**HUNGARIAN UNIVERSITY OF
AGRICULTURE AND LIFE SCIENCES**

INVESTIGATION OF MICROPLASTICS IN THE ENVIRONMENT

Ph.D. Thesis

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1. INTRODUCTION AND AIM

Global plastic production is continuously increasing, as these materials are present in all field of our lives. Without the use of plastics, the current standard of living and convenience, i.e., personal daily time saving is unimaginable. Plastics are truly contributing in many fields to improve the quality of our life (e.g., medical accessories, infrastructure pipeline systems), however in many cases the necessity of their extensive use and our consumer habit is considerable (e.g., disposable packaging).

Due to not appropriate manufacturer and consumer habits and the lack of well-developed waste treatment technologies plastics are globally present in the environment. Beyond the waste observable by the naked eyes, plastic particles under 5 mm, the so called microplastics (MPs) causes further problems. In the past couple of years, the occurrence and potential harmful effects of these tiny particles became not only an important field of environmental researches, but also got public and media attention.

To establish a detailed risk analysis, it is necessary to evaluate microplastic loads in different environmental matrices. This is especially complex, as microplastics are not a well-described material, but pollutants with wide ranges of certain parameters (e.g., size, shape, polymer type and density). Their occurrence has been reported globally from surface waters, sediments and biota as well, but the information on their presence in freshwater environments is limited.

The evaluation and comparison of monitoring data is also hindered by not unified sampling, sample preparation and analysis methods. This is caused by reporting results in different size ranges of MPs and also resulting in altering units of the MP values (e.g., numer or mass of particles in volume of water sample or area of water surface).

Due to the above-mentioned gaps in freshwater MP investigations the goal of my research was to collect surface water samples and analyse their MP content in Hungary, for the first time in Central and Eastern Europe. During my work, in favour

of standardisation, I aimed to develop methods for sampling and evaluate the efficiency of the sampling methods. After sampling, the samples are prepared in multiple steps for analysis, that are not unified as well and certain bias can occur. To be able to obtain more trustful and precise results, I aimed to develop a novel equipment for water sample preparation and to evaluate its efficiency as well.

The goals of my Ph.D. research were:
- to collect water and sediment samples from fish ponds and natural waters and evaluate their MP content
- to develop a sampling method that is capable of filtering large volumes of water on small pore size filters and evaluate its recovery efficiency for quantitative and qualitative MP analysis
- to develop a small volume, cost efficient, chemical thrifty separator equipment for sample preparation and evaluate its recovery efficiency for quantitative and qualitative MP analysis.

2. MATERIALS AND METHODS

2.1. Environmental samples

2.1.1. Sampling locations

There is no available data on the occurrence of MPs in freshwaters of Central and Eastern Europe and the information on MP load of aquaculture systems are globally lacking, so my goal was to sample fish ponds is Hungary. The sampling locations are highlighted in **Figure 1**.
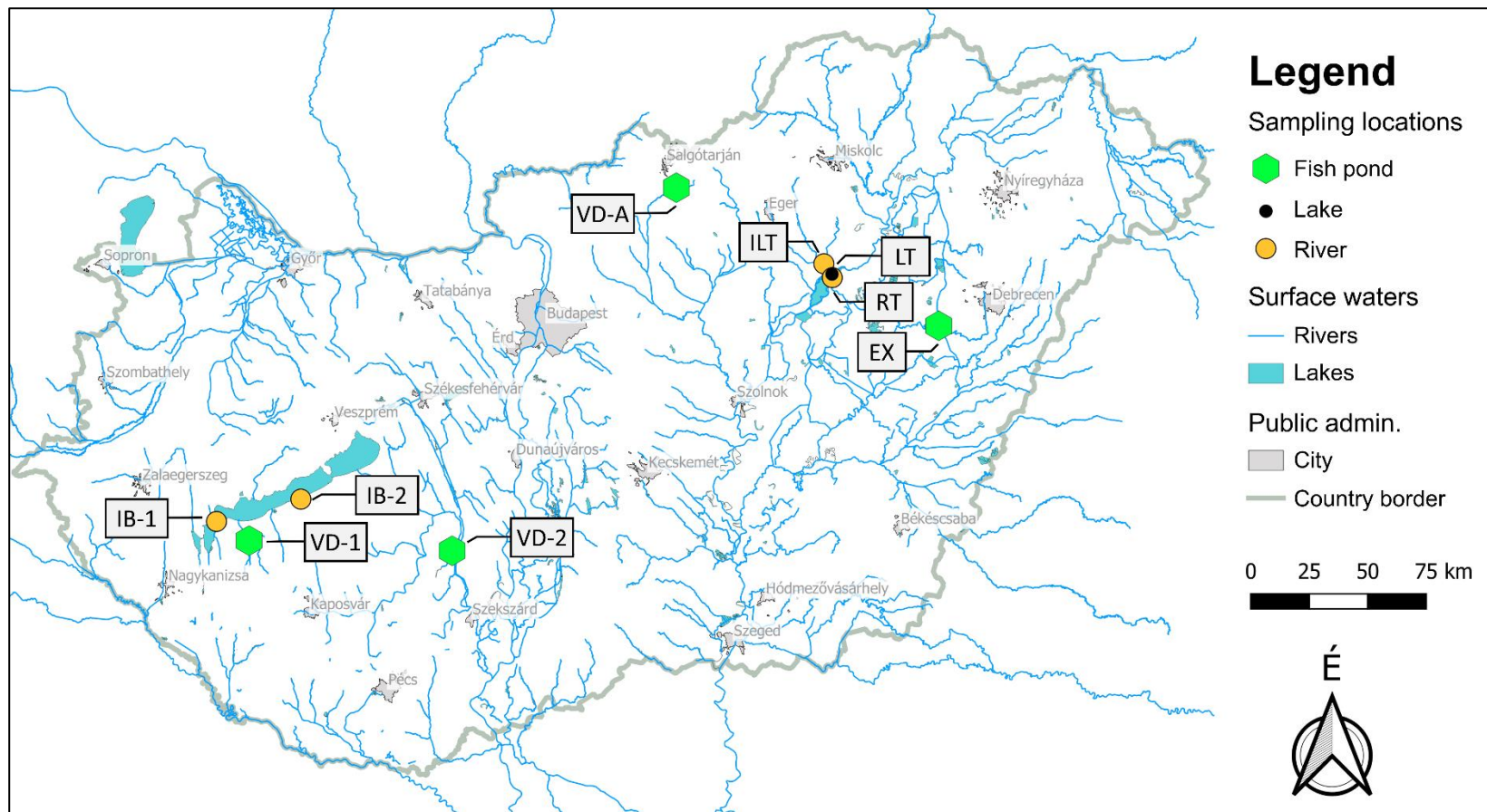
2.1.2. Sampling methods

2.1.2.1. Surface water

After a thorough literature review, a fractionated sampling system connected to a jet pump (Pedrollo JRCm 2A) has been set up, that was operated by a gasoline generator (Honda EU 20i). A 1" PVC hose with a brass foot valve including a 2 mm mesh size strainer is put right under the water surface (at a depth of 10 to 20 cm) and is connected to the pump. The function of the 2 mm mesh size strainer is pre-filtration to prevent early clogging of the fine mesh size filters where samples are retained. This helps to obtain larger sample volumes through the fine mesh size filters. Water is filtered through 10" filter cartridges (Infiltec GmbH, Germany), similar to the one applied by MINTENIG ET AL. (2014) and MINTENIG ET AL. (2017), first on 300 µm, then on 100 µm pore size filters. Water quantity is measured by a flowmeter (GMB MID R100-H, 0.1 L precision). In average 1.5 m^3, but at least 1 m^3 water was sampled (during 30-40 minutes) to obtain representative results.

