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Study on the assessment of microplastic measurements under different conditions in fluvial systems

Work Package T1

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Abbreviations

ATR	Attenuated Total Reflection
FTIR	Fourier Transform Infrared
JDS4	4th Joint Danube Survey
MaP	Macro plastic
MP	Microplastic
NR	Natural rubber
PA	Polyamide
PE	Polyethylene
PET	Polyethylene terephthalate
PMMA	Polymethyl methacrylate
PP	Polypropylene
PS	Polystyrene
SDS	Sodium Dodecyl Sulfate
SOP	Standard procedure operation
SPM	Suspended particulate matter
SRB	Styrene butadiene rubber
TED-GC/ MS	Thermal Extraction and –Desorption- Gas Chromatography/ mass Spectrometry
EVAc	Ethylen-Vinylacetat-Copolymere

1. Introduction

Environmental plastic pollution is emerging and imposes a rising environmental risk to humans and the environment that cannot yet be accurately assessed. Microplastics (MPs) with sizes < 5 mm are found in the environment in various compositions, shapes, morphologies, and textures. The fraction of MPs in total weight of plastic accumulation around the world is predicted to be 13.2% by 2060 (Sharma et al., 2021).

To better understand microplastic situation in rivers, which are main pathway of marine plastic pollution, within Tid(y)Up project microplastic pollution will be measured at selected sampling points in the Danube and Tisza river in the water column. Various methods will be tested and evaluated to be able to compare the measurements carried out by different countries as a basis for microplastic pollution monitoring which helps to fight against transboundary plastic pollution. Best practice options of sampling and analytics for varying boundary conditions will be derived. Thereby, the focus will be on practicality and user-friendliness of the sampling and analytical methods as well as field application of the tested devices. Recommendations for sampling and analysis methods under certain boundary conditions are given considering also the cost-benefit ratio and used as input for output OT1.3. Also, a rough estimation of the microplastic pollution situation along Danube and Tisza river will be provided.

Currently, there is no standard method used to sample microplastics from riverine systems. Most microplastic research focuses on quantity, in particular on particle numbers, and composition of microplastics. The complexity of microplastics and the lack of harmonization in sampling methodology make it difficult to compare different studies (Dris et al., 2015; Koelmans et al., 2019; Kooi & Koelmans, 2019) cited in (van Emmerik and Schwarz, 2020).

However, assessing possible threats attributed to MP requires fast, reliable and at least representative sampling, sample preparation and detection methods that will eventually be harmonized. Only then, a comparison of findings will be possible and avoidance strategies or regulative measures to decrease the unintended entry of plastics into the environment can be discussed (Bannick et al., 2019).

Sampling MPs in a riverine system is different from collecting particles in the marine environment. Several factors, including hydrological conditions of the water body (e.g. water density, wind, currents, waves and tides), temporal and geographical factors determined by the shape of the river, the morphology, and the meteorological situation will influence the pathway of microplastics in the catchment area. These natural elements should be considered when developing a sampling strategy and monitoring of MPs (González-Fernández cited in (Campanale et al., 2020).

2. Status of microplastic-sampling in Danube-River-Basin

Within fourth Joint Danube Survey (JDS4) Environment Agency Austria investigated microplastic pollution at 15 sampling sites along the entire river by means of passive samplers (sedimentation-box), which were originally designed for the sampling of suspended particulate matter. By this principle, suspended material is allowed to settle in a specialised box that has been placed in the river which can be analysed later on (Hohenblum, 2019).

A comprehensive screening of microplastics was carried out over the entire course of the river. Sampling was performed by means of deploying sedimentation boxes into the river for 14 days; followed by thermo-analytical detection (TED-GC/ MS) for determination of the total content of various plastic polymers in the collected suspended particulate matter samples. In all samples almost, all analysed polymers were detected and quantified, whereas there is no clear trend along the Danube with increasing or decreasing contents. The contents ranged between 0.05 – 22.24, 0.00 – 0.45, 0.00-1.03 and 0.00 – 3.32 for PE, PP, SBR and PS [µg/mg] suspended particulate matter (SPM), respectively.

2.1.1. Austria

In spring 2014, Environment Agency Austria was invited to design and lead a survey in order to determine the transport of plastic and microplastic in the Danube River. The main aim of the survey was to produce sound data on the transport of plastic particles at two sampling sites. Within this survey, the Institute of Water Management, Hydrology and Hydraulic Engineering (IWHW) at the University of Natural Resources and Life Sciences adapted two existing techniques used for determining suspended sediment and bed load transport for the new methodology. The method considered the vertical, horizontal and temporal variability of plastic transport in the flow of the river but requires a truck with a crane to lower the massive construction. The construction allowed for simultaneous skimming of the surface and sampling of a middle layer and the river bed with nets of 500 µm and 250 µm mesh size. Sampling took place at the sites of Aschach (Upper Austria) and Hainburg (Lower Austria) from road bridges and was carried out five times at each site at different discharges. For every sampling point, the plastic transport and concentration (mg/1000.m³) was determined for both microplastic and the total amount of plastic.

Samples were processed in the laboratory and plastic particles were separated manually. The plastic material was identified by means of attenuated total reflection (ATR) infrared spectroscopy and by Fourier transform (FT) Infrared Micro-Spectroscopy in the accredited testing laboratory at Environment Agency Austria.

As result of the study an annual average of the plastic transport load could be calculated amounting to a range of between 6 kg and 66 kg per day for particles smaller than 5 mm and a range of between 7 kg and 161 kg for the total plastic load. It has to be taken into account that because of the mesh size mainly particles smaller than 250 µm or 500 µm respectively are underrepresented. The annual load was calculated using the average annual hydrographs of the years 2009 to 2014 for both sites. The annual load for microplastic amounts to < 17 tonnes/year at Hainburg and the total plastic load amounts to < 41 tonnes/year at the same site.

Furthermore, the study revealed that addressing the whole waterbody is of major importance for sampling a river's cross section, since plastic particles have the properties of suspended particles rather than floating particles. They are encountered in the entire river profile, depending on the hydro-morphological conditions; thus, multi-spot sampling is indispensable to acquiring sound results. The majority of the plastic particles consisted of polyethylene and polypropylene. Both compounds account for approximately 80% of global plastic production.

Around 10% of the particles found in the Danube River were identified as pellets, which unambiguously were attributed to industrial activities such as production processes, conversion

and transport. Some 90% of the plastic particles in the Danube River, however, are emitted by diffuse sources, these being littering, fragmentation and transport by wind, run-off from sealed surfaces (roads, parking spaces and residential areas), inappropriate use of products, use of cosmetics, construction activities and so forth. By the same pathways, plastic and microplastic can reach soil, air and other environmental compartments and, once ingested by organisms, move up within the food chain (ICPDR, 2016).

Microplastics was also analysed during Joint Danube survey (JDS), which is an important TransNational Monitoring Network tool under the Danube River Protection Convention (DRPC), whose Contracting Parties are committed to co-operate in the field of monitoring and assessment of water quality (ICPDR, 2020). The sedimentation box method was used for sampling, leaving the boxes in the river for each 14 days.

Thermo-analytical detection (TED-GC/ MS) for determination of the total content of various plastic polymers was applied on the collected suspended particulate matter samples. A baseline of pollution by microplastics in the Danube River Basin has been established for the first time. In total 22 were taken, prepared and analysed with TED-GC/MS in 2019 (ICPDR, 2020, Kittner et al., 2022).

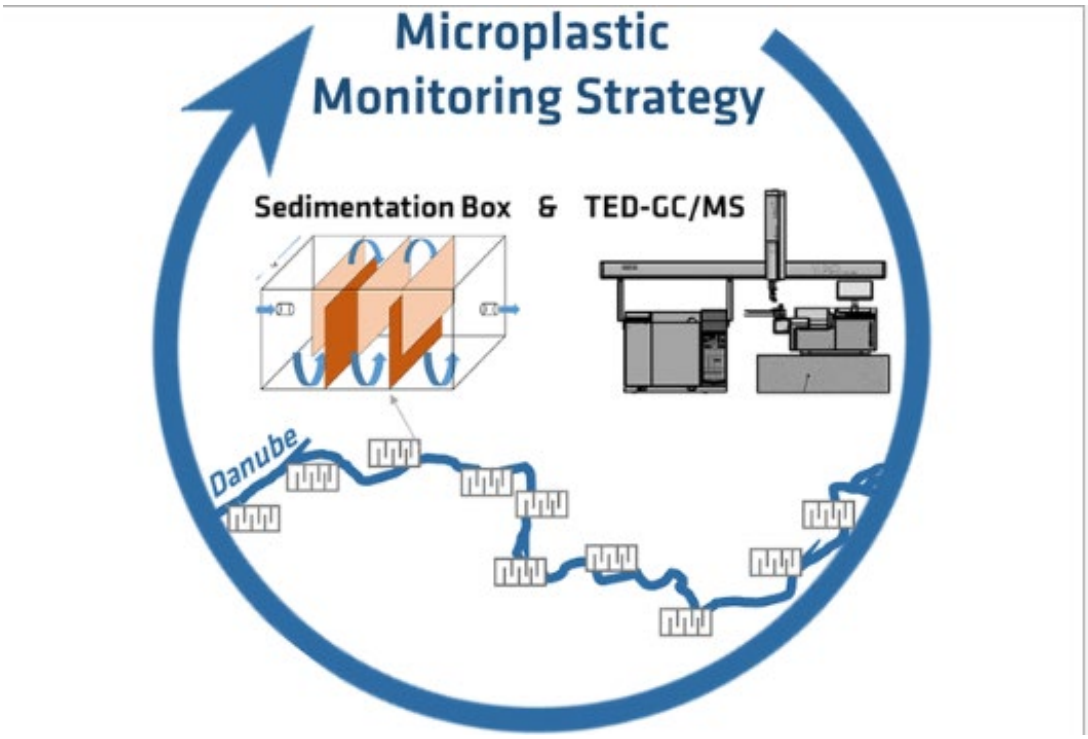


Figure 1: Microplastic Monitoring Strategy within the framework of Joint Danube survey 4

Figure 2: Sampling sites for JDS4, 2019

The basic principle of the sedimentation box is the retardation of the flow velocity within the box through blades to induce the sedimentation of particles. Identical models of sedimentations boxes are also used for the collection of suspended particulate matter (SPM) from water bodies. The inlet openings of the box are 1cm, therefore captured particle size fractions are considered to be < 1cm (ICPDR, 2020).

In Austria samples were taken in Klosterneuburg (JDS4-9) from 11.06.2019-25.06.2019 and in Hainburg from 25.06.2019-9.07.2019 (JDS4-10).

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 °C. A schematic illustration of the filtration process can be found in Figure 3.

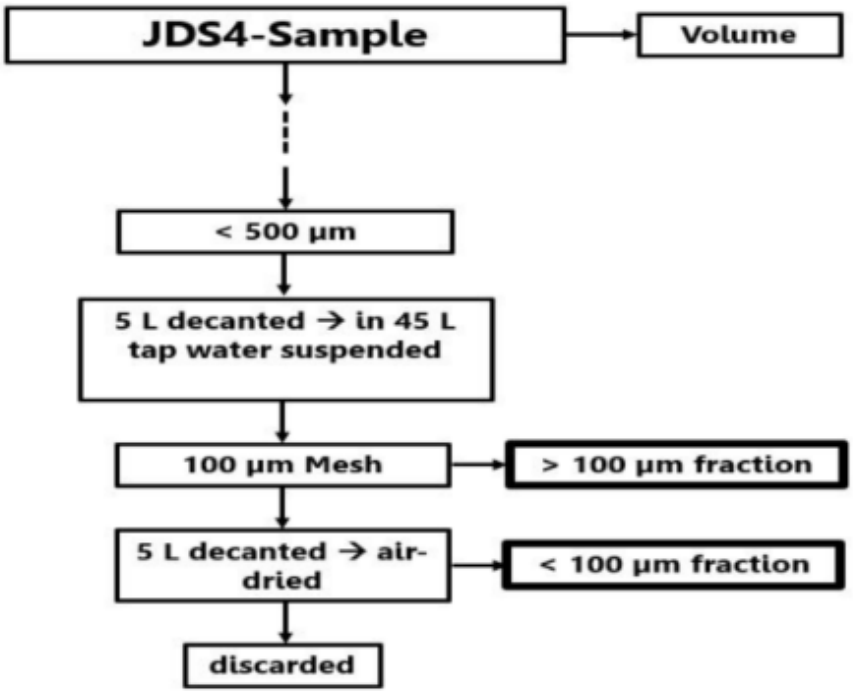


Figure 3: Overview sample preparation during Joint Danube survey 4

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. (Kittner et al., 2022). After further pre-treatment steps (e.g. density separation), thermogravimetric analyses (TGA) of 10 mg of samples were performed to quantify the respective pyrolysable organic matter contents (orgpyr) (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. Results for Austria are listed in Table 1.

Table 1: Microplastic detection results of Austrian sedimentation box samples (JDS4) analysed by TED-GC/MS

Code Location	Fraction	PE (µg/mg)	PS (µg/mg)	SBR (µg/mg)	PP (µg/mg)	PMMA (µg/mg)	NR (µg/mg)
JDS4-9 Klosterneuburg	>100 µm <100 µm	0.49 0.16	0 0	0 0	0 0	0 0	0 0
JDS4—10 Hainburg	>100 µm <100 µm	1.32 0.17	0 0	0 0	0 0	0 0	0.02 0

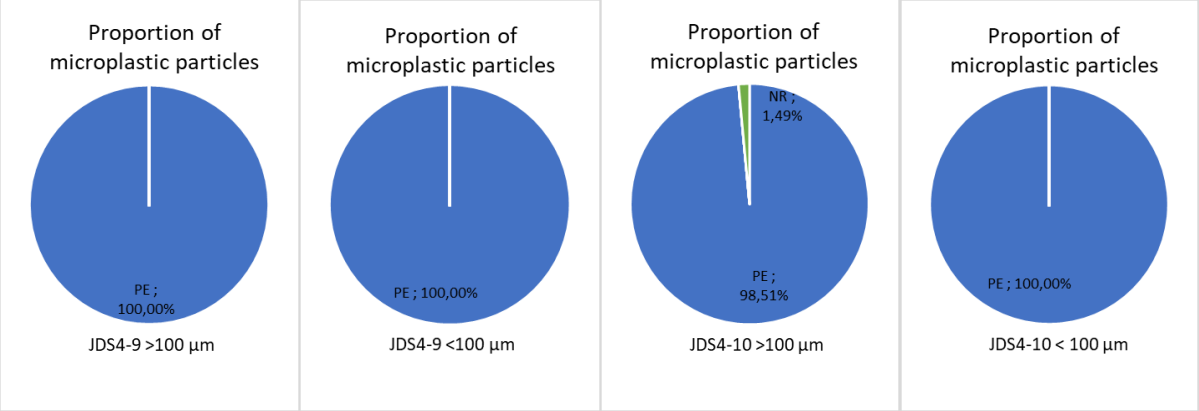


Figure 4: Plastic types of MP in SPM of Sedimentation boxes in Austria (JDS4-9 Klosterneuburg, JDS4-10 – Hainburg)

2.1.2. Slovakia

Microplastic sampling in Slovakia was performed twice in in Bratislava within JDS4 (4th Joint Danube Survey) using a Sedimentation-Box from 27.06 till 18.11.2019 (ICPDR, 2020, Kittner et al., 2022).

Table 2: Microplastic detection results of Slovakian sedimentation box samples (JDS4) analysed by TED-GC/MS

Code Location	Fraction	PE (µg/mg)	PS (µg/mg)	SBR (µg/mg)	PP (µg/mg)	PMMA (µg/mg)	NR (µg/mg)
JDS4-14 I Bratislava	>100 µm <100 µm	3.18 0.36	0.03 0	0 0	0.08 0	0 0	0.06 0
JDS4-14 II Bratislava	>100 µm <100 µm	1.60 0.59	0.02 0.01	0.02 0.05	0 0	0 0	0.04 0

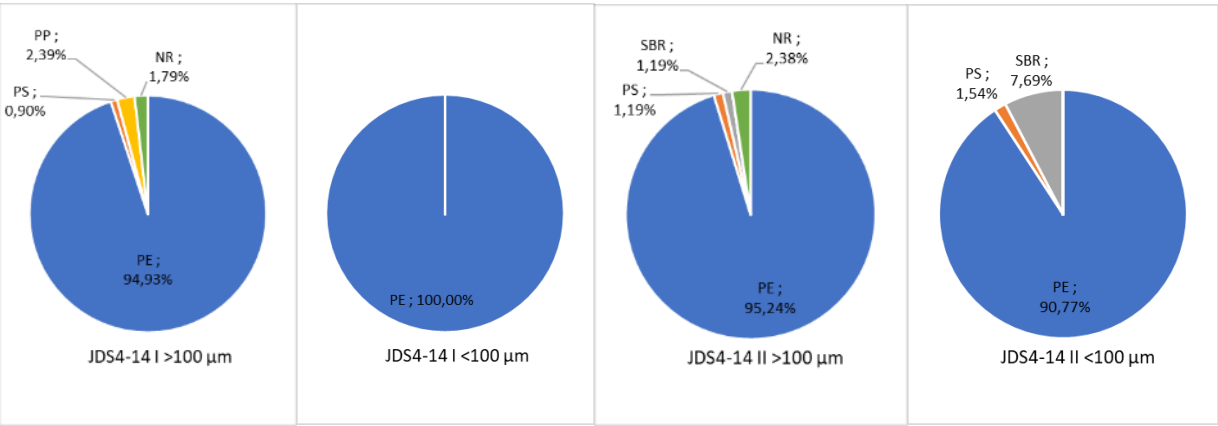


Figure 5: Plastic types of MP in SPM of Sedimentation boxes in Slovakia (JDS4-14 I, JDS-14 II – Bratislava)

2.1.3. Hungary

The first microplastic assessment in Hungary was carried out by WESSLING Hungary Ltd. in 2017 with the sampling of rivers, lakes and fish ponds. For this, fractionated filtration device was used with 300 and 100 µm mesh size filters. Samples (1000-2000 L/location) were taken from the shoreline on a single point, ca. 10 cm below water surface. The samples were prepared with the Micro Plastic Sediment Separator with 1.2 g/cm^3 NaCl solution, and after oxidation with H_2O_2 these were measured with a Bruker Lumos ATR-FTIR microscope. Altogether 13 surface water samples were taken and 12 of them contained microplastics under 2 mm, ranging from 3.52 to 32.05 particles/m^3 with a mean value of 13.79±9.26 particles/m^3. Six different polymer types have been identified in the water samples: polyethylene (PE), polypropylene (PP), polystyrene (PS), polytetrafluoroethylene (PTFE), polyacrylate (PAC) and polyester (PES). In spite of the fact that some of them may have higher densities than the separation solution, the particles have been considered as they could not originate from the sampling and sample preparation equipment, and care was also taken to eliminate airborne pollution. Out of the 12 sediment samples collected parallel to the water samples (one river sediment could not be sampled due to the rocky bottom), 9 showed contamination ranging from 0.46 to 1.62 particles/kg, with a mean value of 0.81±0.37 particles/kg. In the case of the sediments of fish ponds and natural waters of Hungary, similarly to surface water samples, the most common polymer type was PP. PS and PES were also typical, but PE was not as widespread; as the other detected materials, it showed up in only one sample. Results and methods ware detailed by (Bordós et al., 2019).

In 2018, the Tiny Plastic Puzzle campaign organised by WESSLING Hungary Ltd. was measuring MP concentration in the Danube and some of its tributary. Samples were collected with fractionated filtration device with a smallest pore size of 60 µm. During sampling, the size of the pre-filter was 2 mm, so MPs are considered under this size range. Samples were taken along the river cross-section with slow, continuous cross-movement of the sampling vessel. Samples were prepared and measured as described above. Samples were prepared and measured as mentioned above. Danube river sample upstream Budapest (“Észak-Duna”) contained 45 particle/m^3, while downstream Budapest (“Dél-Duna”) contained 55 particles/m^3. River Rába and Ipoly at the tributary showed 12 and 2 particles/m^3, respectively. PE and PP occurred to be the most dominant plastics, share of different polymer types are presented in Table 6. The results of the campaign were presented at www.mikromuanyag.hu .

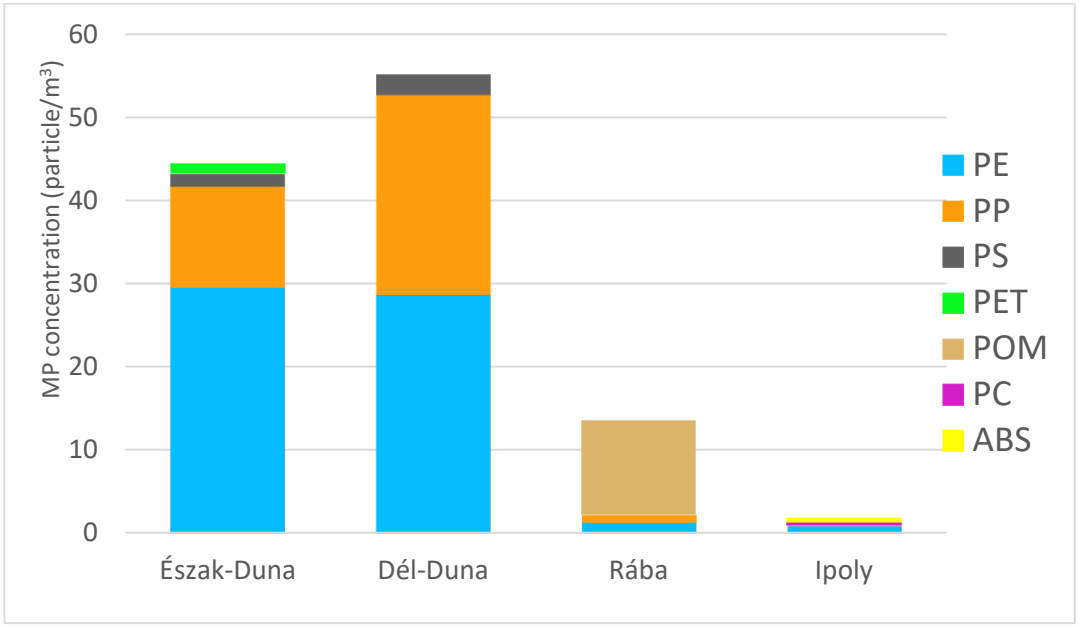


Figure 6: Microplastic concentration and polymer types in samples from the Danube and its tributaries.

In 2019, WESSLING Hungary was commissioned by the Hungarian General Directorate of Water Management (OVF), to conduct MP measurements on five sampling locations at the Danube and river Tisza. Fractionated filtration was carried out between 2 mm and 50 µm. The measurements on Danube was conducted parallelly with the implementation of the sedimentation box during the Joint Danube Survey. Samples (ca. 2000 L) were collected at each Danube locations (upstream and downstream Budapest) twice: once when the box was implemented and once when the box was removed. Afterward the two samples per site were pooled before analysis. Further to Danube samples, 2 spots on Tisza river was designated (KK: Kisköre; VSK: Vásárosnamény). Samples were prepared and measured as mentioned above.

Various concentrations could be observed as presented on Figure 7, but most abundant polymers are PE, PP and PET. One of the reasons of high concentrations at upstream Budapest (Duna-É) could be the high flow event during the first sample collection. Also, high concentrations were measured at Tisza Kisköre (KK). This sampling location is right before the hydropower plant, which means a clearly observable accumulation of macroplastics, and probably microplastics as well.

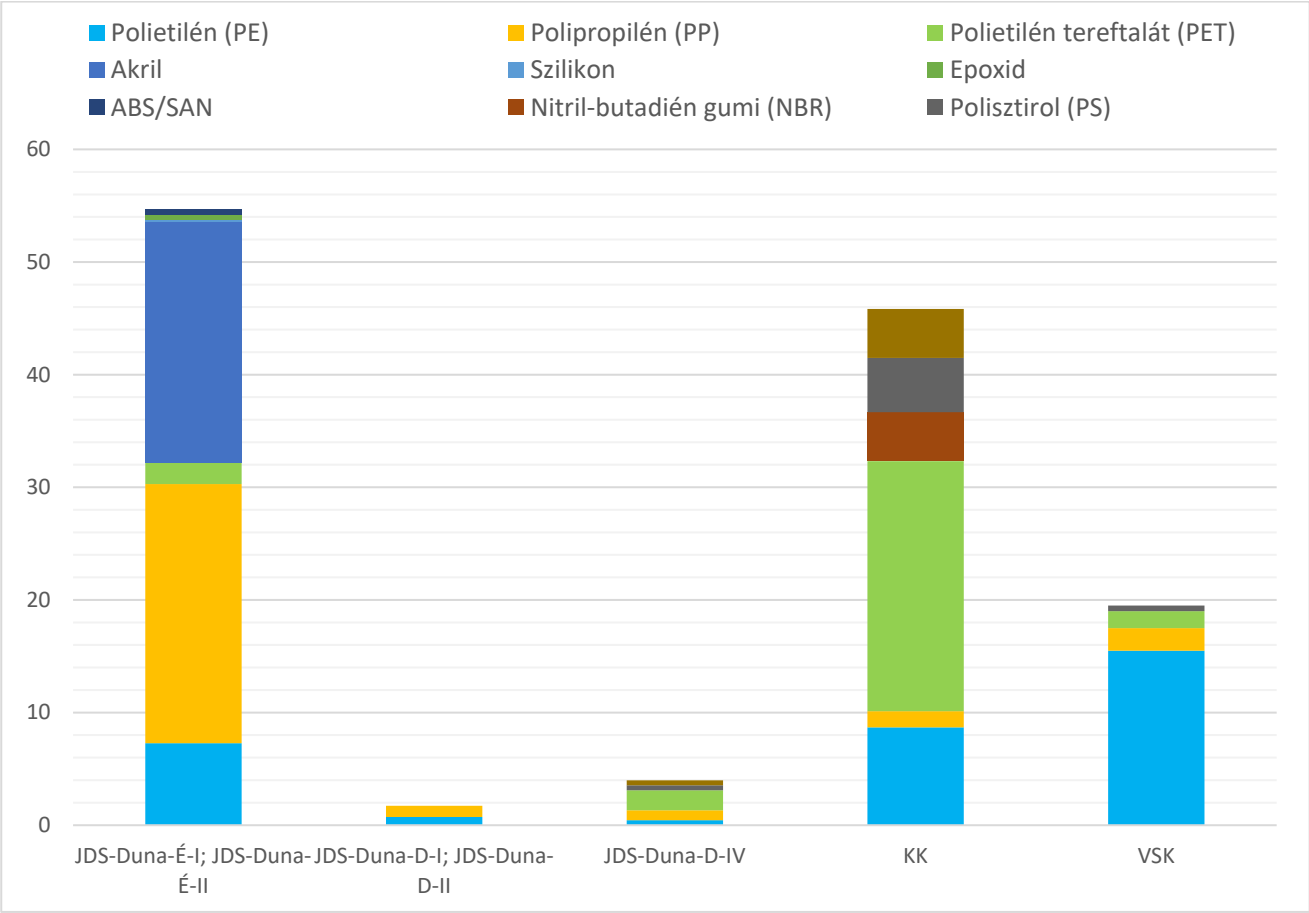


Figure 7: Microplastic concentration on different sampling location on the Hungarian stretch of the Danube and Tisza rivers (Duna-É: Upstream Budapest; Duna-D: Downstream Budapest; KK: Kisköre; VSK: Vásárosnamény).

Within JDS4 sedimentation box sampling was performed three times for sampling in Budapest from 15.07.2019 till 18.11.2019 (Kittner et al., 2022).

Table 3: Microplastic detection results of Hungarian sedimentation box samples (JDS4) analysed by TED-GC/MS

Code Location	Fraction	PE (µg/mg)	PS (µg/mg)	SBR (µg/mg)	PP (µg/mg)	PMMA (µg/mg)	NR (µg/mg)
JDS4-23 Budapest MB	>100 µm	2.97	1.37	0	0	0	0.03
	<100 µm	0.46	0.01	0	0	0	0
JDS4-24 I Budapest MO I	>100 µm	2.50	0.44	0	0	0.02	0
	<100 µm	0.41	0.02	0	0	0	0
JDS4-24 II Budapest MO II	>100 µm	3.10	0.30	0.18	0	0	0.05
	<100 µm	2.33	0.06	0.17	0	0	0.04

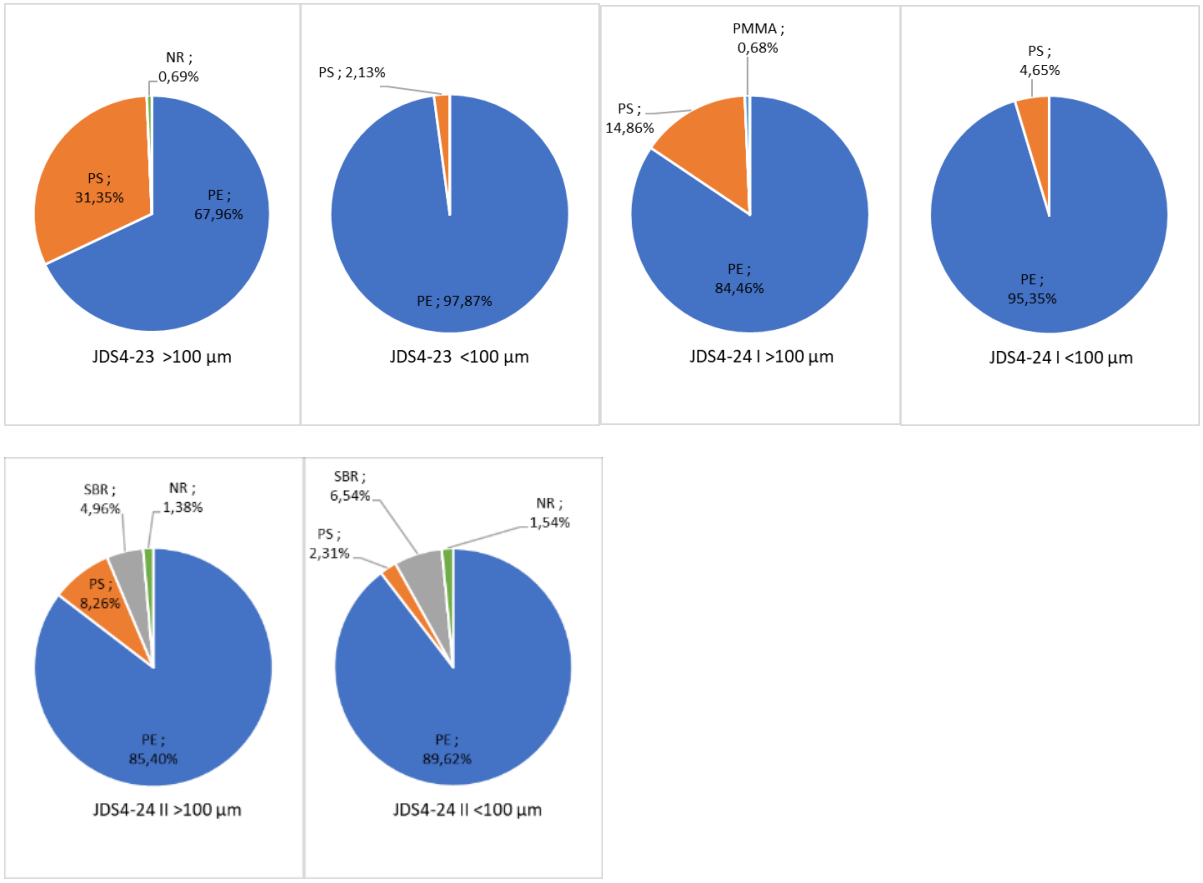


Figure 8: Plastic types of MP in SPM of Sedimentation boxes in Hungary (JDS4-23 - Budapest, JDS4-24I – Budapest MO I, JDS4-24 II – Budapest MO II)

2.1.4. Serbia

Serbian Environmental Protection Agency (SEPA), which is in charge of environmental monitoring is not conducting systematical sampling nor analysis of microplastic in environmental matrices.

During JDS4, for the first time, along the entire length of the Danube River, from Regensburg to the Black Sea, with some tributaries, the contents of microplastics (particles < 1 mm) were analyzed by the same sampling technique and the same detection method.

Along Serbia section, 4 locations - Bezdan, Pancevo and Timok on the Danube River and tributary River, were selected for microplastics analysis. In all samples, almost all analyzed polymers were detected and quantified (Table 4, Kittner et al., 2022).

