Land-Based Solutions for Plastics in the Sea

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D2.4 Sample Preparation | Results | Suggestions for Sampling SMNPs

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7	NATIONAL OCEANOGRAPHY CENTRE	NOC	UNITED KINGDOM	RTO
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ABBREVIATIONS AND ACRONYMS

Abbreviation / Acronym	Description
MP	microplastic
MPs	microplastics
SML	surface microlayer
H_2O_2	hydrogen peroxide
IR	infrared
LDIR	laser direct infrared
PTFE	polytetrafluoroethylene
SDS	sodium dodecyl sulfate
GIT	gastrointestinal tract
PP	polypropylene
PA	polyamide
PE	polyethylene
PTFE	polytetrafluoroethylene
PMMA	polymethyl methacrylate
PVC	polyvinyl chloride
PU	polyurethane
ABS	acrylonitrile butadiene styrene
PS	polystyrene
PC	Polycarbonate
PET	Polyethylene terephthalate
Fig.	Figure
TEP	Transparent Exopolymer Particles
CSP	Coomassie Stainable Particles
PON/C	organic nitrogen and carbon
POM	Polyoxymethylen

1 Introduction

This document corresponds to Deliverable 2.4 Sample Preparation. It also includes the results of the 2nd year and suggestions for sampling SMNPs.

Due to some delay in sampling campaigns especially in the North Sea, some results from the 1st year major sampling campaigns which were not integrated in D2.3 are also described in this deliverable. All samples from the different sampling campaigns (water, sediment, atmospheric, biota) were sent to the different laboratories in France, Germany, Great Britain, Portugal, and Spain. They are all prepared, but some samples still need to be measured. With our analysis going partly down to 10 µm, the measuring time is very time-consuming (depending on the sample from several hours to 14 days). Results that may not be integrated into this deliverable will be part of the final deliverable of WP3 (D3.5). This will be mentioned in the text for the different compartments.

2 Sampling Campaigns

2.1 North Sea Summer

The North Sea results of the summer sampling were integrated into D2.3. However, the text about the sampling campaign was missing.

The summer season sampling took place on RV ALKOR in June/July 2023 (cruise number AL596; https://marine-data.de/?site=expedition&expedition=AL596). The weather was better than during the winter cruise, and more stations could be sampled, again spanning the full study region. The sampling plan was identical at each station, similar to the previous cruise: the “snow catcher” was deployed first to collect microplastics in suspended particles. This was followed by the deployment of a CTD-Niskin rosette to collect water samples and measure the salinity and temperature structure of the water column. In parallel, Garrett screen samplers were deployed from the ship’s bow to collect samples from the sea surface microlayer. A vertically-towed WP2 net (150 µm mesh size) was then deployed three times to collect zooplankton from the entire water column (Fig. 1). The WP2 net was followed by surface sediment sampling using a Van Veen grab. Most stations had sandy sediments with no infauna, so only one sample was collected. Muddy sediments were encountered at Station 1, and the multi-corer was deployed to collect sediment cores. Abundant clams were found in the sediment grab samples at Station 3 and were retained for measurement of microplastics in biota. Finally, once the microlayer sampling was completed, a catamaran net (300 µm mesh size) was deployed to collect zooplankton and particles floating near the sea surface. One catamaran tow sampled a number of planktonic fish at station 3 which were preserved for subsequent microplastic analysis. A large Noctiluca algal bloom extended for 10s of km around stations 1 and 1-int (Fig. 1), and catamaran tow times were reduced by half to avoid clogging the net. Visual surveys for floating macroplastics were conducted during transits between stations, and a few objects were spotted (Fig. 1).

Figure 1: (left) Zooplankton sampling with a WP2 net. (middle) Catamaran net sampling in the middle of a Noctiluca bloom. (right) Macroplastic objects observed during transits.

3 Results

3.1 Surface microlayer

North Sea 2023 Summer/Winter

Sample processing:

The surface microlayer (SML) sampling campaigns took place on RV Alkor in February (AL586) and June/July (AL596) in the North Sea 2023 (Fig. 2). In total, seven SML stations were sampled, of which three were sampled in winter and four in summer (Tab. 1).
The samples were processed in the same way as during the Elbe sampling campaign in 2022/2023. The thickness of the SML was calculated as the volume of water collected per Garrett Screen (GS) dip divided by the total open area of the GS.

The microplastic (MP) samples were sent to UDC. In addition to the samples for MP, we sampled the biological parameters (TEP, CSP, PON/C, Chl *a*, Flow Cells) to get an overview of the biomolecular composition of the SML (presented in the last deliverable). All samples were stored at -80 to -20 °C until the analysis at GEOMAR.
For the gel particles Transparent Exopolymer Particles (TEP) and Coomassie Stainable Particles (CSP) a volume of 20-50 ml was filtered onto 0,4 µm Polycarbonate filters. TEP filters were stained with 1 mL of Alcian Blue (0,02%, pH 2,5) for 3 sec and CSP filters with 1 ml Coomassie Brilliant Blue G (0,04%) for 30 sec. TEP and CSP triplicates were analyzed via photometry. For the microscopical analysis, duplicates were taken and analyzed at a magnification of 200x.

Figure 2: Sampling of the SML using a Garrett Screen on RV Alkor (AL596).

We further collected duplicate samples for particulate organic nitrogen and carbon (PON/C) and Chl *a*. These samples were filtered onto pre-combusted 0.7 µm glass fiber filters (GF/F) with a volume of 150-500 ml. PON/C was measured by gas chromatography in a Euro EA elemental analyzer. Chl *a* was measured via photometry. Flow cytometer samples were fixed with glutaraldehyde and were taken in triplicates. They were measured with a flow cytometer (FACSCalibur). Five different phytoplankton classes can be discriminated via their cell size distribution (0.2-20 µm) and phytopigments. Further samples were taken for the cell abundance of bacteria and viruses, which were determined using a flow cytometer as well.

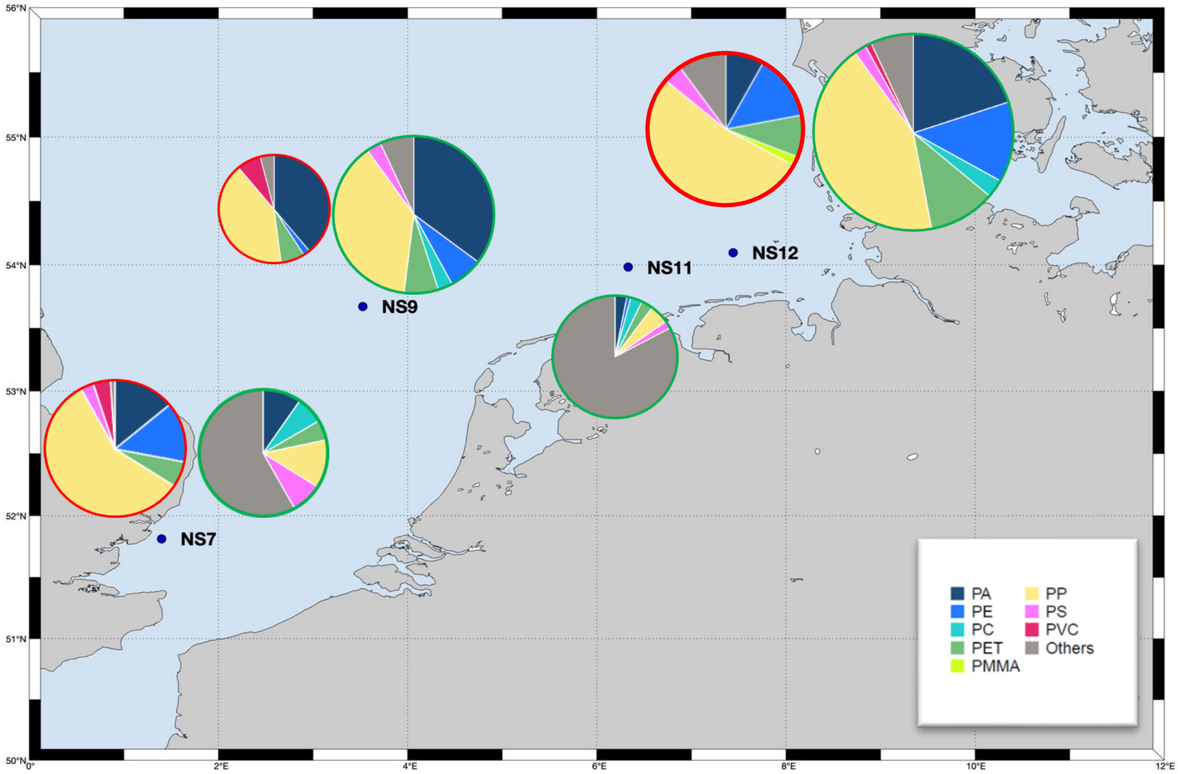


Figure 3: Microplastic concentration [MP/m³] in the SML during the cruises AL586 (red) and AL596 (green). PA: Polyamide, PE: Polyethylene, PC: Polycarbonate, PET: Polyethylenterephthalat, PMMA: Polymethylmethacrylate, PP: Polypropylene, PS: Polystyrol, PVC: Polyvinylchloride.

Figure 3 shows the composition of MP between stations NS7 and NS12 during winter and summer sampling in the North Sea. MP was classified into nine polymers, i.e. PA, PE, PC, PET, PMMA, PP, PS, PVC, and others. The group "others" includes acrylate, alkyd varnish, EVA, polyacetal or POM, PU, tire, ABS, rubber, and PTFE. During the winter sampling campaign (red), the maximum MP concentration was found at the most eastern station (NS12) with 47500 MPs m^{-3}. A decrease in concentrations is visible westwards with the lowest concentration at NS9 (2700 MPs m^{-3}). The average MP concentration is 48537 MP/m³. The dominant plastic polymer that was found is polypropylene (PP), followed by polyamide (PA). Polycarbonate (PC) is the only polymer that was not detected in winter.

During the second sampling campaign in June (green), the maximum polymer concentration was observed at station NS12 (117204 MPs m^{-3}), and minimum MP at station NS7 (41000 MPs m^{-3}). The average concentration was 69284 MPs m^{-3}. In two out of four stations (NS9, NS12) the dominant polymer was polypropylene (PP).

At NS7 and NS11 the group “others” contains the main contributors. This group is characterized by polyacetal or POM. Excluding station NS11, decreasing MP concentrations towards the West were observed similarly to the winter campaign. PC was measured at all stations during summer in comparison to the stations of the winter campaign.

If we compare both cruises, it can be seen that the concentration of MPs at NS9 and NS12 is higher in summer than in winter. Increased winds and wave motion due to winter weather conditions can lead to the mixing of SML water with the underlying water. As a consequence, this could decrease the concentration of MP in the SML. The most western station shows an increase in MP concentrations during winter. Looking at the individual stations across seasons, it is noticeable that the composition of the polymers in NS9 and NS12 changed only slightly. At these stations, one more polymer group was detected during summer than winter. One possible explanation for declining PP and increasing POM at station NS7 during the summer cruise could be the higher concentration of biological biomass in the North Sea in summer.

Elbe sampling campaign 2023

Sample processing

The SML winter sampling campaign at the Elbe 2023 and the processing of the samples will be presented in the last deliverable. The summer results are presented in the Synthesis of this deliverable. In 2023, the sampling campaign comprised only two stations. The first station was sampled in Cuxhaven (E13) at the estuary of the river Elbe and the second station was close to Dessau (E17) in the south-east of Germany.

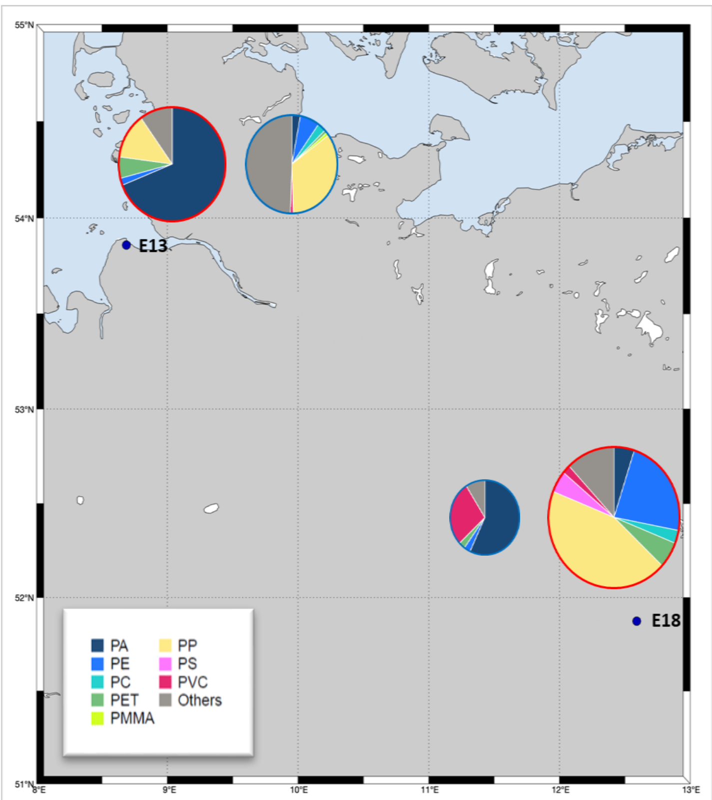


Figure 4: Microplastic concentration [MP/m³] at the SML during the Elbe sampling campaign winter (red) and summer (blue).