2.1.2.2. Sediment

At every location, 2 to 3 kg of bottom sediment samples were also collected using a stainless-steel van-Veen grab sampler and a hand spade. Samples were stored in metal containers until analysis. All sample containers were rinsed with Milli-Q quality water before use.

Figure 1: Water and sediment sampling locations. (VD: valley dammed; EX: excavated; A: angling; IB: inlet Balaton; RT: River Tisza; LT: Lake Tisza; ILT: inlet Laket-Tisza)

2.1.3. Sample preparation and analysis methods

For the density separation, a device described by IMHOF ET AL. (2012), the MicroPlastic Sediment Separator (MPSS, obtained from Hydrobios GmbH) was used. Sieves were rinsed and water samples from the cartridges were poured in the MPSS, then filled up with saturated NaCl solution (1.2 kg/L), stirred for two hours, then they were allowed to settle overnight. In the case of sediment samples, the exact amount measured into the MPSS was recorded, stirring was conducted for four hours, and the remaining steps were the same.

After settlement, the top of the device was disconnected, the supernatant sample (ca. 200 ml) was poured into a beaker and treated with the same volume of 30% H_2O_2 on a laboratory hot plate at 80 °C for 1 hour on 450 rpm. When the oxidation was completed, the discolored solutions were filtered on 0.2 µm pore size Whatman Anodisc (Merck KGaA, Darmstadt, Germany) aluminium oxide filters (25 mm diameter). Filters were analysed under a Bruker Lumos FTIR microscope (Bruker Corporation, Billerica, MA, USA). All suspicious particles selected visually were then automatically analysed in ATR mode. Spectra were compared to the Nicodom FTIR database. Polymeric particles were enumerated and the results were calculated in particles/m^3 or particles/kg, using the sample volumes and mass recorded accurately earlier.

2.2. Sampling method development and efficiency evaluation

2.2.1. On field evaluation of filtered sample volume and filter pore size as efficiency parameters

One of the filters used for the sampling of fish ponds are identical to the widely used manta-net sampling method (300 µm), but application of plankton nets with similar pore size to the other filter (100 µm) also exists. During the efficiency testing of filtering my goal was to establish a system that is capable to filter particles under 100 µm, so I investigated if smaller pore size filters individually or connected

in a raw are capable to filter large volume (1-2 m^3) samples. This was tested in three replications on different types of lakes and rivers. Sampling was considered successful, if individual measures reached the volume of 1 m^3 and the average of the three replicates reached the volume of 1.5 m^3. The below filter setups were tested: 15 µm; 50-15 µm; 50-25 µm; 100-50-15 µm. Beyond decreased pore size of the filter, during the development of the sampling system I strived to exclude plastic spare parts to avoid sample contamination. This was reached by the replacement of the PVC hoses by rubber hoses and the PP filter housings by stainless steel ones. The improved sampling system is presented in **Figure 2**.
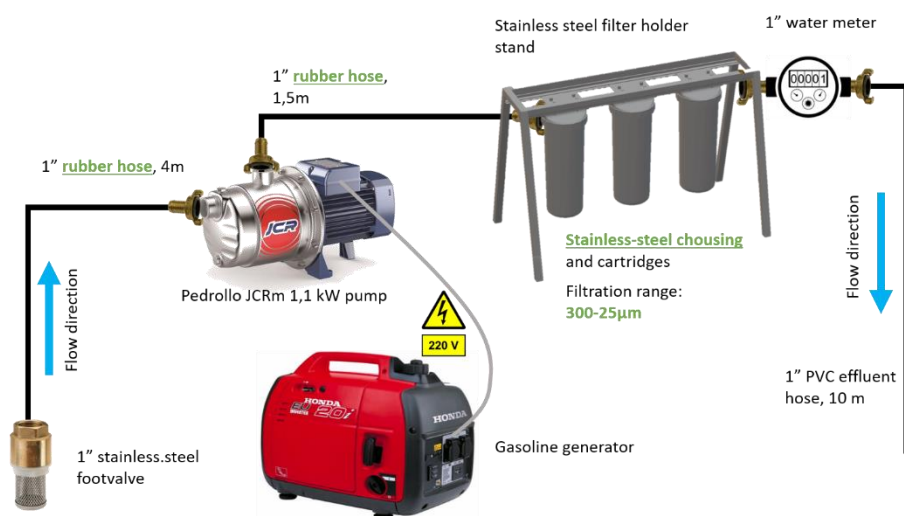
Figure 2: Improved sampling system.

2.2.2. Investigation of microplastic recovery during sampling in controlled test environment

An important feature of the sampling system to collect high volume samples on small pore size filters, but this does not provide information on its recovery efficiency in terms of the representativeness of the samples (i.e., the relation of reported particle concentration and real MP load in the environment). Until now, recovery efficiency of sampling methods were reported in the literature only a few times (ZIAJAHROMI ET AL., 2017; BANNICK ET AL., 2018; FUNCK ET AL., 2020).

2.2.2.1. Preparation of reference microplastics

As only microspheres or powders are available commercially, other types of particles have to be prepared individually. Fragments were generated by cryogenic milling from commercial packaging (PE, PP, PVC PET), and then were sieved to the required fractions. Synthetic fibres (Kaposplast Kft., Hungary) were also used and hand-cut for the desired length (1-1.5 mm). Further to above-described materials, commercially available UV tagged PE microbeads (Cospheric Llc., USA) have been used.

2.2.2.2. Sampling test in controlled environment to determine MP recovery rates

The sampling tests were conducted in a stainless-steel tank (1x1x3 m) to avoid plastic contamination. When the water level reached 1,500 liters in the tank, the content of the prepared reference MP dispersion (30 particles of each material, in total 240 particles) was added and a stirrer was started and operated until reaching the final 2,400 liters, to homogenize the tank's content. The final MP concentration was 100 particles/m^3, which is environmentally relevant in case of freshwaters (ZHAO ET AL., 2014; MANI ET AL., 2015; WANG ET AL., 2017).

Five different setups and a control measurement (all in three replications) have been applied. In all cases 1.5 m^3 water was sampled. These setups were designed with different depths and with different stirring conditions in the stainless-steel tank. The suction valve was either kept constantly 10 cm below the water surface or on halfway between the surface and the bottom (referred to as half-water column sampling). To model different turbulence conditions, during the tests, there were (1) no stirring (N), (2) periodic (P), or (3) continuous stirring (F).