Table 4: Microplastic detection results of Serbian sedimentation box samples (JDS4) analysed by TED-GC/MS

Code Location	Fraction	PE (µg/mg)	PS (µg/mg)	SBR (µg/mg)	PP (µg/mg)	PMMA (µg/mg)	NR (µg/mg)
JDS4-29 Bezdan	>100 µm	2.09	0.04	0.05	0	0	0
	<100 µm	2.38	0.02	0.10	0	0	0.02
JDS4-37 I Pancevo I	>100 µm	9.14	0.22	0.17	0.32	0	0
	<100 µm	0.53	0.04	0	0	0	0
JDS4-37 II Pancevo II	>100 µm	3.44	0	0	0	0	0
	<100 µm	0.79	0.01	0.08	0	0	0
JDS4-36 Sava	>100 µm	6.01	0.48	1.03	0.08	0	0.07
	<100 µm	0.52	0	0.18	0	0	0
JDS4-41 Timok	>100 µm	1.74	0.06	0	0	0	0.02
	<100 µm	0.51	0.01	0	0	0	0

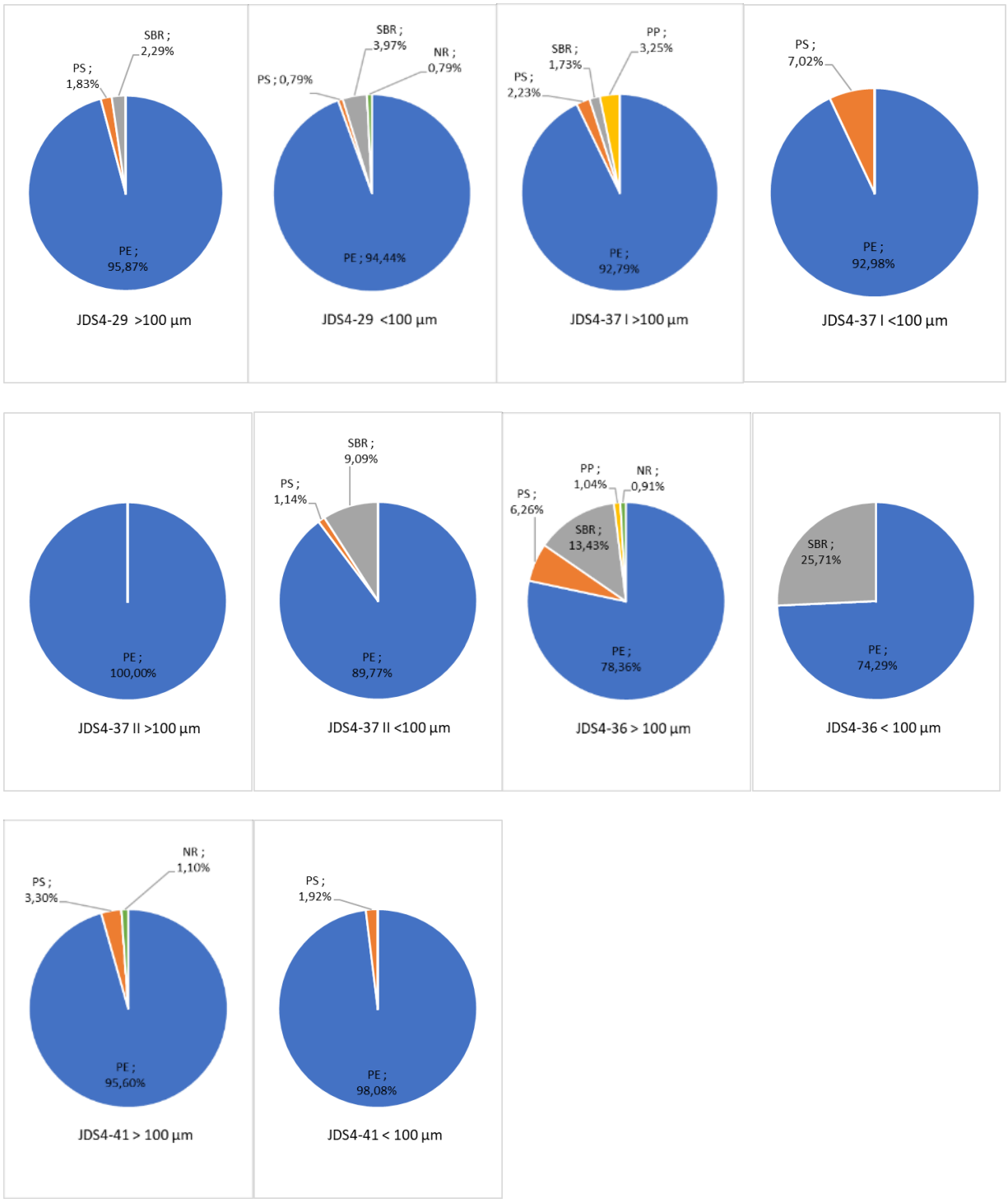


Figure 9: Plastic types of MP in SPM of Sedimentation boxes in Serbia (JDS4-29 – Bezdan, JDS4-37 I – Pancevo I, JDS4-37 II – Pancevo II. JDS4 – 46 – Sava, JDS4 – 41 – Radujevac))

2.1.5. Ukraine

Microplastic in Ukrainian rivers was investigated within JDS4 using a Sedimentation-Box in July and November 2019 (ICPDR, 2020, Kittner et al., 2022) (Table 5).
According to the results of the Joint Danube Research-4, in Tisza (Ukraine) microplastic is 2.42 g per kg of suspended solids (Yurii Shpontak, Ostap Tsapulych, 2020).

Table 5: Microplastic detection results of Ukrainian sedimentation box samples (JDS4) analysed by TED-GC/MS

Code Location	Fraction	PE (µg/mg)	PS (µg/mg)	SBR (µg/mg)	PP (µg/mg)	PMMA (µg/mg)	NR (µg/mg)
JDS4-51 Vilkove – Chilia	>100 µm	2.14	3.32	0	0.07	0	0
	<100 µm	0	0.01	0	0	0	0
NOS-2 Tisza Uzhgorod	>100 µm	1.01	0.02	0	0	0	0
	<100 µm	1.41	0	0	0	0	0

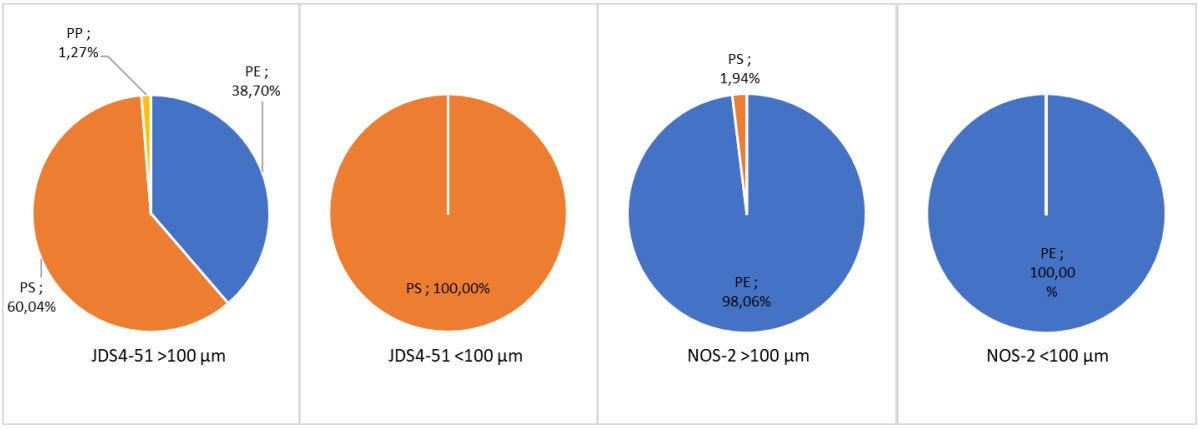


Figure 10: Plastic types of MP in SPM of Sedimentation boxes in Ukraine (JDS4-51 - Vilkove – Chilia, NOS-2 - Tisza Uzhgorod)

2.1.6. Romania

Microplastic sampling in Romania was performed in June 2019 within JDS4 using a Sedimentation-Box. Sampling location was Giurgiu (JDS4-47) (ICPDR, 2020, Kittner et al., 2022). Results are listed in Table 6.

Table 6: Microplastic detection results of Romanian sedimentation box samples (JDS4) analysed by TED-GC/MS

Code Location	Fraction	PE (µg/mg)	PS (µg/mg)	SBR (µg/mg)	PP (µg/mg)	PMMA (µg/mg)	NR (µg/mg)
JDS4-47 Giurgiu	>100 µm	0.17	0	0	0	0	0
	<100 µm	0.30	0	0	0	0	0

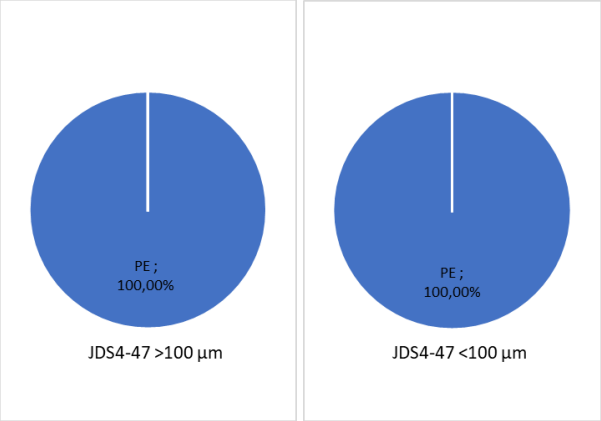


Figure 11:: Plastic types of MP in SPM of Sedimentation boxes in Romania (JDS4-47 – Giurgiu)

2.1.7. Bulgaria

Microplastic sampling in Bulgaria was performed twice within JDS4 using a Sedimentation-Box. sampling location was Ruse (JDS4-46) (ICPDR, 2020, Kittner et al., 2022). Results are listed in Table 7:

Table 7: Microplastic detection results of Bulgarian sedimentation box samples (JDS4) analysed by TED-GC/MS

Code Location	Fraction	PE (µg/mg)	PS (µg/mg)	SBR (µg/mg)	PP (µg/mg)	PMMA (µg/mg)	NR (µg/mg)
JDS4-46p Ruse	>100 µm	5.36	0.19	0.37	0	0	0
	<100 µm	0.42	0	0	0	0	0

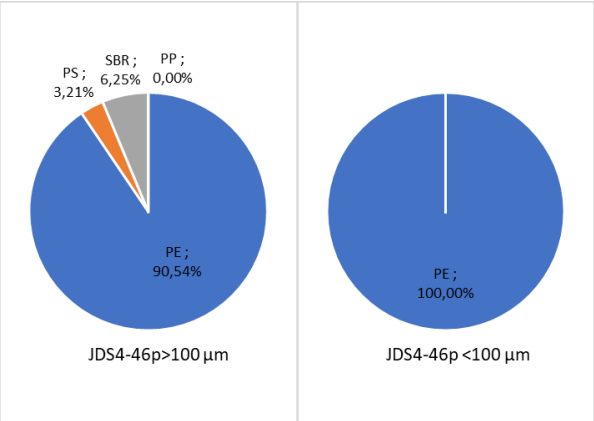


Figure 12: Plastic types of MP in SPM of Sedimentation boxes in Bulgaria (JDS4-46p – Ruse)

3. Sampling-Methods

Until now, no standardized methods exist to detect, identify and quantify microplastics in riverine systems. But to make data about microplastics pollution comparable we need to harmonise sampling, preparation and analysis of microplastics from riverine systems.

(Campanale et al., 2020) provided a collection of procedures concerning the monitoring of microplastics in riverine environments focusing on their sampling and analytical protocols to identify, quantify, and characterise them. Further details regarding the advantages and disadvantages of each analytical technique described, such as general recommendations and suggestions, are provided to give practical support for analytical procedures. In particular, microplastics studies consist firstly of their sampling from the aquatic compartment (aqueous and solid phase). Based on the goal of the research, specific devices can be used to collect particles from different matrices. It follows their quantification after extraction from the environmental matrix, adopting different protocols to isolate microplastics from a large amount of organic matter present in a riverine system. In the end, additional qualitative analyses (e.g., RAMAN and FTIR spectroscopy, GC-MS) are required to identify the chemical composition of particles for a better image regarding the abundance of polymer types, their origin, or other information related to manufacturing processes.

Especially riverine samples are very heterogenous, which makes sample preparation and isolation of microplastics a challenge. Samples from different sampling techniques have different compositions, suggesting that they are complementary rather than substituting methods. But even within the same method, the composition varies greatly depending on the sampling point.

Within Tid(y)Up project three existing and already applied sampling methods were tested under varying boundary conditions:

- Multiple depths net-method: Simultaneously net sampling with mesh sizes of 500 µm and 250 µm in three different depths of water column. Advantages are that within short timeframes huge amounts of water can be investigated in parallel in 3 depths (≈ 3,000 m^3 of water per net and 15,000 m^3 per sampling point within approx. 45 min). Disadvantages

are mainly the need of a bridge or a vessel for sampling and the heterogenous sample composition which greatly increases the effort for sample preparation for analysis.

- Pump-method: sampling with a 1 mm pre-filter with subsequent cascade filtration down to 300µm, 100µm and 50 µm; applicable in varying depths of water column, sample volume 1000-2000 litres depending on suspended solids.
  Considering the BOKU University net method, samples have been taken in 3 locations along the selected cross section in 3 different depths. The suction valve was connected directly in front of the net frame, to ensure sampling in the same time on the same location. Further to this, at every location a sample has been collected through the entire cross section on the surface. According to these, the below sample codes have been implemented.
- Sedimentation-box: sampling close to water surface for approximately 2 weeks; it was also used within the Joint-Danube-Survey.

A detailed description is given in the next chapters followed by a description of the sampling sites, the sampling approach.

3.1. Multiple-depth net-method

3.1.1. General description of sampling method

This relatively new methodology (Liedermann et al., 2018) for measuring microplastic transport at various depths is applicable to medium and large rivers. Compared to established net-measuring methods like the manta trawl, this method offers the possibility of measuring microplastic transport at different depths of verticals that are distributed within a profile. The net-based device is robust and can be used at high flow velocities and discharges. Nets with different sizes (41 µm, 250 µm, and 500 µm) are exposed in three different depths of the water column (at the surface, in the middle of the water column, and at the bottom of the river).

The methodology was tested in the Austrian Danube River, showing a high heterogeneity of microplastic concentrations within one cross section. Due to turbulent mixing, the different densities of the polymers, aggregation, and the growth of biofilms, plastic transport cannot be limited to the surface layer of a river, and must be examined within the whole water column as for suspended sediments. These results imply that multipoint measurements are required for obtaining the spatial distribution of plastic concentration and are therefore a prerequisite for calculating the passing transport. The analysis of filtration efficiency and side-by-side measurements with different mesh sizes showed that 500 µm nets led to optimal results (Liedermann et al., 2018).

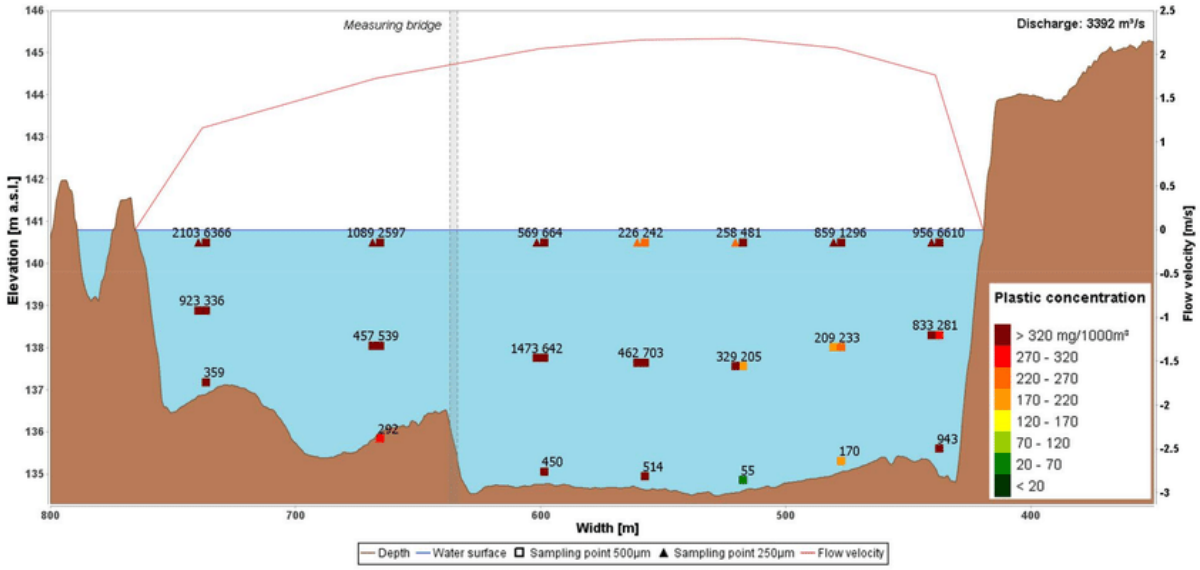


Figure 13: Example of the data gathered for one multipoint measurement performed in the Danube River near Hainburg. The sampling was conducted on 13 January 2015, at a Danube discharge of 3.392 $m^3 s^{-1}$. The plastic concentration (mg/1000 m^3) is displayed for net. a.s.l: above sea level (Liedermann et al., 2018).

The nets, which are attached to a steel rope, are lowered into the water by crane either by truck from a bridge or by ship. The average measuring time per sampling point (e.g. 7 points over cross profile according to figure 1; within TidyUp project 3 points over cross profile are planned) depends on the flow rate and turbidity (clogging occurs at some point). In the above conditions in Austria, this was between 20 and 40 minutes. After removing the nets from river, the sample will be washed with a high-pressure sprayer into a labeled sampling container. The catch can than easily be emptied within comparatively short operational times (30–40 min for all nets).

The discharge (m^3/s) is measured via mechanical flow meter fixed in the middle of the net frame. Additionally, the flow velocity can be determined by an acoustic Doppler current profiler (ADCP) - a hydroacoustic current meter – to get more accurate results. In the end, the plastic transport (e.g. kg/d or t/a) can be estimated.

Table 8: Advantages and Disadvantages of Multi-Depth-Net-sampling

ADVANTAGES	DISADVANTAGES
+ Method covers **depth variance & spatial distribution** over the cross profile of river + **High filtrated water volume** in short time (45min ~3000m^3 per net) + Calculation of **plastic load/transport** possible due to integrated flow meter + reliable and repeatable for higher flow rates + Easy transport of equipment	- Limitation in size distribution - Truck with crane for nets → bridge needed (measurement in flow direction) - Measurement per ship → costly (?) - Official approval may be necessary (ship traffic) - Too high flow velocities & turbulence are a challenge especially for large nets (macro-plastic)

3.1.2. Setup Net-Devise

- **Size:** 2 frames à 60x60cm (max. width ~140cm with buoyant body)
- **Length/height:** Depends on the water level; the nets can be adjusted accordingly on the rope (middle and top);
- **Weight:** Depends on the flow velocity; tests in AT (relatively high velocity compared to eastern Danube region; and positioning of the crane/nets at 90° angles to the flow direction) have shown a compressive force/load capacity of 2 tons; but this can be seen as a maximum!
- **Anchorage:** crane hook is needed
- **Measurement options:**

  a) per truck on bridge

  b) with ship

Detailed device setup:

a) Metal frame à 60x60cm
b) Steel rope and shackles
c) Buoyant body (surface skimming)
d) Long fin and inclination rack
e) Centred single net
f) Upright position when inside the water
g) Sampling container
h) Cleaning per high-pressure sprayer
i) Mechanical flow meter

Figure 14: Net-sampling device (Liedermann, et al., 2018)

3.1.3. Requirements on Vessel or Truck

Measurement by truck

The truck must have an extendable/ telescopic lifting crane. The height of the crane should be at least the depth of the river section to be sampled plus 1-2 meters (this is the depth to which the nets are set on the rope, for example, if the river depth is 6m, a height of 7-8m would be desirable). The maximum lifting capacity depends on the flow velocity. Measurements in Austria, where the flow velocity tends to be higher than in the further eastern course of the Danube, have shown that a bearing load/lifting capacity of two tons is not exceeded, is therefore to be seen as a maximum. The advantage of measuring by truck is that renting a truck is relatively cheap. The disadvantage of this variant, however, is that there must be a corresponding bridge at the sampling location.

Measurement by boat or ship

Basically, the ship must be able to hold the position on the water. Regarding the height of the crane, it should also be considered that an additional 2 meters to the sampled water depth would be desirable to lift the net device onto the boat. If this height were not possible, the first (lowest) net would have to be lowered into the water, and only then can the middle net be attached to the rope. The same applies to the upper net. However, this method would take more time. In addition, it must be considered that the vessel has enough space to handle the net device (cleaning the nets, etc.).

The lifting capacity of the crane would have to be at least 2 tons, because unlike the bridge measurement, where the net hangs in the water in the flow direction, the net would have to be positioned sideways (90° angle) from the ship. The forces acting on the crane increase accordingly.

Compared to measurement by truck, sampling by boat/vessel is much more flexible on the water, but the method is probably also correspondingly more expensive.

3.1.4. Official approvals

The permissions/approvals to be obtained can vary greatly from country to country. The approval requirements listed here apply exclusively to Austrian measurements, but are intended to help partners provide possible indications.

Bridge or road closure

Following requirements concern measurement by truck at bridge:

- During the measurement, at least one lane is blocked for a short distance and would have to be closed off or secured accordingly (traffic signs for speed reduction, etc.)
- Depending on the type of road (responsibility), approval would have to be obtained from the traffic authority (in AT district administration/magistrate or municipal office).
- If the measurement should take place e.g. at the bicycle path on the bridge, an additional permission would have to be obtained from the authority (department of statics)
- A bridge is often a border between two districts, which means that both authorities in the adjacent districts may have to be informed!

National shipping inspectorate

Following requirements refer to **both measuring by truck at bridge or ship**:

- Since the measurement "could affect" shipping traffic (steel rope not visible), notifications often have to be made to the national shipping inspectorate / regulatory authority.
- Normally, 2 persons (upstream and downstream) are required to supervise shipping traffic; depending on the authority's licensing requirements, these persons may also be provided directly by the authority, which may result in additional costs.
- When measuring from ship, the vessel can be directly radioed and informed about the measurement.

3.1.5. Requirements on sampling site

These requirements only refer to the bridge measurement:

- The bridge should essentially not run too high above the river. This could have a negative effect on the requirements of the crane.
- Likewise, priority should be given to bridges with moderate or low traffic volumes.
- The bridge should not have "side walls"

Figure 15: Examples for net-sampling sites (pictures: Wikipedia)

3.1.6. Reconfiguration of net-device

With the newly-developed substructure, it is possible to attach weights on mounting bars to ensure a more stable flow at higher flow velocities.

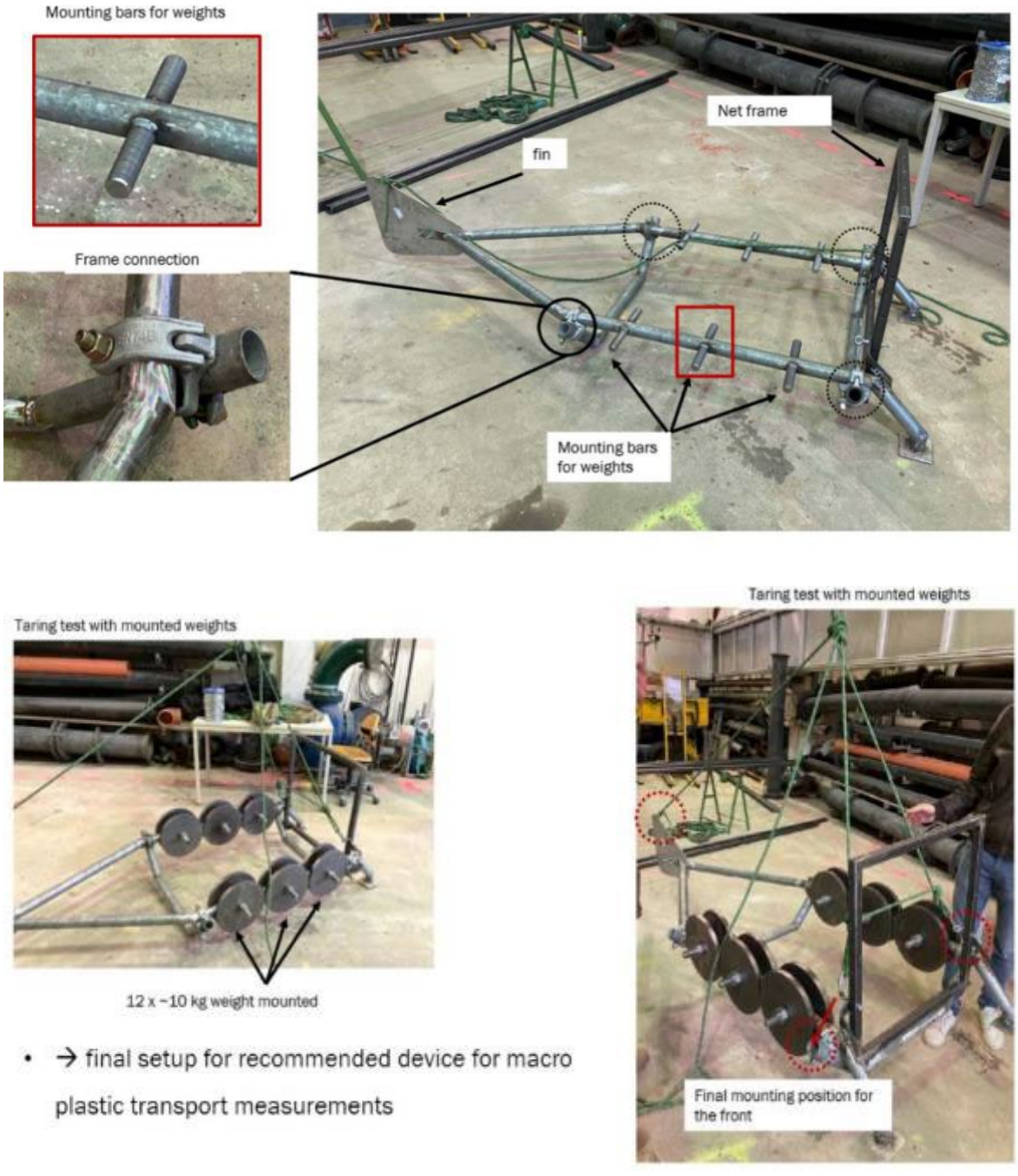


Figure 16:Reconfiguration of net device

3.2. Pump-method and associated sample preparation and analytics

The vast majority of microplastic studies in the aquatic environment apply different nets (e.g. manta net), but the application of different (fractionated) filtration systems operated by pumps are getting more common (Prata et al., 2019; Stock et al., 2019).

A fractionated filtration system has been developed at WESSLING Hungary Ltd. The size of the complete apparatus enables sampling from a smaller boat or from the shore as well. A jet pump is operated by a generator and surface water is transported from a foot-valve (with 1 mm prefilter) through rubber hoses to the stainless-steel filters. Water is filtered through 10" filter cartridges with a mesh size of 300; 100 and 50 µm The sampling concept has been created in strong cooperation with the project partners. In-situ fractionated filtration has been conducted and a minimum of 1000 L of water sample has been concentrated on stainless steel filters with a smallest mesh size of 50 µm. A 1 mm mesh size pre-filter has also been used, therefore reported particles are considered between 50 µm and 1 mm. Sample volume is measured by a flowmeter.

The system is presented in Figure 17. The effectiveness of the sampling apparatus has been tested in controlled environment by (Bordós et al., 2021).

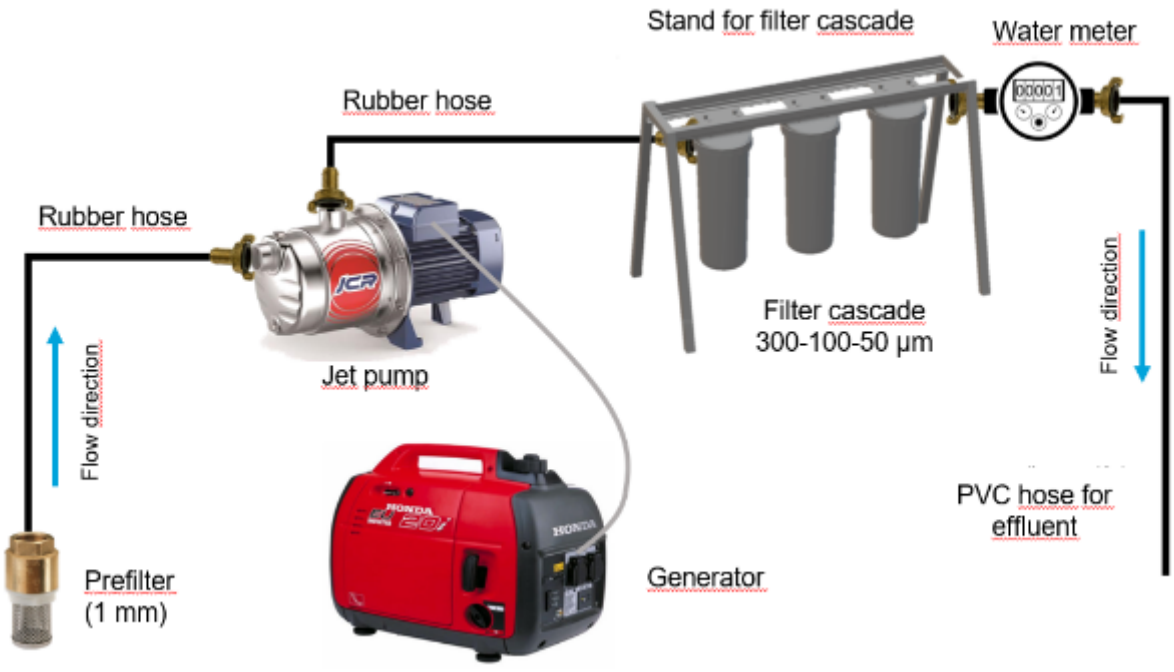


Figure 17: Fractionated filtration device developed by WESSLING Hungary Ltd.

Figure 18: Filter cascade for fractionated filtration

Figure 19: Fractionated filtration device in use.

3.3. Sampling with sedimentation box

The sedimentation box is based on the principle that incoming (river) water enriched with suspended particulate matter (SPM) is slowed down by the chamber-shaped structure, causing the particles to settle down (Figure 20). This passive sampler is placed in around 60 cm below the water surface for 2 weeks There are six 1 cm inlet holes on the front side. The water flows through a total of six chambers before leaving the stainless-steel box at the rear 4 holes. When the box is removed, the holes are closed with silicone stoppers to prevent loss of sample contents. The sample is transferred by ladle into suitable sample containers for transport. Deposits on the bottom are removed from the box by adding water and also emptied into the sample container.

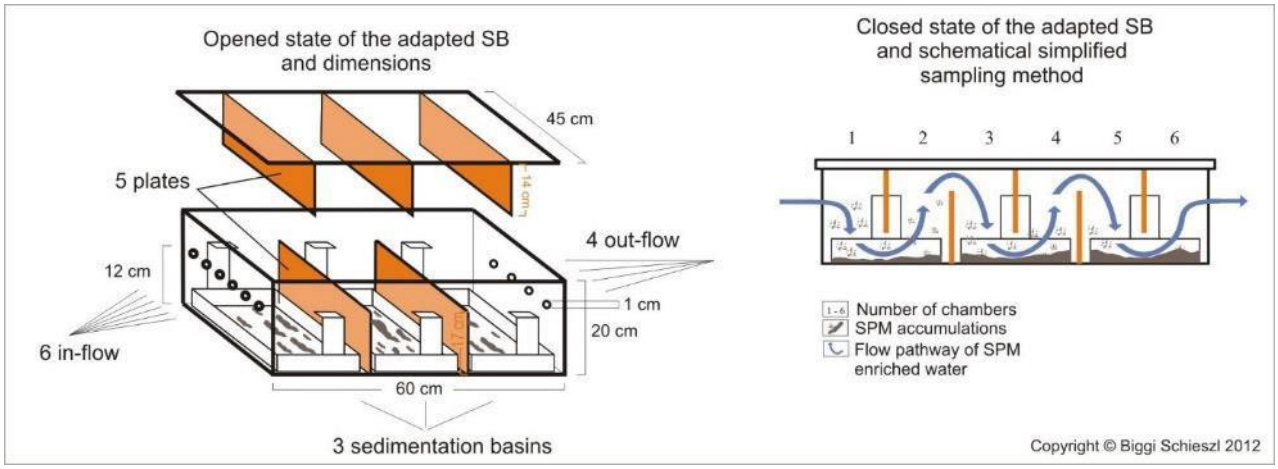


Figure 20: Sedimentation box

The operation of the sedimentation box was described in the Guideline for Sampling and preparation of Suspended Particulate Matter - Standard Operation Procedure (SOP) which was provided by German Environmental Agency during the JDS4 of the Danube river. During this survey, suspended particulate matter samples were taken by means of sedimentation boxes (see chapter 1.2.)

3.3.1. Sampling operation

The sampling has to be performed by trained personal. The sedimentation box is deployed directly in the water body according to the main current by means of stainless-steel ropes, stainless steel chains or in necessary by a fixed stainless-steel construction (Figure 20) The 3-chamber sedimentation box has more wholes for the incoming water masses (six), easy to recognize. The sedimentation box has to be deployed on a dynamic fixing point (e.g. a buoy, a pontoon) for a constant exposition depth of 50 cm below the water surface.