Results

Figure 4 shows the composition of MPs in the Elbe River during 2023. The maximum MP in the SML was found at station E17 in winter (99737 MP/m³). At the same station, MP concentration decreased to the minimum (28417 MPs m^{-3}) in summer. The concentration of polymers was 24% lower in summer than in winter (summer: 28417 MP/m³; winter: 66429 MPs m^{-3}) at station E13. In winter, the composition of MPs changed from a PA-dominated to a PP-dominated composition, with diverse contributors in the group "others". In this group, the main polymer is ethylen-vinylacetat-copolymer (EVA). The increase can therefore not be attributed to biological material and it belongs to plastic polymer.

In total, two more groups of polymers were detected in summer than in winter at station E13. Opposingly, we found five polymer types less in summer than in winter at station E18. In winter, PP was the dominant polymer type, while PA was the main polymer type in summer.

	Sample	Elbe station	North Sea station	Date	Actionlog	Latitude	Longitude	Microplastic	POC/N	Chl a	TEP	CSP	Flow Cells
Elbe Summer 20	Jun-Cux	13		05.07.2022		53,56	8,47						
	Jun-HH	14		22.06.2022		53,33	9,43	x					
	Jun-Ge	15		23.06.2022		53,25	10,22	x			x	x	
	Jun-Dö	16		28.06.2022		53,08	11,14	x			x	x	
	Jun-De	18		29.06.2022		51,51	12,41	x			x	x	
	Jun-LW	17		30.06.2022		51,51	12,13	x			x	x	
Elbe Winter 202	Jan-Cux	13		19.01.2022		53,56	8,47	x	x		x	x	
	Jan-HH	14		20.01.2022		53,33	9,43				x	x	
	Jan-Ge	15		21.01.2022		53,25	10,22	x			x	x	
	Jan-Ge_2	15_2		21.01.2022				x					
	Jan-Dö	16		24.01.2022		53,08	11,14	x	x		x	x	
	Jan-LW	17		25.01.2022		51,51	12,41	x	x		x	x	
	Jan-De	18		26.01.2022		51,51	12,13	x	x		x	x	
Elbe Winter 202	Feb-De	18		22.02.2023		51,51	12,13	x	x		x	x	
	Feb-Cux	13		20.02.2023		53,56	8,47	x	x		x	x	
North Sea Winte	Station 4		NS9	#########	AL586_4-1	53° 17,541' N	03° 52,692'	x	x	x	x	x	x
	Station 6		NS7	#########	AL586_17-1	51° 31,338' N	01° 39,688'	x	x	x	x	x	x
	Station 1		NS12	#########	AL586_28-1	53° 59,392' N	07° 38,197'	x	x	x	x	x	x
Elbe Summer 20	Jul-Cux	13		12.07.2023		51,51	12,13	x	x		x	x	
	Jun-De	18		21.06.2023		53,56	8,47	x	x		x	x	
North Sea Sumn	Station 1		NS12	#########	AL596_18-2	53° 59,740' N	07° 44,232'	x	x	x	x	x	x
	Station 2		NS11	#########	AL596_15-3	53° 48,279' N	06° 33,190'	x	x	x	x	x	x
	Station 4		NS9	#########	AL596_10-3	53° 17,189' N	03° 52,696'	x	x	x	x	x	x
	Station 6		NS7	#########	AL596_5-2	51° 31,353' N	01° 39,762'	x	x	x	x	x	x
	Station 3		N10										

Table 1: Details about sampling and sample preparation of the SML.

3.2 Small microplastics (10 µm–1 mm) pump filtration

Abundance. The samples from the seasonal sampling campaigns (2023) in the Thames (Chapman bouy (T6) and Victoria Docks (T5)), as well as the major sampling campaigns of the North Sea, were all analyzed and data processed; the analysis of Elbe samples is currently in progress with three samples out eight being completed to date. The analysis of the remaining Elbe samples as well as all Mero Barces samples will be completed by the end of 2023 and integrated into D3.5. The pump samples from Mero-Barcés (10-1000 µm) will be sent to NOC shortly and results will also be integrated into D3.5.

The number concentrations of microplastics were on average higher and more variable in the urban Victoria Dock (T5, 1693±1269 MPs m^{-3}) compared to the estuarine Chapman Buoy (T6, 420±371 MPs m^{-3}) and of comparable magnitude with the values recorded at this sites in winter and summer 2022 (detailed in previous deliverable). The MP number concentrations generally increased from winter (1450 MPs m^{-3} in January) to summer (2790 MPs m^{-3} in April), although no MPs were detected in the samples collected at this site in July 2023. No clear trend in MP concentrations was observed at Chapman Bouy, with the highest total MP concentrations (766 MPs m^{-3}) measured in July 2023 and the lowest concentrations observed in April 2023 (27 MPs m^{-3}) (Fig. 5).

The total number concentrations in the North Sea were on average higher and more variable in winter (446±434 MPs m^{-3}) than in the summer (93±60 MPs m^{-3}). Both winter and summer concentrations showed a decreasing gradient away from the Elbe estuary, with the lowest concentrations of 34 MPs m^{-3} in winter and 21 MPs m^{-3} in summer observed at station 6 (farthest from the Elbe outflow but closest to the Thames outflow). The overall average concentrations of MPs in the North Sea (244±317 MPs m^{-3}) were lower than observed in the Thames in 2022 (469±572 MPs m^{-3}, all sites) and in 2023 (1057±1101 m^{-3}, T5 and T6 sites only) but higher than in the Elbe in 2022 (143±202 MPs m^{-3}, all sites) (Fig. 6).

The summary of the MP number concentrations (10 µm–1 mm) observed in winter and summer (years/seasons combined) are shown in Figure 21 (chapter 4 Synthesis).

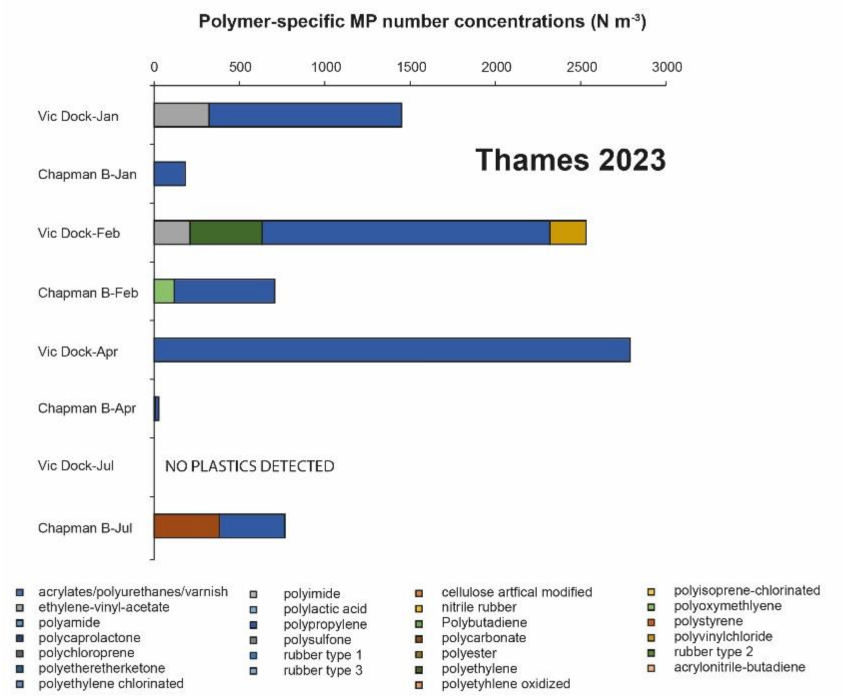


Figure 5: Polymer-specific microplastic number concentrations in the filtered water samples collected in 2023 at two sites in the Thames – Chapman Buoy and Victoria Docks.

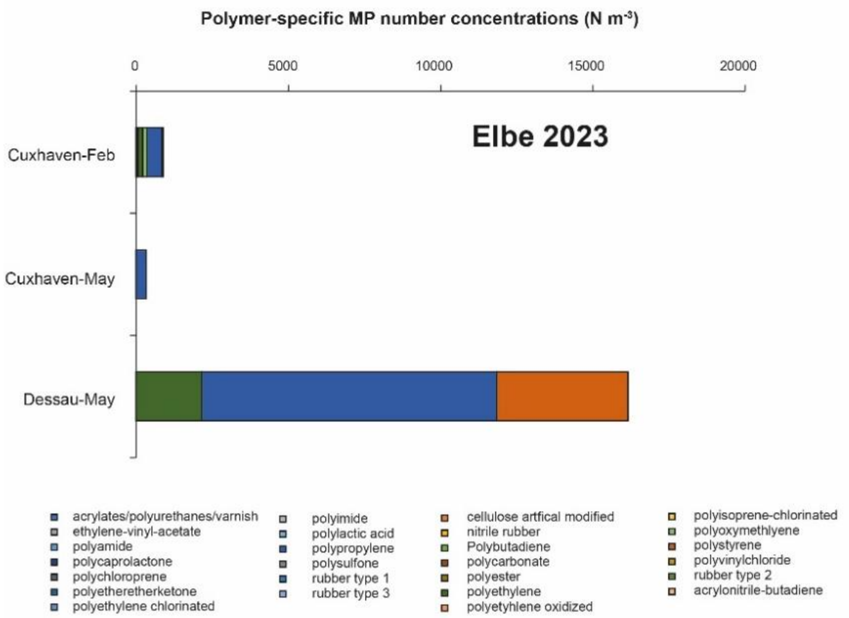


*Figure 6: Polymer-specific microplastic number concentrations in the filtered water samples collected in 2023 at two sites in the Elbe – Cuxhaven and Dessau. **NB:** the analysis of the remaining Elbe 2023 filtered water samples is to yet be complete.*

Polymer diversity. Polypropylene was the most common polymer at Chapman buoy and Victoria Dock accounting for >70% of all polymers detected at these sites, higher, yet consistent with the observations in the Thames in 2022. There were differences in overall polymer composition between the two sites, but remarkably the overall contribution from Acrylates/PUR/Varnishes dropped significantly in comparison to the 2022 data. These polymers were only seen in the samples collected at Chapman Buoy in April 2023.

The polymer diversity in the samples from the North Sea was also dominated by polypropylene (>91%, both winter and summer), with a much smaller input of polyethylene and other polymers. Polypropylene also dominated the polymer composition of MPs in three Elbe samples from 2023 analyzed so far (×2 at Cuxhaven (E13) and x1 at Dessau (E17)). At both locations, polypropylene accounted for ~60% of the detected MPs, higher than reported in the samples collected in 2022. The contribution of polyoxymethylene, which dominated the MP composition of the 2022 samples (28-84%) dropped to 12% (detected in Cuxhaven samples only).

