) of both analyzed fractions. For reference, the shares of the three spiked samples and a neat PE measurement are presented with their mean PE-5 share highlighted (dotted line).

phytoplankton biomass.^{1,2} Mainly centric diatoms with cell sizes $<50 \mu\text{m}$ ³³ have been identified as most abundant and quantitatively most important. Their chloroplasts contain high concentrations of lipids, which consist of several mono-unsaturated fatty acids, e.g., palmitoleic, α -linolenic, or eicosapentaenoic acid.³⁴ Concretely, the abundance of palmitoleic acid in *Stephanodiscus hantzschii*, one of the most dominating diatoms in the Danube River,² can be up to 60% of the total fatty acid content or in sum about $180 \mu\text{g}$ of fatty acids per mg carbon.³⁵

For a possible false-positive PE signal, particularly palmitoleic acid is of interest because it forms dialkenes, namely, 1,14-pentadecadiene, during pyrolysis after decarboxylation, which is equivalent to the PE-5 marker compound. To determine whether potential additional algal-originated PE-5 led to an overestimation of PE, the ratios of the sample weight normalized peak areas of the PE marker compounds were analyzed. To consider possible coelutions within the heterogeneous sample set, three samples of both fractions with low, medium, and high PE contents were investigated (see Figure 3). The three polymer-spiked samples and a neat PE measurement were used as references.

Results showed similar PE-5 contents for the samples of both fractions with no systematic PE-5 increases within the PE content levels. Further, given the relatively homogeneous PE-5 results of the Danube River samples with a maximum difference to the references of $<5\%$, there is no evidence of an additional algal-originated PE-5—neither in the larger fraction $>100 \mu\text{m}$ nor in the smaller fraction $<100 \mu\text{m}$ —which would have been of particular interest with respect to the relevant diatom algae sizes of $<50 \mu\text{m}$.

However, results also showed that some Danube River samples varied greatly in their PE fragmentation behavior. Practice has shown that some other naturally occurring substances containing hydroxycarboxylic acids can form similar thermal decomposition products to the PE marker compounds during pyrolysis, e.g., oxacycloheptadec-8-en-2-one and hex-

adec-7-en-16-olide (t_{R} : 32.9–33.3 min). Detailed analyses detected these compounds in 37 of the 44 Danube River samples. The seven unaffected samples were all in the fraction $<100 \mu\text{m}$ and had low PE contents of 0.00 to $0.96 \mu\text{g}/\text{mg}$. However, the extent of the influence of these substances on PE quantification cannot yet be generalized for environmental samples due to their complex compositions. For instance, in samples with oxacycloheptadec-8-en-2-one compounds originating from coniferous trees, they were calculated to have a PE overquantification of 30% to 100% depending on the tree species.

In conclusion, there is evidence of possible PE overquantification by naturally derived compounds in the majority of the Danube River samples and, consequently, the PE contents of the Danube River detected in this study should be viewed accordingly.

CONCLUSIONS

In this study, a comprehensive MP screening was carried out in the water column of a large European river basin. The aim was to develop principles for a systematic and feasible microplastics monitoring strategy using SB to collect SPM and their subsequent analysis by TED-GC/MS.

A total of 18 sampling sites in the Danube catchment were investigated. The results showed that MP were detected in all samples, PE being the predominant polymer with maximum levels of $22.24 \mu\text{g}/\text{mg}$, $3.23 \mu\text{g}/\text{mg}$ for PS, $1.03 \mu\text{g}/\text{mg}$ for SBR, and $0.45 \mu\text{g}/\text{mg}$ for PP. Additional studies on concentration by density separation and possible interference of PE signals by fatty acids derived from algae were evaluated.

In the context of targeted monitoring, repeated measurements provide more certainty in the interpretation of results for individual sites. Nevertheless, it can be concluded that the chosen approach with integrative sampling and determination of total plastic content has proven successful.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsestwater.1c00439>.

Detailed information on the technical parameters of sampling, sample preparation and detection, and tabular listings of all the results obtained (PDF)

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Notes

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