Close to a weir, lock or dam with a regulated water level the deployment can be realized by a fixed system, keeping in mind the minimal water level throughout the year.

At flow velocities above 1.5 m/s a disturbance or failure of sampling is possible. The sampling efficiency of particles is decreased. In that case, the number of incoming wholes has to be reduced by means of silicon stoppers. A flow velocity between 0.5 – 1.0 m/s is highly recommended to obtain comparable results. Only in an absolute emergency can a sampling location be selected at which s the flow velocity is above 1 m/s. The sampling location must be selected in order to obtain

comparable results. In any case, at increased flow velocities, these must be measured before, during and after exposure and noted in the protocol. Only in these absolutely exceptional cases can inlet openings be closed with silicone plugs. At a flow velocity between 1.0 – 1.5 m/s lock two holes and above 1.5 m/s lock three holes in the inflow section.

4. Estimation of Microplastic Pollution Situation along the Danube river

4.1. Sampling sites and approach

The project area of TidyUP covers seven countries with the focus on Tisza and Danube river. The selection of the sampling sites (Figure 21) was inspired by the Joint-Danube-Survey 4 (JDS4) in 2019 to build on previous results. MP-measurements were performed in Danube river in Hainburg (AT), Budapest (HU), Bezdan (RS), Pancevo (RS), Ruse (RO/BG), Tutrakan (RO/BG) as well as in the Tisza river (upper course, Kisköre, HU) and close to the estuary (Titel, RS) from March to July 2021. Primary objective of the sampling campaign was to compare the different methods under varying conditions (e.g. discharge, flow velocity, water depths, etc.) and to get a rough picture of the MP pollution situation in the Danube/Tisza basin.

Figure 21: TidyUp sampling sites inspired by JDS4 (red marks)

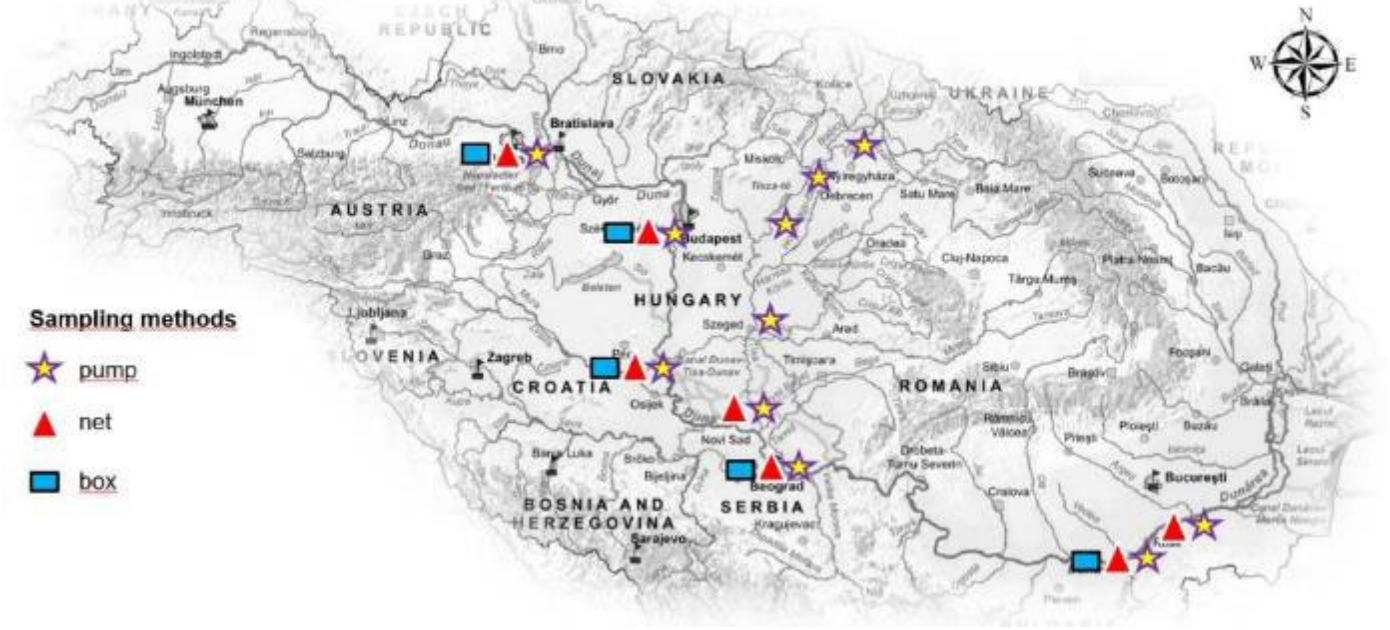


Figure 22: Applied measurement methods at the chosen TidyUp MP sampling sites

According to previous studies (UBA, 2015), the content of MP concentrations in flowing waters can vary greatly depending on the density of the plastic particles as well as the river conditions like the flow velocity or discharge, water depth and positioning in the transverse profile of the river (influence of groyne fields, etc.). To consider the depth variance and spatial distribution of MP, sampling was performed across the river cross-section and at different depths.

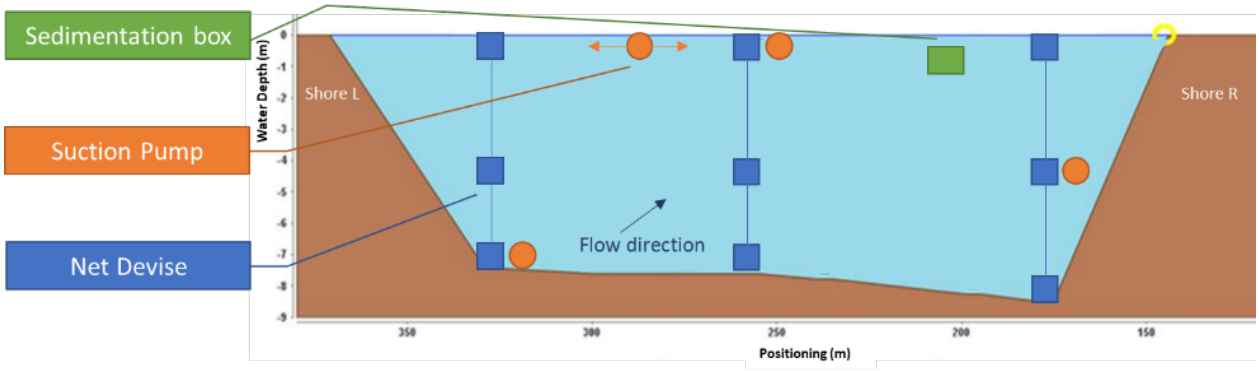


Figure 23: Sketch of Microplastic sampling approach including depth variance and spatial distribution of microplastics

At each sampling site, three measurement points were defined across the river profile (near the river banks and in the middle of the river). The measurements by net and by pumping method were carried out in parallel as schematically shown in Figure 23. Here, the hose of the suction pump was fixed to the frame of the net device in the appropriate river depth. At the end, an additional composite sample was taken with the pump at the water surface over the cross profile. The sampling time (in the water) per sample point was between 30 and 40 minutes. The sedimentation box was deployed before or immediately after the measurement campaign at a depth of about half a meter.

4.1.1. Danube river - Hainburg (AT)

In Austria, MP-measurements with the net-devise (provided by the Institute of Hydraulic Engineering and River Research (IWA) of the University of Natural Resources and Life Sciences, Vienna) and sedimentation box (provided by Austrian Environmental Agency, UBA-AT) were already carried out before the TidyUp sampling campaign (Liedermann et al., 2018, ICPDR, 2020). This means that it was already possible to build on the experience of the two established MP-sampling methods. Also, for sampling with pump experience was available (Bordós et al., 2021) (see chapter 3)

Since a measurement by ship is very expensive in Austria (compare chapter 6.1), especially because of the specifications and requirements for the crane on board, sampling from bridge – in combination with a small boat for the pump - was deliberately chosen. Thus, in Hainburg, parallel measurement was carried out from a bridge (net devise) and by boat (suction pump) on June 30, 2021. This measurement combination between truck and boat was chosen due to existing permits (road closure) and previous experience with bridge measurements. Furthermore, a measurement by ship with crane would be difficult to realize due to bureaucratic and administrative burden and is also financially not feasible in Austria. The sampling campaign was conducted in cooperation with viadonau which provided both the truck and the boat.

Table 9: Information about MP sampling site in AT (net-device + pump-method)

Hainburg (AT)			
Date	30.06.2021	Timeframe:	8:15-15:00
River kilometer	1886,24	Discharge-MQ [m³/s]	1920
	Sampling point 1	***Sampling point 2***	***Sampling point 3***
Coordinates	N 48.14532 E 16.90916	N 48.14611 E 16.90896	N 48.14716 E 16.90784
Time:	10:34-11:08	12:03-12:37	13:28-14:00
Water depth [m]	3.7	4.6	2.8
*Measuring depths [m]	0.3 | 2.5 | 3.4	0.3 | 3.6 | 4.3	0.3 | 1.8 |2.5
Distance right shore [m]	38	121	250
Flow rate [m/s]	2.1	2.15	0.97

*refers to the center of the net frame (60x60cm) at the water surface, the middle of water column and the riverbed

Figure 24: Pictures of net-sampling from bridge close to Hainburg (AT)

Figure 25: Pictures of from sampling with pump attached to the net frame, close to Hainburg (AT)

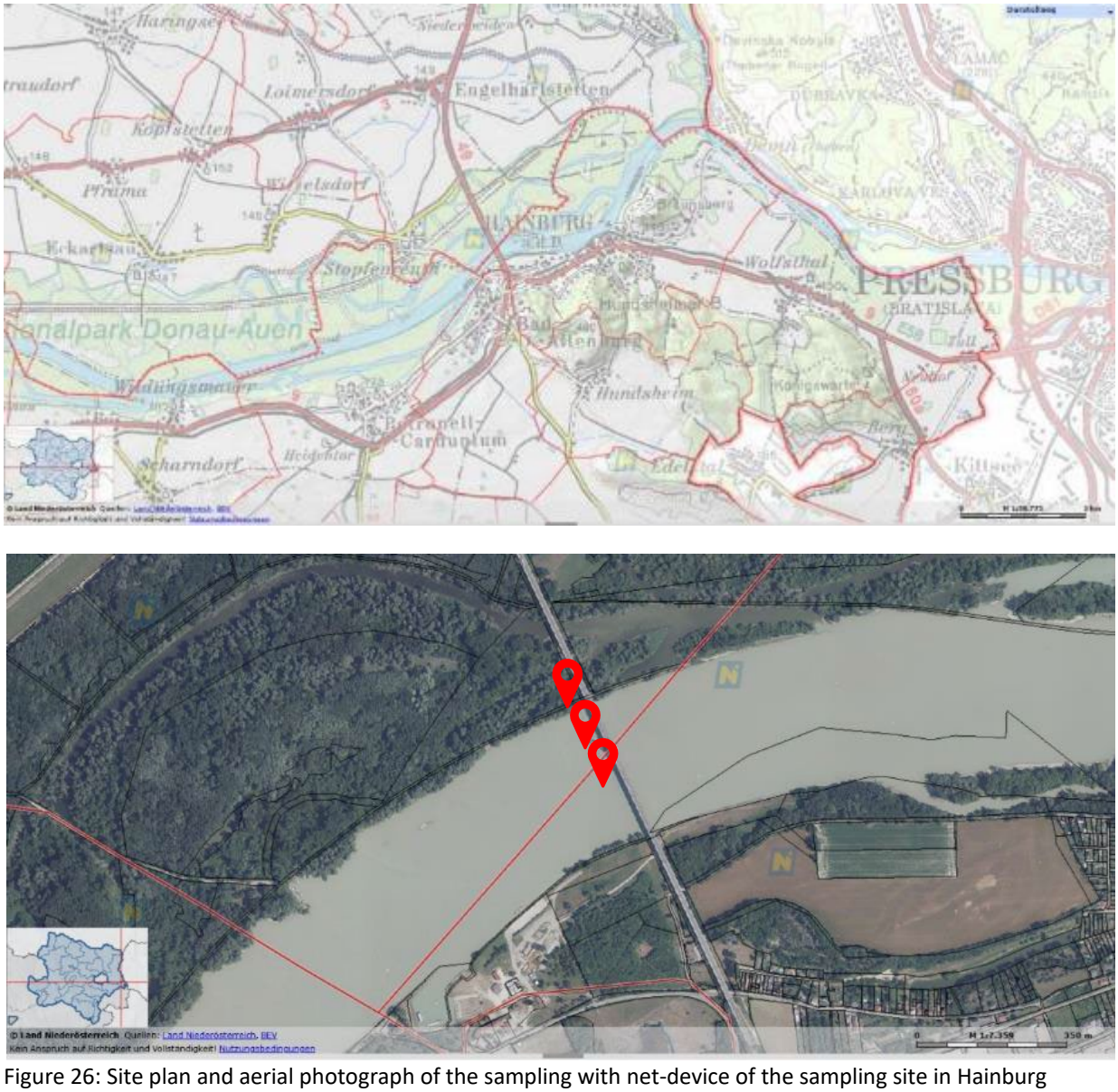


Figure 26: Site plan and aerial photograph of the sampling with net-device of the sampling site in Hainburg

In addition, the sedimentation box was installed two weeks before (16.06.-29.06.21) at a small bridge to the landing stage in Hainburg (Figure 27), around two kilometers downstream to the other measuring site (net + pump method). The taken sample from the sedimentation box was transferred in stainless steel containers to ABF-BOKU and Wessling for subsequent sample preparation and analysis.

Table 10: Information about sampling with sedimentation box in AT

Date of Deployment	16.06.2021	Time:	15:00
Date of removal	29.06.2021	Time:	09:00
River kilometer	1884.15	Measuring depth [m]	0.5
Coordinate	N 48.147694, E 16.936500	Flow rate [m/s]	0.8
Description	On the right bank of the Danube, directly at the "Hainburg" landing stage		

Figure 27: Pictures of deployed sedimentation box in Hainburg (AT)

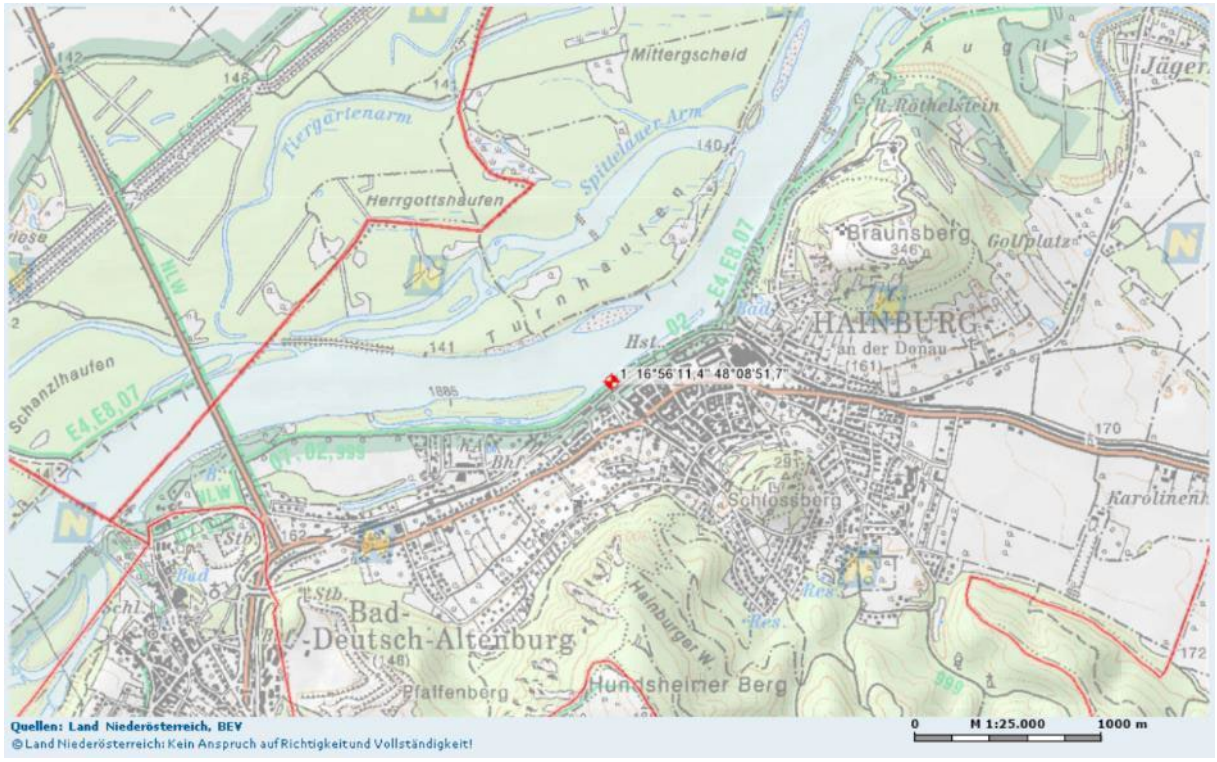


Figure 28: Site plan/coordinates of the sedimentation box and aerial photograph of the sampling site in Hainburg

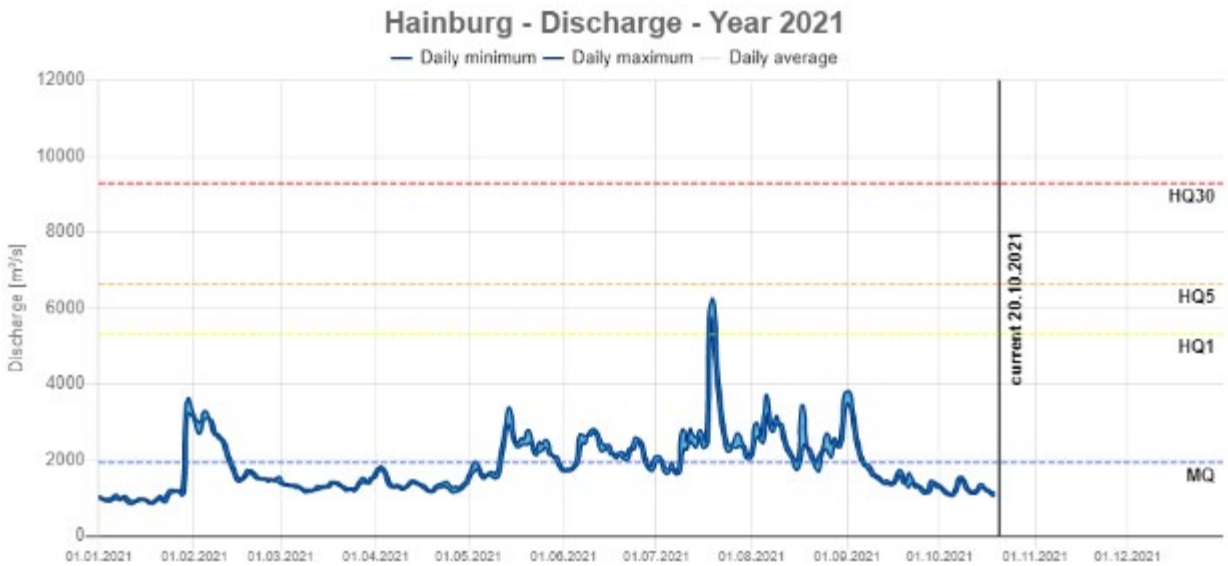


Figure 29: Discharge of Danube river in Hainburg within sampling time frame (Amt der NÖ Landesregierung)

4.1.2. Danube river - Budapest (HU)

The parallel measurement using the 3 sampling methods has been carried out in Budapest at 15th April 2021 (sedimentation box was deployed at 29th March and cleaned during the joint sampling). This was the first occasion, when all these sampling methods was deployed parallelly (Table 11, Table 12, Figure 30). The sampling location was designated downstream Budapest as presented on Figure 31. Previously on this site measurements have been carried out in 2018 and 2019, as described in chapter 2.1.3.

Table 11: Information about MP-sampling site in HU (net-device, pump-method)

Budapest (HU)			
Date	15.04.2021	Timeframe:	8:15-16:00
River kilometer	1630,8	Discharge-MQ [m³/s]	1794
	Sampling point 1	***Sampling point 2***	***Sampling point 3***
Coordinates	N 47.387646 E 18.992328	N 47.385666 E 18.987093	N 47.384961 E 18.991075
Time:	13:06-13:41	11:49-12:24	14:18-14:53
Water depth [m]	6.52	4.71	4.93
*Measuring depths [m]	0.3 | 1.8 | 3.9	0.3 | 1.8 | 3.9	0.3 | 1.8 | 3.9
Distance right shore [m]	70	250	385
Flow rate [m/s]	0.85	0.86	0.91

*refers to the center of the net frame (60x60cm) at the water surface, the middle of water column and the riverbed

Table 12: Information about the sampling site for sedimentation box in HU

Date of Deployment	29.03.2021	Time:	
Date of removal	15.04.2021	Time:	10:10
River kilometer	1631	Measuring depth [m]	0.6
Coordinate	N 47,386404, E 18,989320	Water depth [m]	4.3
Description	Buoy at the right-hand side of the channel		

Figure 30: Deployment of the sedimentation box at Budapest (top left); parallel application of the fractionated filtration and the net device (bottom left & right);

Figure 31: Sampling location at downstream Budapest

Flow rates and water level has been measured by the Hungarian General Directorate of Water Management (OVF). Data before and after 14 days of the sampling are presented on Figure 32. At the day of the sampling, water level was in rising period, but no serious flood event occurred.

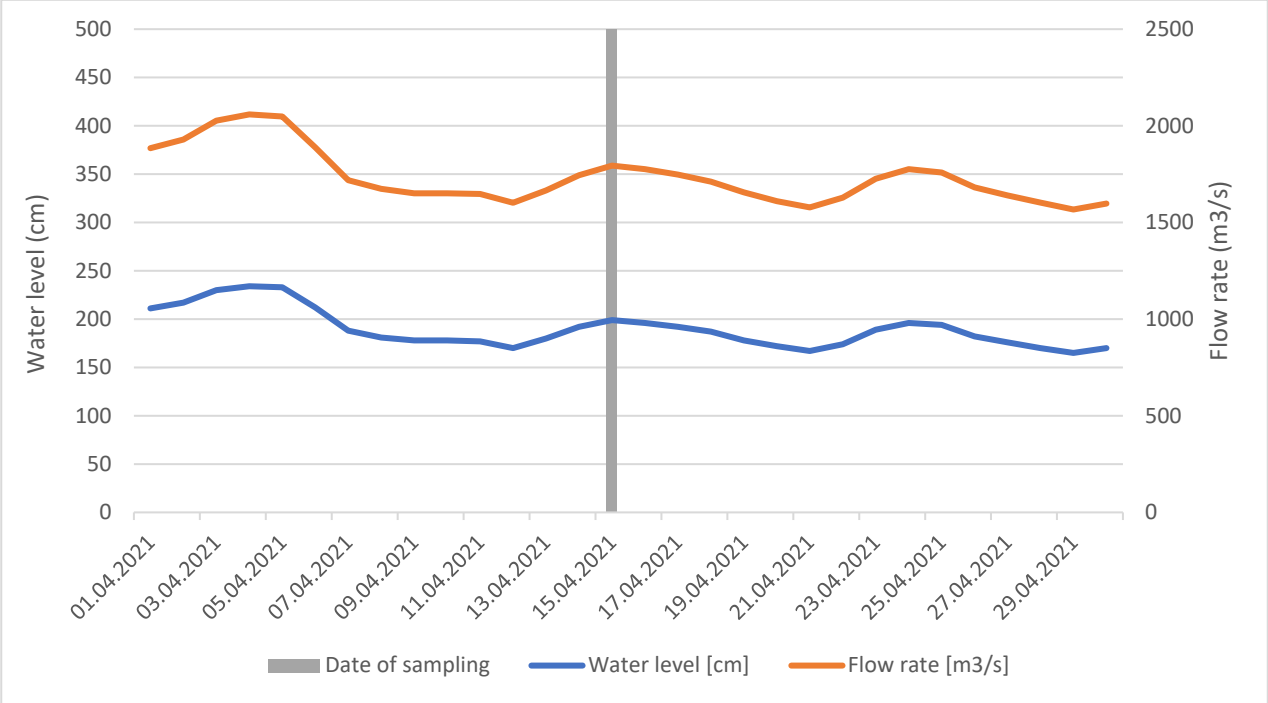


Figure 32: Flow rate and water level data 14 days before and after sampling (sedimentation box deployment on 29th March 2021)

4.1.3. Danube river - Bezdan (RS)

Bezdan was the first of three sampling locations in Serbia. Simultaneous measurement with net devise and suction pump was carried out by boat on May 5th, 2021 in Danube river near “Batina" boarder checkpoint in the vicinity of town of Bezdan. The measurement was carried out from the Argus ship equipped with all necessary tools (electricity supply, crane, open space, laboratory, etc.).

Table 13: Information about MP sampling site 1 in RS (net-device + pump-method)

Bezdan (RS)			
Date	05.05.2021	Timeframe:	9:00-16:00
River kilometer	1424,2	Discharge-MQ [m³/s]	2828
	Sampling point 1	***Sampling point 2***	***Sampling point 3***
Coordinates	N 45.841641 E 18.855617	N 45.842522 E 18.857123	N 45.842866 E 18.859252
Time:	13:59-14:33	15:15-15:35	12:26-13:05
Water depth [m]	6.39	5.47	5.87
*Measuring depths [m]	0.3 | 2.8 | 5.3	0.3 | 2.8 | 5.3	0.3 | 2.8 | 5.3
Distance right shore [m]	115	185	395
Flow rate [m/s]	0.50	0.98	0.76

*refers to the center of the net frame (60x60cm) at the water surface, the middle of water column and the riverbed

Figure 33: Argus ship for microplastic sampling campaign in Serbia

The sampling campaign was conducted in cooperation with the Ministry of Environmental Protection and SEPA (Serbian Agency for Environmental Protection). Two weeks before the sampling campaign, on April 21st, sedimentation box was deployed and fixed on a raft near the coast (Figure 34).

Figure 34: Deployed sedimentation box in Bezdan (RS)

Table 14: Description of sampling site for sedimentation box – Bezdan (RS)

Date of Deployment	21.04.2021	Time:	10:00
Date of removal	05.05.2021	Time:	11:00
River kilometer	1426	Measuring depth [m]	0.8
Coordinate	18°51'29.7"E 45°51'29.6"N	Flow rate [m/s]	

Sedimentation box was lifted from the raft by a crane, then the biweekly collected sample was transferred to a non - plastic container (Figure 35).

Figure 35: Crane use and collecting the sample from sedimentation box

One of the requirements for the ship is to have enough open space in order to conduct simultaneous microplastic sampling using net device and suction pump.

Figure 36: Suction pump and net device sampling, Bezdan (RS)

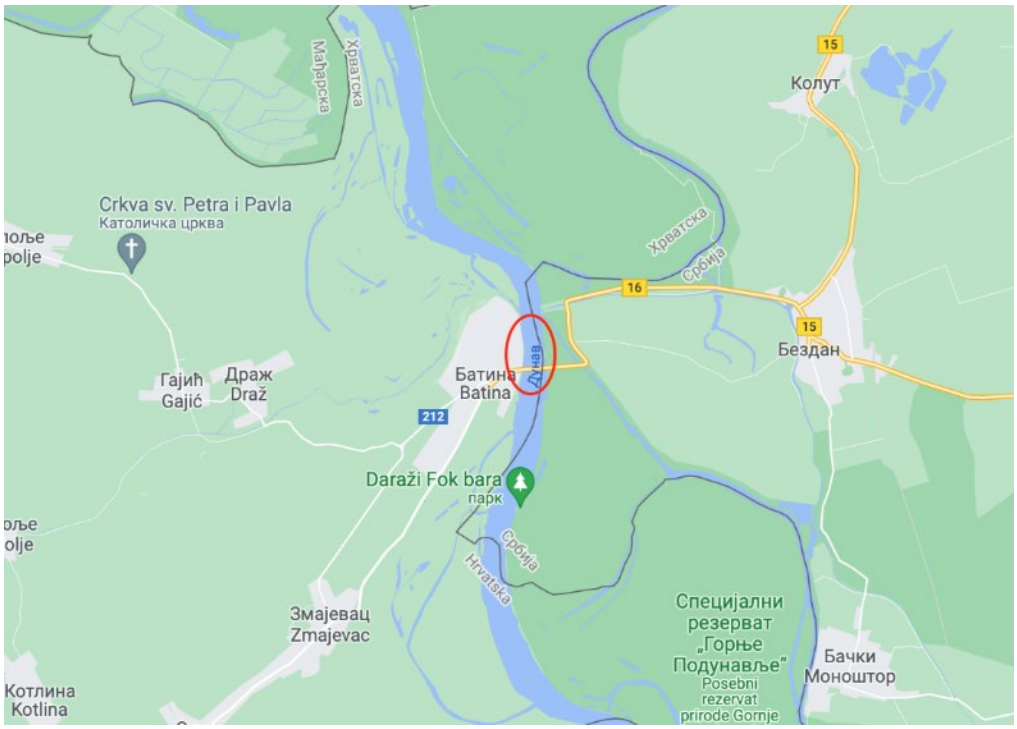


Figure 37: Site plan and aerial photograph of the sampling site in Bezdan

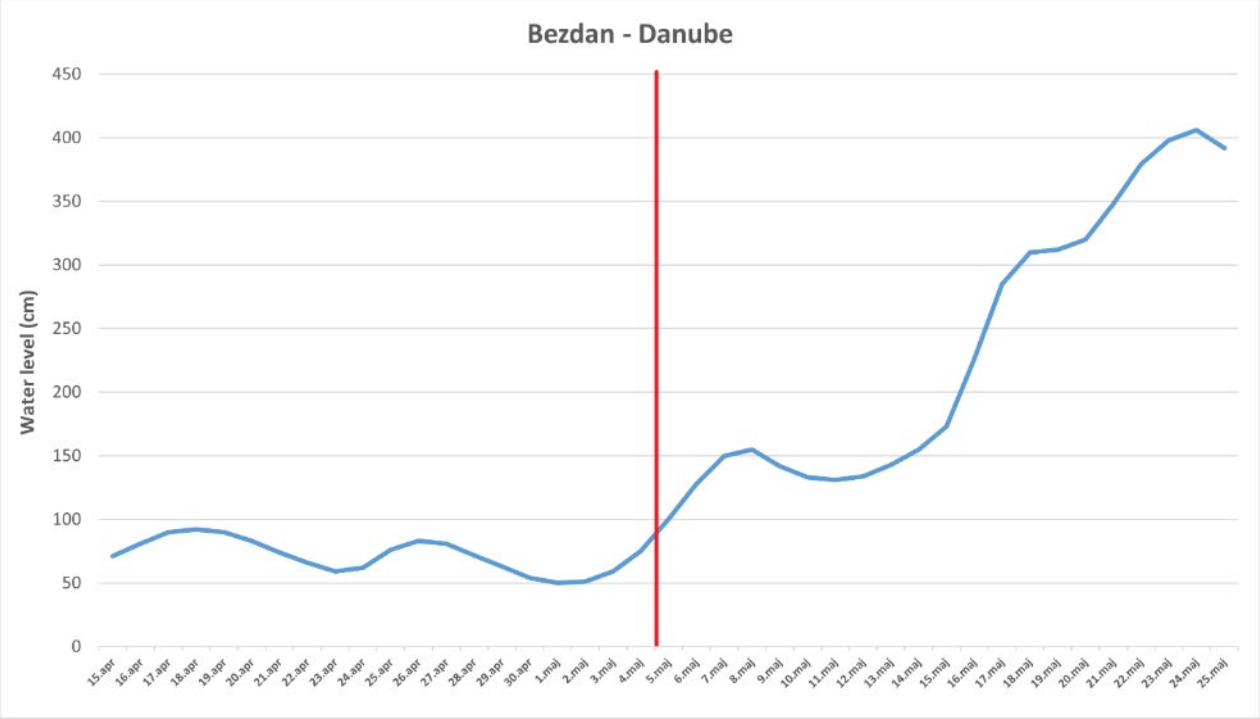


Figure 38: Water level data in Bezdan before and after sampling

4.1.4. Danube river - Pancevo (RS)

In Serbia, near city of Pančevo, on the Danube River, parallel measurement with net devise and suction pump was carried out from the Argus ship on May 7th, 2021. The sampling was similar to the measurement implemented two days earlier near Bezdan (RS).

Table 15: Information about MP sampling site 3 in RS (net-device + pump-method)

	Pancevo (RS)		
Date	07.05.2021	Timeframe:	11:45-18:00
River kilometer	1151	Discharge-MQ [m³/s]	N/A
	Sampling point 1	***Sampling point 2***	[1]***Sampling point 3***
Coordinates	N 44.817806, E 20.643917	N 44.815972, E 20.642111	N 44.816944, E 20.648111
Time:	14:10-14:35	15:56-15:31	12:25-12:49
Water depth [m]	6.60	8.48	5.87
*Measuring depths [m]	0.3 | 2.8 | 5.3	0.3 | 2.8 | 5.3	0.3 | 2.8 | 5.3
Distance right shore [m]	140	190	460/580
Flow rate [m/s]	0.42	0.64	0.52

*refers to the center of the net frame (60x60cm) at the water surface, the middle of water column and the riverbed
[1] Due to high water depth and strong wind net device caught in the anchor of the ship;

Figure 39: Argus ship with net device and suction pump on the Danube River, Pančevo (RS)

At the same time, sedimentation box was placed near the coast (Figure), on the raft. Two weeks after, on May 21st box was collected and sample was placed in a non-plastic container (Figure).

