



Land-Based Solutions for Plastics in the Sea

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101003954

D4.3 1st Report on SMNPs presence in the analysed field samples

Due date of deliverable: 31/05/2024

Actual submission date: 30/05/2024



Horizon 2020
European Union Funding
for Research & Innovation

PROJECT INFORMATION

Project number: 101003954

Project acronym: LABPLAS

Project full title: Land-Based Solutions for Plastics in the Sea

Call: H2020-SC5-2018-2019-2020 submitted for H2020-SC5-2020-2 / 03 Sep 2020

Topic: CE-SC5-30-2020 – Plastics in the environment: understanding the sources, transport, distribution and impacts of plastics pollution

Type of action: RIA – Research and Innovation Action

Starting date: June 1st, 2021

Duration: 48 months

List of participants:

N°	Participant name	Acronym	Country	Type
1	UNIVERSIDADE DE VIGO	UVI	SPAIN	HES
2	UNIVERSIDADE DA CORUÑA	UDC	SPAIN	HES
3	Bundesanstalt fuer Gewaesserkunde	BfG	GERMANY	RTO
4	LABORATORIO IBERICO INTERNACIONAL DE NANOTECNOLOGIA	INL	PORTUGAL	RTO
5	KATHOLIEKE UNIVERSITEIT LEUVEN	KUL	BELGIUM	HES
6	HELMHOLTZ ZENTRUM FUR OZEANFORSCHUNG KIEL	GEOMAR	GERMANY	RTO
7	NATIONAL OCEANOGRAPHY CENTRE	NOC	UNITED KINGDOM	RTO
8	SORBONNE UNIVERSITE	SU	FRANCE	HES
9	OPEN UNIVERSITEIT NEDERLAND	OUNL	NETHERLANDS	HES
10	LEIBNIZ INSTITUTE FOR BALTIC SEA RESEARCH	IOW	GERMANY	RTO
11	ASSOCIACAO PARA O DESENVOLVIMENTO DO ATLANTIC INTERNATIONAL RESEARCH CENTRE	AC	PORTUGAL	RTO
12	UNIVERSIDADE FEDERAL DO SAO PAULO	UNIFESP	BRAZIL	HES
13	BASF SE	BASF	GERMANY	LE
14	TG ENVIRONMENTAL RESEARCH	ER	UNITED KINGDOM	SME
15	CONTACTICA S.L.	CTA	SPAIN	SME
16	STICHTING EGI	EGI	NETHERLANDS	Non-P
17	STICHTING RADBOUD UNIVERSITEIT	RU	NETHERLANDS	HES
18	UNIVERSIDADE FEDERAL DO PARÁ	UFPA	BRAZIL	HES






















The contents of this document are the copyright of the LABPLAS consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

DELIVERABLE DETAILS

Document Number:	D4.3
Document Title:	1st Report on SMNPs presence in the analysed field samples
Dissemination level	PU – Public
Period:	RP2
WP:	WP4
Task:	Task 4.3
Status:	Final
Author:	  UNIVERSIDADE DA CORUÑA
Reviewers:	 UNIVERSIDADE DA CORUÑA
Recommended citation format	2024. 1st Report on SMNPs presence in the analyzed field samples. Deliverable 4.3, LABPLAS Grant Agreement No. 101003954 H2020-SC5-2020-2
Abstract:	<p>We report the first results of the analysis of SMNPs from field samples collected during LAPBLAS sampling campaigns (WP2, D2.2, and D2.3). Water samples from Elbe, Thames, Mero-Barcés, and the North Sea and suspended sediments from water samples from Mero-Barcés Elbe and Thames were processed and analyzed following the guidelines presented in Deliverable 4.1 <i>Guideline form methods for extraction, pre-concentration, and purification of SMNPs</i>. The results shown in this deliverable 4.3 were generated in the framework of LABPLAS Task 4.3. This report describes the first analysis of the field samples by NTA, SEM, fluorescence microscopy, and Raman spectroscopy to determine the presence of SMNPs.</p>

Version	Date	Comments
1.0	16.04.2024	Initial version – 1st Report on SMNPs presence in the analyzed field samples.
2.0	01.05.2024	Reviewed version
3.0	30.05.2024	Final version
4.0	30.08.2024	Reviewed version with changes to address comments by the PO and reviewer

Disclaimer

The views and opinions expressed in this document reflect only the authors' views, and not necessarily those of the European Commission.