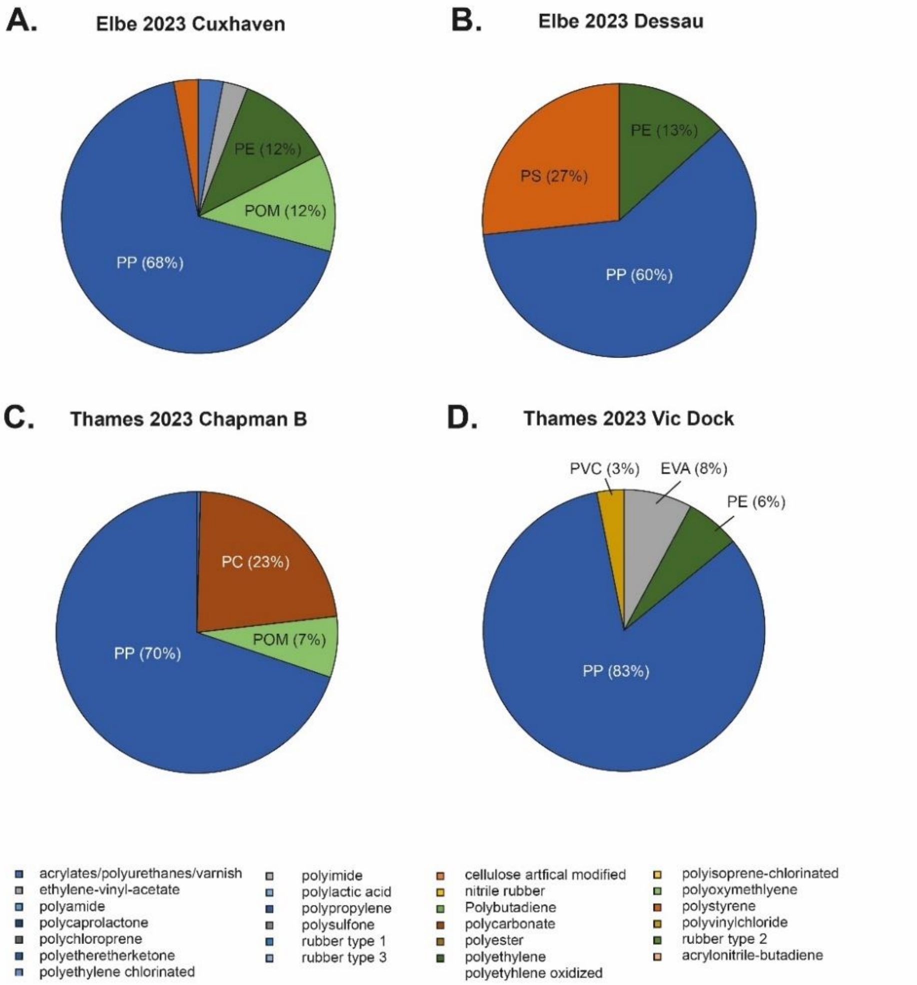


*Figure 7: Polymer composition of microplastic particles (by polymer count) detected in the filtered samples from the Elbe sites (A) Cuxhaven and (B) Dessau (B) and the Thames sites (C) Chapman Buoy and (D) Victoria Docks. All samples were collected in 2023. **NB:** the analysis of the remaining Elbe 2023 filtered water samples is to yet be completed.*

The MP size distribution (as feret diameter) of all the samples analyzed to date are shown in Figures 8 and 9. MPs sizes varied between the rivers and the North Sea and also between times of the sample collection.

The average size of MPs detected in the Thames and in the Elbe in 2022 was 124±125 µm and 118±125 µm, respectively – larger and more variable than the MPs found in the North Sea (69±40 µm). The average size of MPs detected at Chapman Bouy and Victoria Docks in 2023 was also smaller compared to the 2022 observations in the Thames (93±50 µm). The Elbe samples collected in 2023 and analyzed so far, did not show a significant change in size distribution relative to the 2022 data (122±88 µm).

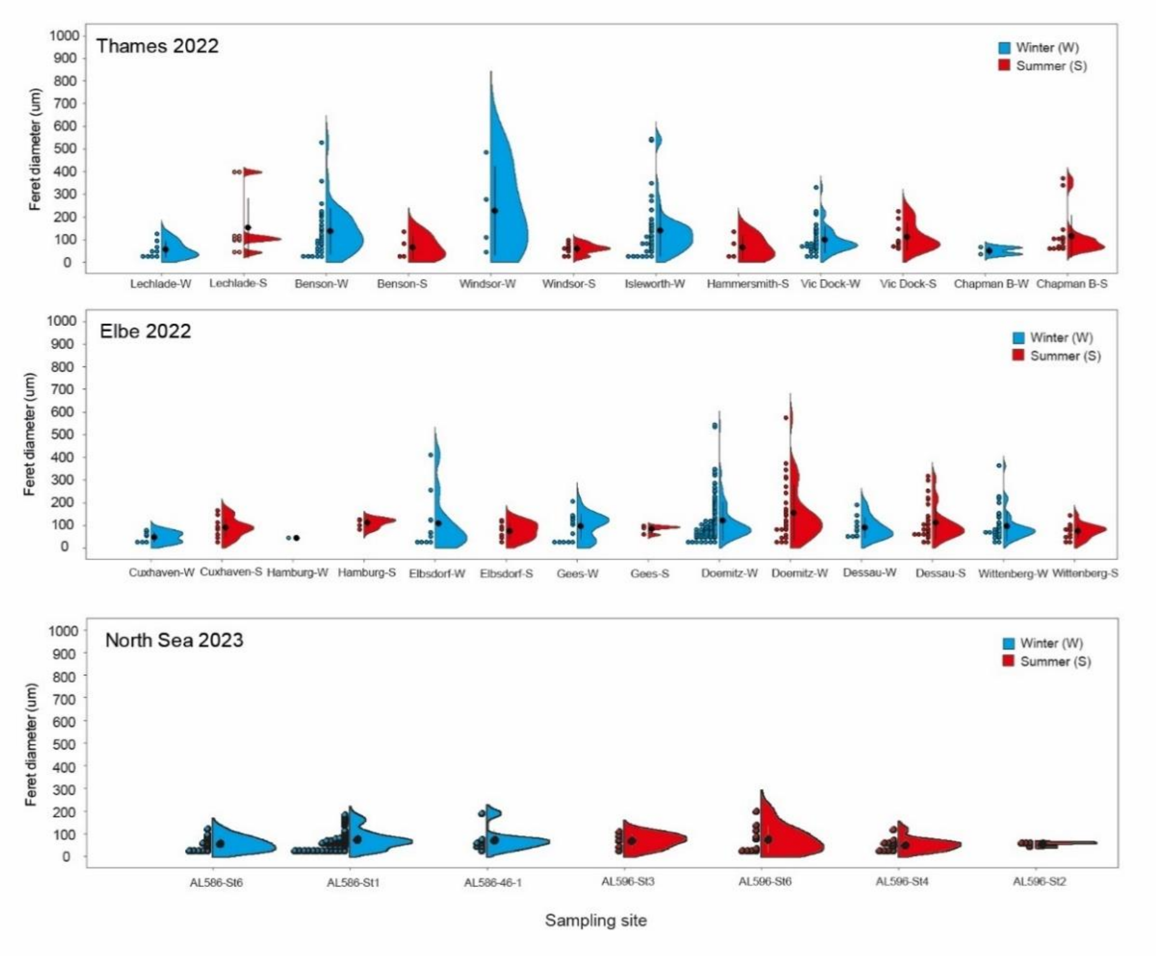


Figure 8: Particle size distribution data (as Feret diameter) for each site sampled in winter and summer 2022 (Elbe and Thames) and 2023 (the North Sea). Data points making up the distributions are shown as circles of the respective color. The width of each distribution was scaled to be equal to allow the inter-comparison of the distributions regardless of the number of data points. The black circles indicate mean particle sizes with horizontal bars as ±standard deviation.

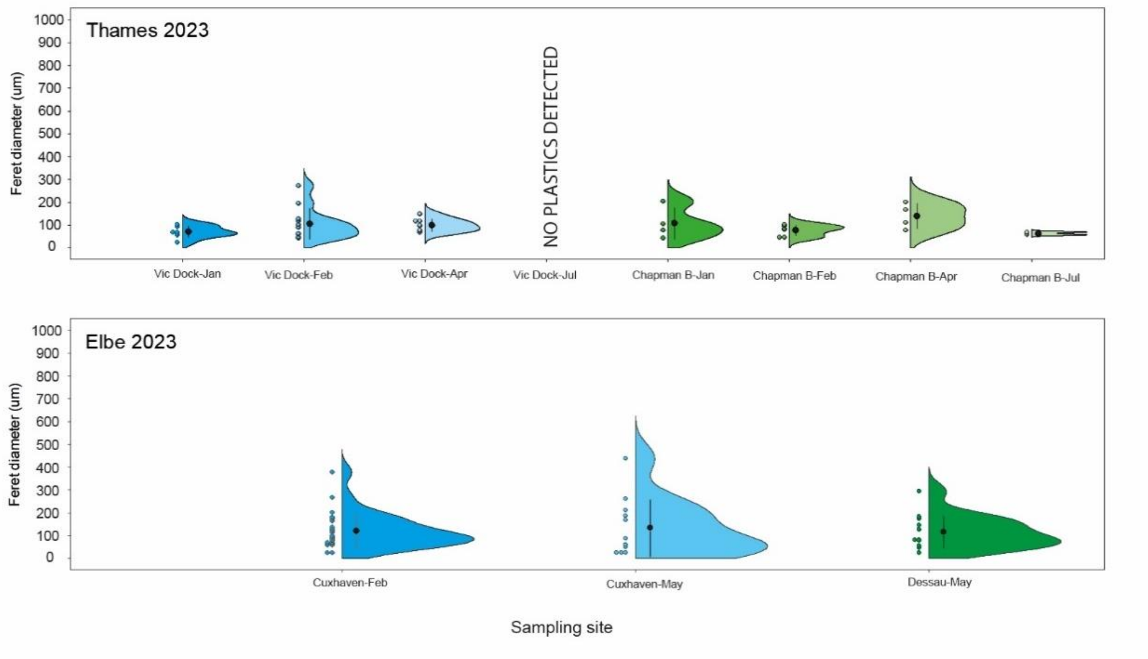


Figure 9: Particle size distribution data (as Feret diameter) for seasonal samples from the Thames (complete) and Elbe (not complete) collected in different months of 2023. Data points making up the distributions are shown as circles of the respective color. The width of each distribution was scaled to be equal to allow the inter-comparison of the distributions regardless of the number of data points. The black circles indicate mean particle sizes with horizontal bars as ±standard deviation.

3.3 Plastic, zooplankton and invertebrates' distribution

A summary of the manta net results is presented in the synthesis (chapter 4).

Analysis of zooplankton showed that these organisms are present across the entire sampled area, with a variation in concentrations and taxonomic composition among stations and seasons. In general, the highest concentration was observed during summer in the North Sea (St9; 10772.3 ind. m^{-3}), whereas the lowest was in the estuarine part of the Thames River in winter 2023 (station T6; 0.07 ind. m^{-3}).

Concentrations of zooplankton were similar between the two seasons in the Thames River in 2022 (average 21.4 ind. m^{-3}), with higher values at stations T4 and T5. However, in 2023, zooplankton concentrations increased tenfold at the two studied stations that year, T5 and T6 (average 228.8 ind. m^{-3}). The North Sea exhibited low zooplankton concentrations in winter (22.35 ind. m^{-3}) and more than 100 times higher concentrations in summer (3496.93 ind. m^{-3}), with no clear pattern on spatial distribution detected. Overall, zooplankton concentrations in the North Sea (average 2194.03 ind. m^{-3}) were higher than those in the Elbe River (471.5 ind. m^{-3}) and the Thames River (228.8 ind. m^{-3}).

For more details on zooplankton results see "Deliverable D3.5. Report on MP and zooplankton indicator from analyzed field samples resulting from Task".

Regarding plastic concentrations, Thames River presents the highest plastic concentration with an average of 10 plastics m^{-3}, followed by Elbe River (0.84 plastics m^{-3}) and North Sea (0.38 plastics m^{-3}) considering all samplings from 2022 and 20233 (see details in chapter 4. Synthesis). Micro (<5 mm), meso (5-20 mm), and macro (> 20 mm) plastics were identified in each station. Microplastics (MP, <5mm) are the most abundant particles representing 91% of the plastics in the rivers and 80% in the North Sea. Considering all the sampling periods, in the three aquatic systems, MP are the most abundant particles with an average of 514.2 MP in Thames River, 97.9 MP in the North Sea and 236.3 MP found in Elbe River. These small particles dominate in number in all stations except for St.12 where 63% (5 plastics) are mesoplastics (5-20 mm) (Fig. 10A). In terms of mass, MP show also the highest mass in the North Sea. However, in both rivers, macroplastics are the ones that present the highest weight (2545 mg and 245 mg in Thames and Elbe, respectively).