Figure 40: Deployment of sedimentation box on the Danube River, Pančevo (RS)

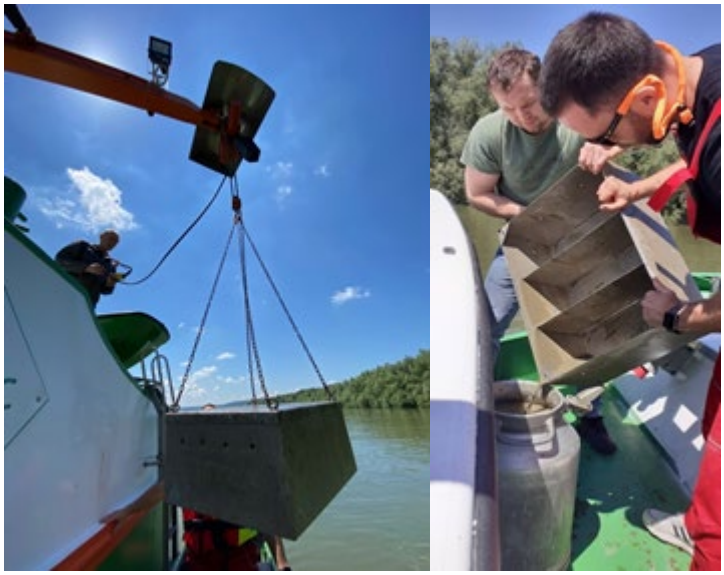


Figure 41: Removal of the sedimentation box on Danube near Pancevo

Table 16: Information about sampling site for sedimentation box in Pancevo (RS)

Date of Deployment	07.05.2021	Time:	13:00
Date of removal	21.05.2021	Time:	12:00
River kilometer	1154	Measuring depth [m]	0.6
Coordinate	N 45.858222, E 18.858250	Flow rate [m/s]	N/A
Description	On the right bank of the Danube, directly at the raft near "Pančevo" coast		

Figure 42: Site plan and aerial photograph of the sampling site in Pancevo

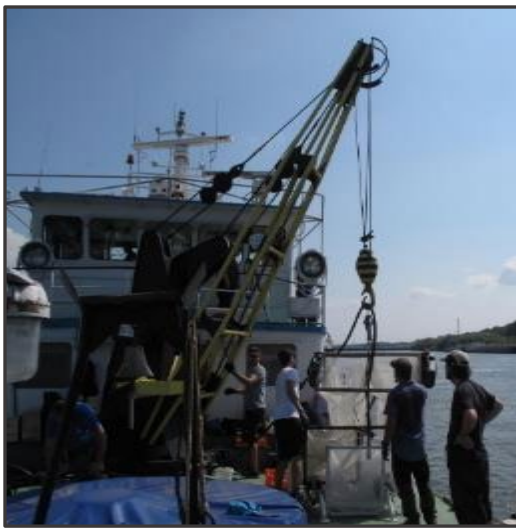
4.1.5. Danube river - Ruse (BG/RO)

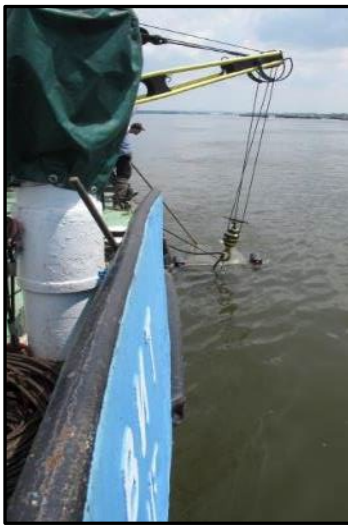
At the beginning of May 2021, an MP parallel measurement was carried out by ship in Ruse, BG. The Danube here is the border river between Bulgaria and Romania. The sampling location in Ruse was chosen for two reasons: first, the location is quite central on the Danube section of the two mentioned countries and second, only in this port an appropriate ship with crane could be organized for the sampling campaign. A bridge measurement as in Austria was out of the question for administrative and space reasons.

Table 17: Information about MP sampling site 1 in BG/RO (net-device + pump-method)

	Ruse (BG/RO)		
Date	07.07.2021	Timeframe:	07:45-14:00
River kilometer	494	Discharge-MQ [m³/s]	
	Sampling point 1	***Sampling point 2***	***Sampling point 3***
Coordinates	N 43.859232, E 25.955650	N43.860187, E 25.953966	N 43.860805, E 25.951335
Time:	11:00-11:36	12:11-12:48	13:20-14:05
Water depth [m]	9.34	9.12	7.31
*Measuring depths [m]	0.3 | 1.8 | 3.3	0.3 | 1.8 | 3.3	0.3 | 1.8 | 3.3
Distance right shore [m]	149	316	515
Flow rate [m/s]	1.00	0.98	0.89

*refers to the center of the net frame (60x60cm) at the water surface, the middle of water column and the riverbed





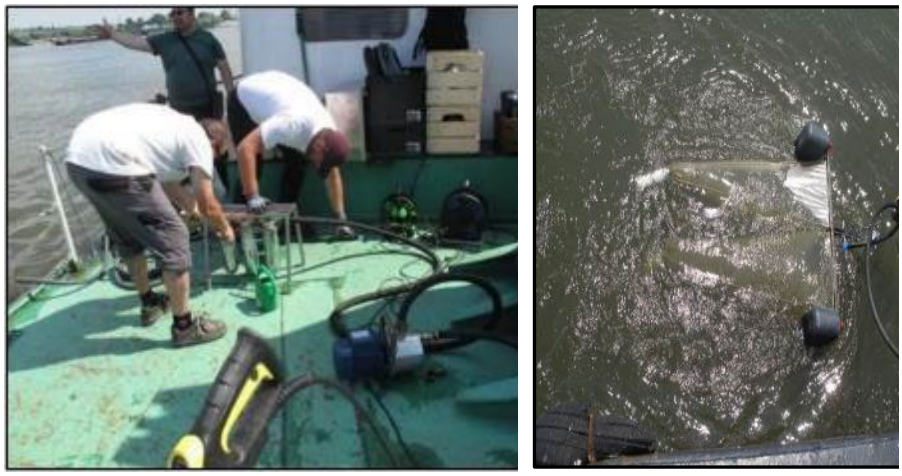


Figure 43: MP Sampling with net and pump devise in Ruse, BG

The sedimentation box was deployed at the same landing stage as in JDS4 for 14 days. Details about the sampling site of the sed.-box and hydrological parameters are summarized in following table.

Table 18: Information about sampling site for sedimentation box in Ruse (BG)

Date of Deployment	07.07.2021	Time:	13:00
Date of removal	21.07.2021	Time:	13:00
River kilometer	494.60	Measuring depth [m]	0.6
Coordinate	N 43.85910 N, E 25.95824	Flow rate [m/s]	0.8
Description	Harbour bridge in Ruse		

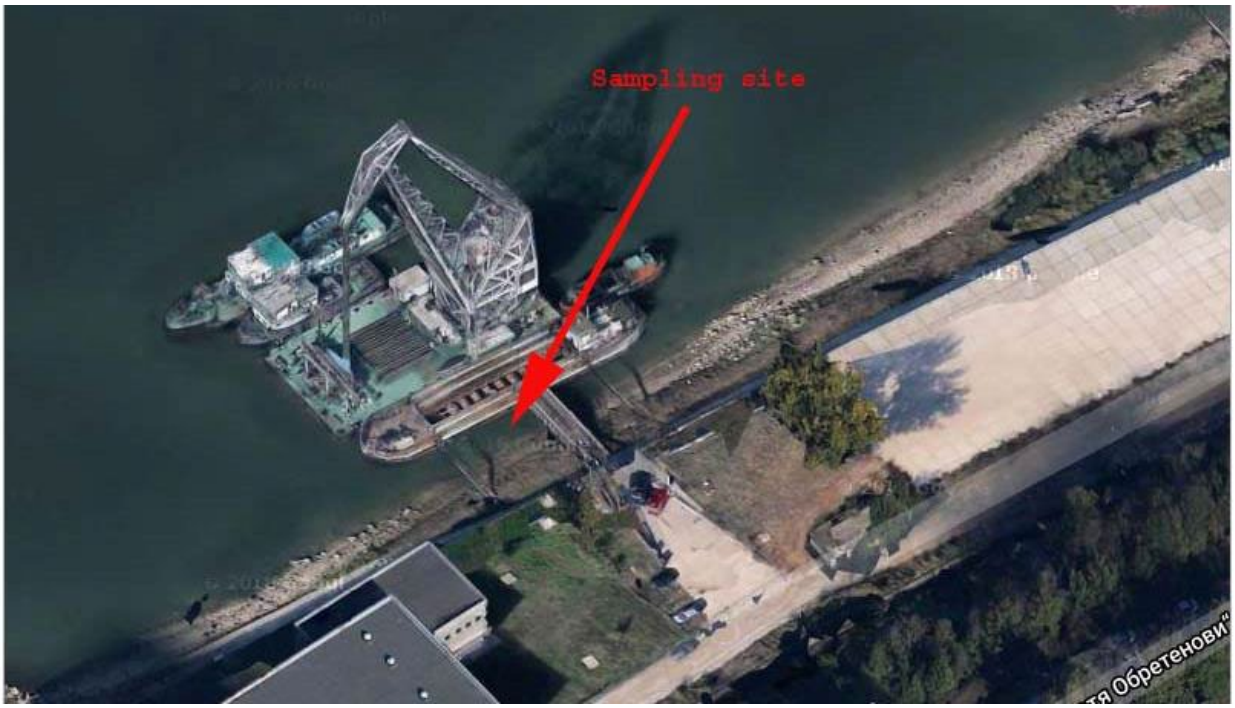


Figure 44: Sampling site at Ruse, sedimentation box

Figure 45: Attaching and removal of the Sedimentation box in Ruse

4.1.6. Danube river - Tutrakan (BG)

In Tutrakan, around 56km from Ruse, a second MP measurement via crane directly on riverbank was performed at the beginning of July, 2021. Only one parallel sample were taken at the right right shore as sampling by net from a ship with crane was not possible. Blades of the flow meter have hardly rotated due to the very low flow velocity.

Additionally, a mixed sampling by pump-method over the river cross section were conducted.

Table 19: Microplastic measurement (Net-sampling device, Pump-method) in Tutrakan (BG)

	Tutrakan (BG)		
Date	08.07.2021	Timeframe:	8:40-11:30
River kilometer	432.6	Discharge-MQ [m³/s]	N/A
	Sampling point 1		
Coordinates	N 44.052015, E 26.611236		
Time:	10:58-11:34		
Water depth [m]	2.52		
*Measuring depths [m]	0.3 | 1.5 | 2.3		
Distance right shore [m]	10		
Flow rate [m/s]	0.37		

Figure 46: Sampling site –net and pump in Tutrakan

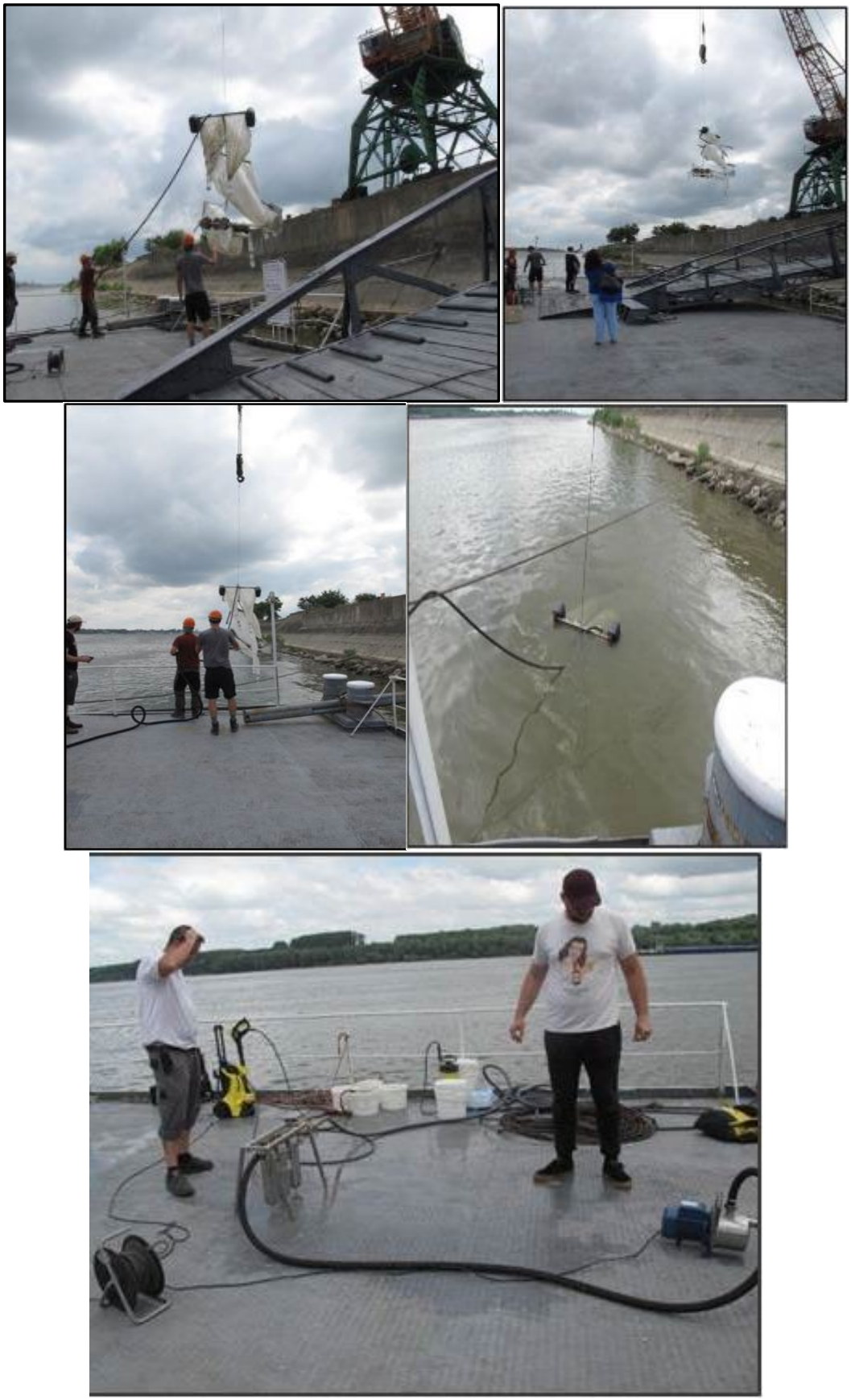


Figure 47: Sampling methods - net and pump applied in Tutrakan, BG

4.1.7. Tisza river - close to the estuary (Titel, RS)

On May 6th, 2021 microplastic sampling campaign with net device and suction pump was conducted on the Tisza River close to the Danube estuary, near the city of Titel (RS).

Table 20: Information about sampling site in Titel (RS)

Bezdan (RS)			
Date	06.05.2021	Timeframe:	16:30-20:15
River kilometer	N/A	Discharge-MQ [m³/s]	1150
	Sampling point 1	***Sampling point 2***	***Sampling point 3***
Coordinates	N 45.196861, E 20.311972	N 45.196333, E 20.312778	N 45.196111, E 20.314000
Time:	18:06-18:38	19:26-19:46	17:10-17:36
Water depth [m]	8.74	8.54	6.10
*Measuring depths [m]	0.3 | 2.8 | 5.3	0.3 | 2.8 | 5.3	0.3 | 2.8 | 5.3
Distance right shore [m]	45	100	220
Flow rate [m/s]	0.55	0.77	0.55

*refers to the center of the net frame (60x60cm) at the water surface, the middle of water column and the riverbed

Figure 48: Microplastic sampling using net device and suction pump on Argus ship – Tisza River (Titel, RS)

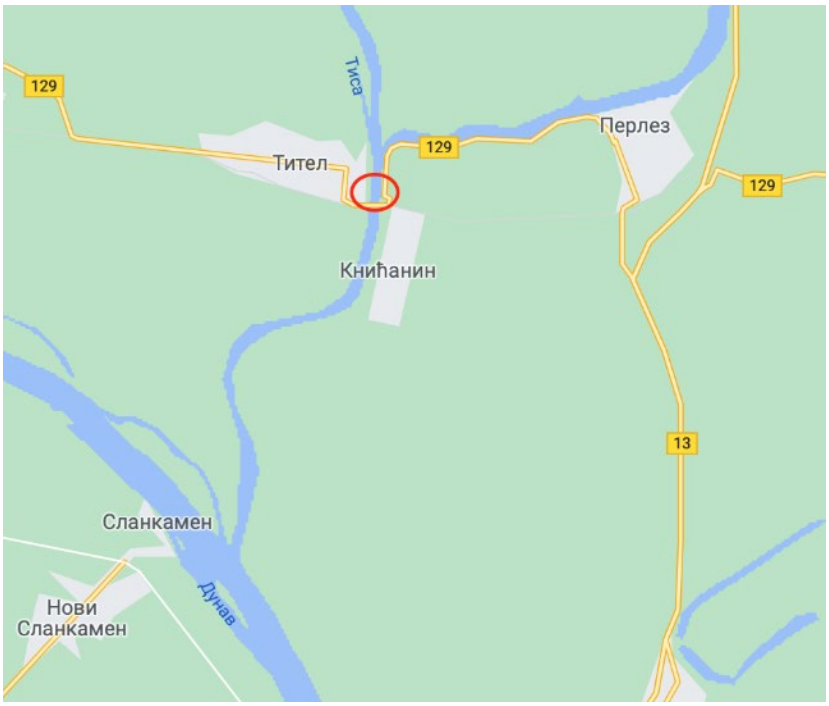


Figure 49: Site plan and aerial photograph of the sampling with net and pump of the sampling site in Titel

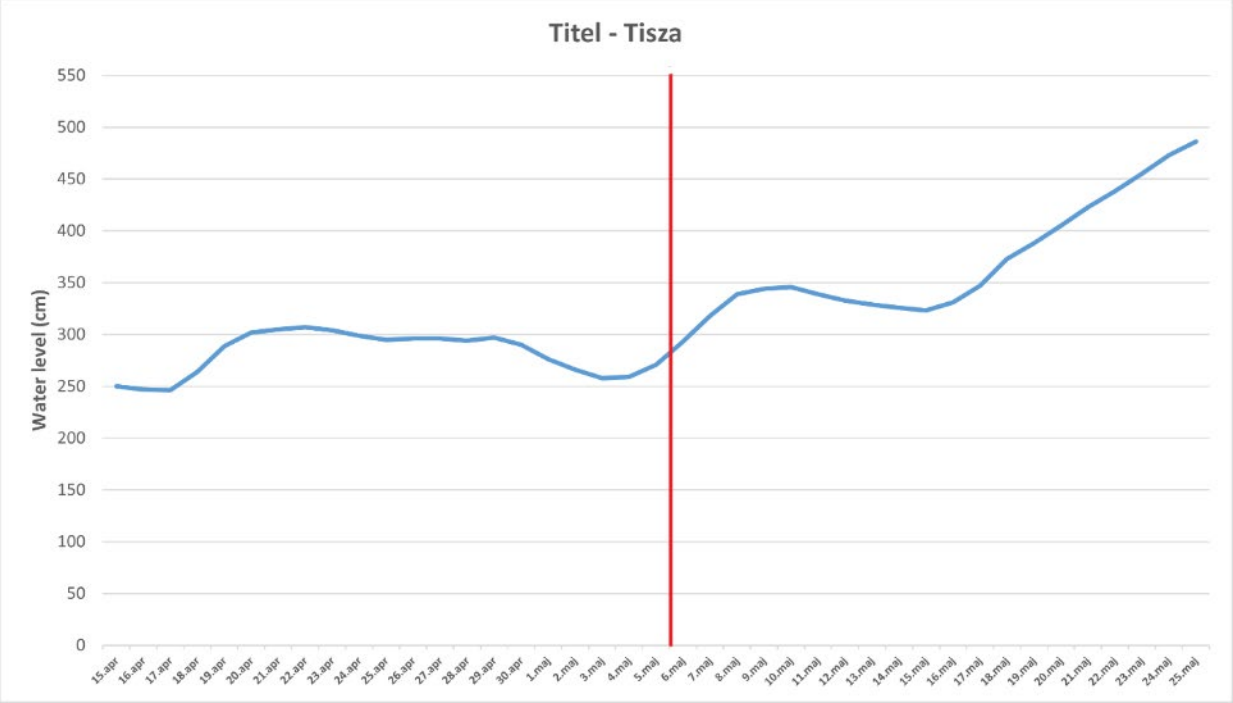


Figure 50: Water level data in Titel before and after sampling

4.2. Sample preparation and analysis within Tid(y)Up

The aim of sample preparation is the isolation of microplastic particles for subsequent detection. Sample preparation turned out to be very time-consuming, especially for net samples, and was tested in a wide variety of ways. Samples varied widely in sample size, particle size ranges, and organic and inorganic content depending on sampling method and sampling location. Various processing steps were therefore tested in different sequences during the project in order to enrich microplastic particles for the subsequent investigations by means of ATR- FT-IR spectrometer as well as FT-IR microscope.

Main challenge of sample preparation originating from net sampling in rivers and subsequent analysis is the fact that only few microplastic particles are captured with a mixture of organic and inorganic by-catches. Searching for plastic particles is like looking for a needle in a haystack. Further processing and analysis of the microplastic particles (e.g. isolation, determination of plastic type, counting and weighing) results is improved by removing non-plastic materials beforehand. Many pre-treatment steps to remove as much as possible organic and inorganic contaminations to isolate microplastics are therefore necessary and have to be done without fragmenting or chemically alteration of the microplastics.

While inorganic compounds can be almost completely be removed due to their higher density, organics have to picked out manually (leaves, branches, etc.) or digested with oxidation agents or enzymes. As digestion of organics may also lead to fragmentation of particles or to chemical alterations recovery rates were also tested during the project.

In order to separate unwanted organic and inorganic bycatch, different processing steps are necessary, which have been evaluated and optimized within the Tid(y)Up project especially for net samples under consideration of quality assurance and control. Physical or chemical degradation of microplastic particles should be avoided as well as secondary contamination. The most practicable methods are summarized in the following chapter to guiding protocols for the three sampling methodologies. For net-samples two analysis methods were compared: measurement with a cost intensive device (FTIR-microscope) with ATR-FITR-spectrometer, with lower investment costs.

Figure 51: Examples of samples (left, middle: samples taken with net; right: sample taken with sedimentation box)

4.3. Protocol for samples taken with nets

Especially the isolation of microplastic particles from net samples from rivers, which are very heterogeneous in terms of composition (woody plants, sediments, aquatic organisms, leaves, etc.) as well as size distribution, is a very time-consuming and laborious task. During the numerous treatment steps of net-samples physical or chemical degradation of microplastic particles should be avoided as well as secondary contamination.

All net samples were analysed at the Institute of Waste management and Circularity (ABF-BOKU); The preparation steps of ABF-BOKU are described below.

Laboratory steps:

From sampling to results, numerous tasks are required to improve results of subsequent analysis of microplastic particles. Removal of by-catches enhances isolation and analysis of microplastics (MPs). If the financial and/or time resources do not allow the analysis of the whole sample, a sample division will be made and sub-samples are analysed. Sequences can be changed depending on the composition of the samples. Furthermore, steps can be repeated if necessary.

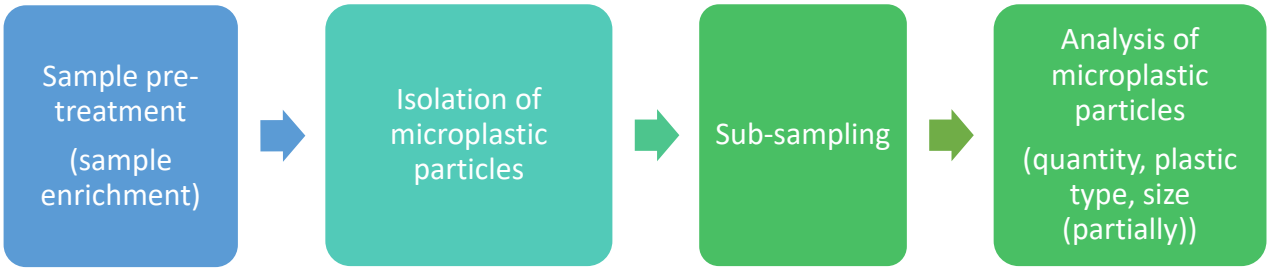


Figure 52: laboratory steps (sample pre-treatment steps based on analysis methods; analysis based on research questions and resources)

The various screening steps which are performed during sample preparation process aim to remove macro plastics and isolate the size fraction of interest for micro plastic detection (i.e. 500 - 5000 µm within this study). This size range is chosen as only particles < 5000 µm are considered as microplastics and as the mesh size during sampling was 500 µm. Only for the size fraction ≥ 500 µm the sampled water volume is known and MP concentrations can be calculated. After clogging of the net, also smaller particles are captured with the net, but cannot be related to a sampled water volume. Nevertheless, samples of size fraction 50 - 500 µm are kept as reserve samples.

Sieving within this study was performed with mesh widths of 50 µm, 500 µm, 1000 µm and 5000µm. Also 20,000 µm mesh size was used for the removal of leaves etc. As above mentioned, the fraction 500 – 5000 µm was further investigated. The splitting into the fractions 500 – 1000 µm and 1000 – 5000 µm is performed to meet analytical requirements of the FTIR measurements.

During the procedures, samples are sieved and dried (50°C). Dry weights are collected after all treatment steps as a means of control and to determine the efficiency of individual treatment steps. Also, wet sieving is performed again and again with the mesh width 50 µm and 500 µm to remove the particles that have degraded during the respective treatment steps

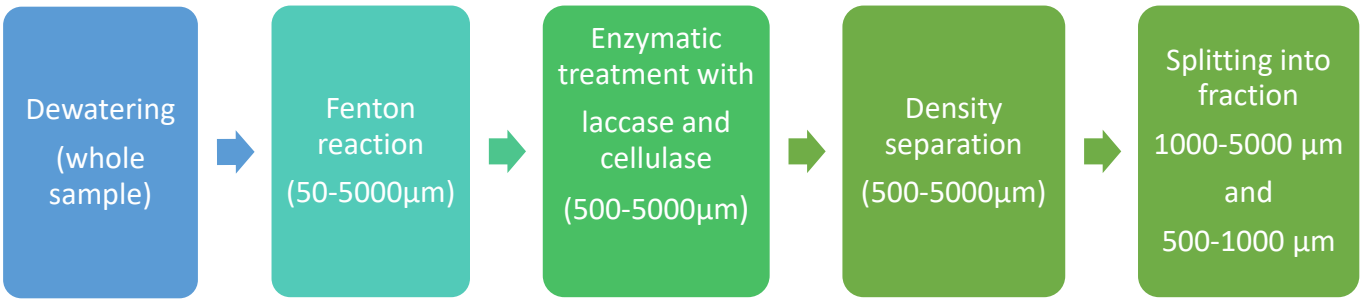


Figure 53: Sample pre-treatment steps for net-samples

Depicted sample pre-treatment steps (Figure 53) may differ depending on the sample composition and the purpose of the study.

Dewatering and removal of macro particles

The entire wet sample is passed over a 5000 µm and 50 µm sieve. The cut off of 50 µm is chosen in order to remove water from the sample. In the presence of many leaves etc., which would quickly clog the 5000 µm sieve, it is recommended to use a 20 mm sieve as well. With the 20 mm and 5000 µm sieve macro particles are removed from the sample. The two sieves are rinsed well with water from above in order to flush out any microplastic particles that have adhered to them. The fraction > 20 mm can also be transferred to a water bath and all big particles are sorted out using tweezers and rinsed onto the 5000 µm sieve using a spray bottle. Microplastic particles that are already clearly visible are picked out and later added to the analysing particles.

Screening into the different size classes, into which the results of the MP analysis are reported prior to pre-treatment steps was tested within the project but discarded, as samples with a high plant fibre content can't be sieved correctly prior to enzymatic digestion.

Figure 54: Removal of macro plastics and visually picking of microplastics (mesh size: left > 20 mm, right 5 mm-20 mm)

Fenton reaction

Fenton reaction is an oxidative process for removing easy degradable organic content (e.g. lignin cellulose and chitin won't be degraded by this method) from the samples by adding hydrogen peroxide (H_2O_2). To accelerate sample digestion, the formation of hydroxyl radicals from hydrogen peroxide will be catalysed via iron (II). This is accelerating the sample digestion and shows good removal rates for organic components.

The samples are placed in beaker. The volume of the beaker must be twice as large as the sample volume because the sample foams up during reaction. Since not only foam but also vapours are generated, the work is carried out under a fume hood. For safety, gloves and goggles are worn when performing the Fenton reaction.

For the digestion per 1 g of sample (dry mass), 5,5 ml deionised water, 10 ml H_2O_2 (30%), 1 ml reagent 1 and 1 ml of reagent 2 (Table 21) are added, the sample is stirred with a glass rod. After digestion for 2-3 days the sample is sieved, rinsed with fresh- and deionised water and dried.

Table 21: Pre-treatment reagents

Reagent	Substance	Molar mass [g/mol]	Concentration [mmol]	Weighing per litre solution [g]	Produces volume. [ml]	Weighing/ volume [mg]
Reagent 1	FeSO4*7 H2O	278.02	2	0.556	100	55.60
Reagent 2	Protocatechuic acid	154.12	2	0.308	100	30.82

Figure 55: Samples before digestion of organics (above left), after Fenton treatment (above right); after treatment, during sieving (below left) and after sieving and oven dried (below right)

Enzymatic treatment

For further removal of the organics which are still present after Fenton reaction, enzymatic treatment is performed. Enzymes were selected based on literature research (e.g. Löder et al., 2017, Campanale et al., 2020) and optimized for river samples according experiences gained during sample treatment within the project.

The following enzymatic treatment protocol is recommended for the removal of organics of Danube river samples. Since samples from rivers do not always have same composition (vegetation

along river, date of sampling, hydrological boundary conditions etc.) the selection of the enzymes should be adapted if necessary. Depending on the mass of plant material the single steps needs to be repeated more than one time. Additional to the laccase and cellulose treatment also an amylase treatment was tested but discarded because of no observed effects.

Enzymes produced by different companies may have different digestion requirements and optima regarding temperature and pH-value, the digestion protocol needs to be adapted accordingly.

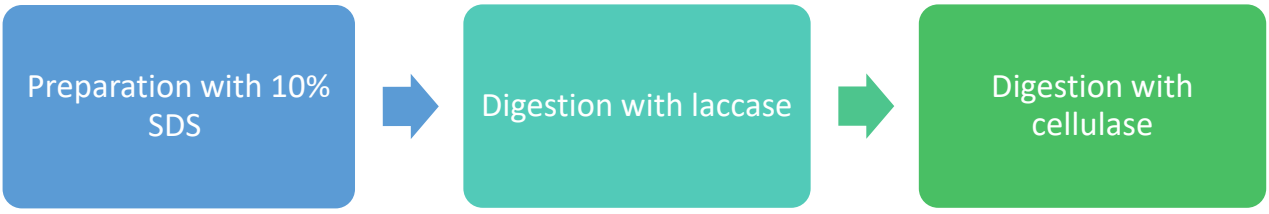


Figure 56: Sequential purification steps during enzymatic treatment

Sodium Dodecyl Sulfate (SDS) Treatment.

The initial incubation is performed using SDS, which is an anionic surfactant. SDS macerates planktonic organisms and animal and plant residues and increased the contact surface for the following enzymatic treatments (Löder et al., 2017).

Performance: 1 g sample is incubated at 50°C in 40 ml SDS (10% strength), after treatment sample needs to be rinsed thoroughly with water before the additional enzymatic digestions. SDS is a denaturing detergent and will destroy added enzymes.

Laccase treatment

The Laccase enzyme is used for the degradation of lignin and a great help for the removal of organics (e.g. Petioles and leaf veins) Zhang et al., 2021.

Performance: 0,1 g sample is incubated at 45 °C for 24 h with 10 ml pH 4 sodium acetate puffer (c = 0,1 mol/L), 5 ml laccase solution (c = 30 U/ml; Laccase F, ASA Spezialenzyme) and 4 ml Hydroxibenzotriazole solution (c = 80 mmol/L).

Cellulase treatment

The cellulase treatment further reduces cell walls and other plant residues.

Performance: 0,1 g sample is incubated at 45 °C for 24 h with 20 ml pH 4,5 sodium acetate puffer (c = 0,1 mol/L) and 10 ml celullase solution (c = 30 U/ml; Cellulase TXL, ASA Spezialenzyme).