The contents of this document are the copyright of the LABPLAS consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

TABLE OF CONTENTS

PROJECT INFORMATION.....	2
DELIVERABLE DETAILS	3
TABLE OF CONTENTS	4
ABBREVIATIONS AND ACRONYMS	8
1 INTRODUCTION	9
2 MERO-BARCÉS RIVER BASIN	9
2.1 Water	9
2.1.1 Detection/Identification/Quantification	10
2.2 Suspended Sediments	15
2.2.1 Detection/Identification/Quantification	15
2.3 Conclusions	18
3 THAMES RIVER.....	19
3.1 Water	19
3.1.1 Detection/Identification/Quantification	19
3.2 Suspended Sediments	23
3.2.1 Detection/Identification/Quantification	23
3.3 Conclusions	25
4 ELBE RIVER	26
4.1 Water	26
4.1.1 Detection/Identification/Quantification	26
4.2 Suspended Sediments	31
4.2.1 Detection/Identification/Quantification	31
4.3 Conclusions.....	33
5. THE NORTH SEA	34
5.1 Water.....	34
5.1.1 Sampling methods	34
5.1.2 Detection/Identification/Quantification	35
5.2 Suspended sediments.....	46
5.3 Conclusions.....	46
GENERAL CONCLUSIONS	47

*The contents of this document are the copyright of the **LABPLAS** consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.*

LIST OF FIGURES

Figure 1. Illustrative photographs for Mero-Barcés River Basin samples from the two first campaigns.	9
Figure 2. Decision tree for water sample preparation.	10
Figure 3. Concentration of particles below 1 µm per millilitre for all water samples from Mero-Barcés received. For the NTA measurements, samples were filtered.	12
Figure 4. Size d90 of the particles obtained by NTA for all water samples from Mero-Barcés received. For the NTA measurements, samples were filtered.	12
Figure 5. SEM images for relevant control data for blanks measured to ensure study quality. Two procedural blanks (without and with 10 µL of ultrapure water) were performed to check the presence or contamination of particles by experimental procedure steps.	13
Figure 6. Representative SEM images for all water samples from Mero-Barcés collected in the second campaign (Winter season). The biggest particles observed in these SEM images were identified as salts, probably calcium carbonate and sodium chloride, crystalized during the drying process.	14
Figure 7. Decision tree for suspended sediments sample preparation.	15
Figure 8. Bright-field and fluorescence images of Anodisc after plastic extraction from suspended sediments of MB4-Meirama sample collected in spring campaign (MB04/03/B0/06/01).	16
Figure 9. Raman spectra of DiO dye, unstained plastic and DiO-stained plastic acquired using 785 nm and 633 nm laser lines. The representative peaks of each plastic are indicated with black dashed lines.	17
Figure 10. Raman spectra of anodisc after plastic extraction procedure from Mero-Barcés sample (MB04/03/B0/06/01), acquired using both 785 nm and 633 nm laser lines.	18
Figure 11. Concentration of particles below 1 µm per millimetre for all water samples from Thames River. For the NTA measurements, samples were filtered.	20
Figure 12. Size d90 of the particles obtained by NTA for all water samples from Thames River. For the NTA measurements, samples were filtered.	20
Figure 13. Representative SEM images for all Thames water samples at high vacuum conditions.	21
Figure 14. Raman analysis of T05 water sample after triggering a filtration procedure. The particles collected on a 200 nm Anodisc were analyzed using a 785 nm laser line.	22
Figure 15. Illustrative photographs for Thames River samples.	23
Figure 16. Images (fluorescent and bright-field) of anodisc after plastic extraction from suspended sediments of Thames samples.	24
Figure 17. Raman spectra of anodisc after plastic extraction procedure from Thames River, acquired using both 785 nm and 633 nm laser lines.	25
Figure 18. Concentration of particles below 1 µm per millilitre for all water samples from Elbe River.	27
Figure 19. Size d90 of the particles obtained by NTA for all water samples from Elbe River.	28
Figure 20. Representative SEM images for water samples collected from the Elbe station “E14” (Hamburg) at different depths: 10, 50 and 20 cm.	29
Figure 21. Representative SEM images for water samples collected from the Elbe station “E16” (Dömitz) and controls for the sampling method (i.e. surface microlayer): ultrapure water used for the washing (i.e. blank) and the ultrapure water collected after washing the surface microlayer machine (i.e. washing).	29
Figure 22. Representative SEM images for water sampled from E13 – Cuxhaven, E15 – Geesthacht and E17 – Dessau.	30
Figure 23. Photograph of water samples collected from Elbe River.	31