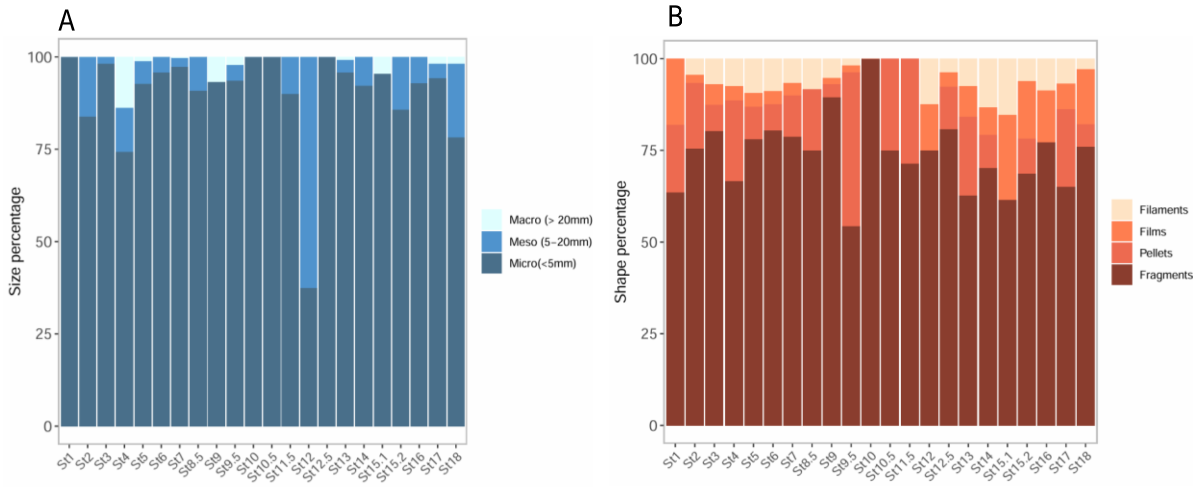


Figure 10: Percentage of plastic particles within three size ranges in each station: macro (> 20mm), meso (5-20mm), and micro(<5mm) (A). Percentage of plastic shapes: fragments, pellets, films, and filaments, found in each station: (B). St1:St6 is Thames River; St7:St12.5 is North Sea and St13:St18 is Elbe River.

Station T4 in Thames River, during the summer of 2022, presented a high concentration of macroplastics that could not be analyzed with ZooScan due to their big size. There is a total of 165 big pieces of debris, which, in terms of mass, correspond to 14160 mg. Thus, 64% of the total plastic weight found in the Thames River corresponds to the weight of macroplastics in that station.

Fragments are the most abundant plastic shape in the three aquatic systems. Overall, considering all the sampling stations, fragments constitute 72.4 %, pellets 14.6 %, filaments 7.9 %, and films 5.1 %. By aquatic systems, the percentage of shapes follows the same previous pattern except for the Elbe River where films (8.8 %) dominate over filaments (7.8 %). The percentages of total fragments are 74.8 % (2863 no. fragments), 76.4 % (1042 no. fragments), and 64.8 % (1289 no. fragments) in Thames River, North Sea, and Elbe River, respectively (Fig. 10B).

These results indicate that MP concentrations can vary significantly across different seasons and locations. Further analysis of plastic distribution will be conducted to compare plastic mass fluxes from rivers to the sea. Additionally, the data will be used to explore the temporal variability with the local hydrodynamics and other environmental parameters that could influence the plastic distribution.

Regarding benthic invertebrates, all the organisms were sorted from freshwater sediment samples and divided into two size classes (500 <x<1000 µm and x > 1000 µm). Number of individuals (total of 1723, average of 17.8 organisms per sample in Elbe and 43.2 in Elbe River), size, and wet weight were measured in each sample. These sorted organisms were stored frozen for later MP ingestion analysis.

Analysis of MP ingestion in benthic invertebrates as well as in zooplankton is currently in progress using a multiple-step enzymatic protocol. Preliminary results on zooplankton show higher ingestion of MP by copepods in the rivers compared with the sea. Completed data on plastic ingestion will be provided in the next report of work package 3 (WP3).

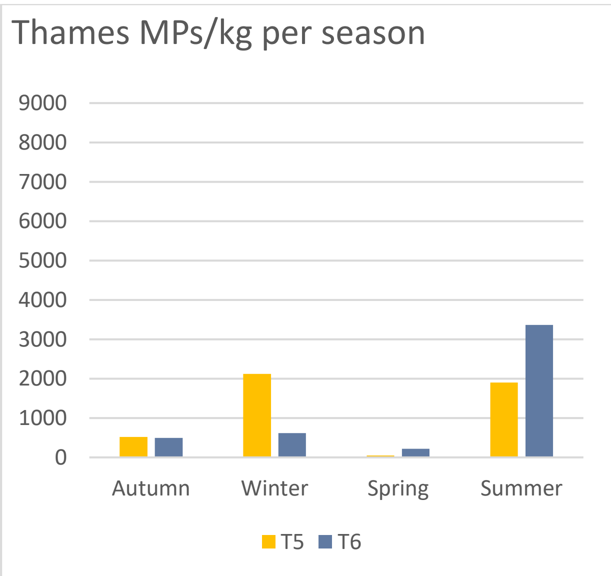
3.4 Sediments

The samples from the seasonal sampling campaigns from Elbe, Thames, and the North Sea are all analyzed. Mero-Barcés samples are processed, but not all results are available yet. Therefore, the results from Mero-Barcés will be integrated into D3.5. For the results of the major sampling (winter and summer sampling 2022) see the Synthesis of this deliverable.

Microplastic abundance

The total microplastics number per kg (MPs/kg) from the Elbe and Thames rivers throughout the four seasons (03=autumn, 04=winter, 05=spring, 05=summer) are shown in Figure 11, the size of the microplastics in Figure 12 and polymer-specific microplastics per sampling site and season in Figures 13 and 14.

In the Thames, the concentrations of microplastics did not differ much. Both sites show comparable data (T5 London 1147±882 MPs/kg; T6 estuary 1172±1272 MPs/kg). In comparison to the winter and summer sampling 2022 (see D2.3), more microplastics were detected, especially in the estuary (T6). Highest concentrations were measured in summer (July; London T5 1902 MPs/kg; estuary T6 3362 MPs/kg) and winter (December; London T5 2117 MPs/kg; estuary T6 615 MPs/kg) whereas lowest concentrations were observed in Spring (May). No clear trend is visible.



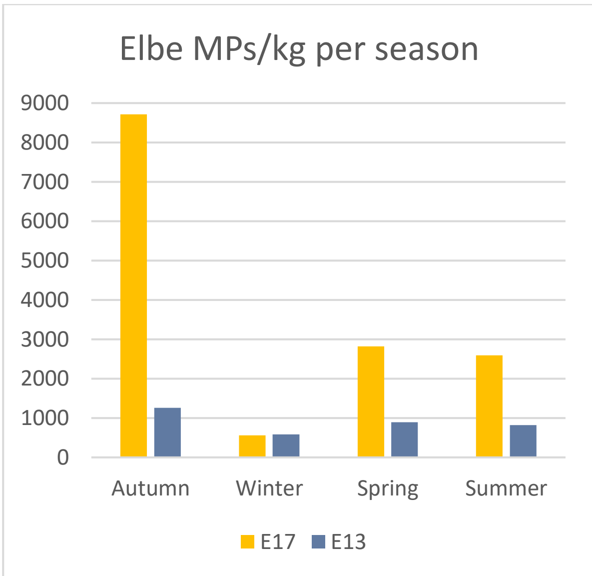


Figure 11: Microplastic abundance in Thames and Elbe rivers (MPs/kg) during the four seasons. Stations: T5 London, T6 Thames estuary, E17 Dessau, E17 Elbe estuary.

For the Elbe, higher concentrations were observed in the freshwater site Dessau (E17; 3676±2679 MPs/kg) than in the estuary (E13; 889±882 MPs/kg). Both sites have highest concentrations in autumn (November) (E17 8713 MPs/kg; E13 1258 MPs/kg) and similar ones in spring (May) and summer (July) (E17 2500 MPs/kg; E13 ~800 MPs/kg). The results from the 1st year sampling also revealed the highest concentrations in winter.

Size distribution

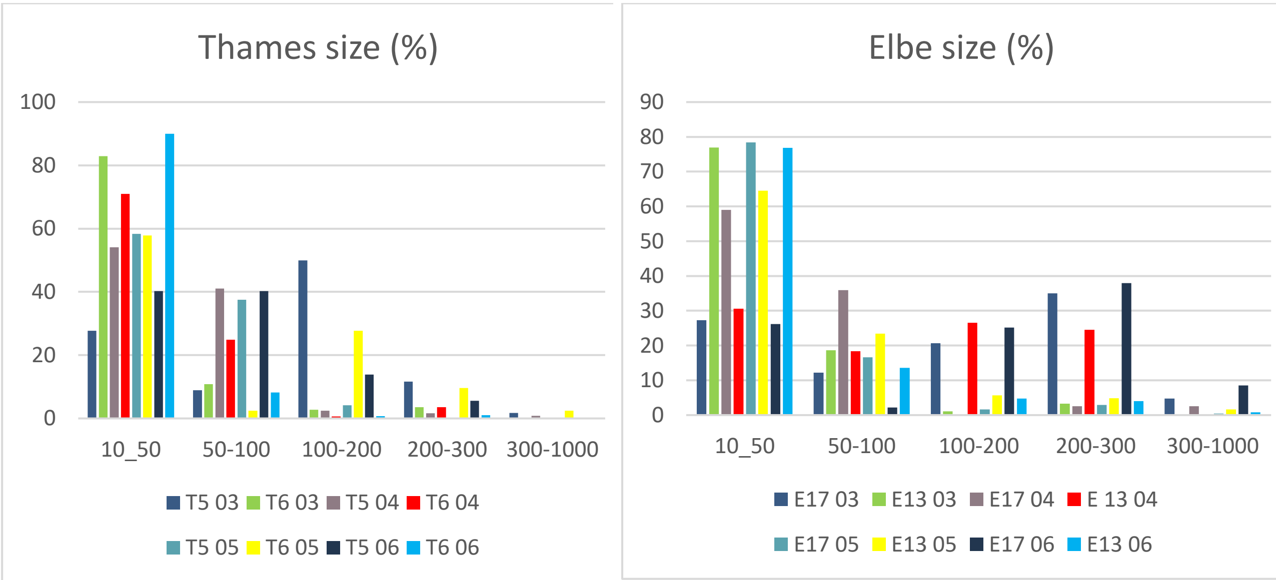


Figure 12: Size distribution of MPs in sediments from the four seasons. 03=autumn sampling, 04=winter sampling, 05=spring sampling, 06=summer sampling. T5=London, T6=Thames estuary, E13=Dessau, E17=Elbe estuary

As already observed for the former results (see D2.3), the abundance increases with decreasing size. The highest amount of microplastics is present in the smallest fraction (10-50 µm). Most microplastics have a size of <100 µm. With some exceptions, this holds for both rivers.

Polymer distribution

In the Thames, PE, PTFE, and PA were the most common polymers in the four seasons. PS represents also a high part but dominates the winter samples in London (>50%, T5 04) and the estuary (~25%, T6 04). For the Elbe, PS, PE, Acrylates/PU/Varnish, and PTFE dominate (Fig. 13).

The different samples throughout the four seasons show that PE and PTFE dominate both sites of the Thames to different extents. In London, PTFE dominates the autumn sample (03), PS in the winter (T5 04), and PE in the summer (T5 06). In the estuary, PE increases from autumn to summer, and the autumn sample (T6 03) accounts for ~60% of POM.

The polymer distribution for the Elbe clearly reveals a domination of PS in the site E17 Dessau. This confirms results from Scherer et al. (2020). The PS spheres are transported from the Mulde River into the Elbe. Moreover, Acrylates/PU/Varnish and PA are present in all samples. The Elbe estuary (E13) is dominated by PTFE, PE, and POM with the domination of Acrylates/PU/Varnish in winter (E13 04).

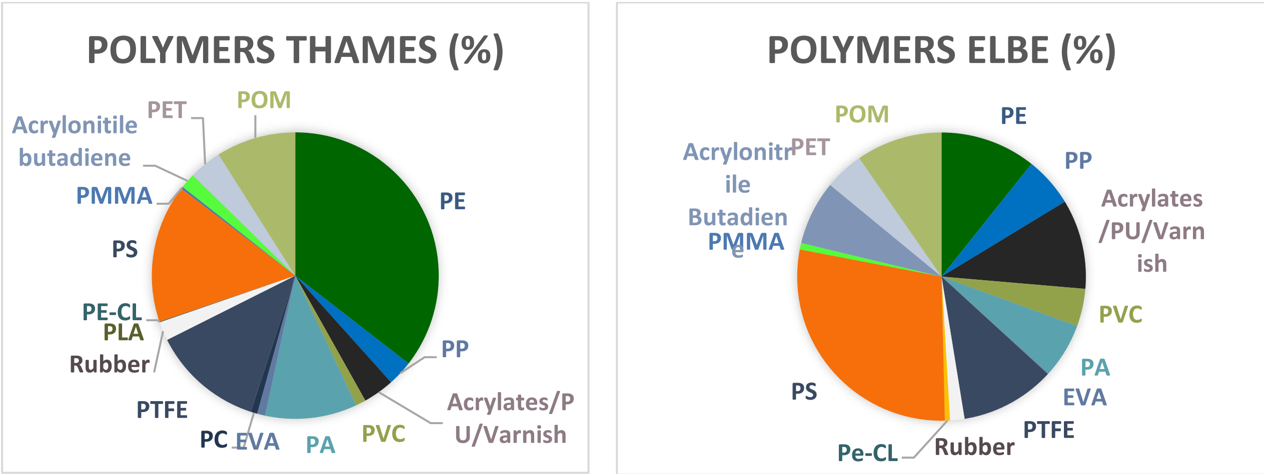


Figure 13: Total polymer composition of all samples from the four seasons from the Thames (left) and the Elbe (right) including two sites per river basin.