Density separation

The sample for density separation is mixed in a beaker with $ZnCl_2$ solution (ρ=1,7g/ml) and dispersed (possibly also briefly in an ultrasonic bath). In a next step the mixture is transferred to separating funnel (Figure 57). To avoid particles sticking in the beaker it should be rinsed carefully several times, because particles are not always well visible to the naked eye. The solution is used sparingly so that the sample volume does not become too large (limited volume in the separating funnel).

After several hours, the settled fraction (= heavy fraction) in the $ZnCl_2$ solution in the separation funnel (mostly inorganic sediments) is gradually drained through the drain tube at the bottom of the separating funnel into a beaker.

The light fraction remaining in the separating funnel is rinsed out with deionized water and separated into two fractions using two sieves with 500 µm and 50 µm sieves, washed thoroughly (collect wash solution and discard in heavy metal waste) dried at 50 °C.

The heavy fraction and the zinc chloride solution will be separated with a 50 µm sieve. The Solution will be collected and reused. The heavy sediment fraction is collected, washed and dried and will be discarded after weighting.

Figure 57: Density separation in $ZnCl_2$-Solution (1.7 kg/l)

Sample splitting – size ranges

The respective detection methods of microplastic particles (i.e. BRUKER LUMOS II FTIR-microscope, BRUKER Alpha ATR-FTIR spectrometer) have certain size ranges where they work optimally. If a microplastic particle is too thick total absorption may occur in case of measurement in transmission mode. Therefore, samples are divided into the fractions 1000-5000 µm, which are

conveniently measurable manually with the ATR-FTIR-spectrometer (BRUKER Alpha) and the fractions 500-1000 µm. As measurements in ATR modus (ATR = Attenuated Total Reflection) occur on the surface of the particles there is no upper limit for sizes. Very thin particles on the other hand (fibres) often result in disrupted spectra.

Within this study for the fraction 500-1000 µm two analytical procedures were compared.

Analytical procedure 1000-5000 µm particles

After pre-treatment, there are still non-plastics in the sample. For the determination of the plastic types with the FTIR-spectrometer, potential plastics of the 1000 - 5000 µm are "picked out" under the microscope and detected by ATR-FTIR spectrometer. The spectra of the investigated particles are matched with databases (within study: BRUKER databases – described in more detail in the Annex) to determine the plastic species. Also, own reference spectra were used during project for comparison (e.g. chewing gum, which is not included within BRUKER database).

Figure 58: Analytical procedure size class 1000-5000 µm

Analytical procedure 500-1000 µm particles

For the fraction 500 - 1000 µm two different approaches were evaluated (method A and B – see Figure 60 and Figure 62) to investigate if cheaper methodologies also lead to reliable results focusing on monitoring in different regions along the Danube river. On the one hand, sub-samples were examined with a powerful, cost-intensive FTIR microscope (BRUKER LUMOS II IMG), on the other hand, a more cost-effective variant was tested by means of "particle picking" under the microscope and subsequent determination of the plastic types by means of an inexpensive and easy-to-operate ATR-FTIR spectrometer (BRUKER Alpha) (method B).

Method A (FTIR-microscope):

The pre-treated oven-dried samples are mixed with deionat and (wait until no more particles stick together) then transferred into a vacuum filtration device, where particles are filtered with an Anodisc-Filter (Whatman, Ø 25mm, pore size 0.2 µm) which is the carrier material of the MPs during measurement with FTIR-microscope.

Figure 59: Vacuum-filtration of the samples (left: whole device, right: view from above of the Anodisc-filter including filter cake)

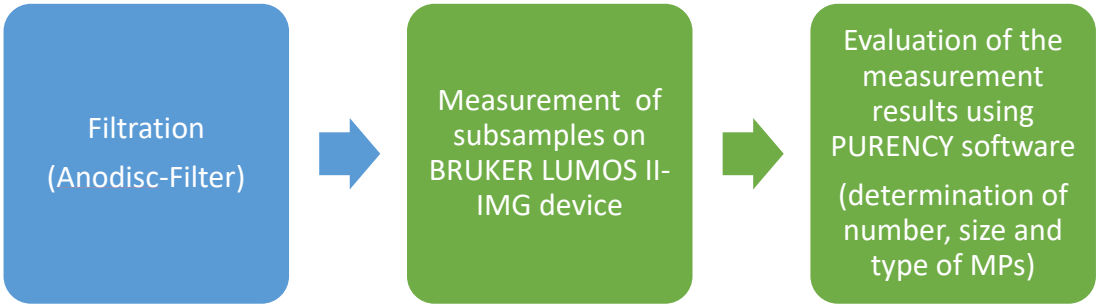


Figure 60: Analytical procedure method A

More detailed information about PURENCY software is provided in the Annex.

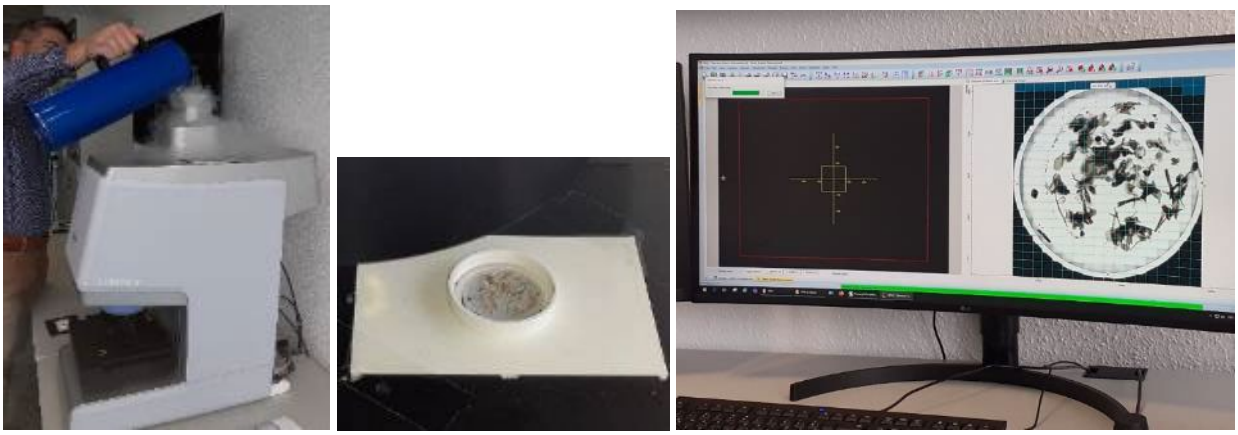


Figure 61: Investigation of MPs with BRUKER LUMOS device

Method B (ATR-FTIR-spectrometer):

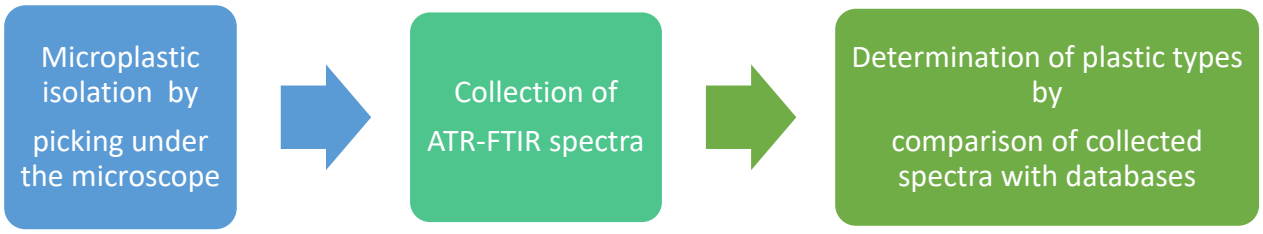


Figure 62: Analytical procedure method B

Figure 64: Sample picking under the microscope and analysis (method B)

Particles of the fraction 500-1000 µm can still be "grasped" with tweezers and examined by means of ATR-FTIR spectrometer. Only in the case of fibres useful spectra may not be collected. Within project, all fibres detected as plastic fibres under the microscope were therefore considered in the microplastic evaluations, but were reported separately due to the uncertainties. Collected spectra are compared with the existing databases (more details in the Annex) and own reference spectra (e.g. chewing gum).

4.4. Protocol for samples taken with pump

The method of sampling results in a laboratory sample of fraction 50-1000 µm. The volume of the water samples has been further reduced by sample splitting in the laboratory then density separation with 1.6 g/cm3 zinc-chloride solution has been applied in the SVGS device (small volume glass separator (Mari et al., 2021)). Organic material content has been oxidised with the use of 30% hydrogen-peroxide and the sample was finally filtered on aluminium-oxide filters (0.2 pore size), to enable FTIR microscopy detection.

The aluminium-oxide filters were analysed by a Thermo Nicolet iN10 MX FTIR microscope to identify polymer types and the number of particles. The spectral data were compared with reference spectra library (https://simple-plastics.eu/) and particles with >80% correlation was

considered as microplastics. The smallest particle size that can be detected during FTIR microscopy analysis was 25 micrometres.

4.5. Protocol for samples taken with sedimentation box

For the collection of sediment samples, the sedimentation box applied in the Joint Danube Survey 4 (see chapter 2) has been applied. The sediment-water suspension collected during the 14 days exposure has been analysed in the laboratory.

While, as mentioned above, large amounts of unwanted organics in particular have to be removed in net samples, inorganic bycatch predominates in sedimentation boxes. Protocol was developed at Institute of Waste management and circularity and WESSLING Ltd.

WESSLING Ltd.:

The sediment suspension samples have been separated in 1.3 g/cm3 calcium-chloride solution in the MPSS (MicroPlastic SedimentSeparator) device. For the large sample volumes in sedimentation boxes with high content of inorganic by-catches $CaCl_2$ solution was used instead of $ZnCl_2$ solution due to environmental and economic reasons. Organic material content has been oxidised with the use of 30% hydrogen-peroxide and the sample was finally filtered on aluminium-oxide filters (Whatman Anodisc 25mm, 0.2 pore size), to enable FTIR microscopy detection. The filter is measured with a Thermo Nicolet iN10mx FTIR microscope, with a linear array detector in transmission mode, where the pixel size is 25 µm. The collected data are then evaluated with the „siMPle“ software where polymer types, particles sizes and numbers are recorded

ABF-BOKU:

In contrast to WESSLING Ltd., ABF-BOKU did not perform sample preparation with a suspended sample, but with the sample that was drained over the 50 µm sieve. Also, despite of high amounts of sediments the more expensive $ZnCl_2$-solution was used for density separation. To keep environmental impact low, the solution was recovered after use. In principal, the sedimentation box samples protocol of ABF-BOKU contains the same treatment steps as the net samples protocol (except enzymatic treatment). However, the sequence is different. Due to the dominance of inorganic impurities, the density separation is ranked first to significantly reduce the sample volume. Due to a high amount of plant fibres (in Danube river samples, plant fibres are found very often - most likely cotton fibres from poplars) sediment particles may be entangled in the sample matrix and cannot be removed sufficiently with only one step. Therefore, density separation is repeated after Fenton treatment, where organic by-catches are removed. As last step further organic by-catches are digested by enzymes.

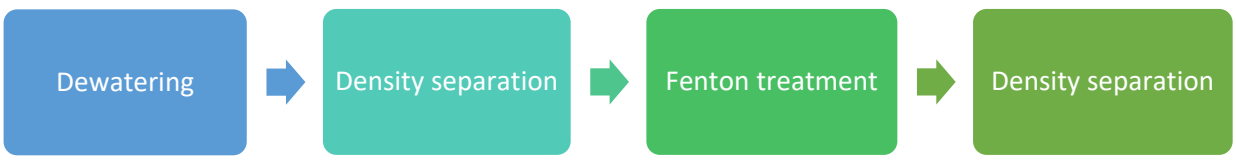


Figure 65: Sample pre-treatment steps of sedimentation box samples

5. Results

5.1. Analytical results

5.1.1. Results from samples taken with nets

Number of microplastics in the size range from 500-5000 µm in net samples was in the same range at all locations and counted up to 4 MP per m^3 (mean values of three nets per location, except for Tutrakan where only in the middle of the water column a sample was taken) in the Danube river and > 8 MP/m^3 in the Tizsa river (Titel). Considering all evaluated fractions, no increase of MP concentration downstream was detected (Figure 66). Looking at the shape of MPs, it turns out, that fibers are very much involved in pollution.

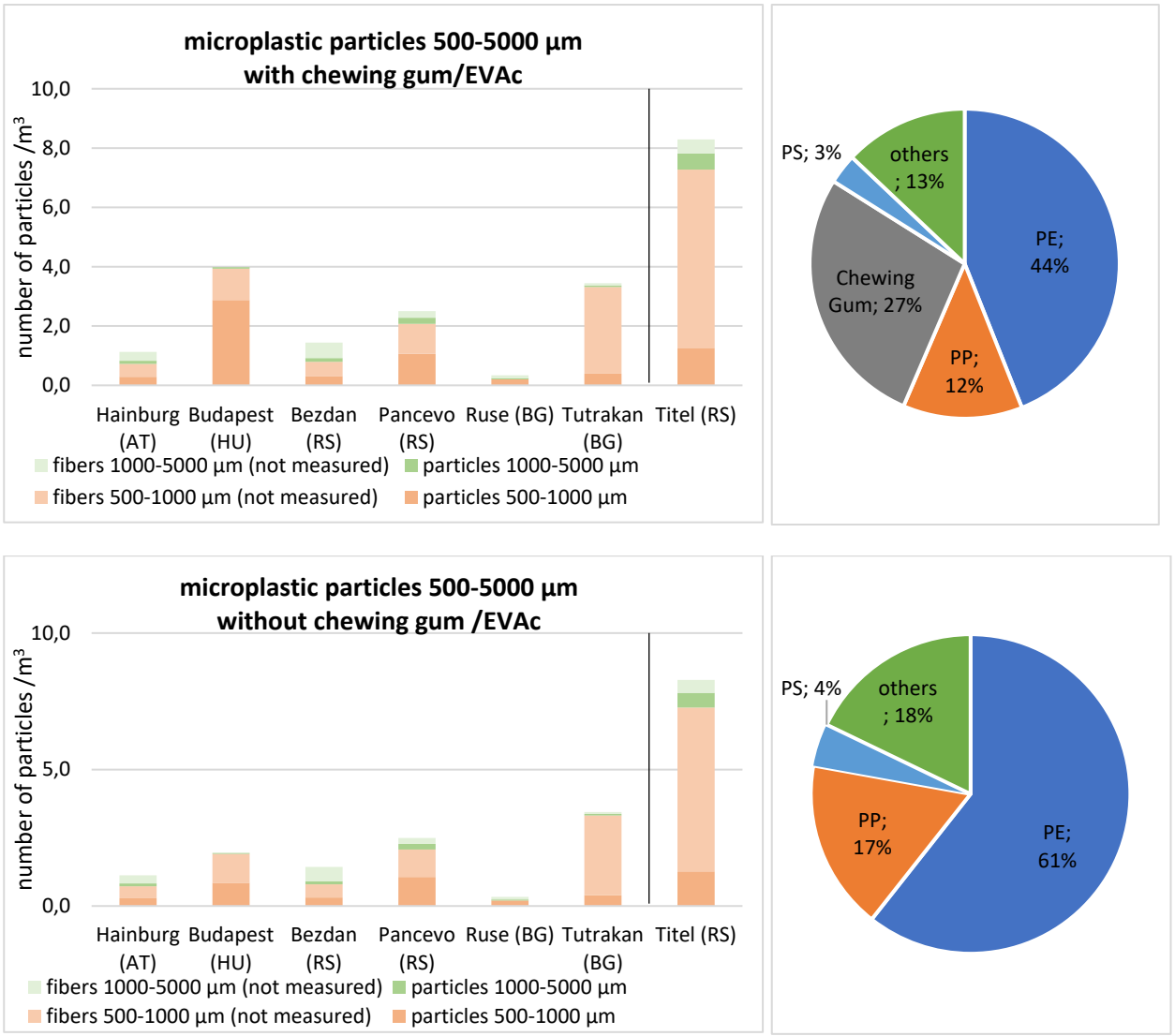


Figure 66: Number of microplastic particles in net-samples (left), proportion of microplastic particles in net samples (right) (above: including chewing gum, bottom without chewing gum) (both: mean values of three net samples at each location)

Looking at the type of plastic, it is obvious that the pollution is mainly caused by PE. PP accounts for the second largest share of pollution and is followed by "other" plastics. Looking at the

proportion of plastic types, chewing gum seems to cause considerable pollution of the Danube river. At a closer look at the data, it turns out that chewing gum was sampled out of the middle of the water column in Budapest. It is not entirely clear whether chewing gum has already been caught as microplastic or whether, for example, a whole piece of gum has been fished out of the water and later degraded during removal from sampling net or sample pre-treatment. Probably a whole piece of chewing gum has been in the net, which has decomposed during the sample pre-treatment and has entered the statistics in the form of numerous microplastic particles.

With smaller particle size, the number of particles increases. Differences in the plastic type distribution are not observed Figure 67.

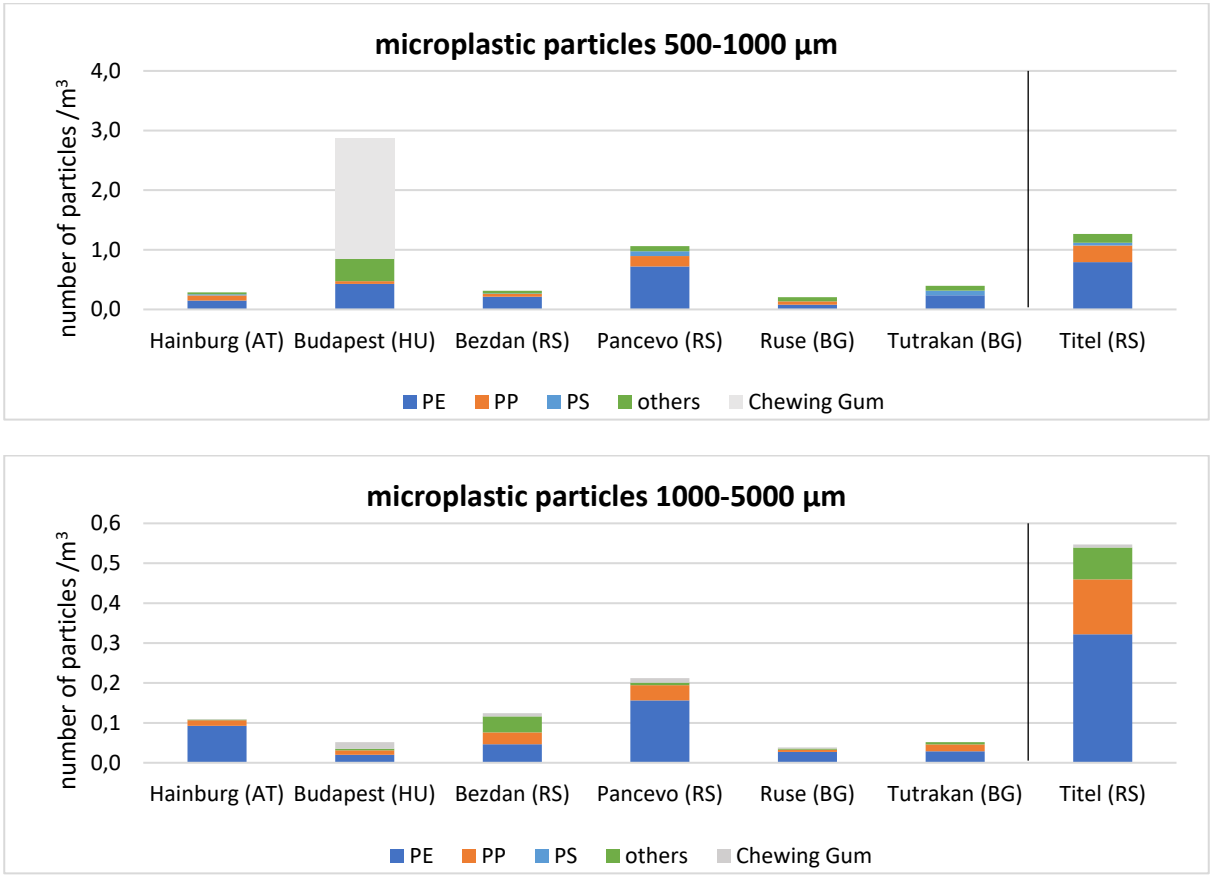


Figure 67: Number of microplastics in the size fraction 500-1000 µm and 1000-5000 µm divided into detected plastic types

In Figure 68 microplastic types are depicted differentiating the three sampling heights in the water column, which are also assigned to the three sampling points across the river section (surface: middle, middle: right shore, bottom: left shore)

In terms of both particle number and plastic distribution, no significant differences in water depth can be identified in these few samples. In Titel (UA) most of the microplastics was detected in the surface and in the middle of the water column, whereas in Pancevo (RS), the water column near the riverbed was more polluted than above it. Whether this is related to the types of plastics present there or to the hydraulic conditions is not known. Looking at the plastic

types, it seems that close to the riverbed PP (ρ= 0.9-1.0 g/cm^3) is more present than above, while PE (ρ= 0.92-0.96 g/cm^3) is relatively evenly distributed throughout the water column.

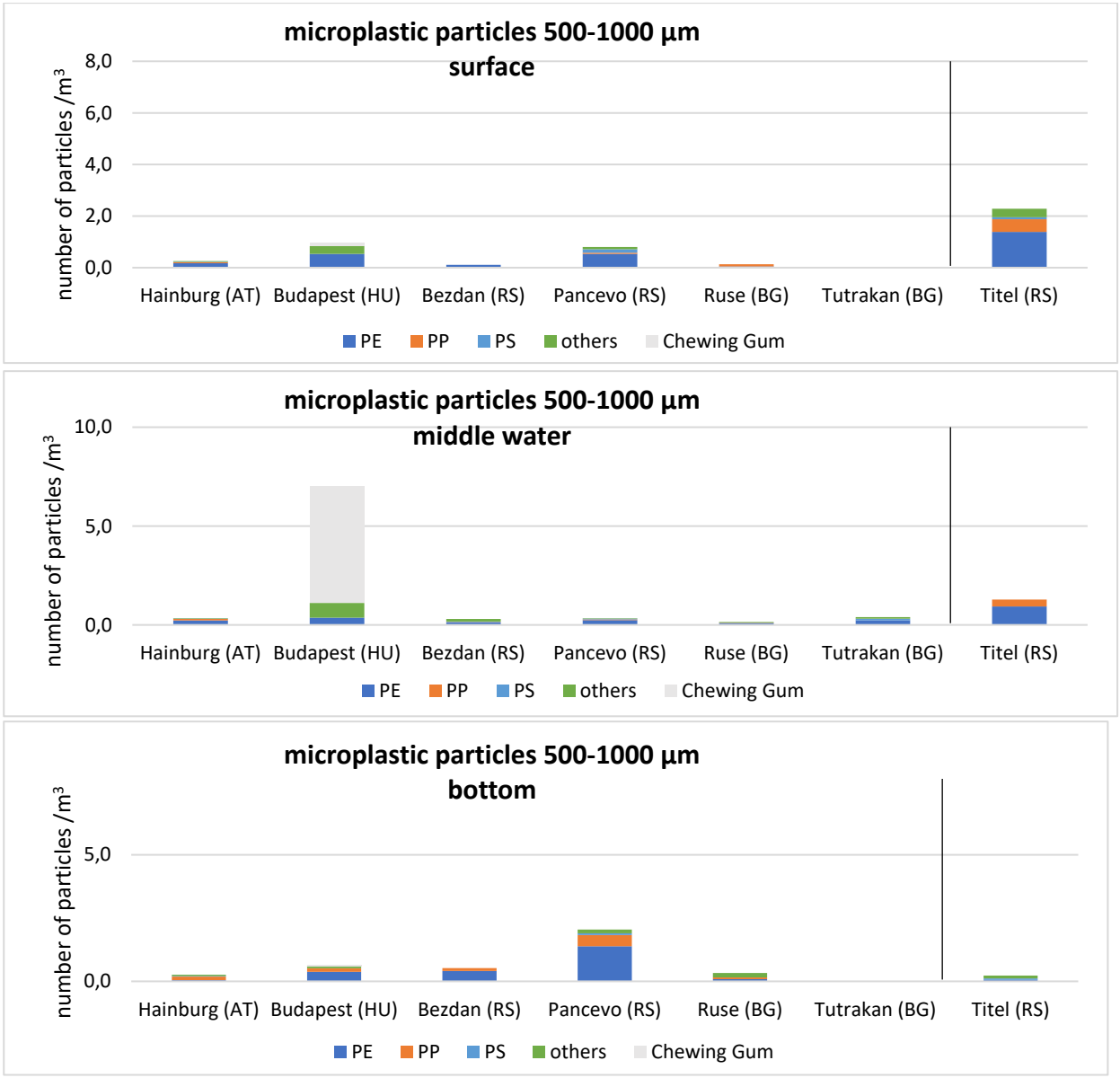


Figure 68: Microplastic particles (size fraction 500-1000 µm) distribution of plastic types in different water depth (in Tutrakan only in the middle of the water column sampling was performed)

Also with regard to the shape of microplastics, no significant differences with increasing water depth could be observed (Figure 69).

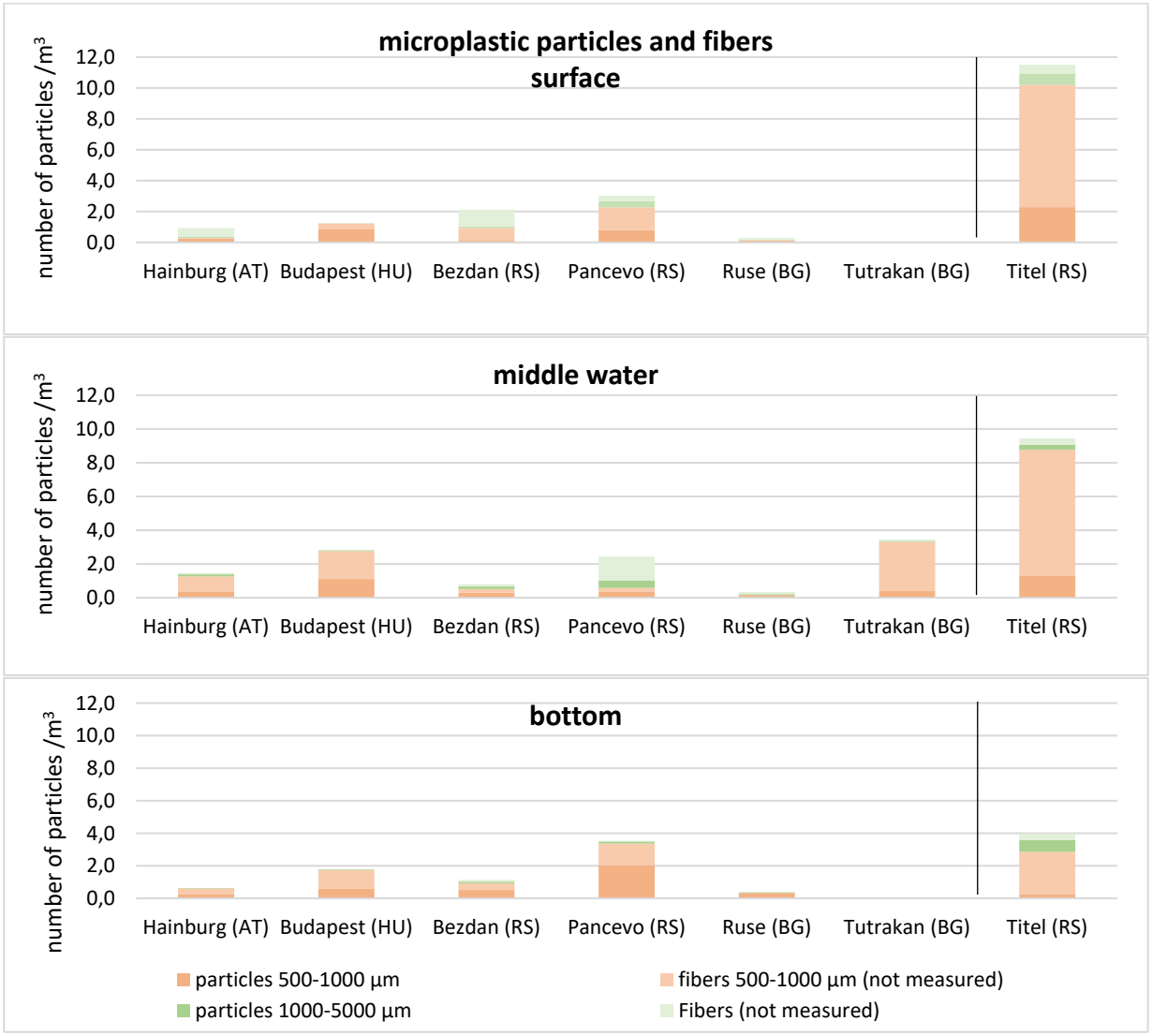


Figure 69: Distribution of particles and fibers (size fraction 500-5000 µm) in different water depth (in Tutrakan only in the middle of the water column sampling was performed) (without chewing gum)

5.1.2. Results from samples taken with pump

Danube

Number of MPs detected cover a broader size range with pump methods are higher than with net-method. However, it must be considered that more smaller particles are detected than with the net method.

Particle numbers identified in water samples are represented in Table 22 and in Figure 70 per polymer type and projected on sample volume. Polymer type distribution in all samples is also represented in Table 22. The distribution of the MPs over the water column is also shown. Differences between the results in sample depth (regarding all locations) are shown in and in Table 23 and Figure 71.

Table 22: Microplastics in water samples of the Danube (CROSS: continuous sampling along the entire cross section on the surface, KF: cross section centre, surface sampling, JK: close to right bank, middle of the water column, BA: close to left bank, bottom layer sampling).

Sample data	Country	Austria				Hungary				Serbia								Bulgaria					
	Location	Hainburg				Budapest				Bezdan				Pancevo				Ruse				Tutrakan	
	Sampling spot	Cross section	Middle point, surface	Right bank, middle water	Left bank, bottom	Cross section	Middle point, surface	Right bank, middle water	Left bank, bottom	Cross section	Middle point, surface	Right bank, middle water	Left bank, bottom	Cross section	Middle point, surface	Right bank, middle water	Left bank, bottom	Cross section	Middle point, surface	Right bank, middle water	Left bank, bottom	Cross section	Right bank, middle water
	Sample code	**AT-CROSS**	**AT-KF**	**AT-JK**	**AT-BA**	**BP-CROSS**	**BP-KF**	**BP-JK**	**BP-BA**	**BEZ-CROSS**	**BEZ-KF**	**BEZ-JK**	**BEZ-BA**	**PAN-CROSS**	**PAN-KF**	**PAN-JK-100**	**PAN-BA**	**RU-CROSS**	**RU-KF**	**RU-JK**	**RU-BA-100**	**TUT-CROSS**	**TUT-JK**
Sample volume (L)		1439	1301	1617	1504	1973	1564	1787	1022	1169	1241	1314	1301	1463	1303	1306	1423	1380	1624	1500	1516	1743	1892
MPs/m3	PE	19,5	26,9	19,2	4,0	62,3	1,3	10,1	6,8	18,0	14,5	8,4	79,9	190,7	117,4	45,7	75,9	2,2	6,2	6,0	9,2	4,0	5,8
	PP	2,1	4,6	7,4	0,0	1,0	18,5	0,0	0,0	2,6	7,3	5,3	2,3	4,1	10,7	7,5	1,4	3,6	3,7	8,0	6,0	6,9	6,3
	PS	3,5	1,5	1,9	0,7	0,5	1,3	0,0	2,0	3,4	1,6	2,3	1,5	1,4	3,8	0,8	3,5	0,0	0,0	0,7	0,0	0,0	2,1
	Polyester	0,0	0,8	0,0	0,0	0,5	0,0	0,0	0,0	0,0	0,0	0,8	0,0	0,0	0,0	0,0	0,0	0,0	1,2	0,0	0,0	0,0	0,5
	ABS	0,0	0,0	0,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,7	0,6	0,0	0,0	0,6	0,5
	PU	0,0	0,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	PA	0,0	0,0	0,0	0,0	0,0	0,6	0,0	0,0	0,0	0,8	0,0	0,0	0,0	0,0	1,8	0,0	0,0	1,2	0,0	0,0	0,0	0,0
	Sum MP	**25,0**	**34,6**	**29,1**	**4,7**	**64,4**	**21,7**	**10,1**	**8,8**	**24,0**	**24,2**	**16,7**	**83,8**	**196,2**	**132,0**	**55,8**	**80,8**	**6,5**	**12,9**	**14,7**	**15,2**	**11,5**	**15,3**

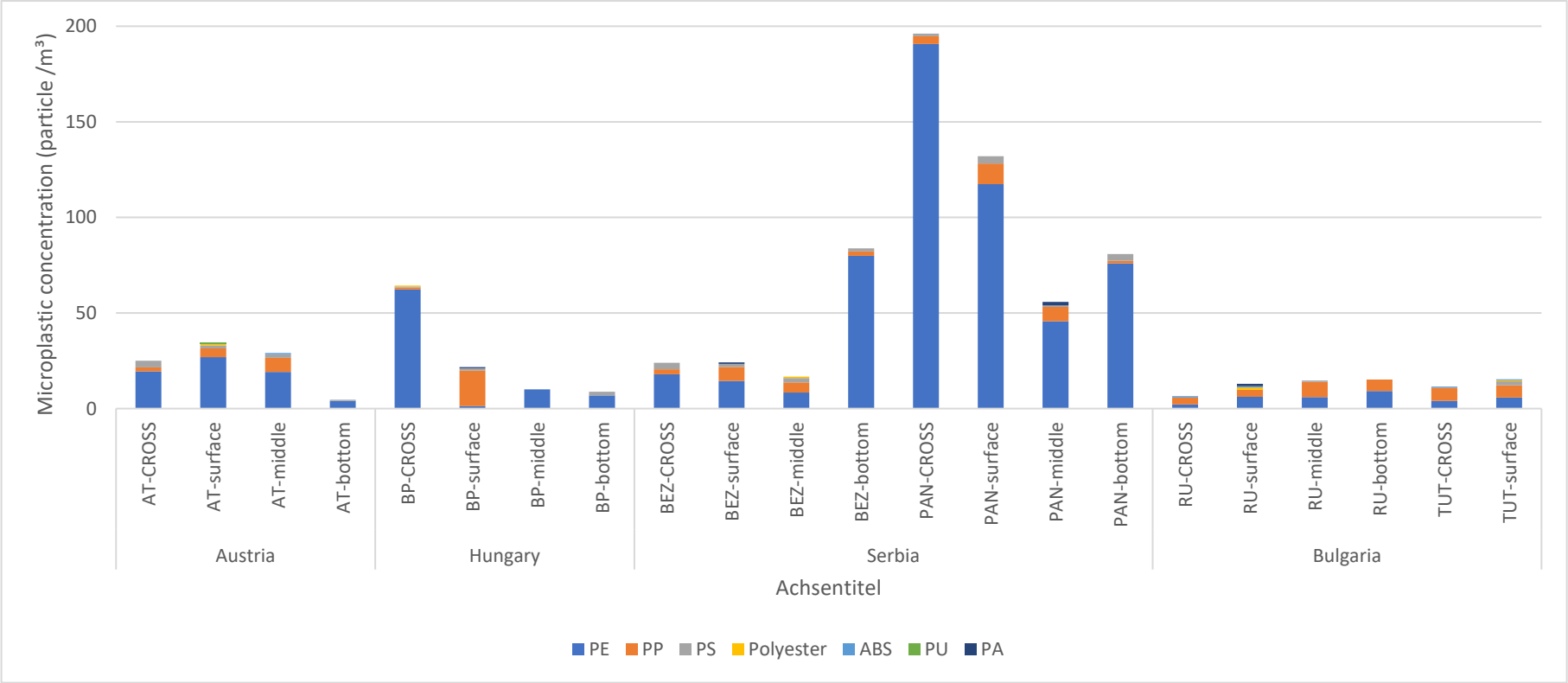


Figure 70**:** Microplastics in water samples of the Danube (CROSS: continuous sampling along the entire cross section on the surface)

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Table 23: Average microplastic concentration in different sampling depth (Danube).