Figure 24. Images (fluorescent and bright-field) of anodisc after plastic extraction from suspended sediments of Elbe samples.....	32
Figure 25. Raman spectra of anodisc after plastic extraction procedure from Elbe River, acquired using both 785 nm and 633 nm laser lines.....	33
Figure 26. Concentration of particles below 1 µm per millilitre for all water samples from the North Sea collected using the CTD method. For the NTA measurements, samples were filtered.	36
Figure 27. Size d90 of the particles obtained by NTA for all water samples from the North Sea collected using the CTD method. For the NTA measurements, samples were filtered.	37
Figure 28. Concentration of particles below 1 µm per millilitre for surface microlayer samples from the North Sea collected using the Garrett screen system.	38
Figure 29. Size d90 of the particles obtained by NTA for surface microlayer samples from the North Sea collected using the Garrett screen system. For the NTA measurements, samples were filtered...	39
Figure 30. Concentration of particles below 1 µm per millilitre for residual water samples from the North Sea collected using a marine snow catcher. For the NTA measurements, samples were filtered.	40
Figure 31. Size d90 of the particles obtained by NTA for residual water samples from the North Sea collected using a marine snow catcher. For the NTA measurements, samples were filtered.	41
Figure 32. Representative SEM images for surface microlayer samples from NS7-Thames estuary, NS10-West Frisian Is. and NS12-Elbe estuary.	42
Figure 33. Representative SEM images for residual water from above the aggregate layer sampled in NS7, NS9, NS10 and NS12 using a marine snow catcher system	43
Figure 34. Representative SEM images for residual water from the aggregate layer sampled in NS7, NS9, NS10 and NS12 using a marine snow catcher system.	43
Figure 35. Representative SEM images of water sampled in the North Sea south of Helgoland.	44
Figure 36. Raman spectra and bright-field images of treated samples from water above the aggregate layer collected in NS7-Thames estuary using a snow catcher method.....	45
Figure 37. Raman spectra and bright field images of treated samples from water above the aggregate layer collected in NS9-Broad Fourteens using the snow catcher method.	45
Figure 38. Raman spectra and bright field images of treated samples from water above the aggregate layer collected in NS10 – West Frisian Is. using the snow catcher method.....	46
Figure 39. Representative photography of water collected from the North Sea.	46