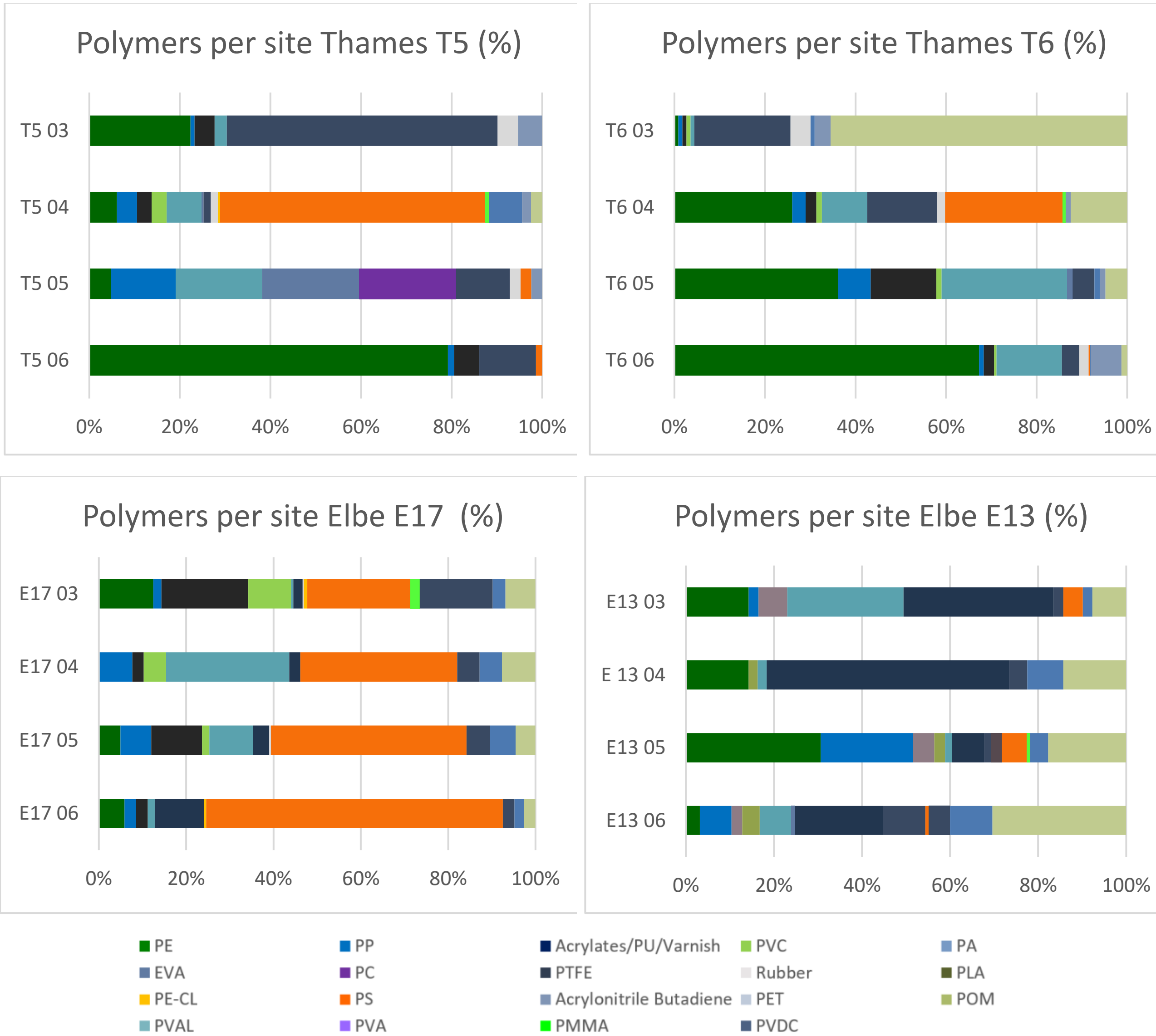


Figure 14: Polymer distribution per site in the Thames (T5 London, T6 Thames estuary) and Elbe (E17 Dessau, E13 Elbe estuary) rivers (%).

3.5 Bivalves

Three different sample preparation protocols for bivalves, using mussels as a matrix, were developed, and validated for microplastic (MP) determination at UDC: two alkali-oxidative methods, and one enzymatic protocol. The latter, using 2% SDS, protease and H_2O_2 was identified as the most suitable digestion approach, with recoveries ranging from 90-97% for PET, PVC, PS, PP, PA, and PE (70-250 µm). Moreover, the two-step alkaline oxidative methods can be reliable, low-cost alternatives yielding highly similar results, although two filtration steps are required, and therefore, the potential loss of MPs should be considered.

A high-throughput determination method based on quantum-cascade laser IR transflectance imaging (QC-LDIR) is proposed, using gold-coated filters for direct analysis after resuspending the filtrate obtained from a stainless-steel metallic filter (10µm). This newly developed analytical method is currently being applied to **clams** from the North Sea (GEOMAR) and invasive Red Swamp **crayfishes** from the Mero-Barcés River basin (UDC) to quantify and characterize MPs (20-5000 µm) in these samples, along with blank procedures.

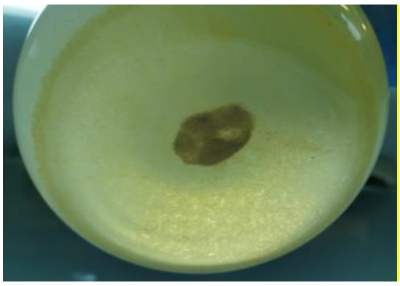


Figure 15: Enzymatic digestion extract of clams from the North Sea at UDC.

It should be noted that sediment is present in the enzymatic digestion extracts of clams (Fig. 15). Therefore, a further density separation will need to be performed before LDIR analysis (Agilent 8700 LDIR using Clarity Software 1.6 Beta). Results for bivalves will be presented in D3.5 and a publication in a peer-reviewed scientific journal is in preparation.

3.6 Fish

The fish samples from the Elbe sampling in September 2023 were sent to NOC, but unfortunately were lost in the UK customs and could not be recovered. Therefore, only results from 2022 are available for the Elbe (integrated into D2.3).

3.7 Atmospheric deposition

3.7.1 Elbe river

Atmospheric samples from four seasons (January, April, June, and October 2023) were collected from two sites in Hamburg (Altona and Veddel). In the laboratory, the samples were vacuum-filtered through 10 µm stainless-steel filters, H_2O_2 was added for the destruction of organic matter, again filtered, and transferred to a gold filter which was then analyzed by means of LDIR (Agilent 8700 LDIR). Results will be integrated into D3.5.

3.7.2 Thames river

Atmospheric samples from the 2023 seasonal campaign have been collected from two sites in the Thames River. They are currently being processed at UDC and the results will be presented in D3.5.

3.7.3 Mero-Barcés

All deposition samples from the Mero-Barcés River basin collected in 2023 (winter, spring, summer, and autumn) were processed at UDC using the low-to-moderate digestion protocol based on SDS and H_2O_2 (surfactant-oxidative method), previously described in D3.2 and published by López-Rosales *et al.* (2024). After digestion, the extracts from the atmospheric deposition, along with corresponding blank procedures, are being analyzed by LDIR (20-5000 µm). A complete comparison of all the results from the 1st and 2nd year will be presented in D3.5.

Figure 16: a) Depobulk® system (circular collecting surface = 0.038 m2, 10 L) and b) EnviroPlaNet sampler (circular collecting surface = 0.009 m2, 2,5 L).

In this deliverable, results from the first two sampling campaigns of the 2nd year, winter and spring 2023, are shown, where two passive sampling devices for collecting atmospheric bulk deposition (wet and dry deposition), namely EnviroPlaNet, a prototype from the Spanish Network of Researchers working on plastic pollution (www.enviroplanet.net), and the commercial Depobulk® system, are compared (Fig. 16).

Results of total MPs in winter 2023 were (213 ± 61 MP/m^2/day) in the Depobulk® system, and (491 ± 115 MP/m^2/day) in the EnviroPlaNet sampler, while in spring 2023, the results were (247 ± 96 MP/m^2/day) and (400 ± 149 MP/m^2/day), respectively. Figure 17 shows the average of 2 replicates using both passive samplers, classified by shape (fiber or particle), size, and polymer type.

In winter 2023, data from the Depobulk® and EnviroPlaNet samplers agreed, showing that approximately 63% of the collected MPs were in the 50-20 µm range. However, the composition of these MPs varied between the different passive devices. In the aforementioned size range, the Depobulk® system showed a distribution dominated by PET (16%), PP (11%), PU (8%), PE (2%) and rubber (7%), while the EnviroPlaNet system showed a distribution primarily composed of PP (20%), PET (17%), PA (16%), PE (2%) and rubber (1%). Considering the total MPs (500-20 µm range), the Depobulk® sampler indicated a higher proportion of PET (33%), followed by PP (16%), PU (12%), rubber (9%), PS (6%) and PE (3%), while the EnviroPlaNet device revealed a predominance of PP (35%), followed by PET (29%), PU (16%), PS (5%), PE (3%) and rubber (1%). Therefore, in winter 2023, the Depobulk® system recorded a higher percentage of PET in both the 50-20 µm range and total MPs, while the EnviroPlaNet device indicated a predominance of PP in both categories. Additionally, both Depobulk® and EnviroPlaNet passive samplers identified PP and PET as the main polymers present during the winter. Although there were some small differences, both samplers showed a similar prevalence of these polymers.

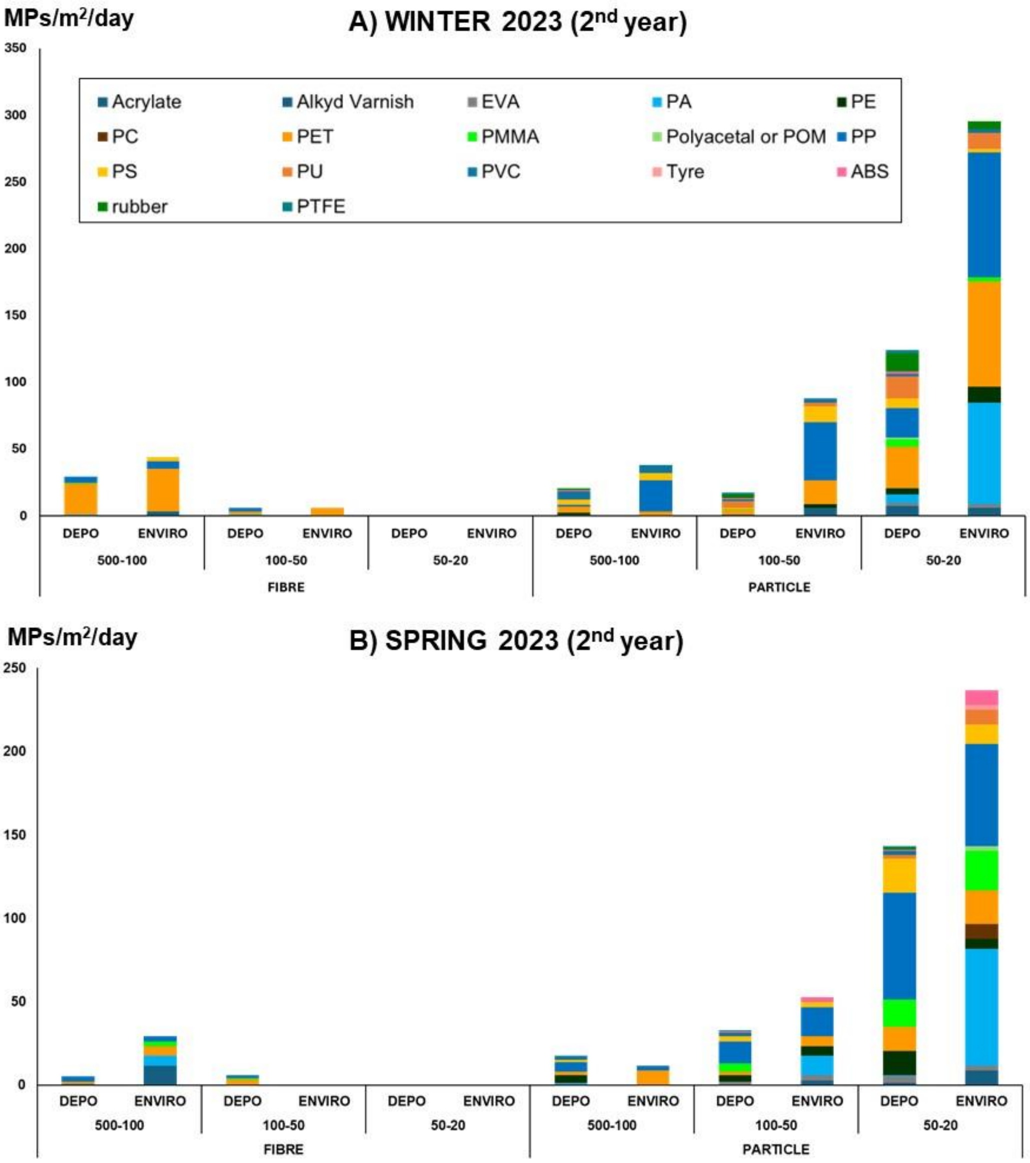


Figure 17: Average deposition rates (MP/m2/day) of airborne MP (n = 2) using the two sampling devices classified by shape (fiber or fragment), size (µm), and polymer type. A) 2nd year (2023) winter sampling campaign and B) spring 2023 sampling. 'Depo' and 'Enviro" stand for the Depobulk® and EnviroPlaNet systems, respectively."