		Cross section	Middle point, surface	Right bank, middle water	Left bank, bottom
MP/m3	PE	49,4	28,7	17,9	35,2
	PP	3,4	8,5	5,7	1,9
	PS	1,5	1,7	1,1	1,5
	Polyester	0,1	0,4	0,2	0,0
	ABS	0,2	0,2	0,1	0,0
	PU	0,0	0,1	0,0	0,0
	PA	0,0	0,4	0,4	0,0
	Sum of MP	**54,6**	**40,1**	**25,3**	**38,7**

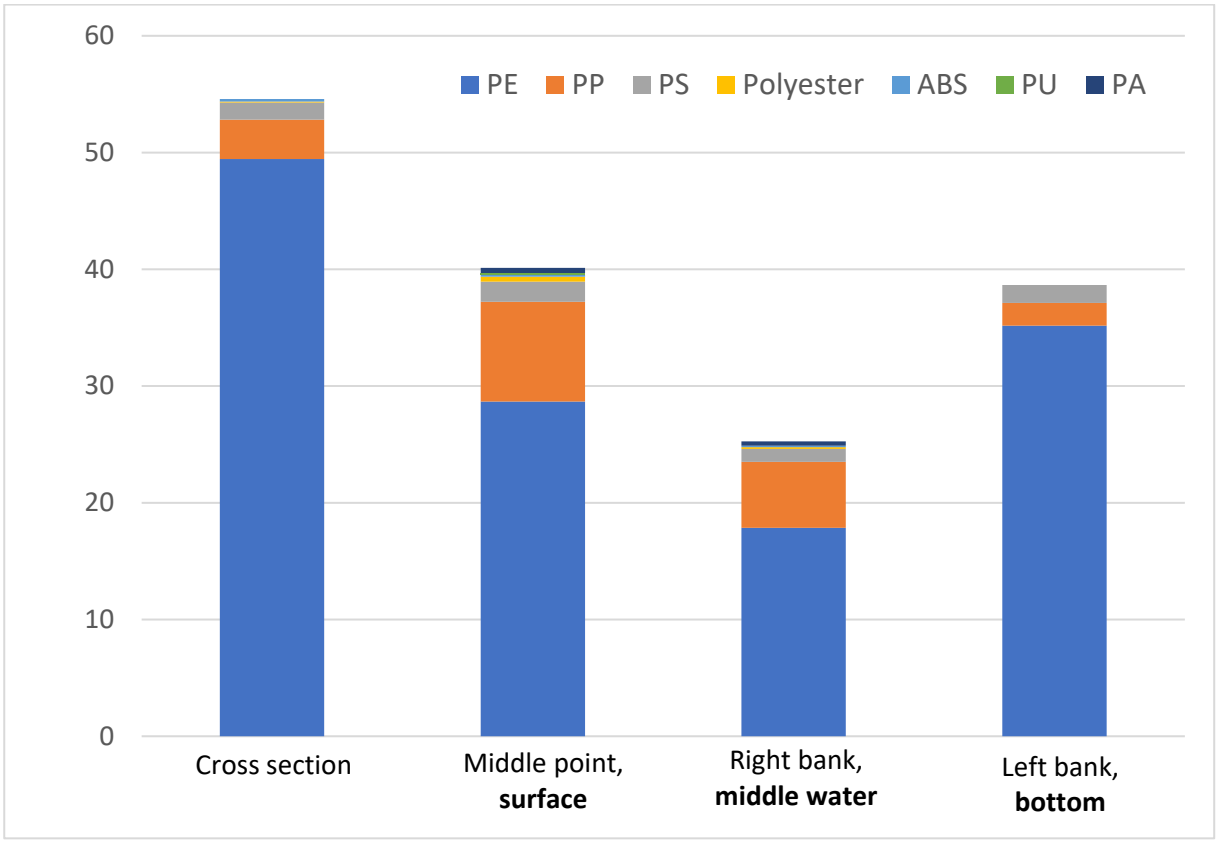


Figure 71: Average microplastic concentration in different sampling depth (Danube).

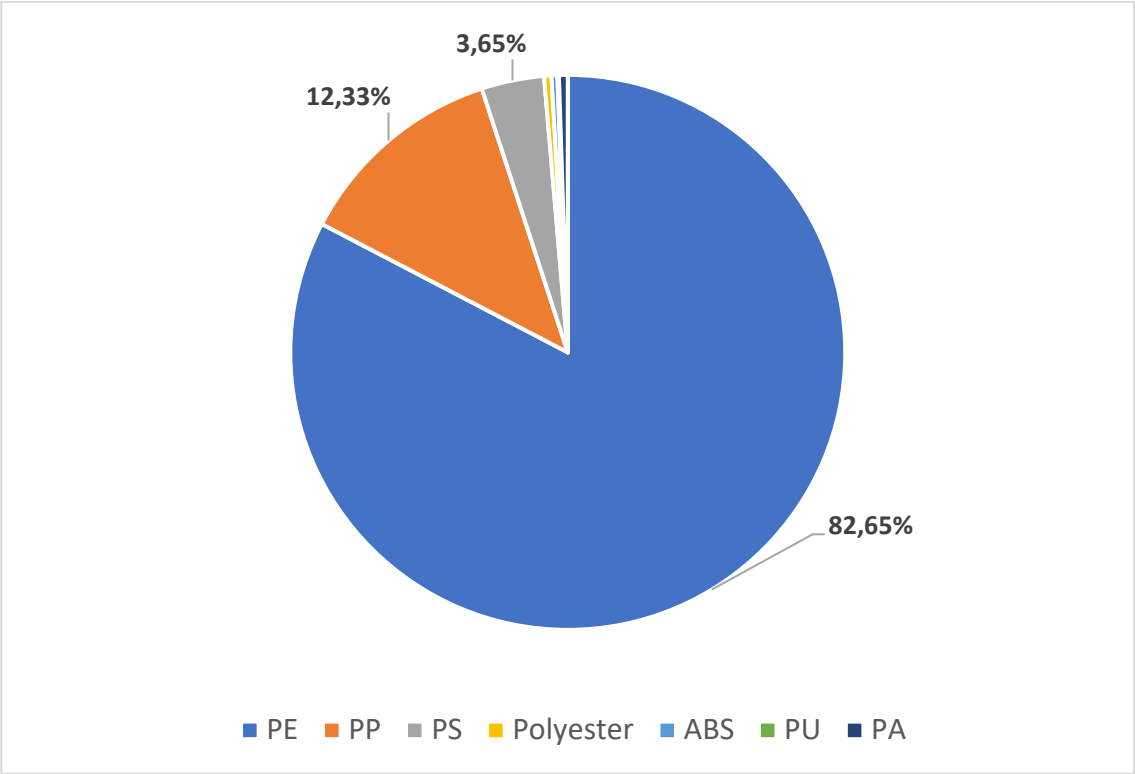


Figure 72: Polymer type distribution in water samples (Danube).

Tisza (water samples)

Water samplings on river Tisza was focusing on one hand on the Hungarian river stretch, and on the other hand samples have been collected in the Tisza River estuary at Titel, Serbia. Hungarian sampling points have been designated in strong cooperation with the General Directorate of Water Management, considering national monitoring spots and local Water Directorates capacities as well, because boat for the samplings have been rented from them. Designated points are:

- Zemplénagárd (AEQ057): below the ferry at Tuzsér, sample code: TUZS
- Tokaj: downstream Bodrog estuary at Mező street, sample code: TK
- Kisköre: upstream the hydropower plant, sample code: KK
- Tiszasziget (AEQ056): at the border, sample code: TSZ

Exact coordinates of the sampling spots are presented on the sampling reports. At the above locations 3 sampling campaigns have been carried out on a way, that on the same location samples were taken every 3 weeks. These repetitions ensure better representativity and reducing the possible failure in data. Samples were taken with a continuous sampling along the entire cross section on the surface (please refer to “CROSS” sampling method at the Danube River samples).

Particle numbers identified in water samples are represented in Table 24 and in

Figure 73 per polymer type and projected on sample volume. Polymer type distribution in all samples is represented in Figure 74.

Table 24**:** Microplastics in water samples of the Tisza.

Sample data	Country	Hungary												Serbia			
	Location	Tuzsér			Tokaj			Kisköre			Tiszasziget			Titel			
	Sample code	**TUZS-1**	**TUZS-2**	**TUZS-3**	**TK-1**	**TK-2**	**TK-3**	**KK-1**	**KK-2**	**KK-3**	**TSZ-1**	**TSZ-2**	**TSZ-3**	**TIT-CROSS**	**TIT-KF**	**TIT-JK**	**TIT-BA**
	Sample volume (L)	2087	2046	1892	1963	2004	2004	2004	2002	2081	2002	2001	2009	1516	1503	1005	644
MPs/m3	PE	35,8	24,9	1,1	1,0	1,0	0,5	1,5	0,0	0,5	4,0	5,0	1,5	38,9	22,0	33,8	20,2
	PP	5,9	20,0	3,2	3,6	1,0	0,0	4,0	2,0	4,8	2,0	9,0	1,0	11,2	11,4	5,0	15,5
	PS	7,2	4,7	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,5	0,0	2,6	0,7	6,0	4,7
	Polyester	0,0	2,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,7	0,0	0,0	1,6
	ABS	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,0	0,0	0,0	0,0	0,0
	PU	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
	PA	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,7	0,0	0,0
	Sum of MP	**48,9**	**52,5**	**4,8**	**4,6**	**2,0**	**0,5**	**5,5**	**2,0**	**5,3**	**6,0**	**16,0**	**2,5**	**53,4**	**34,7**	**44,8**	**41,9**

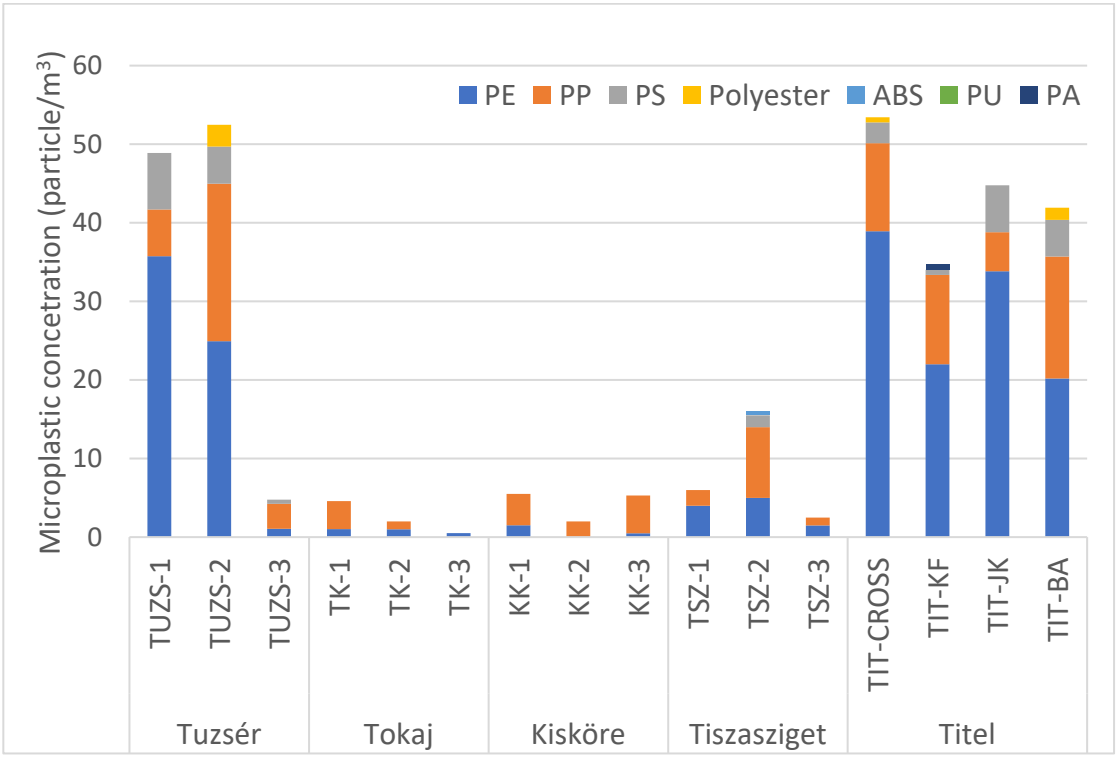


Figure 73: Microplastics in water samples of the Tisza.

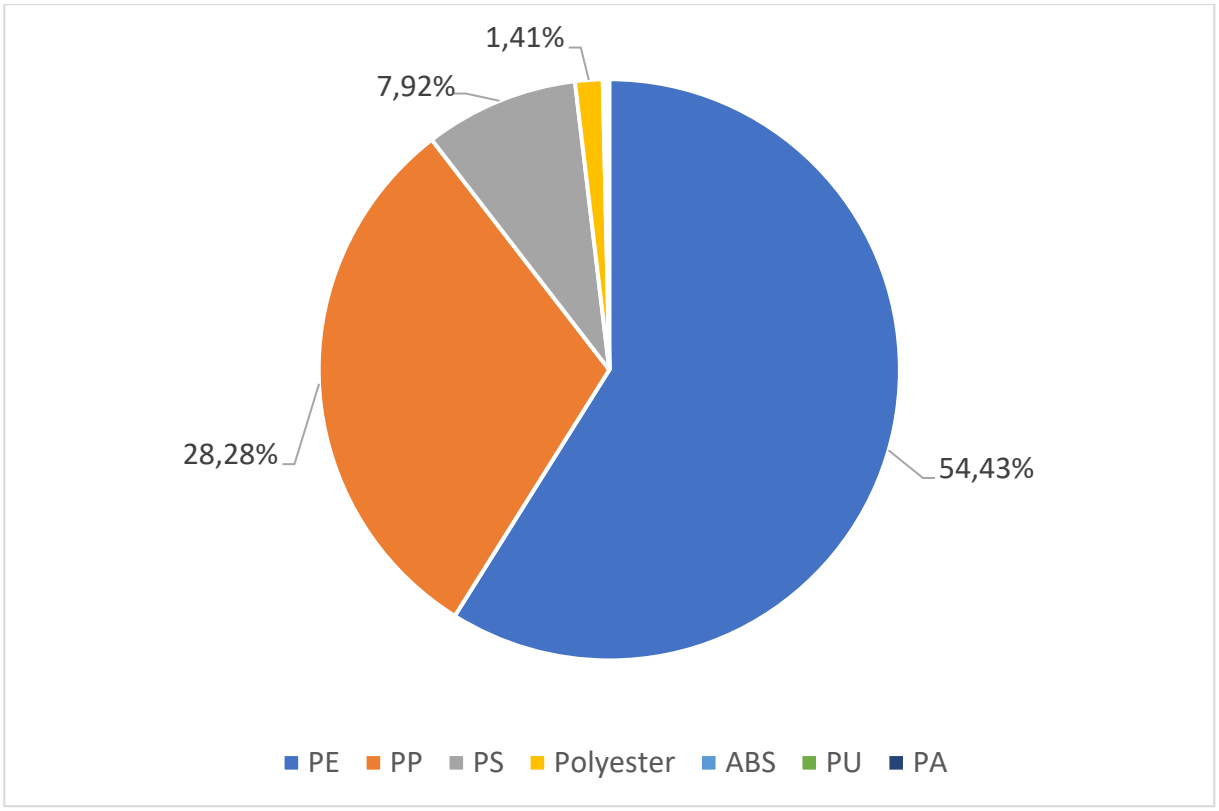


Figure 74: Polymer type distribution in water samples (Tisza).

5.1.3. Results from samples taken with sedimentation box

Samples collected in the sedimentation box are more integrated in time (14 days exposure) compared to water samples, but due to the lack of sample volume (water flow) measurements results cannot be projected on sample volume. It is also not possible to project the results on a weight basis, as samples are prepared in a form of a suspension (compare 2.1.1). Reporting on sample mass basis can be improved in the future, as after thorough homogenisation dry matter content of a representative subsample can be measured. During this process an issue might arise from the non-homogeneous distribution of MP particles in the sediment suspension, that might result in non-homogeneous subsamples.

Particle numbers identified in sediment samples are represented in Table 25 and in Figure 75 per polymer type per sampling location. Figure 75 depicts results from Sedimentation-box survey in 2019 (JDS4 – Kittner et al., 2022). Results also show plastic type distribution. Unlike current study, only low shares of PS were detected. Main detected plastic type was PE. Polymer type distribution in all samples of current study is represented in Figure 77.

Table 25: Microplastic content of sediment samples of the Danube river.

Sample data	Country	Austria	Hungary		Serbia	Bulgaria
	Sampling location	Hainburg	Budapest		Bezdan	Ruse
	Sample code	**AT**	**BP-1**	**BP-2**	**BEZ**	**RUS**
MPs/sample	PE	46	17	10	39	15
	PP	34	38	0	19	25
	PS	175	4	8	16	9
	Polyester	0	0	0	0	0
	ABS	2	0	0	3	0
	PU	0	0	0	0	0
	PA	0	0	0	0	0
	Sum of MPs	**257**	**59**	**18**	**77**	**49**

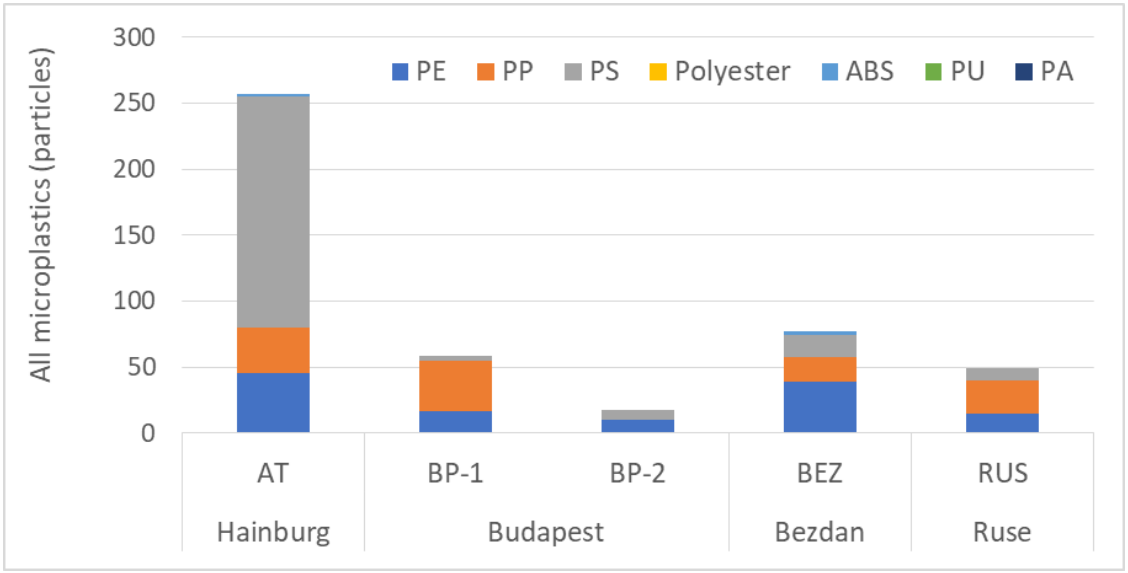


Figure 75: Microplastic content of sediment samples of the Danube river.

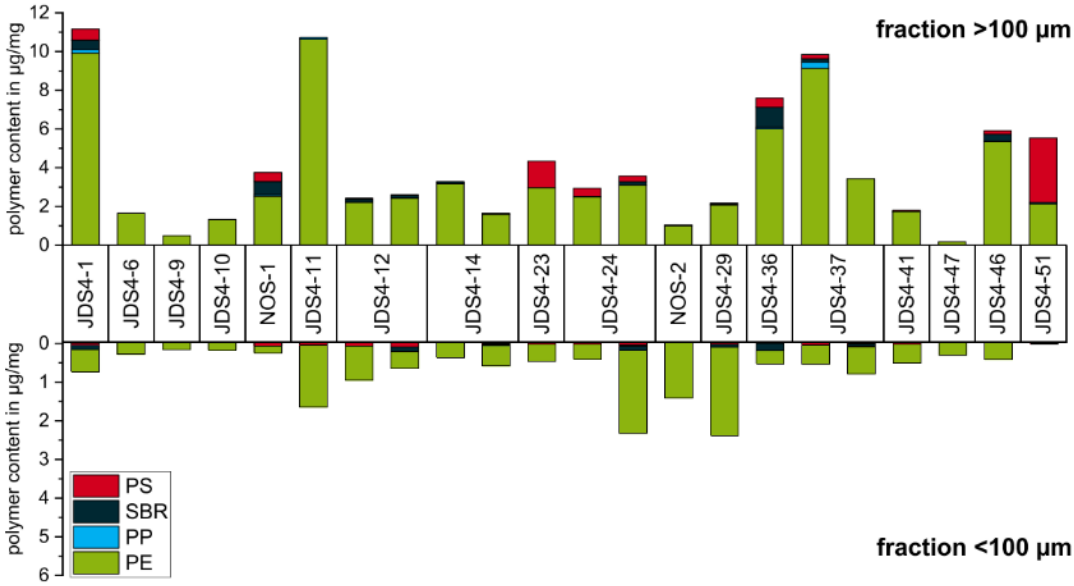


Figure 76: Detected MP contents in the samples from JDS4 survey whose contents were above the corresponding LOD values (Kittner et al., 2022)

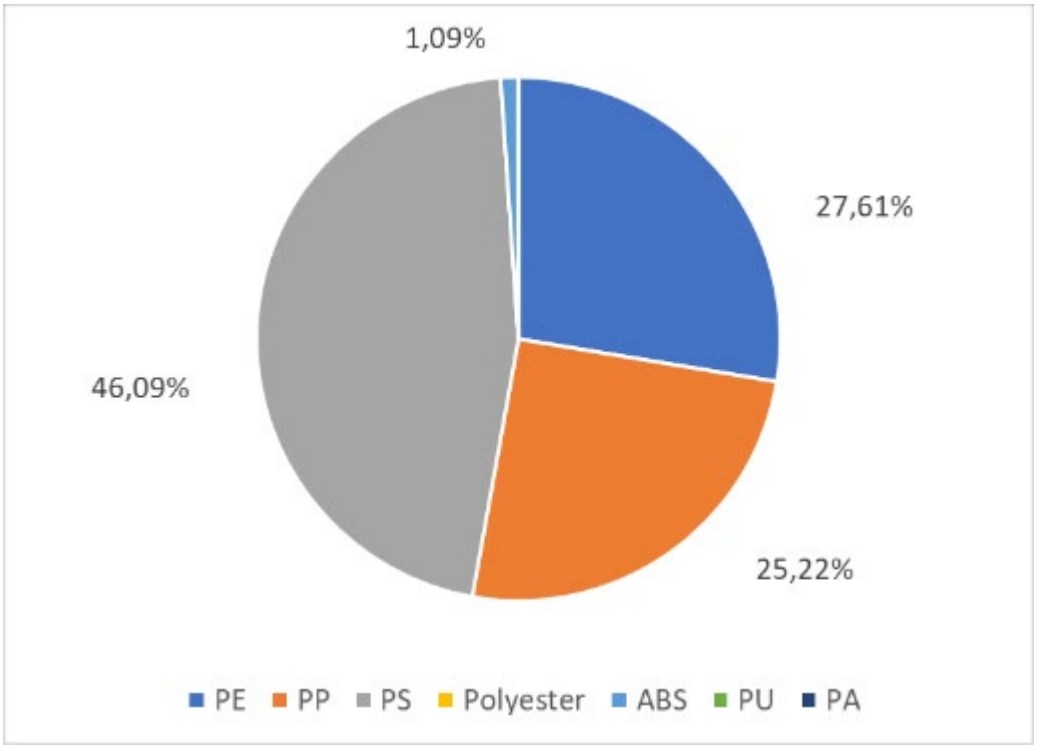


Figure 77: Polymer type distribution in sedimentation box samples (Danube).

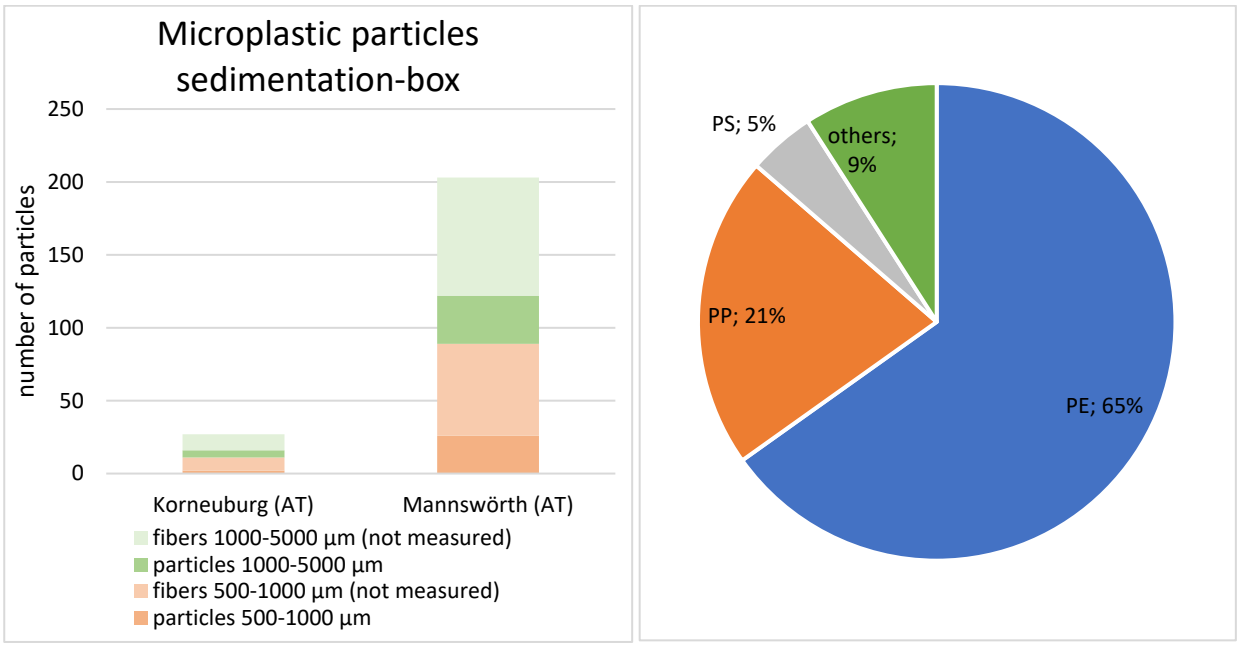


Figure 78: Microplastic content of sediment samples of the Danube (Korneuburg) and Schwechat (Mannswörth) river (left) and polymer distribution

The MPs in Korneuburg and Mannswörth (AT) were analysed by ABF-BOKU whereas all other locations were analysed by WESSLING Ltd. Particle numbers in Hainburg are higher than at the rest of the locations but can be compared with particle numbers of Mannswörth. The difference between Korneuburg (Danube river) and Mannswörth (Schwechat) could possibly be explained due to a higher pollution situation in Schwechat, but needs to be further investigated.

Samples investigated by WESSLING have a higher proportion of PS than those analysed by ABF-BOKU. This is mainly influenced by the high amount of PS in the Austrian Hainburg sample. Conversely, the proportion of PP in the BOKU samples is much higher. PE is in the same range. To estimate whether this is random, due to the pollution situation at the sites or due to the sample preparation method more samples have to be taken. Previous evaluations (JDS4 – see chapter 2 and Figure 76) showed a different picture with the predominant presence of PE in the sedimentation boxes. PS in JDS4 study was only in sample from Ukraine in higher shares (JDS4-51 = Vilkove- Chilia UA).

6. Comparison of evaluated sampling and analytical methods

6.1. Comparison of sampling procedure (practicability, costs etc.)

In the following performed sampling methods are compared considering important aspects like practicability or costs. These aspects of the respective methods may be assessed differently in the individual partner countries. On the whole, however, the advantages and disadvantages can be well distinguished from each other for the entire Danube region. In Table 26 the comparison of the aspects is summarized.

In terms of practicality (handling) of the sampling device, the net method is probably the most cumbersome. Due to the enormous dimensions of the nets (60x60 cm) high flow velocities and turbulences are a big challenge during measurements. With measuring device in this design measurements are only possible with a certain water depth (at least 2-3 m). Both the pump method and the sedimentation box perform significantly better than the heavy, very large nets. Most practicable method is the pump method. It turned out to be a clean, uncomplicated and practicable MP sampling technique. The effort can be classified as low. The setup of the measuring device is very user-friendly, the filter cylinders are quickly assembled and the pump is connected immediately.

The duration of the measurement for the net and the pump-method (30 to 40min) is only slightly lower than with the net (~45 min). But, the preparation time for measurements with nets is longer than for sampling with pump. Also, it is necessary to thoroughly clean the net after each sampling due to the “sticky” deposit. Sampling with pump can be performed after a short preparation time. Sampling with the sedimentation-box takes the longest time in total, but it is not necessary to control the measurement during two weeks, but you have to visit the sampling location twice. Time for installation and removal of the box is low.

Table 26: Comparison of sampling methods

	Net-sampling	Pump-method	Sedimentation box
Practicability/ handling			
Duration of preparation, measurement and cleaning			
Sampling requrements and costs			
Necessary skills			
Official approvals (e.g. bridge sampling necessary)			
Representative sampling over water column			
Representative sampling over the river cross section			
Captured particle size range	(250) 500-5000 µm	50 µm -1000 µm	< 1cm
Sampled water volume per sample (m^3)			 unknown

Especially the costs of the measurements differ a lot between the partner countries and also have to be adapted to the current economic situation. Sampling requirements also vary, depending on the boundary conditions of the various countries. In the Annex costs and requirements for equipment are listed in detail for project period. In terms of costs and requirements the sedimentation box is the best option, whereas net sampling is most expensive method. Also, sampling with pump is due to fees for shipping inspectorate and rental fees for boats quite high in most countries. The high costs of net sampling result from the high number of working hours and also from fees for the inspectorate and the rental of truck and crane.