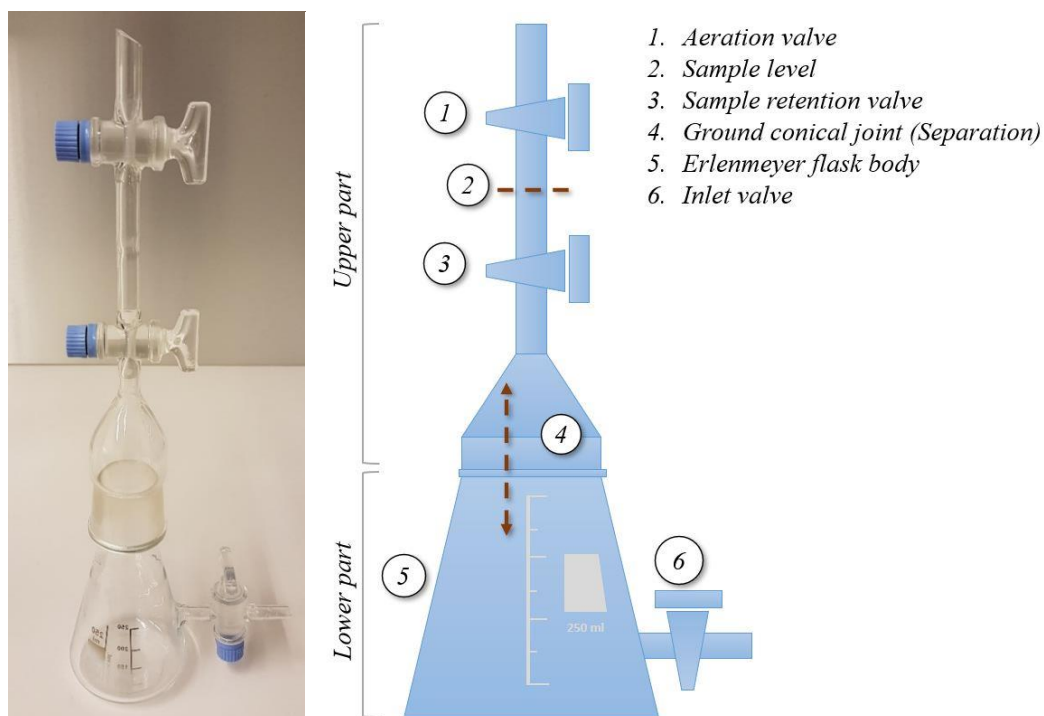After filtration tests, the content of the filters and filter housings were thoroughly rinsed, then it was filtered on a Whatman Anodisc (25 mm in diameter, 0.2 µm pore size, Merck KGaA, Darmstadt, Germany). The filters were analysed with a Fourier transform (FT) spectrophotometer – microscope system in the near-infrared (NIR) region. After correlating reference spectra, particles with a correlation of over 80% were accepted as MPs.

2.3. Development of sample preparation and determination of MP recovery rates

The evaluation of MP recovery during sampling means more precise results, however losses can also occur during sample preparation. This indicates a need for method development and recovery measures in this process as well.

2.3.1. Development and operation of the "Small Volume Glass Separator (SVGS)"

A major step during sample preparation is density separation. After reviewing and unifying the advantages of methods described in the literature, I've designed an equipment that enables fast and cost-effective sample preparation. After submitting a request for utility model protection in 2019, the equipment received utility model protection this was warded later by the Hungarian Intellectual Property Office (reference nr. 5155). The SVGS – that is presented in **Figure 3** – consists of two parts made of borosilicate glass that are attached with a ground-surface conical joint.

1. Aeration valve
2. Sample level
3. Sample retention valve
4. Ground conical joint (Separation)
5. Erlenmeyer flask body
6. Inlet valve

Figure 3: The Small Volume Glass Separator (SVGS).

The sample is added to the bottom part through the top opening of the bottom part with a glass-coated magnetic stirring rod. Afterwards the upper part is attached, and the brine solution (ca. 500 ml) is filled in through the side inlet valve. The separated sample (ca. 10 ml) is collected between two valves in the upper part. After the separation the sample retention valve is closed, the brine solution is discarded on the side valve for recycling, and the upper part containing the sample is detached. The upper part is turned upside down, and the sample is transferred with thorough rinsing.

2.3.2. Determination of MP recovery rates during sample preparation

The SVGS equipment compared with a widely used preparation method, described by MASURA ET AL. (2015). For this, fluorescently tagged microspheres (FMP) were used, and the recovery efficiency of the SVGS was also tested with the application of environmentally more relevant particles (in terms of sizes and shapes).

2.3.2.1. Comparison of sample preparation methods

For the method comparison test commercially available microspheres (Cospheric Llc.) have been used. To ensure standard quality and avoid unknown naturally occurring microplastics of the test medium, artificial solutions were prepared based on international guidelines (OECD 201 artificial freshwater, OECD 3030 artificial wastewater).

2.3.2.2. Determination of MP recovery rates using the SVGS

The SVGS was evaluated for the recovery capacity of environmentally relevant (size, shape and polymer type) microplastics as well. For this, 1 L of OECD 201 solution was spiked with PE, PP, PET, PVC fragments and PA fibre (10 of each, 50 in total). Test were run in triplicate. After sample preparation (density separation and oxidation) samples were filtered on 0.2 µm pore size Whatman Anodisc (Merck KGaA, Darmstadt, Germany) aluminium oxide filters (25 mm diameter) and analysed by FT-NIR microspectroscopy.

3. RESULTS AND DISCUSSION

3.1. Microplastics in environmental samples

3.1.1. Water samples

Altogether 13 surface water samples were taken (in average 1492 ± 326 L) and 12 of them contained microplastics between 2 mm and 100 µm ranging from 3.52 to 32.05 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). The results are presented in **Figure 4**, emphasizing that until these measurements none of the studies have been indicated MPs in fish ponds globally. As it is highlighted in **Figure 4**, only one valley dammed lake effluent sample (VD-2-OUT) was free of microplastics over 100 μm, but all fish pond influents – that are originating from natural surface waters – were loaded with MPs.
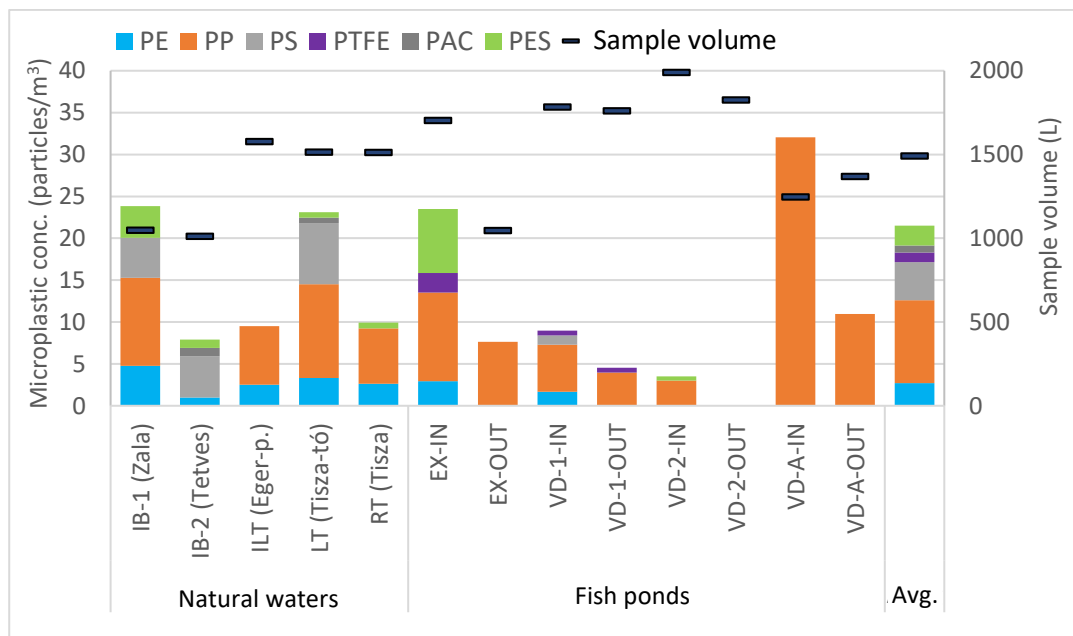
Figure 4: Microplastics in surface waters. (IN: inlet, OUT: outlet, VD: valley dammed fish pond, EX: excavated fish pond, PE: polyethylene, PP: polypropylene, PS: polystyrene, PTFE: polytetrafluorethylene, PAC: polyacrilate, PES: polyesther)

The results show different MP loads in the samples, but **it is important to note that MPs in all influents presented higher values than in the effluents**. Although these results are in a similar range that of other freshwaters in Europe, results are still not evidently comparable due to the lack of unified sampling and analysis methods. While on the river Rhein 1-10 particles/m^3 (MANI ET AL., 2015) was measured, in Italian lakes and Swiss rivers 0.2-4.1 particles/m^3 and 0.1-6.5 particles/m^3 was found, respectively (FAURE ET AL., 2015; FISCHER ET AL., 2016).