In spring 2023, the results showed an increase in the proportion of smaller MPs (50 to 20 µm), reaching 70% for the Depobulk® system and 72% for the EnviroPlaNet device. In this size range, the Depobulk® sampler´s main polymers were PP (31%), PET (7%), PS (10%), PMMA (8%), and PE (7%), while the EnviroPlaNet sampler´s distribution was PP (19%), PET (6%), PA (21%), rubber (7%) and PE (1.5%). Examining the total MPs (500-20 µm range), the Depobulk® data indicated 43% PP, 12% PET, 11% PMMA, 12% PS, and 11% PE, whereas the EnviroPlaNet system showed 26% PP, 12% PET, 27% PA, 4% PS and 3.5% PE. Despite the differences, both Depobulk® and EnviroPlaNet systems identified PP as one of the predominant polymers present in the collected MPs in spring. Like the findings from winter 2023, both samplers showed a comparable distribution regarding the presence of PP, PE, and PET, with some variations in the proportions of other polymers such as PA and PS.

Overall, both samplers identified similar predominant polymers, such as PP and PET, in the collected MPs. However, there were notable differences in the specific composition of MPs between the two samplers (e.g. PA), including variations in the proportions of certain polymers and the presence of unique polymers in each sampler. These differences may be attributed to variations in the design and performance of the samplers, as well as the environmental and geographical conditions under which the measurements were conducted.

In both sampling campaigns, PP was one of the most common polymers, followed by PET. Additionally, differences in the composition of MPs between seasons were observed. For example, PET was more abundant in winter, while in spring there was a significant increase in PA and PS. These variations may reflect seasonal changes in human activities and weather patterns that affect the dispersion and deposition of MPs in the air. It is important to note that PA can sometimes be confused with natural polyamide (NPA), i.e. pollen, which increases significantly in spring. Therefore, expanding the pollen database is crucial to avoid possible false positives.

Changes in the particle size distribution between seasons were also observed. For example, the proportion of MPs in the 50-20 μm range increased slightly in spring compared to winter, suggesting possible changes in emission sources or atmospheric processes affecting MP transport and deposition.

Overall, PP was the most abundant polymer across both seasons and samplers (Depobulk® and EnviroPlaNet). Although there was some variation in the proportion of PP between sampling campaigns and samplers, this polymer consistently remained one of the most abundant in all samples analyzed.

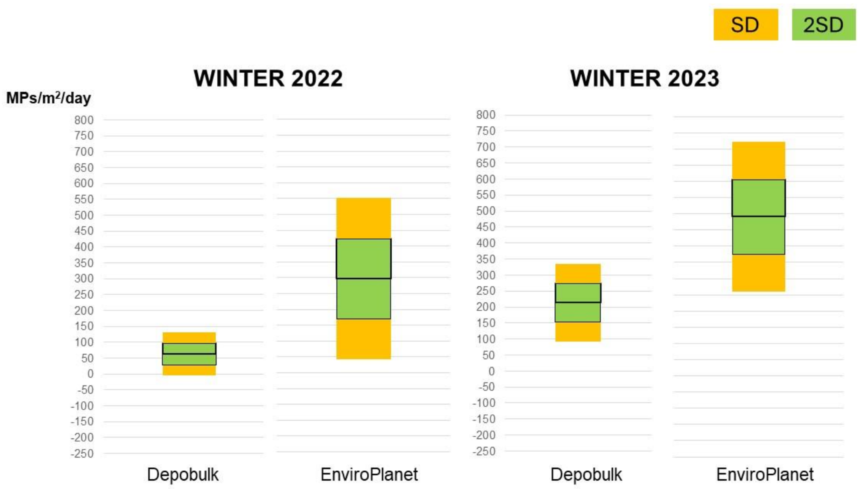


Figure 18: Data comparison of winter sampling campaigns from the 1st and 2nd year. Average ± SD (green) and average ± 2 ·SD (yellow) of total MPs deposition (MP/m2/day) encountered for each sampling device in each sampling month (n = 2).

A comparison was conducted between the results of all MPs collected during two different winter seasons. The data from winter 2022, as published by López-Rosales et al. (2024), were analyzed alongside those from winter 2023. The comparison was made based on averages and 95% confidence intervals (X ± 2 SD) (Fig. 18). The results indicated a slight increase in airborne MPs over the last year.

Figure 19 presents data from the sampling campaigns conducted during year 1 (summer 2021 - winter 2022) and year 2 (winter and spring 2023). As discussed in Lopez-Rosales et al. (2024), the EnviroPlaNet system tends to exhibit a tendency toward over-representation. However, upon comparing the plots of the 95% confidence intervals, it appears that they may not be significant, except for autumn 2021 (for further explanation, refer to the publication). Except for summer 2021, the Depobulk® system exhibits generally reduced data dispersion. This observation aligns with expectations, as the system's hopper has four times the surface area. Such a design feature likely contributes to the enhanced data coherence, indicating a plausible relationship between design characteristics and data consistency.

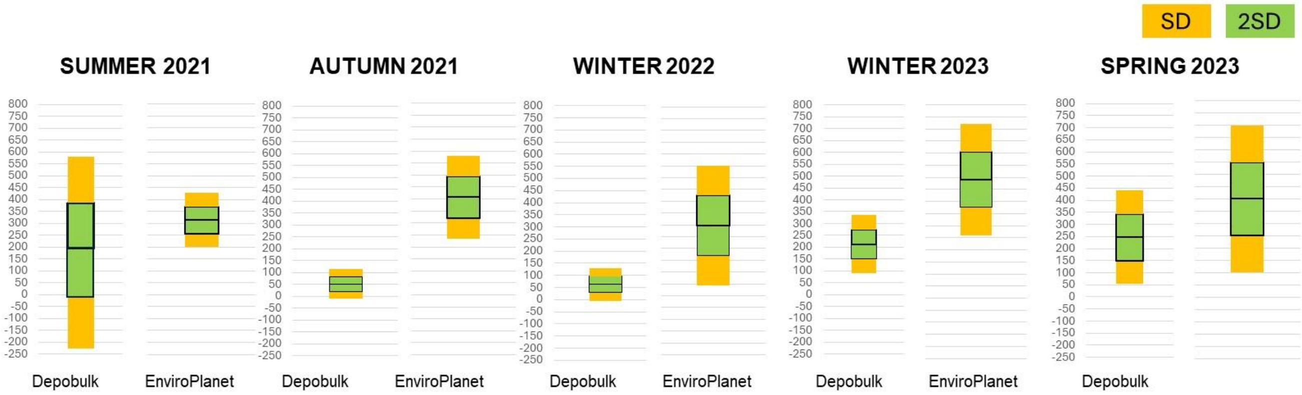


Figure 19: Data comparison of sampling campaigns from the 1st and 2nd year. Average ± SD (green) and average ± 2·SD (yellow) of total MPs deposition (MP/m2/day) encountered for each sampling device in each sampling month (n = 2).

4 SYNTHESIS

4.1 Thames-Elbe-North Sea

Surface microlayer

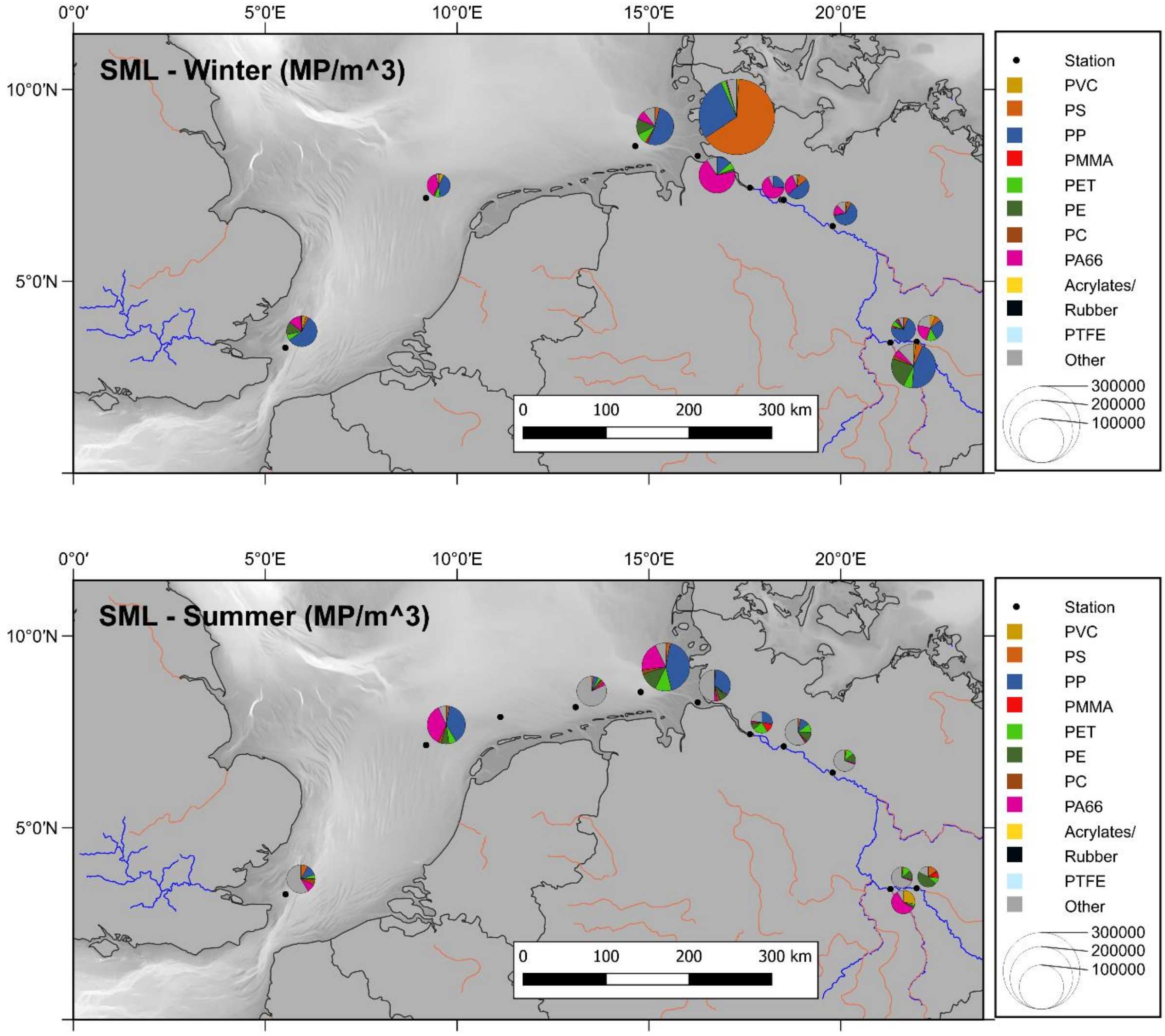


Figure 20: Microplastics abundance and polymer diversity of the surface microlayer from the North Sea and the Elbe from the first-year sampling campaigns (winter above, summer below).

The results of the SML from the winter and summer sampling campaigns in 2022 show that most microplastics occur in the Elbe estuary and the North Sea in both seasons (Fig. 20). In winter, one freshwater sample from the Elbe (E17 Dessau) also has a higher number of microplastics. The polymer composition is dominated by PA and PP (with the exception of the winter sample from the Elbe estuary containing >60% PS) in winter and PP, PA, and others in summer.