Table 27: Comparison of estimated total working hours (personnel times working hours per working step) and total sampling costs for above described measurements with net, pump, sedimentation box in partner countries

Method	Estimated working hours in Austria	Estimated total Costs in Austria in 2021	Estimated working hours in Hungary	Estimated total costs in Hungary in 2021	Estimated working hours in Serbia	Estimated total costs in Serbia in 2021	Estimated working hours in Bulgaria	Estimated total costs in Bulgaria in 2021
Net-sampling (bridge)	58 h	€ 3,315	56 h	€ 2.153	78 h	€ 9.940	58 h	€ 3.091
Pump-method	26 h	€ 2,645	26h	€ 766	25h	€ 600	26h	€ 3.460
Box-sampling without boat	14 h	€ 350	17 h	€ 270	13h	€ 150	14 h	€ 310
Box-sampling with boat		€1.785		€ 540		€ 450		€ 370

In terms of necessary skills, sampling with net is most demanding method, which requires experience and knowledge that is not necessary for measurements with pump or box. Although, no special knowledge is necessary for sampling with pump, it nevertheless requires a short instruction.

Required permits (e.g. for measurements by truck including crane from bridges or a stable ship) are considered as disadvantage of the net method. In Austria e.g. where the measurements were performed from the bridge, in addition to the temporary road closure (at least one lane), which requires approval by the responsible district administrative authority (district administration/magistrate or municipal office), the measurement is notifiable to the shipping inspectorate, which is responsible for the safety of shipping traffic. Depending on the duration of the measurement campaign (hours to days) and the frequency (multiple measurement per month), it takes longer or shorter to obtain the necessary permissions. A lead time of at least two to four weeks for one measurement should be included. Due to the space-saving equipment, sampling with pump is also possible from a smaller boat Permits for measurements by small boats (pump) or installation of the box at already floating bodies are easier to get.

Captured particle sizes are > 500 µm (>250 µm) for net samples, 50-1000 µm for pump samples and < 1000µm for sedimentation box samples. Sampling with 250 µm nets and under certain boundary conditions even 41 µm have been performed in a previous study (Liedermann et al., 2018) but the analysis of filtration efficiency and side-by-side measurements with different mesh sizes showed that 500 µm nets led to optimal results. A disadvantage of net sampling is the downward limitation of the size distribution. Too small mesh sizes lead to rapid clogging of the net. A 41 µm net was also previously tested (Liedermann et al., 2018) and data were successfully gathered at lower discharges, but failed at higher discharges as high suspended sediment and

organic loads stressed the net material. At flow velocities of around 2–3 m/s for the mean flow condition, the application of meshes that are too fine is not feasible.

The high infiltrated water volume of net sampling in a short time (45min ~ 3000m³ per net) allows a comparatively high sample volume. Integrated flow measurements allow a calculation of the plastic load (number of particles /m^3) in the river. The sample volume of the pump is about 1,000-2,000 litres in 30 to 40min (see sampling protocols from AT), depending on the concentration of suspended solids and the filter sizes used. The sample volume taken can be read from a water meter. Box The box itself does not seem to be isokinetic (possible deflection of particles), furthermore only the MP concentration at the water surface at a single point in the river cross section is considered. While the "filtered" discharge or sample volume can be determined for the other two sampling techniques, this is not possible for the Sedimentation box due to the lack of measuring instruments (e.g., flowmeter or water meter). Transport volumes can therefore also not be estimated. In summary, this method is very simple and quick to implement, but its validity is limited to the sole detection of surface floating microplastic contamination with a temporal resolution.

In terms of sampling representativeness, net-method and pump-sampling perform better than the sedimentation-box. The applied net-method generally allows measurements at all points of the river cross-section, but no composite samples across the cross-section (nets cannot be moved during the measurement). Simultaneous sampling at different depths, as well as sampling with two different net diameters at the same depth is possible. Simultaneous sampling at different depths at the same measuring point is certainly important for comparing the results. Construction of sampling equipment allows to place the first level of net directly below the water surface, as well as at two more depths (preferably at medium depth and at the bottom) up to a maximum depth of about 10 meters due to construction reasons.

The pump can be used at any point in the river (multi-point sampling), and can in principle also include the depth variance or the spatial distribution in the cross-section. However, a multipoint measurement under these aspects would take a lot of time (3 times compared to the net method since the measurements are performed sequentially and not in parallel; for parallel measurements 3 pumps are needed). For deeper locations (riverbed), however, a special fixture would have to be used to hold the suction tube in position as well. Otherwise, the hose would be carried away by the current. Due to the 1 mm pre-filter on the intake, larger MP particles (up to 5mm) are not considered. However, modifications for a larger prefilter would theoretically be possible.

Sampling with the sedimentation box is only possible outside of shipping channels and close to the water surface due to the measurement period of up to two weeks (single-point measurement).

The advantages and disadvantages of the three evaluated methods are briefly summarized:

Sampling of microplastics with **multi-depth-net device** turned out to be the most complex procedure, primarily in terms of providing the necessary conditions (a vessel of larger dimensions equipped with a crane, official approvals etc.), as well as a long-term procedure of cleaning the nets after sampling. The applied method with net enables simultaneous sampling at different depths, as well as sampling with two different net diameters at the same depth.

The **sedimentation box** is a very practical, passive, economically viable monitoring tool that is easy to install in a water body and does not require any special prior knowledge. A prerequisite for the measurement is a load-bearing, floating object to which the box can be attached during the sampling period. However, this is also the sampling methodology is the most inaccurate and many parameters cannot be recorded due to the simple setup. The temporal aspect is probably the biggest advantage of this method (measurement period over 2 weeks), but derivations on the degree of pollution cannot be made due to the low coverage of the river.

Compared to the other two methods, the **pumping method** is moderately complex. Except of a power source and a vessel, there are no other essential requirements for conducting the sampling. No special prior knowledge is required, and measurements at all heights in the water column and at all points across the river cross section allow representative sampling. Pump sampling is the only method that allows composite sampling across the river cross section (movement of the pump from one bank to the other, during the measurement).

With the current setting of the net sampling apparatus, **pump sampling is clearly recommended.**

6.2. Comparison of sample preparation and analysis

Since the sample preparation causes a very large share of costs and working time when analysing MPs, it is important to evaluate the advantages and disadvantages of the differently composed samples resulting from the different sampling methods. Evaluation criteria for the three sampling methods and two different lab-procedures (costly FTIR-microscope (lab-method A) vs. simple ATR-FTIR-spectrometer (lab-method B)) are listed in Table 28.

Table 28: Comparison of sample preparation and analysis

	Net-sampling + Lab-method A	Net-sampling + Lab-method B	Pump-method + FTIR-microscope	Sedimentation box + FTIR-microscope
Captured particle size range	(250) 500-5000 µm	250) 500-5000 µm	50 µm -1000 µm	< 1cm
Sample composition	Heterogenic sample composition (size, material), mainly organic impurities	Heterogenic sample composition (size, material), mainly organic impurities	Homogenic size distribution, little bycatch	Homogenic size distribution, mainly inorganic impurities
Time for sample preparation				
Time for measurement of microplastic particles				
Estimated costs of sample preparation and analysis per sample				

The three compared sampling methods refer to different particle size ranges. While net-samples have the disadvantage, that particles < 500 µm cannot be related to the sampled water volume, pump method have the disadvantage of not covering MPs > 1000 µm. The sedimentation box captures particles < 1 cm.

For subsequent sample preparation it is of great importance how the sample is composed. Especially net samples which are very heterogenic in both size distribution as well as composition,

are challenging later on. Excluding larger MPs as is done with the pump and with the box results in more homogeneous samples, from which MPs can be more easily extracted for further analysis.

Sample preparation time correlates, as mentioned, greatly with sample composition but also with method of analysis. While measurements with the FTIR-microscope are performed automatically, measurements with a simple FTIR-ATR-spectrometer require that each individual particle is manually placed on the measuring cell. FTIR-microscope measurements are also time consuming, but do not do not require the continuous presence of the examining person.

Costs of sample preparation and measurement are high for all mentioned methods. Net-sampling + Labor method B incurs higher personnel costs than all other methods, but lower costs in measurement (no expensive equipment rentals and no costly Anodisc filters are necessary.

The advantages and disadvantages of the evaluated methods are briefly summarized:

When comparing the methods, the **net method (in the current setting)** performs worst due to the extensive sample preparation steps and the associated costs. Due to the large sample volumes and the large amount of unwanted bycatch, numerous treatment steps are necessary, resulting in high costs. However, a great advantage of the method is the large volume of water that is examined. **Method A** does not require each single MP to be isolated by hand, as measurement is performed automatically. Investment costs of this method are high compared to Method B. **Method B** requires all particles to be picked out individually under the microscope and applied manually, one at a time, to the measuring cell of the FTIR-ATR spectrometer.

Hand collection and individual identification (lower investment costs) is a more cost-effective option for larger particles (in combination with net sampling).

Many treatment steps carry the risk of reducing or crushing particles as well as a higher risk of secondary contamination. Although recovery rates for plastics were very high in the tests performed, these were carried out with "new" plastic particles. The recovery rate of the added particles that went through the above sample preparation steps with the sample was 81.96%. The recovery rate of PE was 84.59%, that of PP 76.77% (Berghammer, 2022). It should be noted that the tests were performed with new plastics. Tests with plastic films that have already been exposed to environmental influences such as solar radiation or abrasion (as was the case, for example, with many macro-plastic films found in the Danube in a previous project) are recommended.

The preparation of **sedimentation box** samples represents a moderate effort and thus only causes lower costs. However, the detected plastic particles cannot be compared to any volume flow and are therefore not suitable for the determination of loads. A comparison between sampling locations or sampling times as well as the analysis of the composition plastic types is nevertheless possible and useful.

For **pump-method samples**, due to the pre-filter, no leaf debris or other macro particles are sampled and needs to be removed prior to analyses. As the diameter becomes smaller, the number of micro plastic particles in the water increases. Due to a not too large sample quantity and a rather homogeneous sample composition, preparation and measurement efforts are kept within limits.

Therefore, apart from the investment costs, the measurement costs are also not too high. On the other hand, particles > 1000 µm are excluded from the analysis and particles that adhere to leaves, for example, are also not taken into as they are excluded by the pre-filter.

With regard to sample preparation and analysis, the pump method is recommended as the more practicable method for long term monitoring especially with more sampling sites in different countries with different framework conditions. The pumping method also offers the possibility to detect particles smaller than 250 µm. This is all the more important as this fraction accounts for up to 2/3 of the detected particles. However, this method is only useful if appropriate laboratory equipment is available for the automated detection of such small particles. If this is not available and manual selection with tweezers is increasingly required, the advantages of the net method can be seen.

6.3. Comparison of results

Due to the different particle size fractions in the samples gained with the three sampling methods, no direct comparison of the results of the all samples is possible. However, a comparison of the results is possible for the sampling methods with net and pump for MPs in the size fraction 500-1000 µm. In Figure 79 results from net (method A and B – see chapter 4.3) and pump method are depicted. The reported particle count is mostly an average of 3 samples per site.

No significant differences in the results could be found between the two analysis methods A and B concerning number of detected MPs. However, the particle count is slightly higher with method B. Surveys with more samples to compare are recommended.

Samples collected by pump and processed and analysed according to the protocol described above resulted in higher microplastic concentrations than samples collected by net. As already mentioned, no concentrations can be calculated for sediment box samples.

With all three methods, the pollution situation along the Danube is represented approximately the same (same order of magnitude). Except in Bezdan and Pancevo significant more particles were found with the pump method. The sampling site in the Tisza River (Titel) is more polluted than the sampling sites in the Danube River.

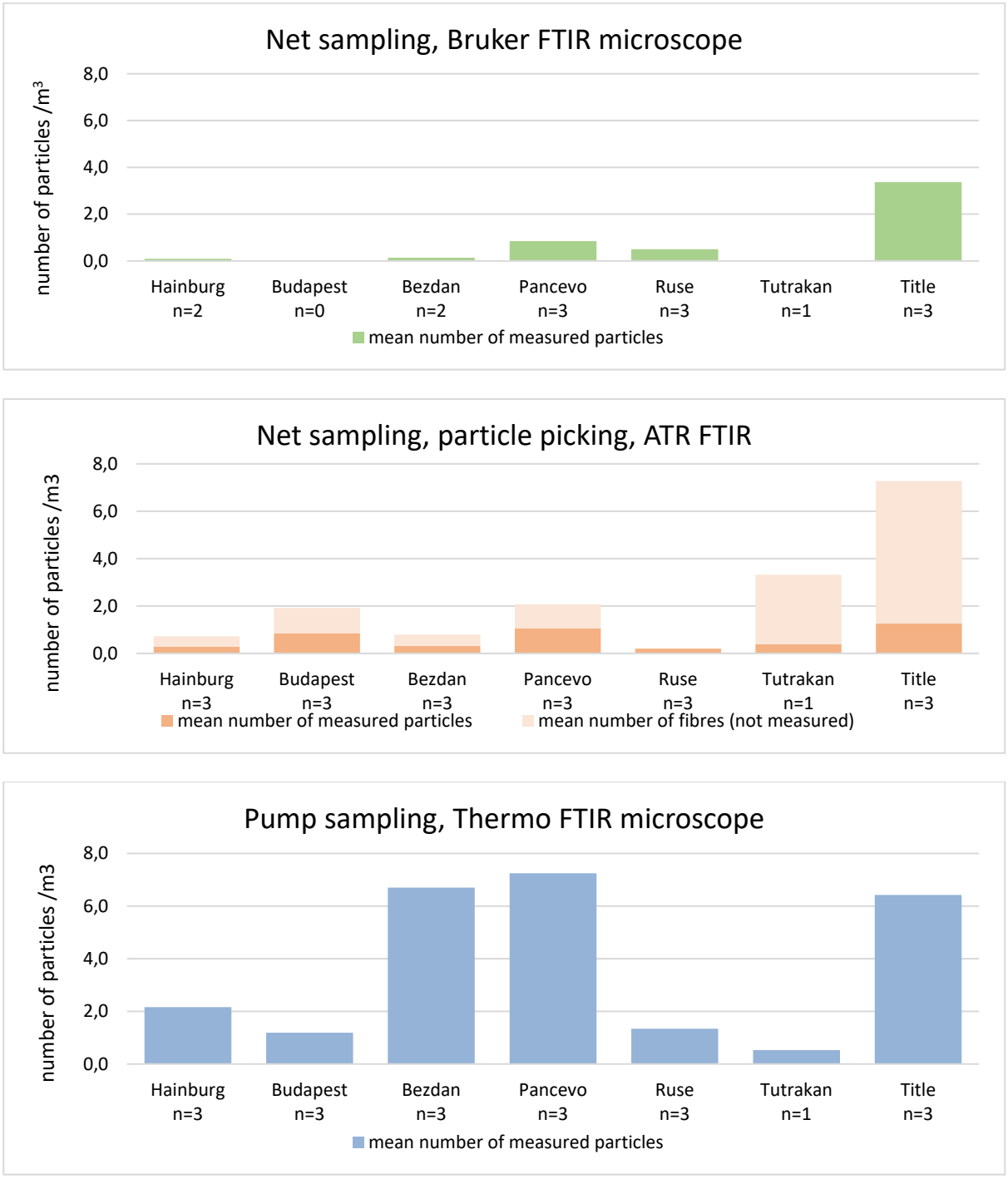


Figure 79: Comparison of MPs in the size fraction 500-1000 µm (net sampling + FTIR-microscope, net sampling + particle picking + ATR-FTIR spectrometer, pump sampling + FTIR microscope)

A comparison of plastic type distribution in samples of the location Hainburg sampled with net, pump and sedimentation box is depicted in Figure 80.

While the different methods come to similar results in terms of concentration, there are large discrepancies in the determination of the plastic types. Net samples analysed with BRUKER

microscope were assigned to PE and EVAc, whereas net samples analysed with the simple ATR-FTIR also showed considerable proportions of PP and other polymers. It should be noted that the sub-sample size at the analysis was much smaller due to economic reasons and only 3 particles were detected. The probability of a more diverse composition is clearly higher with 99 particles analysed by simple, cheap ATR-FTIR spectrometer.

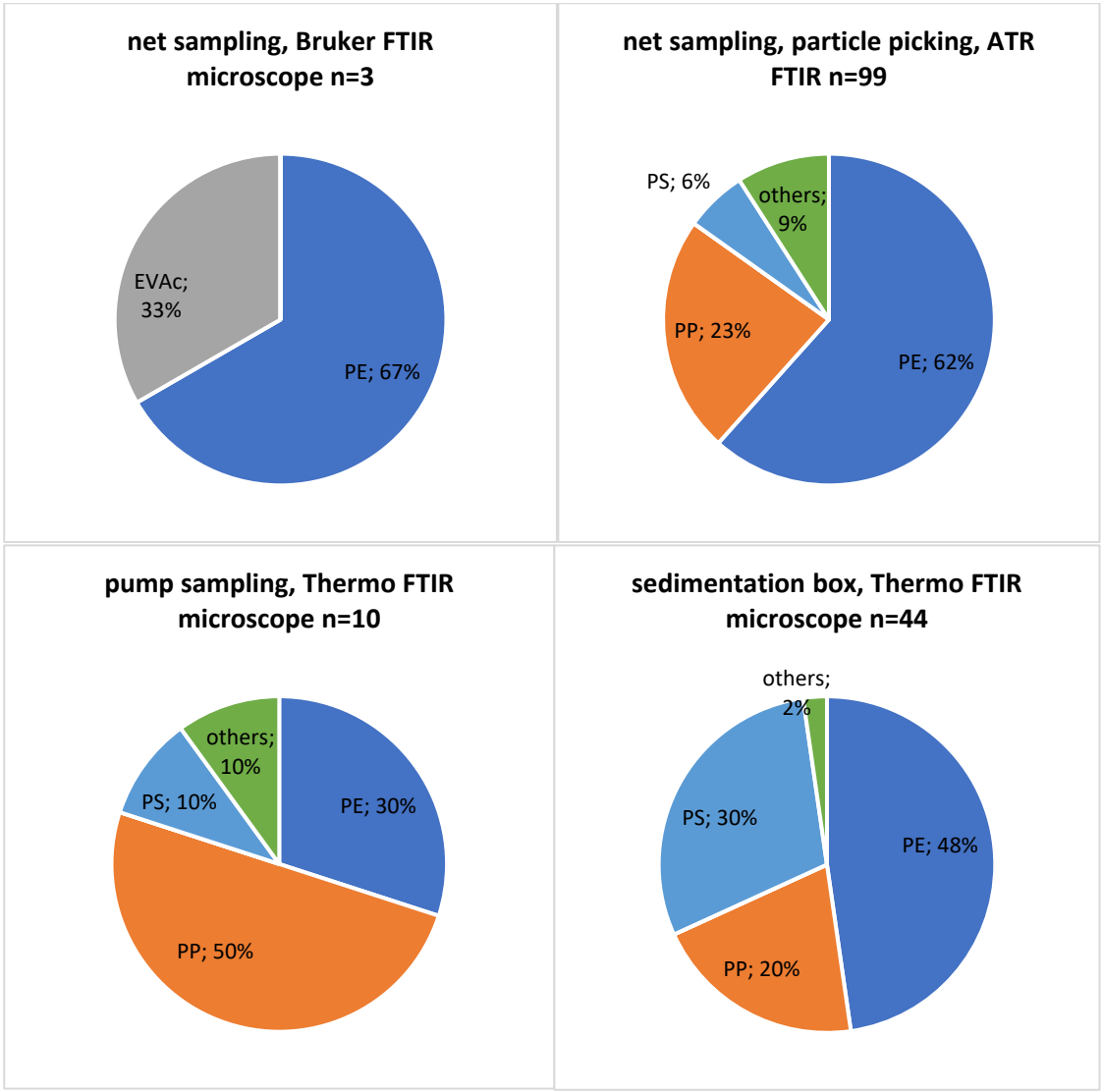


Figure 80: Comparison of plastic type distribution of net sub-samples (analysis according method A and B), pump method samples as well as sedimentation box samples at the Hainburg site (the strongly fluctuating particle counts (n) are also due to the different sizes of the sub-sample)

Table 29: Comparison of results of different sampling and preparation methods

	Net-sampling + method A	Net-sampling + method B	Pump-method +FTIR-microscope	Sedimentation box + FTIR-microscope
Detectable MP size range	(250) 500-5000 µm  *	(250) 500-5000 µm  *	50 µm -1000 µm  *	50-5000 µm  *
Determination of MP number				
Particle number /m³ sampled water volume (MP concentration)				
Particle shape /size				
Particle Weight		 **		
Detection of plastic type		 ***		

* the evaluation of the particle size ranges assumes that the lower size range is more significant due to the higher number of particles (in most publications results are given in MP numbers /m³). If the focus lies on MP masses, the net method performs better.

** at least for the particles of the fraction 1000-5000 µm the determination of the mass by means of analytical balance is possible, for smaller particles only sum values can be recorded

*** subjectivity of examiner may result in underestimation of MPs – it is recommended to pick out all potential particles as generously as possible, if non-plastics are isolated, they will be rejected again after the FTIR measurement

Detectable particle size ranges depend both on sampling and analysis method. While net-sampling only allows determination of concentrations for MPs > mesh size (500 µm) pump method also covers small particle size ranges. Determination of plastic types is possible also for very small particles with the FTIR-microscope. Measurements of fibers and particles < 500 µm

with ATR-FTIR-spectrometer mostly do not yield spectra of the required quality for determination of plastic types.

The pumping method also offers the possibility to detect particles smaller than 250 µm. This is all the more important as this fraction accounts for up to 2/3 of the detected particles. Analyses down to a lower particle size of 50 µm are possible.

Net samples also catch particles smaller than the mesh size, but they cannot be related to a sampled water volume as it is not known when the net starts clogging. The box captures all particle sizes downward - but again, the particles cannot be compared with a sampled water volume.

MP numbers, sizes and shapes can be determined for all samples, concentrations for all except the box-samples. Weight of individual MPs can only be determined conveniently when using ATR- FTIR-spectrometer for analyses, because each MP is picked and measured one by one. Of course, also after FTIR-microscope measurements bigger particles can be isolated and weighed, but this leads to an immense additional effort. During project weights were detectable with analysis balance for net-sample MPs > 1000 µm.

Detection of MPs types was performed using databases /software's descried in the Annex. Software like PURENCY offers advantages like high reliability and traceability and robust analysis results for a wide range of matrices, including very polluted environmental samples, whereas database of BRUKER ATR-FTIR-microscope and own reference spectra do not contain spectra of weathered plastics, which is a disadvantage. Also, the subjectivity of the examiner may result in underestimation of MPs with this method. Standardized databases for microplastics, which guarantee comparability of results of different studies should be aimed. Databases should also contain spectra of weathered plastics. Also, it may be helpful to have reference spectra of common non-plastic natural polymers (in this study e.g. beetles, seed shells etc.) to avoid false positive assignments. Since only plastic types, which are already contained within databases, can be determined it is recommended to have visual looks (microscope) at the potential particles from time to time.

Comparison of plastic types gained by different sampling and analysis methods may possibly indicate the problem of different databases, but can of course also be caused by the different PN procedures - more samples are recommended.

Considering all aspects, results from pump-method + FTIR-microscope seems to be best option due to the possibility to detect also small MPs.

7. Summary & Conclusions

The optimal sampling method depends on the respective boundary conditions. But only the net method and the pump method allow calculations of microplastic concentration and load. Each of the methods studied has certain advantages and disadvantages. Weighing the advantages and disadvantages should be done in the context of the particular problem as well as framework conditions. Advantages of a large sampled water volume (net method), for example, go hand in hand with higher sample processing costs.

The composition of the sample, particle sizes as well as examined water quantity, the time expenditure of the sample preparation and measurement depend on the kind of the respective sampling. Thus, the sample preparation effort also depends on the sampling method.

Sample preparation must not be ignored and is usually more complicated and time-consuming than the measurement of the particles themselves. The major challenge here is to isolate all plastic particles from all other unwanted organic and inorganic contaminants in the sample and thus make them detectable without altering or even destroying the microplastic particles in any way. In river samples, there are comparatively few microplastic particles in a heterogeneous and complex matrix of organic and inorganic bycatch, so the processing is very challenging compared to other environmental samples

Pre-treatment steps performed can influence results (lost and undetected particles, secondary contamination, etc.) Porous plastics can be crushed by the preparation process, leading to an overestimation of the number of particles. Thus, different preparation steps and analytical methods lead to results of different quality and significance and thus prevent comparability of MP studies in rivers.

Therefore, for the respective sampling methods, the sample preparation and measurement procedure for different sampling methods were optimized within the Tid(y)Up project and recorded in protocols for future standardization. However, harmonized protocols or standardized approaches for quality assurance and quality control in sampling and evaluation of microplastics need to be (further) developed.

Comparing the three-sampling methods together with sample preparation and analysis procedure it turns out that each method has own advantages and disadvantages which can compensate each other. For a comprehensive scientific monitoring, a combination of net and pump sampling would be recommended as as in combination of both methods' MP concentrations can be detected over a range of 50-5000 µm.

Sampling of microplastics with **multi-depth-net device (in current design)** turned out to be the most complex procedure, primarily in terms of providing the necessary conditions (a vessel of larger dimensions equipped with a crane, official approvals etc.), as well as a long-term procedure of cleaning the nets after sampling. The applied method with net enables simultaneous sampling at different depths, as well as sampling with two different net diameters at the same depth. Also, sample preparation of net samples is challenging. Due to the large sample volumes and the large amount of unwanted bycatch, numerous treatment steps are necessary, resulting in high costs. However, a great advantage of the method is the large volume of water that is examined. **Lab**

Method A (analysis with FTIR-microscope) does not require each single MP to be isolated by hand, as measurement is performed automatically. Investment costs of this method are high compared to Method B. **Method B (analysis with ATR-FTIR-spectrometer)** requires all particles to be picked out individually under the microscope and applied manually, one at a time, to the measuring cell of the FTIR-ATR spectrometer.

The **sedimentation box** is a very practical, passive, economically viable monitoring tool that is easy to install in a water body and does not require any special prior knowledge. A prerequisite for the measurement is a load-bearing, floating object to which the box can be attached during the sampling period. However, this is also the sampling methodology is the most inaccurate and many parameters cannot be recorded due to the simple setup. The temporal aspect is probably the biggest advantage of this method (measurement period over 2 weeks), but derivations on the degree of pollution cannot be made due to the low coverage of the river. The preparation of sedimentation box samples, prior to analysis, represents a moderate effort and thus only causes lower costs. However, the detected plastic particles cannot be compared to any volume flow and are therefore not suitable for the determination of loads. A comparison between sampling locations or sampling times as well as the analysis of the composition plastic types is nevertheless possible and useful.

Compared to the other two methods, sampling with **pumping method** is moderately complex. Except of a power source and a vessel, there are no other essential requirements for conducting the sampling. No special prior knowledge is required, and measurements at all heights in the water column and at all points across the river cross section allow representative sampling. Pump sampling is the only method that allows composite sampling across the river cross section (movement of the pump from one bank to the other, during the measurement). For pump-method samples, due to the pre-filter, no leaf debris or other macro particles are sampled and needs to be removed prior to analyses. As the diameter becomes smaller, the number of micro plastic particles in the water increases. Due to a not too large sample quantity and a rather homogeneous sample composition, preparation and measurement efforts are kept within limits. Therefore, apart from the investment costs, the measurement costs are also not too high. On the other hand, particles > 1000 µm are excluded from the analysis and particles that adhere to leaves, for example, are also not taken into as they are excluded by the pre-filter.

With regard to sample preparation and analysis, the **pump method is recommended** as the more practicable method for long term monitoring especially with more sampling sites in different countries with different framework conditions. The pumping method also offers the possibility to detect particles smaller than 250 µm. This is all the more important as this fraction accounts for up to 2/3 of the detected particles. However, this method is only useful if appropriate laboratory equipment is available for the automated detection of such small particles. If this is not available and manual selection with tweezers is increasingly required, the advantages of the net method can be seen.

Hand collection and individual identification (lower investment costs) is a more cost-effective option for larger particles (in combination with net sampling). To avoid large volume, heterogenic samples taken with net it is recommended to develop scaled down net sampling device

Literature

Bannick, C.G., Szewzyk, R., Ricking, M., Schniegler, S., Obermaier, N., Barthel, A.K., Altmann, K., Eisentraut, P., Braun, U., 2019. Development and testing of a fractionated filtration for sampling of microplastics in water. Water Research 149, 650-658.

Berghammer Julian, 2022: Mikroplastik in Flussproben - Qualitätssicherung und Qualitätskontrolle im Zuge der Probenaufbereitung und -analytik. Universität für Bodenkultur Wien, Institut für Abfall- und Kreislaufwirtschaft. Wien, 2022.

Bordós, G., Gergely, S., Háhn, J., Palotai, Z., Szabó, É., Besenyő, G., Salgó, A., Harkai, P., Kriszt, B., Szoboszlay, S., 2021. Validation of pressurized fractionated filtration microplastic sampling in controlled test environment. Water Research 189, 116572.

Bordós, G., Urbányi, B., Micsinai, A., Kriszt, B., Palotai, Z., Szabó, I., Hantosi, Z., Szoboszlay, S., 2019. Identification of microplastics in fish ponds and natural freshwater environments of the Carpathian basin, Europe. Chemosphere 216, 110-116.

Campanale, C., Savino, I., Pojar, I., Massarelli, C., Uricchio, V.F., 2020. A Practical Overview of Methodologies for Sampling and Analysis of Microplastics in Riverine Environments. Sustainability 12.

Hohenblum, P., 2019. What is the Magic Silver Box Telling Us?, DANUBE WATCH- THE MAGAZINE FOR THE DANUBE RIVER. ICPDR - International Commission for the Protection of the Danube River.

ICPDR, 2016: https://www.icpdr.org/main/publications/plastics-and-microplastics-danube-river#%20 (site requested at 28.7.2021)

ICPDR, 2020. JOINT DANUBE SURVEY 4 SCIENTIFIC REPORT: A SHARED ANALYSIS OF THE DANUBE RIVER.

Kiessling, T., Knickmeier, K., Kruse, K., Brennecke, D., Nauendorf, A., Thiel, M., 2019. Plastic Pirates sample litter at rivers in Germany – Riverside litter and litter sources estimated by schoolchildren. Environmental Pollution 245, 545-557.

Liedermann, M., Gmeiner, P., Pessenlehner, S., Haimann, M., Hohenblum, P., Habersack, H., 2018. A Methodology for Measuring Microplastic Transport in Large or Medium Rivers. Water 10, 414.

Löder, M. G. J., et al. (2017). "Enzymatic Purification of Microplastics in Environmental Samples." Environmental Science & Technology 51(24): 14283-14292.

Mári, Á., Bordós, G., Gergely, Sz., Büki, M., Háhn, J., Palotai, Z., Besenyő, G., Szabó, É., Salgó, A., Kriszt, B., Szoboszlay, S. (2021): Validation of microplastic sample preparation method for freshwater samples. Water Res. 202. 117409. https://doi.org/10.1016/j.watres.2021.117409

Miller, S.D., Haddock, S.H., Elvidge, C.D., Lee, T.F., 2005. Detection of a bioluminescent milky sea from space. Proc. Natl. Acad. Sci. U. S. A. 102, 14181.

Prata, J.C., da Costa, J.P., Duarte, A.C., Rocha-Santos, T., 2019. Methods for sampling and detection of microplastics in water and sediment: A critical review. TrAC Trends in Analytical Chemistry 110, 150-159.

Sharma, S., Basu, S., Shetti, N.P., Nadagouda, M.N., Aminabhavi, T.M., 2021. Microplastics in the environment: Occurrence, perils, and eradication. Chemical Engineering Journal 408, 127317.

Stock, F., Kochleus, C., Bänsch-Baltruschat, B., Brennholt, N., Reifferscheid, G., 2019. Sampling techniques and preparation methods for microplastic analyses in the aquatic environment – A review. TrAC Trends in Analytical Chemistry 113, 84-92.

UBA (2015). Mikroplastik in der Umwelt. Vienna, Umweltbundesamt.

van Emmerik, T., Schwarz, A., 2020. Plastic debris in rivers. WIREs Water 7, e1398.