All these results were obtained by 300 µm mesh size plankton nets, and no comprehensive FTIR analysis has been conducted. Compared to these studies the current work means a step forward as

- particles have been investigated with a lower cut-off (100 µm)
- all suspected particles (based on morphology) have been analysed by FTIR to reduce false positive results
- indicative MP measures of fish ponds were lacking globally.

If we analyse the abundance of the different polymer types in the samples, we can conclude that **occurrence of PP and PE is the most dominant**. These are the most commonly identified materials in European freshwaters (FAURE ET AL., 2015; MANI ET AL, 2015; SCHERER ET AL., 2020). This is not surprising, as collectively they take almost 50% proportion of the global production (HTTP 1). The occurrence of different polymer types in the positive samples is highlighted in **Figure 5**.

3.1.2. Sediment samples

The average processed sample mass was 2703 ± 493 g. Out of the 12 sediment samples (the River Zala could not be sampled due to the rocky bottom at the site), 9 showed contamination ranging from 0.46 to 1.62 particles/kg, with a mean value of 0.81 ± 0.37 particles/kg. These data indicate were much lower concentrations than in previous European (KLEIN ET AL., 2015; FISCHER ET AL., 2016; LESLIE ET AL., 2017; SCHERER ET AL., 2020) or international freshwater studies. (SU ET AL., 2016; WANG ET AL., 2017). The results of the current measurements are represented in **Figure 6**.
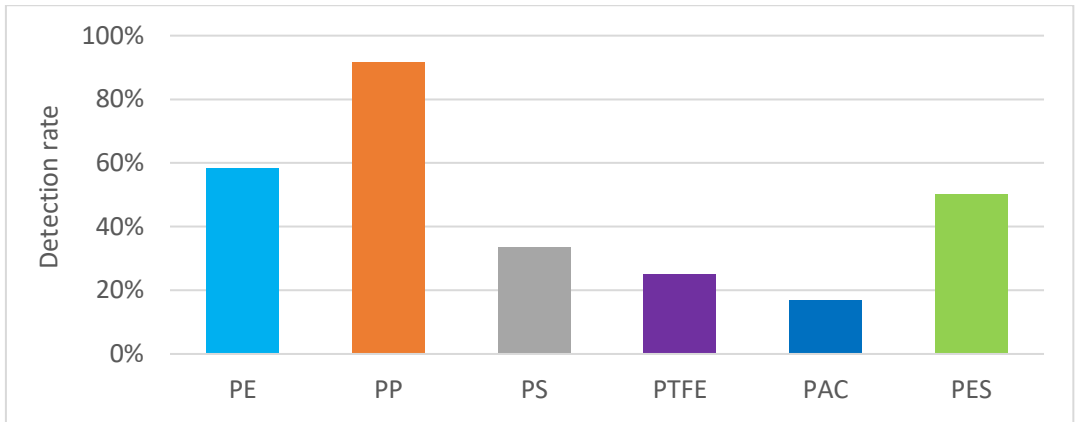
Figure 5: Detection rate of different polymer types in the positive samples. (PE: polyethylene, PP: polypropylene, PS: polystyrene, PTFE: polytetrafluorethylene, PAC: polyacrilate, PES: polyesther)
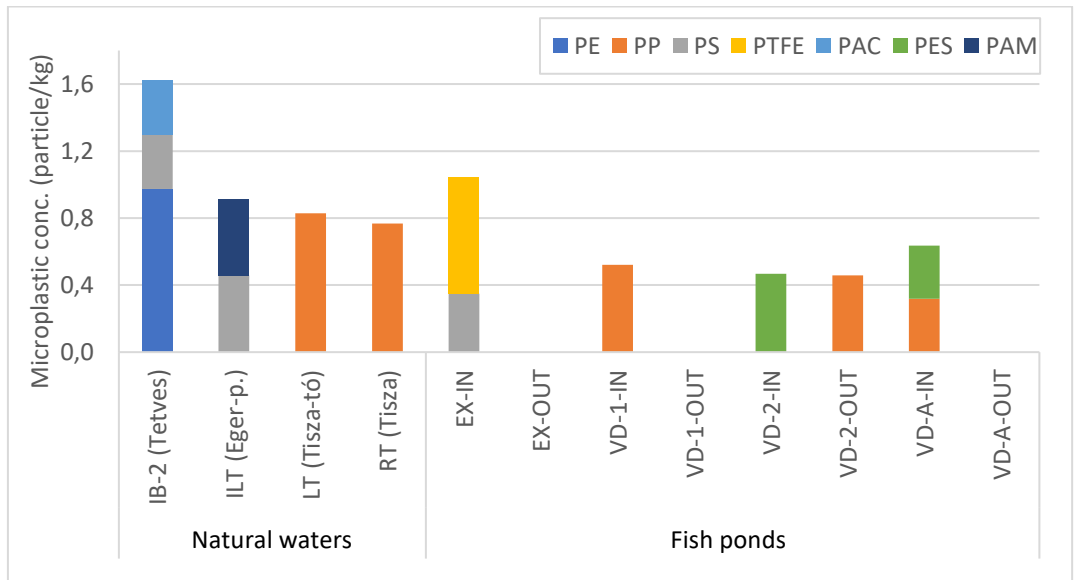
Figure 6: Microplastic concentration (particles/kg) in sediment samples. (PE: polyethylene, PP: polypropylene, PS: polystyrene, PTFE: polytetrafluorethylene, PAC: polyacrilate, PES: polyesther; PAM: polyamide)

In the sediment samples the most common polymer type was PP. In bottom sediments, based on previous European studies beyond PP, PE was commonly identified as well (VIANELLO ET AL., 2013; SCHERER ET AL., 2020). While the results of the fish pond water samples are comparable to the international freshwater studies, the results of the sediment samples are much lower. One reason for this might be the

density of the used brine solution (NaCl; ~1.2 g/cm^3) during the density separation step. This conclusion was considered later, so when the development of sample preparation was conducted, denser brine solution was used to improve recovery of denser polymers.

## 3.2. Sampling method development and efficiency evaluation

The first MP sampling series resulted in versatile experiences, that has drawn attention on the need of sampling method development and its efficiency evaluation. To improve filtering efficiency, smaller pore size filter cascades were tested on the field. Primary goal was to sample large amount of water (optimally 2 m^3) while keeping the filter pore size lower. The MP recovery efficiency of the whole sampling system was finally tested in a model system, that was spiked with microplastics.

3.2.1. On field evaluation of filtered sample volume and filter pore size as efficiency parameters

The field tests were conducted with the sampling system, that has been developed based on the fish pond sampling experiences. The improved system contains no plastic spare parts, as rubber hoses and sealings, such as stainless-steel filters and filter housings were applied. The average filtered sample volumes and flow rates are shown in **Figure 7**. We can conclude, that the 50-25 µm filter cascade was the only one, that fulfilled the requirements at all locations.