LIST OF TABLES

Table 1. NTA results for all the water samples from Mero-Barcés received. D90: 90% of the total particles are smaller than this size; D50: 50% of the total particles are smaller than this size; D10: 10% of the total particles are smaller than this size. Control samples and Milli-Q water as procedural blank were also analysed.....	11
Table 2. NTA results for all the water samples from Thames River received. D90: 90% of the total particles are smaller than this size; D50: 50% of the total particles are smaller than this size; D10: 10% of the total particles are smaller than this size. Control samples and Milli-Q water as procedural blank were also analysed.....	19
Table 3. NTA results for all the water samples from Elbe River received. D90: 90% of the total particles are smaller than this size; D50: 50% of the total particles are smaller than this size; D10: 10% of the total particles are smaller than this size. Control samples and Milli-Q water as procedural blank were also analysed.....	27
Table 4. NTA results for water samples from the North Sea collected using CTD. D90: 90% of the total particles are smaller than this size; D50: 50% of the total particles are smaller than this size; D10: 10% of the total particles are smaller than this size. Control samples and Milli-Q water as procedural blank were also analysed.	36
Table 5. NTA results for surface microlayer samples from the North Sea collected using the Garrett screen system. D90: 90% of the total particles are smaller than this size; D50: 50% of the total particles are smaller than this size; D10: 10% of the total particles are smaller than this size. Control samples and Milli-Q water as procedural blank were also analysed.	38
Table 6. NTA results for water samples from the North Sea collected using a marine snow catcher. D90: 90% of the total particles are smaller than this size; D50: 50% of the total particles are smaller than this size; D10: 10% of the total particles are smaller than this size. Control samples and Milli-Q water as procedural blank were also analysed.	40

ABBREVIATIONS AND ACRONYMS

Abbreviation / Acronym	Description
ASTM	American Society for Testing and Materials standards organization
CCD	Charged Coupled camera
cLSM	Confocal Laser Scanning Microscopy
CPE	Could Point Extraction
CTD	Conductivity, Temperature, and Depth
D	Deliverable
DiO	3,3'-Di Octadecyloxacarbocyanine perchlorate
E	Elbe
ETD	Electron Detectors
LoD	Limit of Detection
MB	Mero-Barcés
NIST	National Institute of Standards and Technology
NS	North Sea
NTA	Nanoparticle Tracking Analysis
OECD	Organisation for Economic Co-operation and Development
PE	Poly Ethylene
PET	Poly Ethylene Terephthalate
PP	Poly Propylene
PS	Poly Styrene
Py-GC-MS	Pyrolysis Gas Chromatography coupled to Mass Spectrometry
SD	Standard Deviation
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscopy
SERS	Surface-Enhanced Raman Scattering
SMNPs	Small Microplastics and Nano Plastics
SRMs	Standard Reference Materials
T	Thames
TEM	Transmission Electron Microscopy
WTP	Water Treatment Plant

1 INTRODUCTION

Currently, the detection and identification of the smallest small microplastics (<10 µm – 1000 nm) and nanoplastics (1000 nm – 1 nm) (SMNPs) in environmentally relevant samples is highly challenging because of the lack of standardized analytical procedures.

This deliverable describes the analysis of the field samples of water and suspended sediments collected during the sampling campaigns (D2.2 and D2.3) to determine the presence of SMNPs. This analysis was carried out following the guidelines described in D4.1. The results reported here are based on the analysis of the samples using nanoparticle tracking analysis (NTA), scanning electron microscopy (SEM), fluorescence microscopy, and Raman spectroscopy.

2 MERO-BARCÉS RIVER BASIN

2.1 Water

The water samples for SMNP analysis were collected at approximately 0.5 – 1.0 m depth in 6 different locations:

1. Location 1 – Cecebre reservoir bridge 1 – Mero River (MB1)
2. Location 2 – Cecebre reservoir bridge 2 – Barcés River (MB2)
3. Location 3 – Guiliade (MB3)
4. Location 4 – Meirama – Reservoir or “Cleaned Spot” (MB4)
5. Location 5 – Cecebre beach (MB5)
6. Location 6 – Dam WTP “A Telva” (MB6)