Zhang W, Wang W, Wang J, Shen G, Yuan Y, Yan L, Tang H, Wang W. Isolation and Characterization of a Novel Laccase for Lignin Degradation, LacZ1. Appl Environ Microbiol. 2021 Nov 10;87(23):e0135521. doi: 10.1128/AEM.01355-21

ANNEX

Tables and Figures

ANNEX

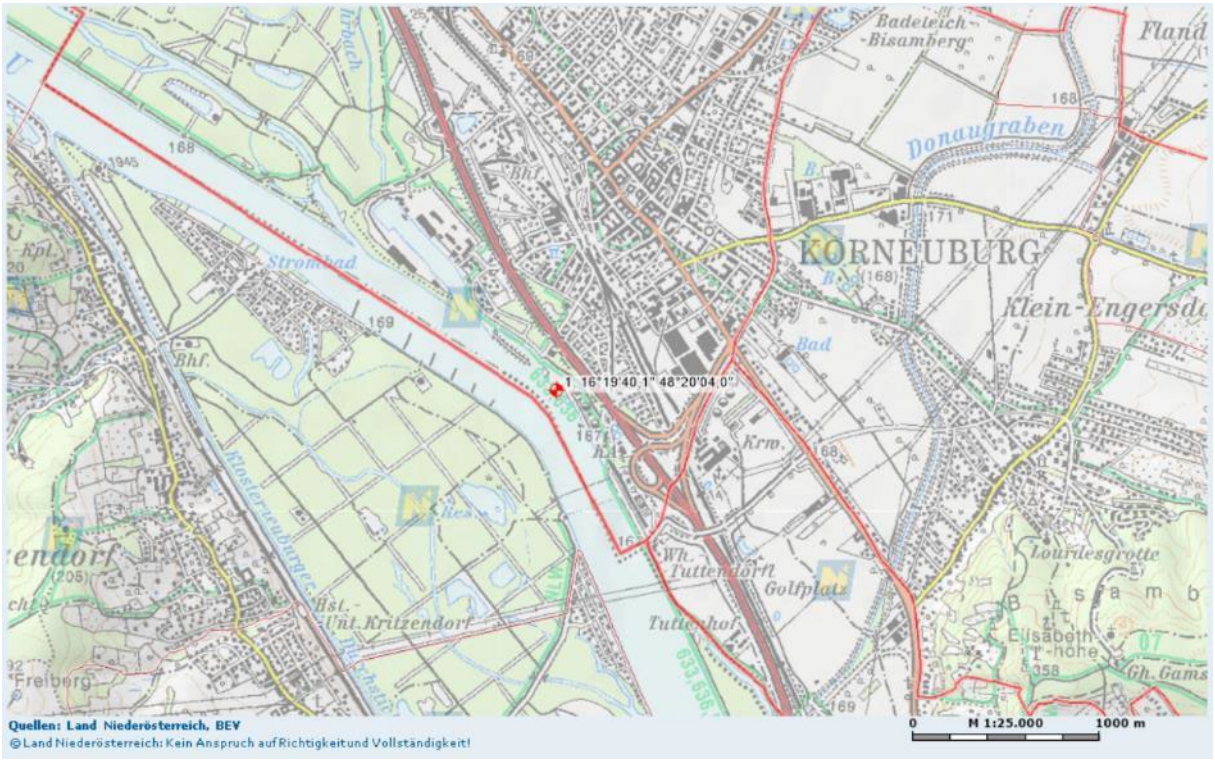
Sampling locations in Austria where sedimentation box was applied

Hainburg

See chapter 4.1.1

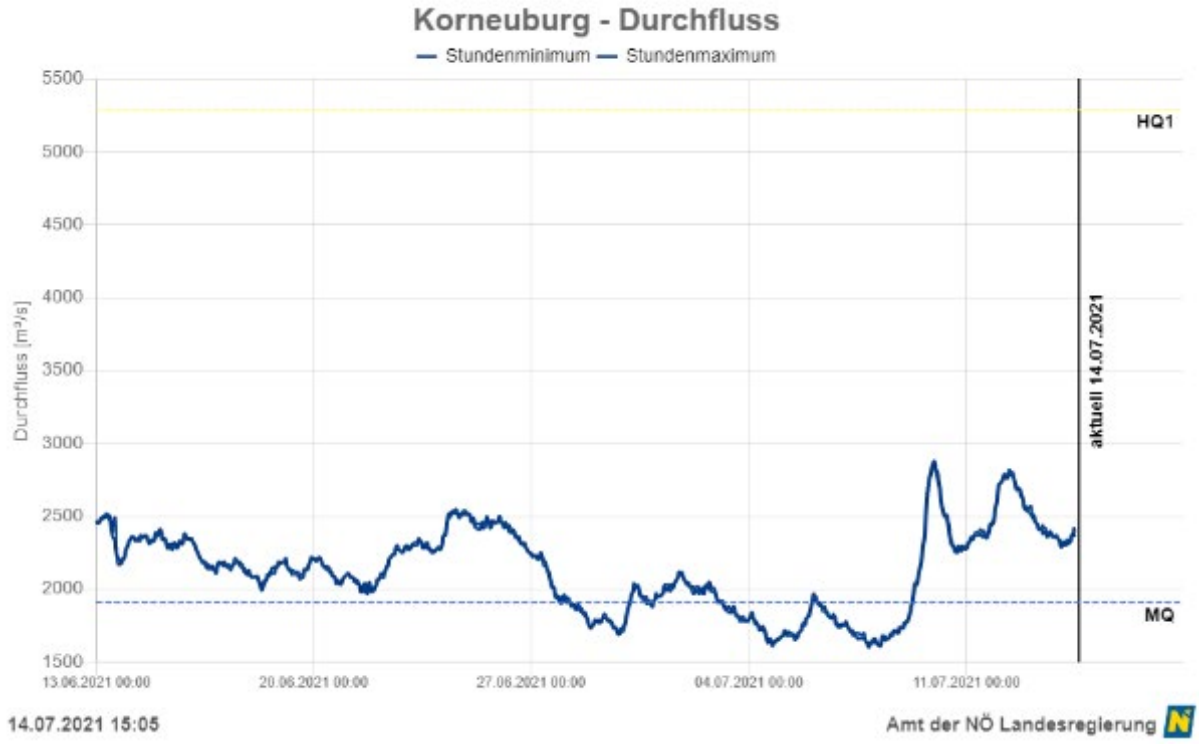
Korneuburg

Date of installation	29. June 2021	Time:	11:00
Flow rate [m/s]	0,8	Depth [m]	0,5
Date of removal	14. July2021	Time:	11:00
Flow rate [m/s]	1,2	Depth [m]	0,5
Coordinates	Longitude	16° 19' 40.1'' O	
	Latitude	48° 20' 04.0'' N	
River kilometres	1942,35		
Description	On the left bank of the Danube, directly at the landing stage "Korneuburg ".		



Annex 1: Site plan/coordinates of the sedimentation box and aerial photo of the sampling site in Korneuburg

Annex 2: Photos of the installed sedimentation box in Korneuburg

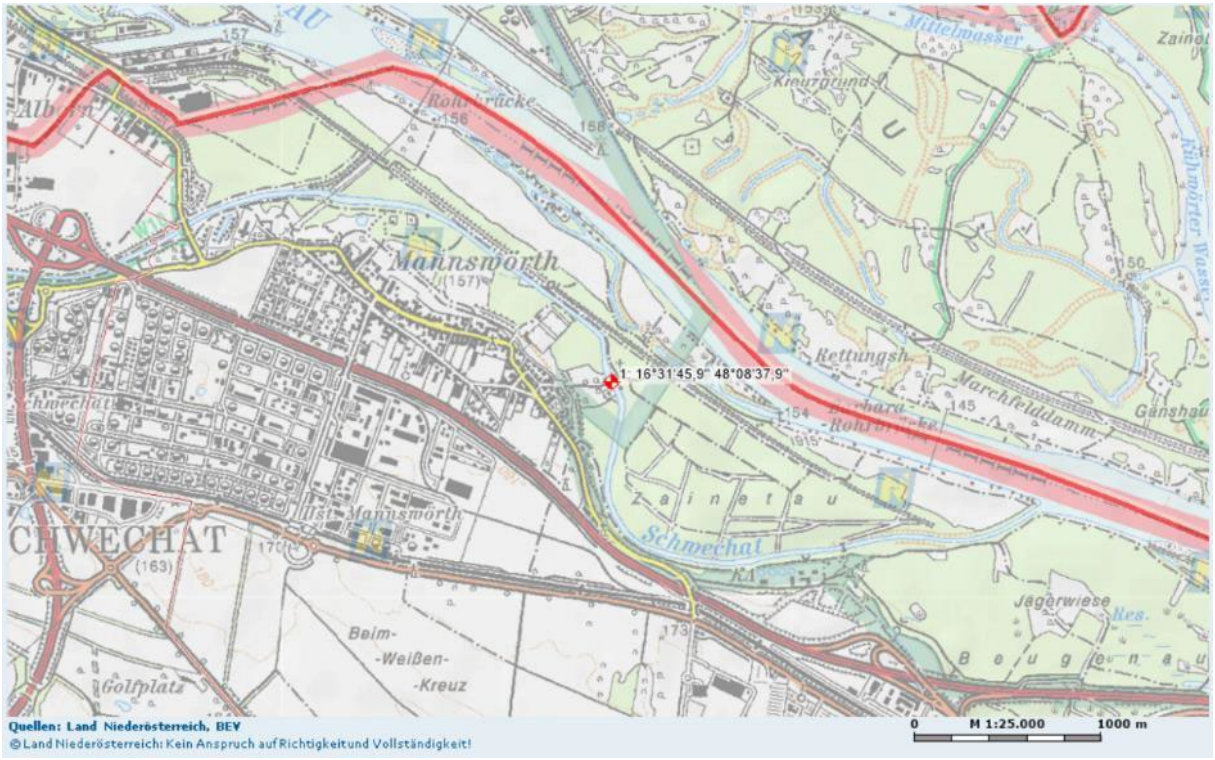


Annex 3: Flow rate of Danube river at Korneuburg during sampling period

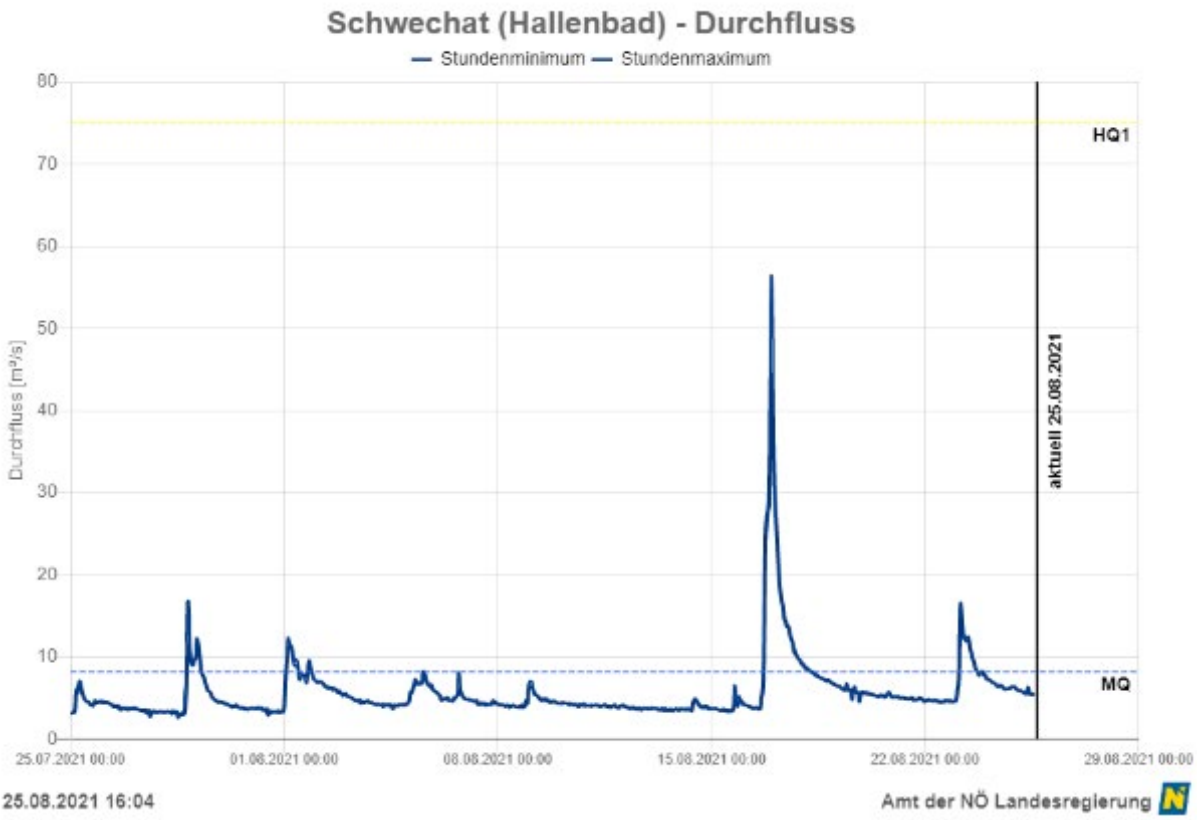
Schwechat

Date of installation	2. August 2021	Time:	13:30
Flow rate [m/s]	0,5	Depth [m]	0,5
Date of removal	25. July 2021	Time:	12:30
Flow rate [m/s]	0,5	Depth [m/s]	0,5
Coordinates	Longitude	16° 31' 45.9'' O	
	Latitude	48° 08' 37.9'' N	
River kilometres	1942,35		
Description	In the middle of the Schwechat, under the "Zainethbrücke" in Mannswörth		

Annex 4: Photos of the installed sedimentation box under the Zaineth Bridge on the Schwechat River



Annex 5: Site plan/coordinates of the sedimentation box and aerial photo of the sampling site in Mannswörth (Schwechat)



Annex 6: Flow rate of the Schwechat at Korneuburg over the sampling period

FTIR-Databases

BRUKER FTIR-microscope

Measurements performed with BRUKER LUMOS II FTIR-microscope were evaluated using PURENCY software (https://www.purency.ai/blogs-news/microplastics-finder-why-what-and-how):

The identification of microplastics is based on the comparison of the measured spectra with reference spectra which come from particles of known composition. Oxidation, the presence of biofilms, total absorption for large particles, residues of the sample matrix and much more can influence the infrared spectra of microplastics to varying degrees. Consequently, these must be considered when selecting the reference spectra for reliable detection of microplastics. For this purpose, an extensive collection of spectra must be available. In a classical database comparison, each spectrum in the acquired image is compared with every reference spectrum from the database. The total computing time quickly adds up to hours, which limits the number of reference spectra. Therefore, there is a trade-off between time and analytical quality when using spectral libraries. Furthermore, there is no standardized database for microplastics analysis, which raises questions of comparability.

The limitations of conventional spectral libraries when it comes to large data files and high data variability, which we observe in the case of microplastics analysis, opened up the search for more advanced, alternative data analysis solutions. The key word being machine learning. Unlike "classical" database matching, model-based machine learning analysis can contain practically any number of reference spectra and substance classes. These reference spectra, now called the training data, are the basis for deriving statistical models by means of machine learning algorithms.

Purency applied this method to create a robust data analysis solution for microplastics analysis based on microFTIR images: the Microplastics Finder. The current version of the Microplastics Finder (R2021a) is based on a unique, expert-curated training dataset of more than 12000 reference spectra. About 50% of the data consist of polymer spectra and 50% of matrix spectra. The training data comes from real-life samples and, therefore, also includes “imperfect” spectra. For example, spectra with (partial) total absorption and those from different environmental matrices such as wastewater, sediment or sewage sludge are considered. Therefore, the solution is robust to these challenging effects.

The analysis of new, unknown spectra and the decision whether a spectrum is microplastic takes a fraction of a second. With a typical file size of a spectroscopic image (approx. 5 - 10 GB), the overall results are available in a few minutes. Furthermore, the Microplastics Finder has a broad applicability: more than 20 polymer types in a wide range of environmental matrices can be measured.

https://www.purency.ai/blogs-news/microplastics-finder-why-what-and-how

Plastic types to be matched in Purency were:

PP, PE, PVC, PU, PET, PS, ABS, PA, PC, PMMA, POM, CA, EVAc, EVOH, PAN, PBT, PEEK, PPSU, PSU, silicone, PLA

Thermo Nicolet iN10mx FTIR microscope

siMPle combines the interface of MPHunter with AWI Automatic Pipeline for MP analysis, creating a freeware program for MP data analysis (https://simple-plastics.eu/).

Following polymers are included in the software

PE, PP, Polyester, PA, Acrylic, SAN, PVC, Vinyl copolymer, EVA, PVA, PVAC, PVDC, PU, PS, SBR, ABS

Polycarbonate, Epoxy, Phenoxy resin, Diene elastomer, POM, PEG, PTFE, EPDM, PLA, Aramid, Polyimide, PEBAX, Cellulose acetate, Alkyd

BRUKER Alpha ATR-FTIR-Spektrometer

Following databases were used for interpretation:

BRUKER BPAD, SR (Bruker Demo) Database and an own created database including chewing gum and non-plastics (e.g. chitin).

BPAD BRUKER ATR-Polymer Library contains following polymers:

BPAD BRUKER ATR-Polymer-Library

Entry No.	Compound
1	Polyurethane (PUR-WS)
2	Polyurethane (PUR-WS)
3	Polycarbonate / Acrylonitrile Butadiene Styrene Blend (PC + ABS)
4	Polycarbonate / Acrylonitrile Butadiene Styrene Blend (PC + ABS)
5	Polycarbonate / Acrylonitrile Butadiene Styrene Blend (PC + ABS)
6	Structural Reaction-Injection Moulding (S-RIM)
7	Structural Reaction-Injection Moulding (S-RIM)
8	Polyurethane-Reaction Injection Molded Polyurethane-Glycol (PUR-RIM-GLYCOL)
9	Polyurethane-Reaction Injection Molded Polyurethane-Glycol (PUR-RIM-GLYCOL)
10	Polyurethane-Reaction Injection Molded Polyurethane-Amine (PUR-RIM AMINE)
11	Polyurethane / Reaction Injection Molded Polyurethan / Amin-Polymer (PUR-RIM AMIN)
12	Polyurethane-Reaction Injection Molded Polyurethane-Amine (PUR-RIM AMINE)
13	Polyvinyl Chloride-Unplasticised (PVC-U)
14	Polyvinyl Chloride-Unplasticised-Acrylonitrile / Butadien / Styrene (PVC-U-ABS)
15	Polyvinyl Chloride-Unplasticised-Graphite Fibril (PVC-U-GF)
16	Polyvinyl Chloride-Phenolic (PVC-P)
17	Polyvinyl Chloride-Plasticized (PVC-P)
18	Polyvinyl Chloride-Phenolic-Nitrile Butadiene Rubber (PVC-P-NBR)
19	Polybutylene Terephthalate (PBT)
20	Polybutylene Terephthalate (PBT)
21	Polypropylene (PP)
22	Thermoplastic Polyurethane (TPU)
23	Thermoplastic Polyurethane (TPU)
24	Thermoplastic Polyurethane (TPU)
25	Thermoplastic Polyurethane (TPU)
26	Polyamide 66 (PA 66)
27	Polyamide 66 (PA 66)
28	Polyamide 66 (PA 66)
29	Polyamide 6 (PA 6)
30	Polyamide 6 (PA 6)
31	Polyamide (PA 6)
32	Polyamide 66 (PA 66)
33	Polyurethane-Reaction Injection Molded Polyurethane-Amine (PUR-RIM AMINE)
34	Polyurethane-Reaction Injection Molded Polyurethane-Amine (PUR-RIM AMINE)
35	Polyurethane-Reaction Injection Molded Polyurethane-Glycol (PUR-RIM-GLYCOL)
36	Low-Density Polyethylene (PE-LD)
37	Polyvinyl Chloride - Hard (PVC-Hard)
38	Polyvinyl Chloride - Hard (PVC-Hard)
39	Polyvinyl Chloride - Hard (PVC-Hard)
40	Polypropylene (PP)
41	Polypropylene (PP)
42	Polypropylene (PP)
43	Polypropylene (PP)
44	Polypropylene (PP)
45	Polypropylene (PP)
46	Polypropylene (PP)
47	Blend of Polypropylen and Ethylen / Propylen (PP + EPDM)

48	Blend of Polypropylen and Ethylen / Propylen (PP + EPDM)
49	Polyoxymethylene (Acetal) (POM)
50	Polyoxymethylene (Acetal) (POM)
51	Polyoxymethylene (Acetal) (POM)
52	High Density Polyethylene (PE-HD)
53	Polypropylene (PP)
54	Polypropylene (PP)
55	Polypropylene (PP)
56	Blend of Polypropylen and Ethylen / Propylen (PP + EPDM)
57	Blend of Polypropylen and Ethylen / Propylen (PP + EPDM)
58	Polypropylene (PP)
59	Polypropylene (PP)
60	Polypropylene (PP)
61	Polypropylene (PP)
62	Blend of Polypropylen and Ethylen / Propylen (PP + EPDM)
63	Sheet Molding Compound (SMC)
64	Sheet Molding Compound (SMC)
65	Polymethylmethacrylate (Acrylic) (PMMA)
66	Polymethylmethacrylate (Acrylic) (PMMA)
67	Low-Density Polyethylene (PE-LD)
68	High Density Polyethylene (PE-HD)
69	High Density Polyethylene (PE-HD)
70	Styrene Acrylonitrile (SAN)
71	Styrene Acrylonitrile (SAN)
72	Acrylonitrile Styrene Acrylate (ASA)
73	Acrylonitrile Styrene Acrylate (ASA)
74	Acrylonitrile Styrene Acrylate (ASA)
75	Acrylonitrile Styrene Acrylate (ASA)
76	Polyphenylene Ether + High Impact Polystyrene (PPE + HIPS)
77	Polyphenylene Ether + High Impact Polystyrene (PPE + HIPS)
78	Polyphenylene Ether + High Impact Polystyrene (PPE + HIPS)
79	Polycarbonate (PC)
80	Polycarbonate (PC)
81	Polycarbonate (PC)
82	Polycarbonate (PC)
83	Polyamide 66 (PA 66)
84	Polyphenylene Ether (PPE)
85	Blend Of Polyphenylene Ether And Polyamide (PP + PA)
86	Blend Of Polyphenylene Ether And Polyamide (PP + PA)
87	Polyphenylene Ether (PPE)
88	Acrylonitrile Butadiene Styrene (ABS)
89	Acrylonitrile Butadiene Styrene (ABS)
90	Acrylonitrile Butadiene Styrene (ABS)
91	Acrylonitrile Butadiene Styrene (ABS)
92	Polyethylene Terephthalate (PET)
93	Polybutylene Terephthalate (PBT)
94	Polybutylene Terephthalate (PBT)
95	Polycarbonate / Polybutylene Terephthalate (PC + PBT)
96	Polycarbonate / Polybutylene Terephthalate (PC + PBT)
97	Polybutylene Terephthalate (PBT)
98	Polycarbonate / Polybutylene Terephthalate (PC + PBT)
99	Polystyrene (PS)
100	High Impact Polystyrene (HIPS)

101	Polyurethane (PUR-WS)
102	Polyurethane (PUR-WS)
103	Polyurethane (PUR-WS)
104	High Density Polyethylene (PE-HD)
105	Styrene Acrylonitrile (SAN)
106	Styrene Maleic Anhydride Copolymer (SMA)
107	Styrene Maleic Anhydride Copolymer (SMA)
108	Polyamide 6 (PA6)
109	Sheet Molding Compound (SMC)
110	Acrylonitrile Butadiene Styrene (ABS)
111	Acrylonitrile Butadiene Styrene (ABS)
112	Blend of Polypropylen and Ethylen / Propylen (PP + EPDM)
113	Polyoxymethylene (Acetal) (POM)
114	Polyamide 6 (PA6)
115	Polyamide 6 (PA6)
116	Polyamide 6 (PA6)
117	Blend Of Polyphenylene Ether And Polyamide (PP + PA)

Sampling requirements and costs

Austria

Annex 7: Personnel effort for bridge-boat sampling

	Net-devise		Pump Method		Sedimentationbox	
	min	recomm.	min	recomm.	min	recomm.
Staff	4[1]	5	1	2	2	2
Truck Driver	1	1	-	-	-	-
Vessel Crew	-	-	-	-	-	-
Boat Crew	-	-	1	1	0[2]	1
SUM ∑	5	6	2	3	1	3

[1] Two persons handle the devise; two persons have to control the ship traffic from bridge looking upstream and downstream[2]if the box is deployed at an accessible bank-near site

Annex 8:: Cost estimate for MP-sampling with net in AT (3 sampling point over transverse profile)

Cost center	Description	Staff	Hours	Costs* per h	Total costs
Preparation and Follow-up	Loading equipment, etc.	2	2	€ 35.00	€ 140.00
Transport of equipment	incl. staff arrival and return	6	2	€ 35.00	€ 420.00
Road closure and securing sampling site	Set up traffic signs, attaching barrier tape, etc.	3	2	€ 35.00	€ 210.00
Set up net devise for truck		2	1	€ 35.00	€ 70.00
Rental fee truck	Including truck driver	1	8	€ 75.00	€ 600.00
Net measurement	3 sampling points	3	5	€ 35.00	€ 525.00
Supervision traffic	incl. ship traffic	2	5	€ 35.00	€ 350.00
Required permissions				-	-
Fee shipping inspectorate		1	1	€ 1,000.00	€ 1,000.00
Estimated Total Costs					€ 3,315.00

*Average gross wages of participated staff (no overhead included)

Table 6: Cost estimate for MP-sampling with pump method in AT (3 sampling point over transverse profile)

Cost center	Description	Staff	Hours	Costs* per h	Total costs
Preparation and Follow-up	Loading equipement, etc.	1	2	€ 35.00	€ 70.00
Transport of equipment	incl. staff arrival and return	2	2	€ 35.00	€ 140.00
Loading equipment on boat	incl. enloading afterwards	2	1	€ 35.00	€ 70.00
Pump measurement	3 sampling points + mixed sample across river profile	2	5	€ 35.00	€ 350.00
Rental Boat	Incl. boat crew	1	7	€ 145.00	€ 1,015.00
Required permissions					-
Fee shipping inspectorate		1	1	€ 1,000.00	€ 1,000.00
Estimated Total Costs					€ 2,645.00

Annex 9:: Cost estimate for MP-sampling with sedimentation box in AT (1 sampling point)

Cost center	Description	Staff	Hours	Costs* per h	Total costs
Preparation and Follow-up	Loading equipement, etc.	1	2	€ 35.00	€ 70.00
Transport of equipment	incl. staff arrival and return	2	2	€ 35.00	€ 140.00
Deployment of Sedbox	site accessible from shore	2	1	€ 35.00	€ 70.00
Removal Sedbox	incl. decanting of sample	2	1	€ 35.00	€ 70.00
Rental Boat	incl. boat crew	1	3	€ 145.00	€ 435.00
Required permissions					-
Fee shipping inspectorate		1	1	€ 1,000.00	€ 1,000.00
Estimated Total Costs	bank-near sampling without boat			MIN	€ 350.00
Estimated Total Costs	incl. boat and fee			MAX	€ 1,785.00

Hungary

Annex 10: Cost estimate for MP-sampling with net method in HU (3 sampling point over transverse profile)

Cost center	Description	Staff	Hours	Costs* per h (€)	Total costs (€)
Preparation and Follow-up	Loading equipment, etc.	2	2	27	108
Transport of equipment	incl. staff arrival and return	6	2	27	324
Road closure and securing sampling site	Set up traffic signs, attaching barrier tape, etc.	3	2	27	162
Set up net devise for truck		2	1	27	54
Rental fee boat with crane	Boat with full staff	1	8	1100	1100
Net measurement	3 sampling points	3	5	27	405
Supervision traffic	incl. ship traffic	2	5	-	-
Required permissions				-	-
Fee shipping inspectorate		1	1	-	-
Estimated Total Costs					**2153**
From this, personnel					1053
From this, rental					1100

Annex 11: Estimated investment costs for PUMP method

Generator	830
Pump	230
Water meter	60
Rubber and PVC hoses, foot valve with 1 mm prefilter	300
Filter stand	240
Filter cartridges (3 pieces)	210
Filter mesh (3 pieces)	170
Total investment (€)	**2040**

Annex 12: Cost estimate for MP-sampling with pump method in HU (3 sampling point over transverse profile)

Cost center	Description	Staff	Hours	Costs* per h (€)	Total costs (€)
Preparation and Follow-up	Loading equipement, etc.	1	2	27	54
Transport of equipment	incl. staff arrival and return	2	2	27	108
Loading equipment on boat	incl. enloading afterwards	2	1	27	54
Pump measurement	3 sampling points + mixed sample across river profile	2	5	27	270
Rental Boat (smaller, without crane)	Incl. boat crew	1	7	40	280
Required permissions					-
Fee shipping inspectorate		1	1	-	-
Estimated Total Costs					**766**
From this, personnel					486
From this, rental					280

Annex 13: Estimated investment costs for BOX method in HU

Preparation of the sedimentation box	420
Stainless steel barrel	140
Chains and other acessories	140
Total investment (€)	**700**

Annex 14: Cost estimate for MP-sampling with BOX method in HU (3 sampling point over transverse profile)

Cost center	**Description**	**Staff**	**Hours**	**Costs* per h (€)**	**Total costs (€)**
Preparation and Follow-up	Loading equipement, etc.	1	2	27	54
Transport of equipment	incl. staff arrival and return	2	2	27	108
Deployment of Sedbox	site accessible from shore	2	1	27	54
Removal Sedbox	incl. decanting of sample	2	1	27	54
Rental Boat (smaller, with small crane)	incl. boat crew	1	6	90	540
Required permissions					-
Fee shipping inspectorate		1	1	-	-
Estimated Total Costs	**bank-near sampling without boat**			**MIN**	**270**
Estimated Total Costs	**incl. boat and fee**			**MAX**	**810**
From this, personnel					270
From this, rental					540

Serbia

Annex 15: Cost estimate for MP-sampling with net method in RS (3 sampling point over transverse profile)

Cost center	**Description**	**Staff**	**Hours**	**Costs* per h (EUR)**	**Total costs**
Preparation and Follow-up	Loading equipment, etc.	2	2	15	60
Transport of equipment	incl. staff arrival and return	6	6	15	540
Loading equipment on boat	incl. unloading afterwards	3	2	15	60
Rent of boat with crane	Including boat crew	1	8	1100	8800
Net measurement	3 sampling points	3	8	20	480
Estimated Total Costs					9940

*Average gross wages of participated staff

Annex 16:: Cost estimate for MP-sampling with pump method in RS (3 sampling point over transverse profile)

Cost center	Description	Staff	Hours	Costs* per h	Total costs
Preparation and Follow-up	Loading equipment, etc.	1	2	15	30
Transport of equipment	incl. staff arrival and return	2	2	15	60
Loading equipment on boat	incl. unloading afterwards	2	1	15	30
Pump measurement	3 sampling points + mixed sample across river profile	2	5	20	200
Rental Boat	Incl. boat crew	1	7	40	280
Estimated Total Costs					600

Annex 17:Cost estimate for MP-sampling with sedimentation box in RS (1 sampling point)

Cost center	Description	Staff	Hours	Costs* per h	Total costs
Preparation and Follow-up	Loading equipement, etc.	1	2	15	30
Transport of equipment	incl. staff arrival and return	2	2	15	60
Deployment of Sedbox	site accessible from shore	2	1	15	30
Removal Sedbox	incl. decanting of sample	2	1	15	30
Rental Boat	incl. boat crew	1	3	100	300
Estimated Total Costs	bank-near sampling without boat				150
Estimated Total Costs	incl. boat and fee				450

Bulgaria

Annex 18:Cost estimate for MP-sampling with net in BG (3 sampling point over transverse profile)

Cost center	Description	Staff	Hours	Costs* per h	Total costs
Preparation and Follow-up	Loading equipment, etc.	2	2	25	100
Transport of equipment	incl. staff arrival and return	6	2	70	840
Road closure and securing sampling site	Set up traffic signs, attaching barrier tape, etc.	3	2	12	72
Set up net devise for truck		2	1	25	50
Rental fee truck	Including truck driver	1	8	60	480
Net measurement	3 sampling points	3	5	35	525
Supervision traffic	incl. ship traffic	2	5	35	350
Required permissions					
Fee shipping inspectorate		1	1		350**
Estimated Total Costs					3091

*Average gross wages of participated staff

**shipping cost to the lab abroad

Annex 19:Cost estimate for MP-sampling with pump method in BG (3 sampling point over transverse profile)

Cost center	Description	Staff	Hours	Costs* per h	Total costs
Preparation and Follow-up	Loading equipement, etc.	1	2	35	70
Transport of equipment	incl. staff arrival and return	2	2	70	280
Loading equipment on boat	incl. enloading afterwards	2	1	35	70
Pump measurement	3 sampling points + mixed sample across river profile	2	5	80	800
Rental Boat	Incl. boat crew	1	7	320	2240
Required permissions					
Fee shipping inspectorate		1	1		
Estimated Total Costs					3460

Annex 20:Cost estimate for MP-sampling with sedimentation box in BG (1 sampling point)

Cost center	Description	Staff	Hours	Costs* per h	Total costs
Preparation and Follow-up	Loading equipement, etc.	1	2	35	70
Transport of equipment	incl. staff arrival and return	2	2	25	100
Deployment of Sedbox	site accessible from shore	2	1	35	70
Removal Sedbox	incl. decanting of sample	2	1	35	70
Rental Boat	incl. boat crew	1	3	20	60
Required permissions					
Fee shipping inspectorate		1	1		
Estimated Total Costs	bank-near sampling without boat				310
Estimated Total Costs	incl. boat and fee				370