Filtration of surface water samples down to 25 µm with large sample volumes is a remarkable improvement, as in previous studies on smaller pore size filters (3-10 µm) large volumes (200-1100 L) were only obtained in cleaner matrices, such as drinking water (FUNCK ET AL., 2020; KIRSTEIN ET AL., 2020; MINTENIG ET AL., 2019). From surface water only 12-20 L water samples could be filtered on smaller, 32-50 µm mesh (ZHAO ET AL., 2014; WANG ET AL., 2017) and larger volumes was passed through only on larges mesh (100-250 µm) filters LUSHER ET AL., 2014; SETÄLÄ ET AL., 2016).
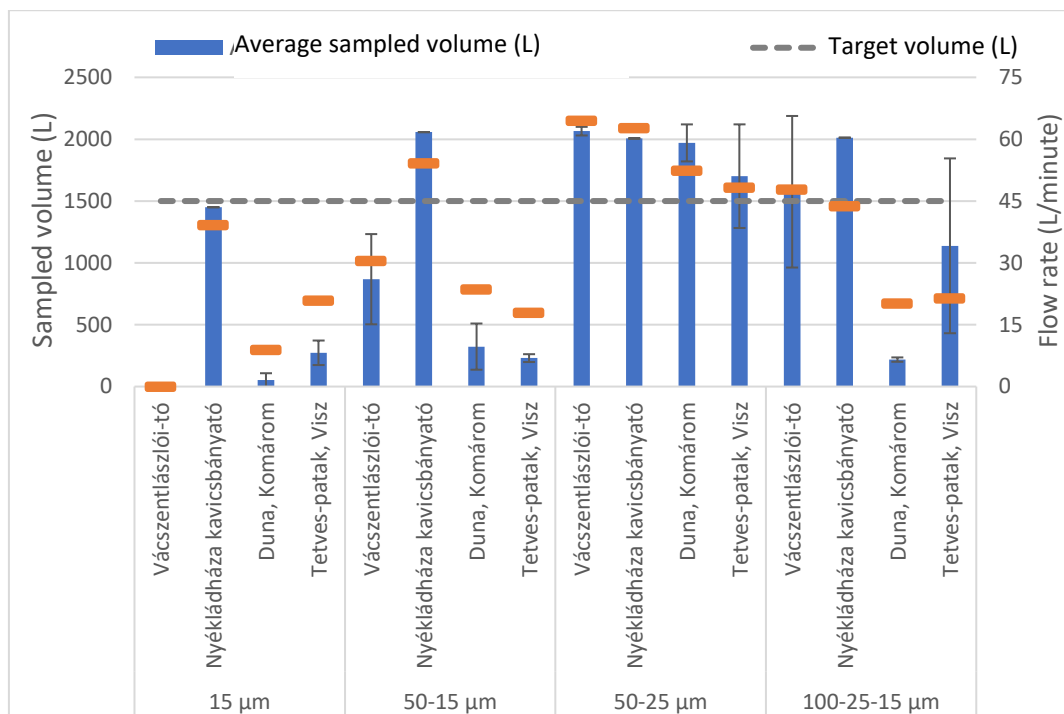
Figure 7: Average sampled volumes in the field test (mean of three replications ± SD) and the specific flow rates.

3.2.2. Investigation of microplastic recovery during sampling in controlled test environment

The field tests proved the sampling capacity of the 50-25 µm filter cascades, so microplastic sampling efficiency of this setup was also tested. This was conducted in a larger scale system, where a determined MP concentration was set in the water and samples were taken and analysed to determine recovery rates.

Compared to previous studies a novelty of this test, that different sampling depth and turbulence conditions were compared. Different turbulence conditions were meant to represent different lakes and rivers, where the distribution of MPs is not only described by their density but other parameters, such as turbulences as well. The highest recovery results occurred during the continuous stirring of the tank. When sampling depths are compared, it can be concluded, that **surface sampling resulted in higher recovery values** under all turbulence conditions, results are represented in **Figure 8**.

Regarding polymer types and their density, PE particles with low density clearly showed the highest recovery in all setups (in average 29.7% at fragments [0.95 g/cm^3] and 24.9% at spheres [1.2 g/cm^3]). Without considering polymer shape, descending order of average recoveries based on three replications are: PE (27.3±18.7%, range 11.5-62.2%) > PA (16.9±11.9%, range 7.1-40.4%) > PVC (8.5±8.4%, range 1.8-24.6%) > PET (7.4±6.6%, range 2.7-20.2%) > PP (3.2±3.3%, range 0.9-9.7%). Except PP, these **results indicate decreasing tendency of recovery for MPs with higher density**: PE (0.95 g/cm^3) > PE (1.2 g/cm^3) > PA (1.14 g/cm^3) > PET (1.3 g/cm^3) > PVC (1.29 g/cm^3) > PET (1.37 g/cm^3). This indicates a particle loss due to sedimentation. The results are highlighted on **Figure 8**.

In our study there is a limitation to compare the effect of polymer size on recovery, as only PP and PET were used in different sizes (100-300 µm fragments and 1000-1500 mm fibres). Similar to earlier studies (HENGSTMANN ET AL., 2018; HURLEY ET AL., 2018; SILVA ET AL., 2018; WIGGIN & HOLLAND, 2019) average percentage of recovery decreased parallelly with the size class of particles. There is a further limitation as particle shape is also different, even though if fibres are rather thick and not simulating laundry originated MPs. Fragmentation of polymers might cause an error as well, but this has not been tackled in any sampling validation study yet and neither could be measured in the frame of this research.

The goal was to cover the three main shapes of microplastics (spheres, fragments, fibres) during the experiments and thus to represent environmentally relevant particles as well. This is an important step, as currently commercially available spheres/pellets are probably the most popular in not only the sample preparation validation (QUINN ET AL., 2017; HURLEY ET AL., 2018; WIGGIN & HOLLAND, 2019) but in sampling validation tests as well (BANNICK ET AL., 2018; HILDEBRANDT ET AL., 2019; FUNCK ET AL., 2020).

When we look at the recovery results of the current sampling validation considering the shape of the polymers, we can conclude that microspheres have the highest average recovery rates (24.9±34.4%). Recovery for fibers and fragments are