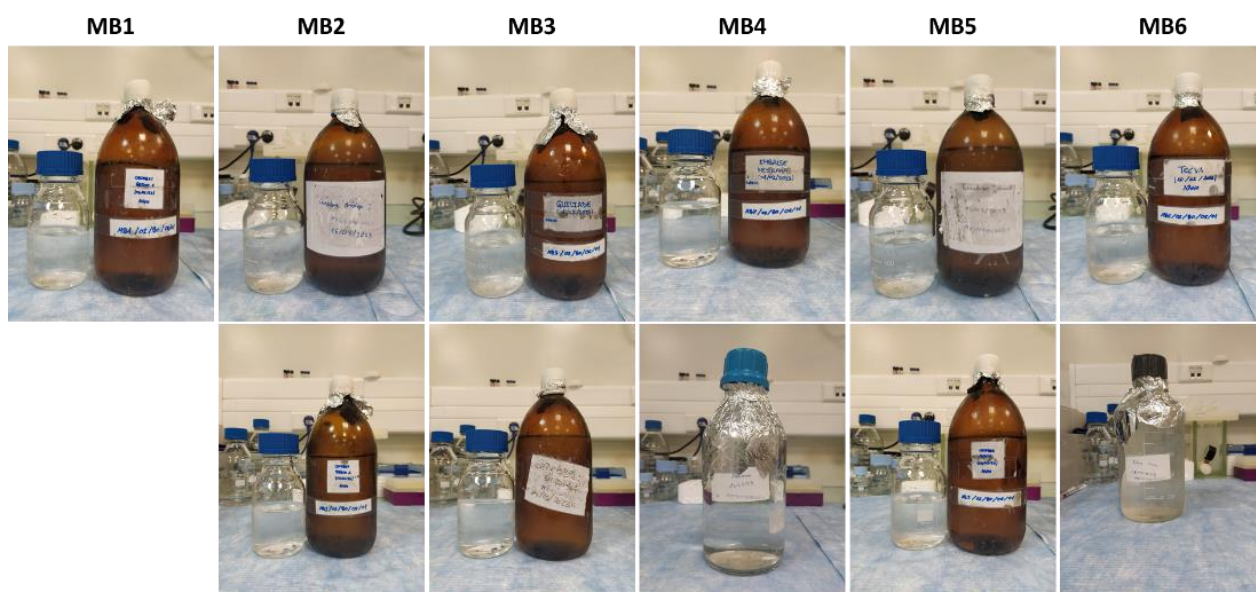


Figure 1. Illustrative photographs for Mero-Barcés River Basin samples from the two first campaigns.

The volume was 1 – 1.5 L and stored in amber borosilicate bottles at 4 °C until further use (more details in D2.2 and D2.3). The water samples were prepared for the analysis following the decision tree established in deliverable 4.1, as shown in Figure 2.