Pump filtration

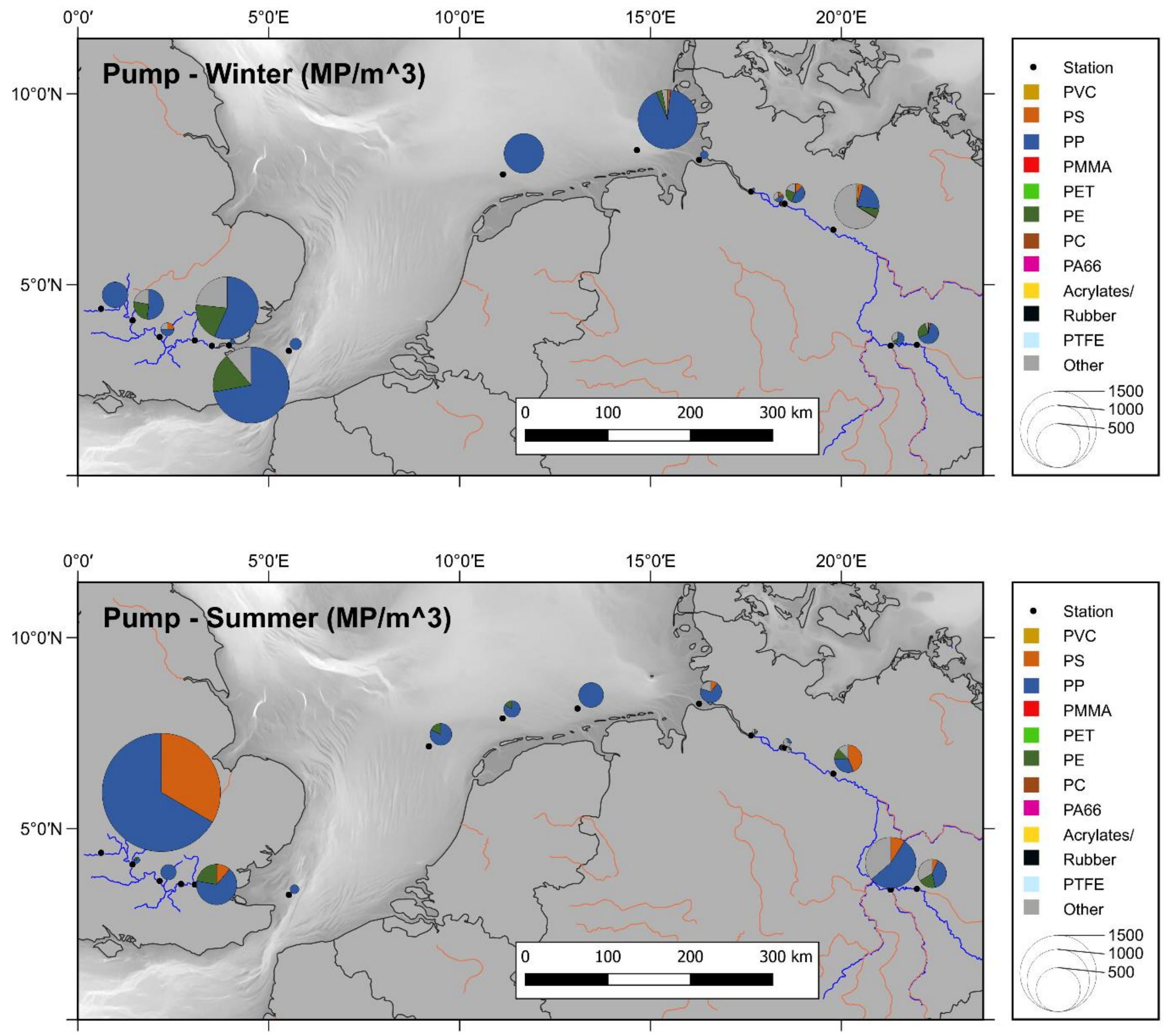


Figure 21: Microplastics abundance and polymer diversity of pump filtration (10-1000 µm) from the Thames-North Sea-Elbe from the first year sampling campaigns (winter above, summer below).

Clear differences in the MP loads and their composition were observed between the Thames, Elbe, and the North Sea, as well as a lot of variability between the sampling domains and the specific sites therein (Fig. 21). Polypropylene dominated the MP composition at all locations and seasons sampled. The contribution of other polymers such as acrylates (Thames) and POM (Elbe) varied between sampling seasons. Once the full dataset becomes available, we will assess i) the causes of the observed inter-river variability of MP concentrations, ii) differences and changes in polymer composition in different domains and seasons, iii) the links between the MP loads and composition measured in two rivers and the North Sea. The observations of 10 µm–1 mm MPs will also be placed in the context of other observations (surface layer, manta nets, sediments, biota).

Manta net

The following data are the results of the analysis of plastic and zooplankton samples collected along 18 stations with a manta net (>335 µm) in Thames River, Elbe River, and North Sea during different seasons in 2022 and 2023. Plastic and zooplankton were scanned using the ZooScan Hydroptic system for characterization and abundance calculations. Polymer identification was performed using infrared hyperspectral imaging.

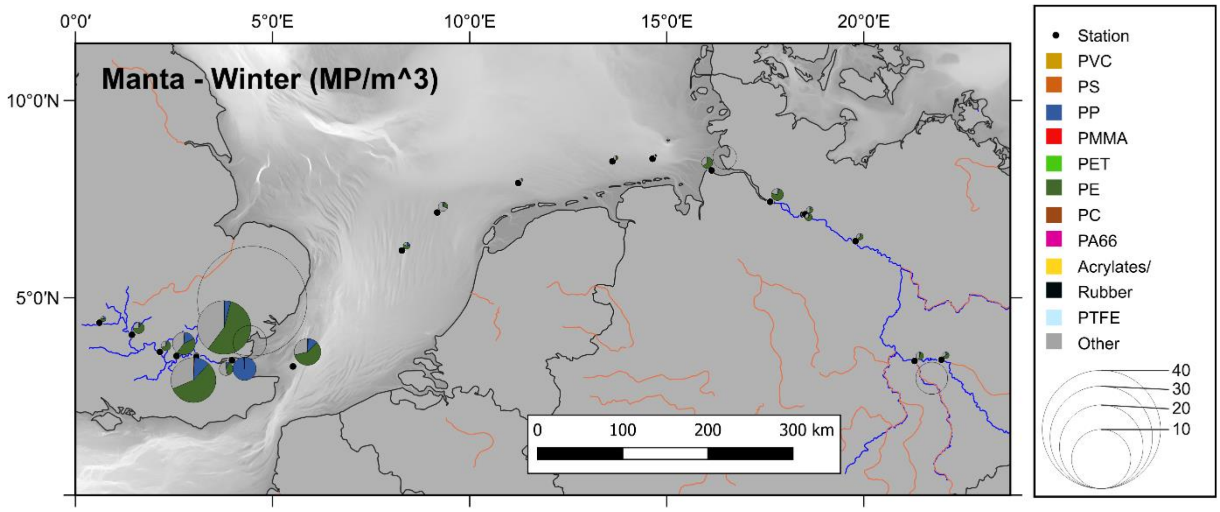


Figure 22: Plastic concentration (number m-3) and polymer identification of samples collected with manta (>335 µm) during 2022 and 2023 in the winter season. The size of the pie chart corresponds to the total plastic concentration and colors represent the different polymer types. Black dots mark the continuous stations from T1 upstream of Thames River to E18 upstream of Elbe River. Empty circles represent areas where we have total plastic concentration values but not polymer identification.

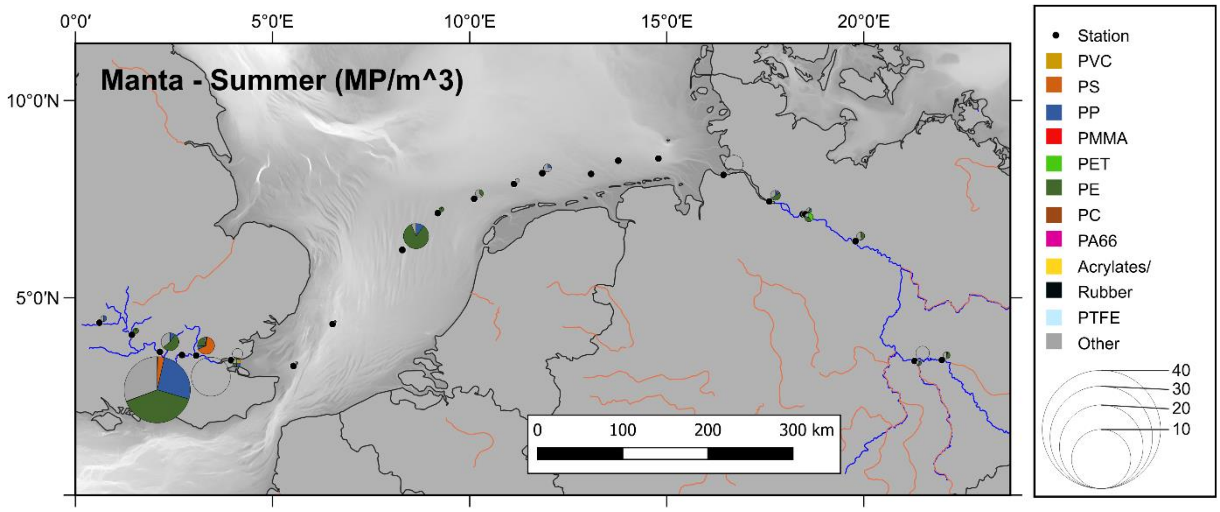


Figure 23: Plastic concentration (number m-3) and polymer identification of samples collected with manta (>335 µm) during 2022 and 2023 in the summer season. The size of the pie chart corresponds to the total plastic concentration and colors represent the different polymer types. Black dots mark the continuous stations from T1 upstream of Thames River to E18 upstream of Elbe River. Empty circles represent stations where we have total plastic concentration values but not polymer identification.

Thames River presents the highest plastic concentration with an average of 10 plastics m^{-3}, followed by Elbe River (0.84 plastics m^{-3}) and North Sea (0.38 plastics m^{-3}) considering all samplings from 2022 and 2023. In

Thames, the highest plastic concentrations are found at the stations closer to London (T4 and T5) and in the estuarine (T6). The average plastic concentrations found in the Thames in 2022 were 2.53 plastic m^{-3} in winter (Fig. 22) and 2.44 plastic m^{-3} in summer (Fig. 23). However, especially high concentrations were observed during the 2023 sampling in station T5 in winter (34.32 plastics m^{-3}) (Fig. 22) and T6 in spring (118.4 plastics m^{-3}). These stations receive a high input of plastic from local sources in a highly urbanized area such as London.

Elbe River plastic is more abundant in the areas influenced by the tide (E13 and E14). In the Elbe, average plastic concentrations were 0.21 plastic m^{-3} in winter and 0.17 plastic m^{-3} in summer 2022 (Fig. 23). In 2023, higher concentrations were observed at an upstream station E17 (average of 1.34 plastic m^{-3}) potentially due to local sources of pollution, and at station E13 close to the sea (average of 3.65 plastic m^{-3}).

Overall, both rivers present the highest concentration in spring. However, when comparing summer and winter, the three aquatic systems have higher concentrations in winter than in summer. Thames River has an average in winter of 6.3 plastics m-3 and in summer is 2.4 plastics m-3. Elbe River has an average in winter of 1.2 plastics m-3 and in summer is 0.5 plastics m-3. In the North Sea, the average in winter is 0.43 plastics m-3 and 0.34 plastics m-3 in summer.

Polyethylene (PE) is the most abundant identified polymer type in all stations along the three aquatic ecosystems with a maximum of 99.8% in summer in Thames River (Figs. 22 and 23). Polypropylene (PP) is also present in most of the stations especially in Thames River in summer (26%) where polystyrene (PS) also has relevant concentrations (66%) (Fig. 23). Polyethylene terephthalate (PET) was observed only in Elbe River during the summer of 2022 (Fig. 23) which could potentially be due to a temporary local discharge. Other polymer types such as polyvinyl chloride (PVC) and polyamide (PA66) were also identified, but in a few stations and low concentrations.

Sediments

The synthesis from winter and summer sampling campaigns from the Thames, North Sea, and Elbe show that more microplastics occur in sediments of the rivers than in the North Sea (Fig. 24). In both seasons, the concentration is higher in winter than in summer. The total microplastic concentration varied between and within the seasons and the different sites. The polymer composition also differed between the river basins. The most common polymers were Acrylates/PU/Varnish, POM, PE, and PTFE for the Thames and PTFE, rubber and PE for the Elbe. In the Thames, PE was much more present in winter while this was the case for PA in summer. PS dominated the freshwater samples in summer. In the North Sea, Acrylates/PU/Varnish, PE, and PTFE dominated the winter sampling whereas PA, PTFE, and rubber were the most dominant polymers during the summer sampling.