much lower, 11.0±10.4% and 10.8±3.8%, respectively (as presented on **Figure 8**). The results support the opinion that **studies applying only microspheres as reference material might be optimistic and overestimate the recovering capacity** of the method for environmentally more relevant (regarding shape and size) particles.
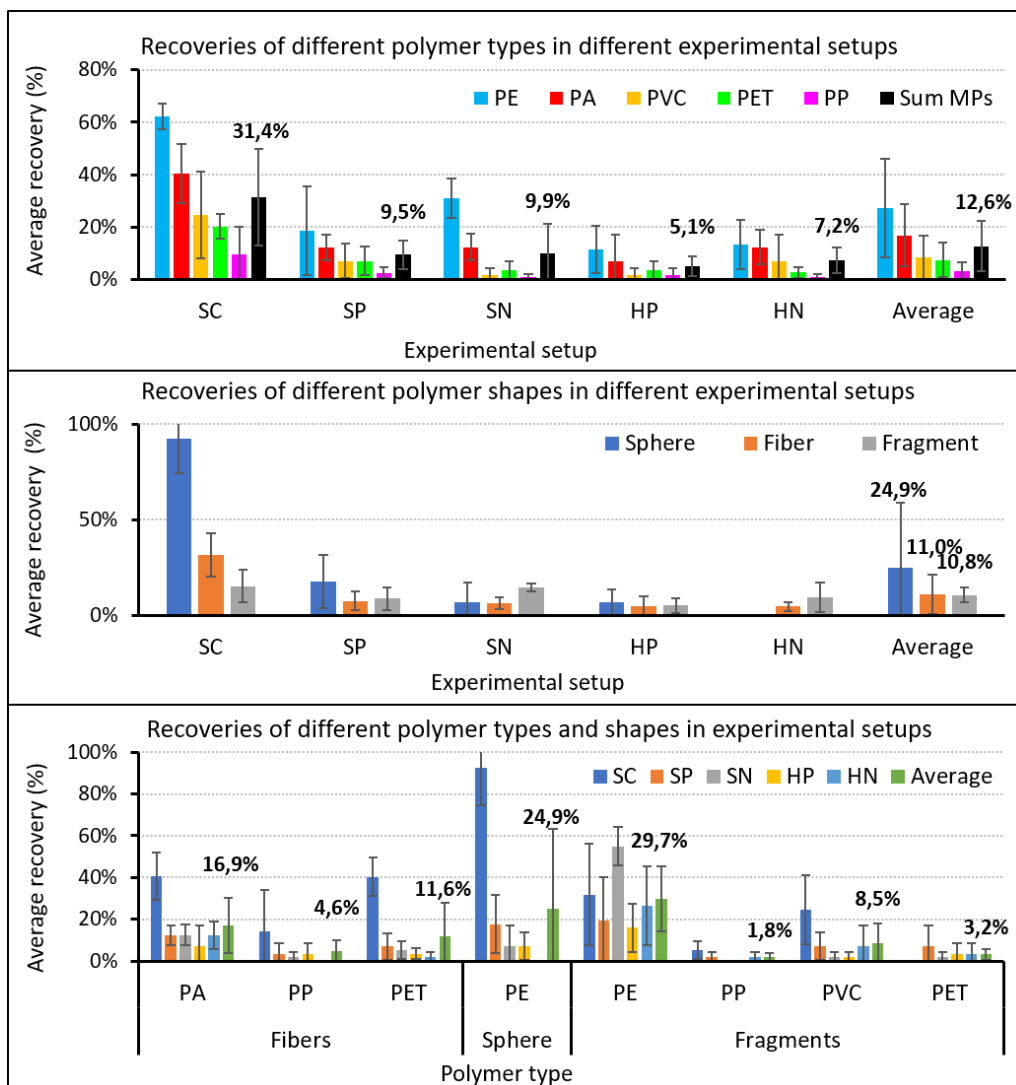
Figure 8: Average recovery rates (%) of different polymer types and shapes in different experimental setups. Data are expressed as mean ± SD based on three repetitions. (SC- surface, continuous stirring; SP – surface, periodical stirring; SN – surface, no stirring; HP – half water column, periodical stirring; HN – half water column, no stirring. PE – polyethylene; PP – polypropylene; PVC – polyvinyl chloride; PET – polyethylene terephthalate; PA – polyamide)

3.3. Determination of MP recovery rates during sample preparation

Beyond investigating sampling efficiency, my aim was to also determine recovery rates during sample preparation, because losses or sample contamination can occur at these steps as well, which can influence the precision of the final results.

An equipment called the **Small Volume glass Separator (SVGS) was designed to improve sample preparation. This device enables fast sample preparation in a cost-efficient way, with low volumes of brine solution and few sample transfer steps**, that helps to prevent both contaminations of the sample and particle loss. The SVGS and the related procedure was compared to a widely used sample preparation method with the use od fluorescently tagged microspheres and standard artificial solutions. The recovery efficiency of the SVGS was tested with environmentally more relevant (regarding shape and size) particles as well.

3.3.1. Comparison of sample preparation methods

As it is highlighted on Figure 9, the results of the five replicates show that **the method described by MASURA ET AL., 2015 resulted in 45-70% recovery, while the use of the SVGS resulted in 81-93% recovery**. All polymers used in this test are made of PE and all are spherical. This allows a comparison of the effects of density and size on the recovery. A clear correlation between density and recovery could be observed, as the highest-density particles (300-355 µm; 1.2 g/cm^3) had poor recovery (average 14%±20%, range 4-34%) in every scenario when the samples were prepared with the method of MASURA ET AL. (2015), while less dense particles (300-355 µm; 1.13 g/cm^3) had higher recovery (average 65%±14%, range 24-88%) and the low-density particles (300-355 µm; 0.97 g/cm^3) showed the highest values (average 92.5%±7%, range 86-98%). In case of the SVGS method effect of density is not observable as all particles in the size range of 300-355 µm (with different densities) showed similarly high recovery results in each medium (average 92.5%±11% - 94.5%±8%, range 86-100%).

In the case of particles with similar densities (0.97 – 0.98 g/cm^3), but with different size (90 – 106 µm; 300 – 355 µm) there is a clear difference in recovery rates, too. Bigger particles are recovered better, both methods yielded an average of 92.5±7% recovery. Smaller particles were recovered with the SVGS in average 60%±23% (range 48-78%), which is slightly lower, than the recovery of the other method (average 69%±20%, range 50-74%). All results are presented in **Figure 9**.

3.3.2. Determination of MP recovery rates using the SVGS

The recovery efficiency of the SVGS was tested not only with the use of microspheres but environmentally more relevant fragments (PE, PP, PET, PVC in 100-300 size range) and fibres (PA, 1000-1500 µm length). As shown in **Figure 10**, based on three replications on average of all polymers, 64%±28% was recovered. From all five materials, PA (1.14 g/cm^3) had the highest recovery rate consistently, all replicates yielded over 90% (on average 96.7%±4.7%). The second highest recovery regarding the PVC (1.28 g/cm^3) particles, on average 90%±8.2% was recovered. The results of the other three particle types vary: the highest density polymer, PET (1.37 g/cm^3), yielded on average 50%±14.1%, similarly to the low-density PE (0.95 g/cm^3) where on average 53.3%±20.6% of the particles were recovered. Interestingly, the worst recovery occurred in case of PP, where the density is the lowest amongst the tested materials (0.9 g/cm^3). PP was recovered only in an average of 30%±14.1%.
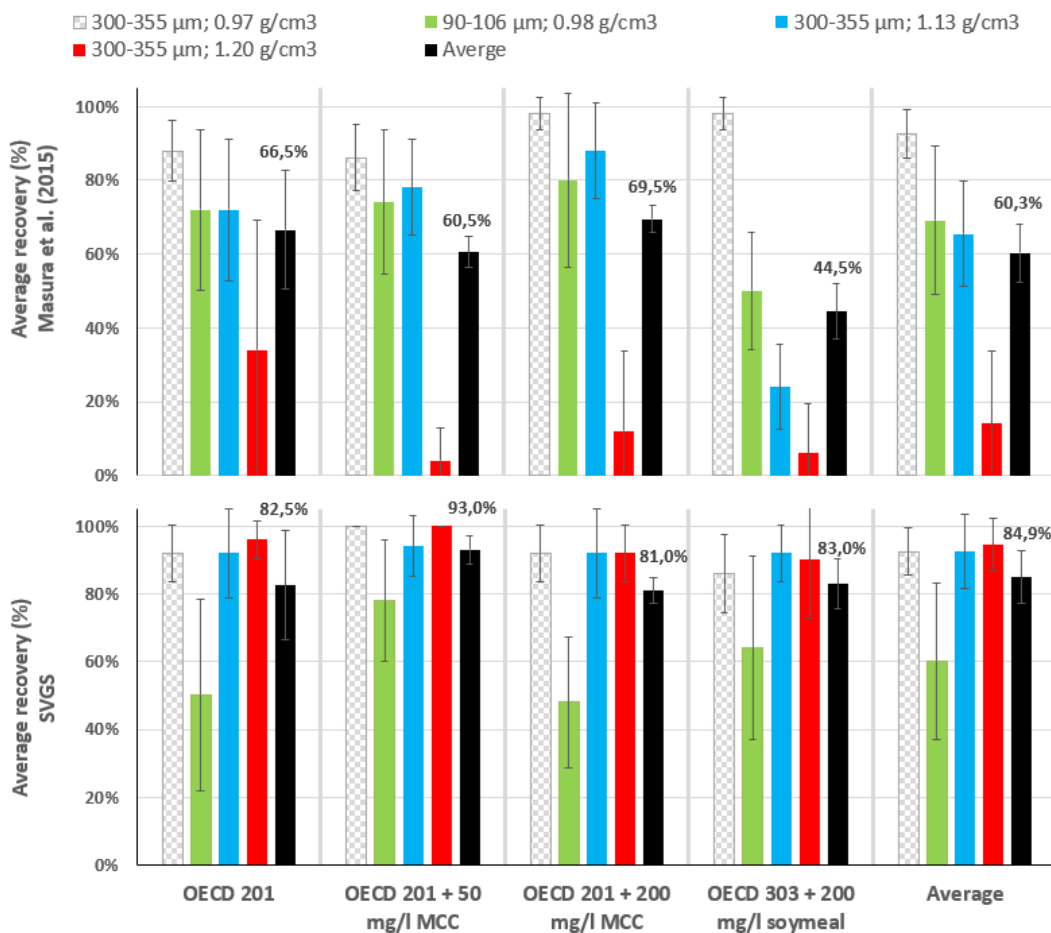
Figure 9: Recovery efficiencies (mean ± SD based on fife replications) of the method by MASURA ET AL. (2015) and the Small Volume Glass Separator (SVGS) using fluorescent microspheres of 90-106 μm és 300-355 μm size and 0.97-1.2 g/cm^3 density. (MCC – microcrystalline cellulose).