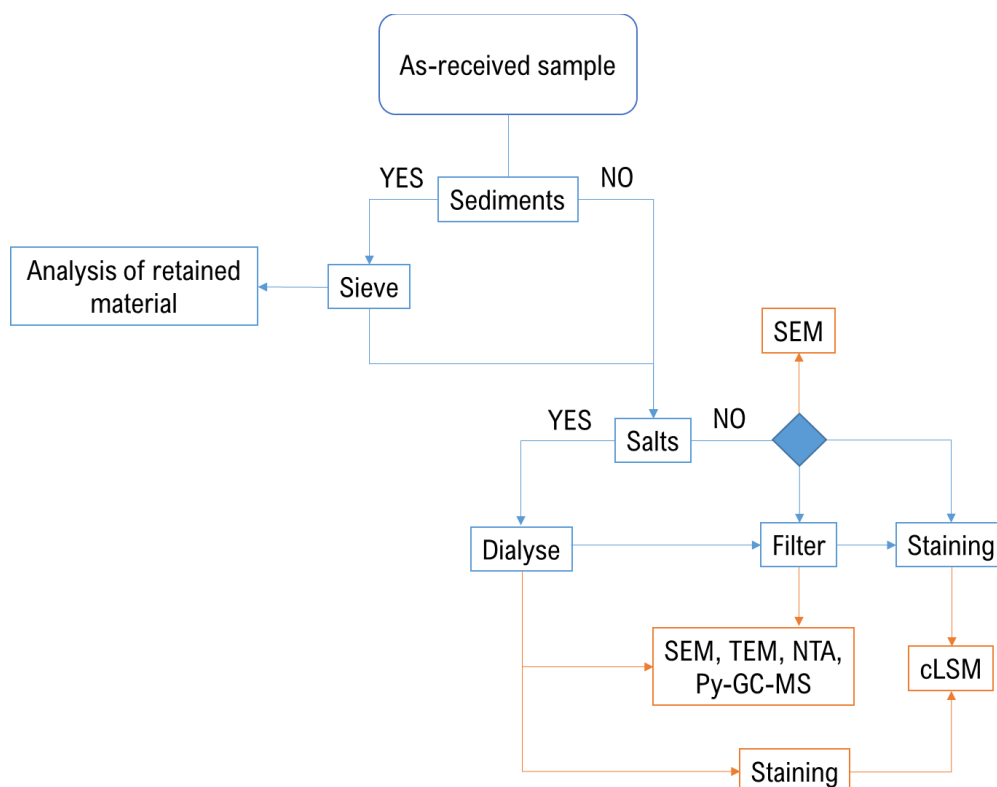


Figure 2. Decision tree for water sample preparation.

Thus, the sample preparation for these water samples was different according to the sampling station:

- MB4-Meirama (MB04/03/B0/06/01) sampled in the spring campaign contained suspended sediments and therefore, a sieving step was performed to separate these sediments from the water. Then, the sample was filtered prior to the analytical technique required. This sample was not dialyzed since it is freshwater.
- MB2-Cecebre reservoir bridge 2 – Barcés River, MB4-Meirama – Reservoir, and MB5-Cecebre beach were dialyzed because they contained high concentrations of salts. Then, samples were filtered when the analytical technique required so.
- MB1 – Cecebre reservoir bridge 1 – Mero River, MB3-Guiliade, and MB6-Dam WTP “A Telva” were only filtered when the analytical technique required so since their source is freshwater.

2.1.1 Detection/Identification/Quantification

2.1.1.1 NTA analysis

The water samples were filtered by 1 μm nylon membrane before NTA analysis. Size, size distribution, and concentration of particles in the sample (particles/mL) were estimated within the range of 1 μm – 50 nm. Therefore, this analysis will allow assessing the presence of nanoplastics (herein defined as particles with a size below 1 μm^1).

The sample was loaded in a 1 mL plastic syringe for analysis. The infusion rate for the NTA measurement was adjusted to 100. Five videos with a duration of 60 seconds (total frames analyzed = 1498) were recorded for

¹ N. B. Hartmann, T. Hüffer, R. C. Thompson, M. Hassellöv, A. Verschoor, A. E. Daugaard, S. Rist, T. Karlsson, N. Brennholt, M. Cole, M. P. Herrling, M. C. Hess, N. P. Ivleva, A. L. Lusher and M. Wagner, Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris, *Environ. Sci. Technol.*, 2019, **53**, 1039–1047.

The contents of this document are the copyright of the LABPLAS consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

each sample. The instrument was rinsed with 1 mL of ultrapure water between replicates and un-mounted the cell holder after each sample analysis. The parameters for NTA analysis were selected according to the better visualization of plastics spiked in ultrapure water as reported in our recent publication².

NTA results obtained from all water samples are shown in Table 1. In all samples, the particle per frame was lower than 5 particles/frame. The highest particle/frame obtained was 4.4 for the water sample sampled in Cecebre beach (MB5) in winter (February 2023). It is worth noticing that the particle/frame obtained for these samples is not in the ideal range for accurate and reproducible values in NTA. According to the NTA manufacturer, the ideal range is 10 – 100 particles per frame. OECD 125³ and ASTM E2834-12⁴ guidelines, recommend measuring at least 700 particles for polydispersed samples. As 1498 frames were recorded, more than 2500 particles are measured even with 2 particles per frame. However, we must take into account that the values obtained for these samples may have less quality due to the low number of particles per frame detected.

Table 1. NTA results for all the water samples from Mero-Barcés received. D90: 90% of the total particles are smaller than this size; D50: 50% of the total particles are smaller than this size; D10: 10% of the total particles are smaller than this size. Control samples and Milli-Q water as procedural blank were also analyzed.