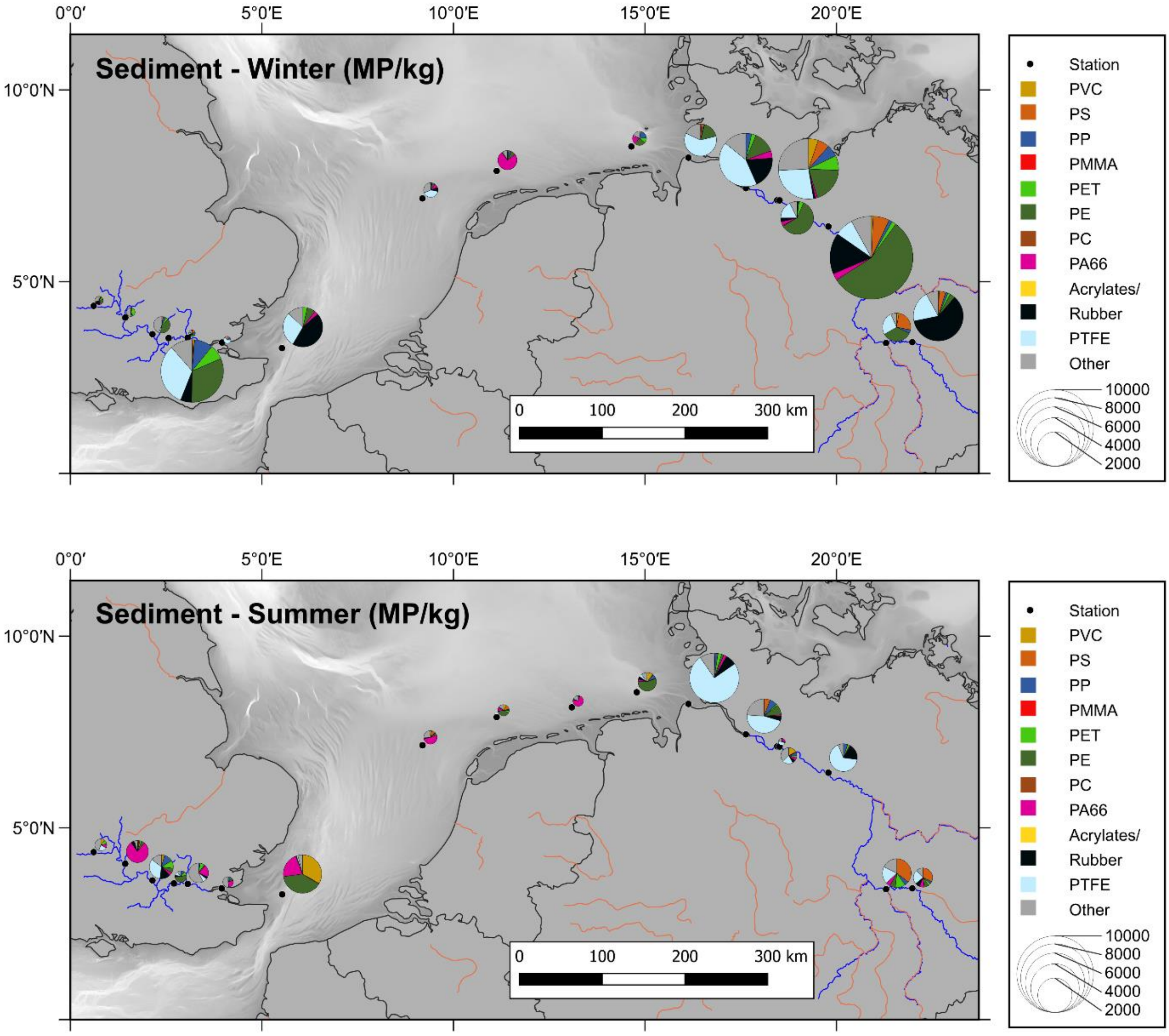


Figure 24: Microplastics abundance and polymer diversity of sediments (10-1000 μm) from the Thames-North Sea-Elbe from the first year sampling campaigns (winter above, summer below).

5 Calculation of Mass

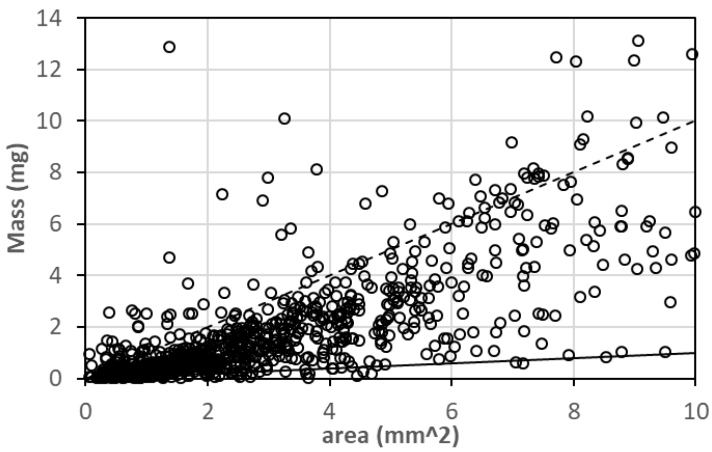
In many articles, only the number, polymer, and size of microplastics are reported. MP can either be expressed as the number of particles present in a sample or as the mass concentration of the respective polymer. However, further information about mass might also be relevant for monitoring, ecotoxicological analyses, and risk assessment. Moreover, it gives a better idea of how much microplastic is accumulated and thus it is easier to compare different study areas. In the Guidance Document for Marine Litter, only mass-based information is not suggested for monitoring microliter (MSFD 2023). Therefore, it would be ideal to have both information.
In the following, different possibilities to calculate the mass of microplastics are suggested:

- Direct information about the mass of MP (for different polymers) can be obtained through the thermal degradation of the polymers. Currently, two main thermoanalytical techniques are used for mass-quantitative MP analysis: pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) and thermo-extraction desorption GC/MS (TED-GC/MS) (Primpke *et al.,* 2020). Unlike spectroscopic methods, these thermoanalytical techniques are destructive but allow the determination of the chemical nature and the amount of the polymer, such as rubber, plastics, biomass, etc. in solid matrices. The sample is thermally decomposed into smaller stable fragments/components under defined conditions using a pyrolyzer or a thermogravimetric system, respectively. The pyrolysis products are then chromatographically separated via GC/MS, producing a characteristic pyrogram. Each polymer type is identified by its unique decomposition products. Thermoanalytical methods can be performed qualitatively on individual MP particles and their organic additives, as well as on complex environmental samples, to simultaneously identify and quantify MP polymer types (Reichel *et al.,* 2020).
  In the LABPLAS project, only selected samples from tire wear and wastewater treatment plants have been analyzed by Py-GC/MS (WP3). A new analytical approach of combined thermodesorption and Pyrolysis (TD-GCMS + Py-GC/MS) in a single run has been developed and implemented by UDC (D3.3 Characterization of the contribution of road dust and water run-off in MP emissions to the aquatic environment). This method allows for the identification of sorbed organic chemicals, such as additives, and the type of polymer in the same sample. Firstly, trace organic chemicals are desorbed from the particles via thermodesorption and analyzed by GC/MS. Then, the polymers are decomposed by pyrolysis, and the resulting decomposition products (polymer and their additives) are identified via GC/MS. For polymer quantification, specific markers are selected to confirm their presence, with one or two markers used for quantification through external calibration.
  UDC has recently participated in an interlaboratory comparison (ILC) on the detection and quantitative assessment of MPs by spectroscopic and thermo-analytical methods organized by VAMAS TWA 45 "Micro- and Nano Plastics in the Environment" (Project 2) (http://www.vamas.org/twa45/). UDC applied the proposed Py-GC/MS method for the identification and quantification (mass fraction) of two unknown MPs in a water-soluble matrix. All participating laboratories correctly identified the polymer types. For UDC, the results of the determination of the MP mass fraction demonstrated good consistency across thermo-analytical methods. This confirms the validation of UDC´s MP mass quantification approach.

- For the pump filtration samples of 10-1000 µm, the particle volume, and mass were calculated by the siMPle software that was used for the identification and characterization of MPs (Primpke et al., 2020)

- The mass of the individual microplastic particles can be calculated by first estimating the particle volume using the particle dimensions (length and/or area) measured on the IR image and assuming a range of particle shapes (i.e. third dimension). Then the estimated particle volume is multiplied by polymer-specific particle density to calculate the mass (see Pabortsava and Lampitt, 2020 for details).

The challenge to estimate particle mass from a 2-dimensional area is that the third dimension must be estimated (or perhaps more accurately, "invented"). Because plastic polymer densities vary less than this third spatial dimension (less than a factor of 2 vs. a potentially unlimited factor), the unknown particle height introduces more uncertainty in the mass estimate than the polymer type. However, direct measurements of individual particle mass show an indication of correlation with particle area (Fig. 25a; here, particle area is from analysis of the near-infrared hyperspectral camera images of large-size particles). Most of the data fall within an envelope between 0.1 and 1 mg mm-2, suggesting that it is possible to at least constrain the microplastic mass-area relationship within an order of magnitude (Fig. 25b).



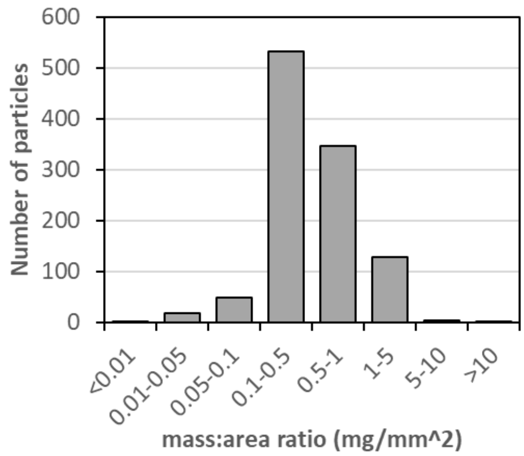


Figure 25: (left) Relationship between particle area measured with the near-infrared hyperspectral camera and individual particle mass measured on a microbalance. Slopes of 0.1 and 1 mg mm-2 are indicated by the solid and dashed lines, respectively. (right) Histogram of mass:area ratios.

6 RECOMMENDATION FOR SAMPLING

During the Labplas project, two major sampling campaigns took place in the first year with six sampling sites in the different study areas (Thames, Elbe, and Mero-Barcés rivers, North Sea) which were sampled in winter and in summer. The second year sampling included two sites in the rivers with a seasonal sampling in autumn, winter, spring, and summer. In every sampling location, one sample was taken per site due to time constraints.

Many papers have been published about recommendations for sampling, sample preparation, and analyses in the last years (e.g. Stock et al. 2019). The MSFD Technical Group on Marine Litter published an update of their detailed guidance document 2023. In there, manta nets (300 µm) are mentioned as well as pump systems (300 down to 20 µm) for water and grabs for sediments.

As many microplastics have a size <100 µm and the abundance increases with decreasing size, the LABPLAS project decided to sample down to 10 µm by pump filtration and analyze as many samples as possible within the 10-1000 µm size class (sediment, biota, atmosphere. Moreover, different compartments and the entire water column down to the sediment were sampled to better understand the distribution of plastics in the water column: nanoplastics on selected sites, the surface microlayer, small microplastics with a pump (10-1000 µm), larger microplastics with a manta net (>300 µm) and sediments with a Van Veen grab.

To prevent external contamination, the filters from the pump filtration were stored in glasses and only cleaned in the lab. Moreover, control blanks were collected every time a sample was processed. These are all suggestions which make the results more confident.

The project also showed that sampling is very time- and labor-intensive. For every sampling station, a boat is needed as well as at least two people to take the different samples. As the sample preparation and analysis are also very time intensive, only one sample per site and compartment was taken. The results of these constraints result in data that are highly variable and differ partly between the seasons. Therefore, having more samples and replicates would be ideal if the economic, environmental, and time limitations allow it. For the pump filtration e.g., the amount of water filtered could be increased. However, as we filtered the water through 10 µm, the filters clogged quite quickly so several filters are needed.

For detailed monitoring, more samples need to be taken. It is suitable to choose one or two sites and to take samples in different depths or once per month over a longer period. As the distribution is quite heterogeneous, all compartments need to be sampled in order to better understand where the plastics are transported. For calculating the microplastic load, pump filtrations are not enough. Therefore, nets are a good addition. In order to facilitate the results, particles could also be measured by weight.

Moreover, more attention should be paid to the seasons e.g. heavy rain events and tidal fluctuations as with more water more plastics are likely to be present. To better understand the transport of microplastics, models of suspended matter and sediment transport can be used. The results show varying degrees of horizontal and vertical gradients in the microplastic distribution in the river. The best way is to connect the results of models and verify them with field data.

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