Both with fluorescently tagged microspheres and environmentally more relevant particles and fibres it was found, that recovery of smaller particles is worse than that of the larger particles. Effect of polymer density on recovery was clearly shown in test conducted with FMPs, while no correlation was indicated with environmentally more relevant particles.
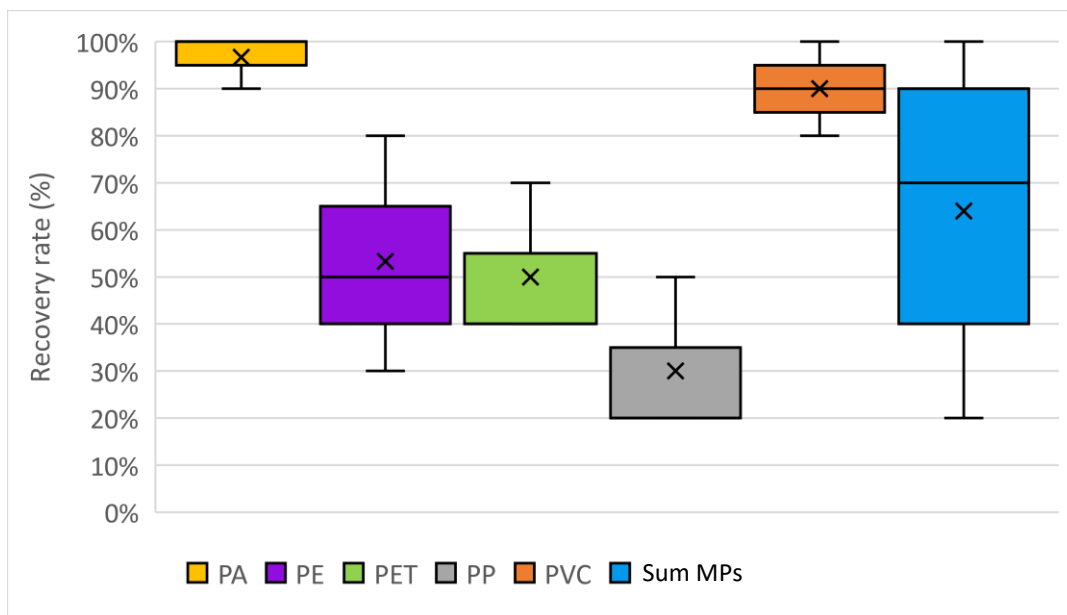
Figure 10: Recovery of environmentally relevant microplastics with the SVGS based on three replications. On the box-plot upper and lower whiskers represent the minimum and maximum values, in the interquartile range average is highlighted with x and median with a line. (Polyamide [PA]; Polyethylene [PE]; Polyethylene terephthalate [PET]; Polypropylene [PP]; Polyvinyl chloride [PVC].)

Similarly to the sampling method validation test results, it was observed during the sample preparation with the SVGS that the recovery rate of spherical MPs are higher (in average 93±2%), than that of environmentally more relevant fibres and fragments (in average 64±28,7%). **This highlights that possible overestimation can occur in the recovery when only microspheres are used during the test.** Despite this fact, fluorescently tagged microplastic beads are a viable alternative for standard addition purposes, helping to build strong QA/QC measures. These serve as an optimal surrogate standard as they are not present in the environment so these can be well distinguished from natural ones, thus excluded from the final results. Furthermore, FMPs provide valuable information about particle losses, as these are easily tracked during preparation processes.

4. CONCLUSIONS AND RECOMMENDATIONS

During my Ph.D. research I have investigated microplastics in environmental samples (surface water and sediment) and developed sampling and sample preparation procedures to improve the currently diverse methods, which in the end enhances unification, that is necessary to help the comparison of the results in the future. The different steps of method development and its results are presented in **Figure 11**.
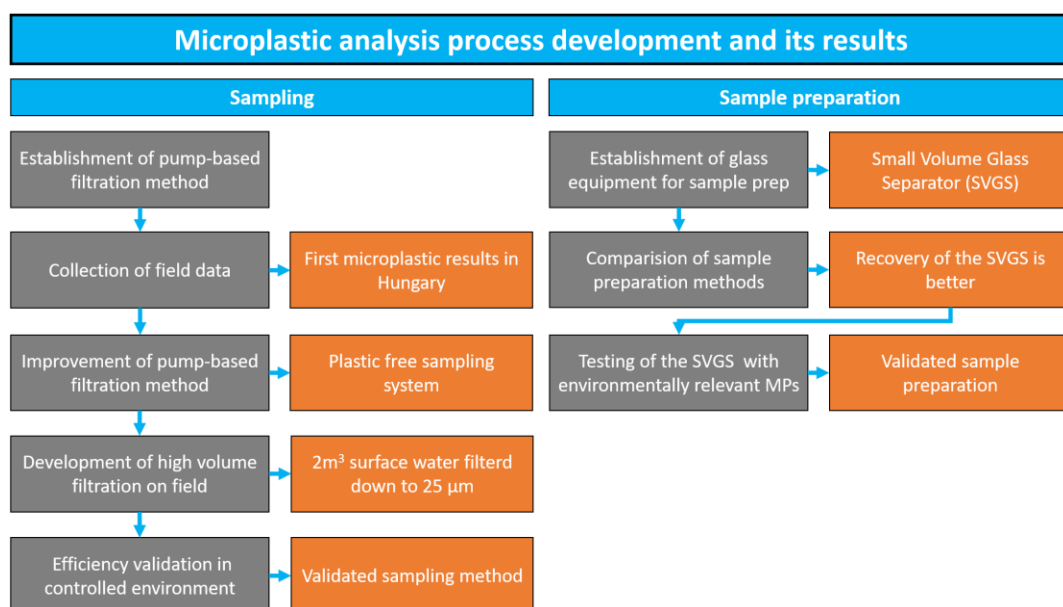
Figure 11: Different steps of microplastic sampling and sample preparation method development conducted during my research and its results.

My research resulted in the indicative analysis of microplastics in the environment for the first time in Hungary. These are the first results in Central and Eastern Europe as well. As currently published sampling and sample preparation methods are not unified, I have contributed to develop these procedures and evalute its recovery capacity.

The results showed, that all of the natural waters (lakes, rivers) and all of the fish pond inlets – that are originating from natural freshwaters – were polluted with MPs. regarding effluents, only one valley dammed reservoir was contaminated with

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MPs. Results are between 5-25 particles/m^3 (average 13.79 particles/m^3, min.: 3.52 particles/m^3, max.: 32.05 particles/m^3, based on 13 samples). However comprehensive comparison with previous results is not possible (due to different sampling strategies and analysis methods), it is observable that current results are in the same range as in the international literature. The most commony polymer types that were identified during my work were PE, PP and PS, similarly to earlier studies. The results of the sediment samples yielded much lower MP concentrations than that of the previous studies. None of the samples contained more than 2 particles/kg. Particles were dominantly identified as PE and PS.

These indicative results are not sufficient to serve as monitoring data, but still valuable as the first results in the region, and also indicates the necessity of well-organised monitoring programs. Only these campaigns can provide in the future sufficient information on exposure and serve as an input data for ecological an human health risk modelling. The setup of monitoring programs is not possible while unified methods are lacking, so an important goal of my research was to develop and evaluate sampling and sample preparation methods. I have designed and applied such equipment and procedures that ease reproducibility of freshwater sampling and sample preparation thus enhancing standardisation.

For the first Hungarian samplings I've establish a compact, easy to use sampling system based on fractionated filtration. The samples can be transported in the filter cartridges that prevents contamination. Sample volume is precisely measured by a water meter to help reproducibility and result comparison. Based on field experiences, the sampling system has been further developed, e.g., all plastic spare parts were excluded. I've examined different filter cascades to collect larger (1-2 m^3) sample volumes on smaller pore size filters. It was concluded that the 50-25 µm cascade was efficient on all lakes and rivers with different trophic levels. Filtration of surface water samples down to 25 µm with large sample volumes is a remarkable improvement, as in previous studies this volume was filtered only on 100-300 µm pore size filters. Further development possibility to reach lower cut-off

level of sampling at 10 µm, but this might need the enlargement of filter surface or periodic reverse flushing of the filters.

It is an important feature of the sampling device that it collects large volume samples on small pore size filters, but this does not characterize its MP particle recovery efficiency. To gather information on this, the whole system was tested in a model environment with the use of the 50-25 µm filter cascade. In this test not only commercially available microspheres were used, but environmentally more relavant particles (fibres, fragments) as well.

Test were conducted in different depth (surface, half water column) and in different turbulence conditions (no stirring, periodical or continuous stirring). Surface sampling was proven the most efficient. Decreasing recoveries parallel with increasing polymer density were indicated. It was also observed, that microspheres are better recovered than other particles.

This highlights that possible overestimation can occur in the recovery when only microspheres are used during the test which means, the use of irregular particles is necessary for better characterisation of recovery efficiency.

The evaluation of MP recovery during sampling means more precise results, however losses can also occur during sample preparation. This indicates a need for method development and recovery measures in this process as well. To improve sample preparation, the Small Volume Glass Separator (SVGS) was designed. With this denser brine solution can be used on a cost-efficient way and recovery of denser particles are improved as well. The method was compared to a widely used method in the literature with the use of FMPs and standard aqueous media. Based on the results of the five replications we can conclude, that the SVGS method yielded better recovery results (81-93%) than the method described by MASURA ET AL., (2015), where recoveries were between 45-70%. With both methods larger particles (300-355 µm) are recovered better.

Based on these results, the SVGS seemed a viable solution for sample preparation, but its efficiency has to be tested with environmentally more relevant particles (fibres, fragments) as well. Average recoveries of these materials were 64±28,7%. the effect of polymer density on recovery was not shown, but it was observable, that the recovery of spheres is better. Th highlights that the sole use of spheres might lead to possible overestimation, as observed similarly during sampling validation.

Researched leading to the above-mentioned recommendations are started in the past years. Complex development of the topic of microplastics could be enhanced with:

- further validation of sampling, sample preparation and analysis methods
- establishment of standardised methods based on applicability, recovery and cost-efficiency
- comprehensive monitoring programs with standardised methods to obtain information on sources and fate of microplastic
- identification of human exposure routes (e.g., drinking water, foodstuff, indoor and outdoor air)
- identification of possibly harmful ecotoxicological and human health effects and establishment of complex risk assessment models
- awareness raising in social, manufacturer and distributor level to avoid use of plastics and generation of plastic waste
- development and application of appropriate waste management systems.

5. NOVEL SCIENTIFIC FINDINGS

Thesis 1

Microplastics between 0.1-2 mm have been identified in surface waters, such as fish ponds with the use of fractionated filtration in Hungary for the first time. The average concentration was 21.5 particles/m^3 (3.52-32.05 particles/m^3 range). After density separation with sodium-chloride polymer types were identified. Most abundant polymers were made of polypropylene and polyethylene.

Thesis 2

During indicative analysis of freshwater sediments microplastic particle numbers and polymer types have been determined after density separation with sodium-chloride. It was observed, that sediment samples of aquaculture systems and natural waters (rivers, lakes) contained significantly less microplastics as reported in earlier studies. In average 0.37 particles/kg (0.46-1.62 particles/kg range) was detected, most dominantly polypropylene.

Thesis 3

A plastic free universal sampling equipment has been constructed for surface water investigations with the use of rubber hoses and sealings, such as stainless-steel filters and filter cartridges. This was tested on different waters and with cascade filtration large volume (up to 2 m^3) sample could be taken on small pore size (25 µm) filters. This is a remarkable improvement, as in previous studies larger volumes was passed through only on larges mesh (100-250 µm) filters, while on smaller pore size filters (32-50 µm) only low volumes (12-20 L) were filtered.

Thesis 4

During my work I have collected data for the first time on the efficiency of the complete sampling process of a fractionated filtration system using microplastics in different shapes, sizes any polymer types in environmentally relevant concentrations. It has been found, that surface sampling was more efficient in all of the test cases. My results also highlight, that validation tests conducted solely with microspheres

might result in overestimated recoveries of microplastics that are environmentally more relevant in terms of shape and size.

Thesis 5

During my research I have designed a fast and effective sample preparation method that reduces brine solution use (500 cm^3 recyclable ZnCl$_2$/sample), while minimizing background contamination and particle losses during the sample transfer steps. With the use of microspheres, the developed method showed significantly higher recoveries (in average 84.9%) for the denser particles as well, than that of a widely used method from the literature (60.3%). The developed method showed 94.5% recovery of the particles with 1.2 g/cm^3 density, while the method from the literature showed only 14% recovery. The new equipment and the related procedure were also validated with microplastics of different shapes (fragments, fibres) and different polymer types (PE, PP, PET, PVC, PA) in environmentally relevant size ranges (100-300 µm fragment and 1000-1500 µm fibre), with the use of OECD standard artificial waters.

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