Sample	particle/mL (xE7)	SD (xE7)	particle/frame	SD	Mean size (nm)	SD	Mode size (nm)	SD	size D10 (nm)	SD	size D50 (nm)	SD	size D90 (nm)	SD
MilliQ water_1umFilter	0.2	0.0	0.1	0.0	180	39	179	40	135	46	179	40	234	36
MB1/01/BO/06/01	2.1	0.1	1.6	0.1	138	2	108	9	84	4	130	3	207	4
MB2/01/BO/06/01	2.9	0.2	2.2	0.1	138	4	91	10	76	4	115	4	223	10
MB3/01/BO/06/01	1.2	0.3	0.9	0.1	172	26	92	14	75	4	131	12	327	104
MB4/01/BO/06/01	2.7	0.2	2.1	0.1	151	10	109	20	87	3	136	9	263	26
MB5/01/BO/06/01	2.9	0.3	2.1	0.1	143	4	128	10	78	4	136	4	218	22
MB1/02/BO/06/01	1.9	0.1	1.4	0.1	148	4	102	6	71	7	111	6	253	17
MB2/02/BO/06/01	2.4	0.2	2.0	0.1	115	3	80	9	67	4	100	4	187	12
MB3/02/BO/06/01	3.0	0.2	2.3	0.1	121	3	90	12	70	6	110	5	187	7
MB4/02/BO/06/01	2.8	0.2	2.0	0.0	111	3	79	4	63	1	95	3	177	13
MB5/02/BO/06/01	5.5	0.3	4.4	0.3	123	3	3	111	69	9	114	4	204	1
MB6/02/BO/06/01	2.7	0.1	2.2	0.1	141	4	86	10	63	3	112	3	248	35
MB1/03/BO/06/01	1.9	0.2	1.7	0.2	130	2	107	13	65	4	119	5	201	21
MB2/03/BO/06/01	1.7	0.1	1.7	0.1	142	6	112	21	72	5	115	6	231	19
MB3/03/BO/06/01	1.1	0.1	1.2	0.1	139	6	95	11	68	10	112	8	265	16
MB4/03/BO/06/01	0.6	0.1	0.6	0.1	143	12	81	5	77	4	124	11	240	46
MB5/03/BO/06/01	1.7	0.1	1.7	0.1	145	7	94	10	74	5	121	3	267	35
MB6/03/BO/06/01	1.9	0.2	1.4	0.2	122	7	105	7	69	5	107	5	194	15

Despite the low concentration detected, some differences in the particle concentrations were observed not only between locations but also between sampling campaigns as shown in Figure 3. The highest concentrations of particles were detected in general in the winter campaign (i.e. 23-Feb in Figure 3) except for the water sampled in both Cecebre reservoir bridge 1 (MB1) and 2 (MB2). The concentration in these was similar in all campaigns. This could be because those are the largest open spaces in this case of study. Moreover, those locations are in the middle of the reservoir where the largest water mass is present (more details in D2.1, D2.2, and D2.3). In contrast, the lowest concentrations were recorded for the sampling campaign made in spring (i.e. 23-May in Figure 3). The highest and lowest concentrations were detected in Cecebre beach (MB5) in winter and

² Sorasan C, Taladriz-Blanco P, Rodriguez-Lorenzo L, Espiña B, Rosal R. New versus naturally aged greenhouse cover films: Degradation and micro-nanoplastics characterization under sunlight exposure. *Science of the Total Environment* 2024, **918**.

³ OECD, OECD Guidelines for the Testing of Chemicals. Test No. 125: Nanomaterial Particle Size and Size Distribution of Nanomaterials, 2022.

⁴ ASTM E2865-12(2018), Standard Guide for Measurement of Electrophoretic Mobility and Zeta Potential of Nanosized Biological Materials, ASTM International, West Conshohocken, PA, 2018, www.astm.org

The contents of this document are the copyright of the LABPLAS consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

Meirama (MB4, reservoir or “Cleaned Spot”) in spring, respectively. The size of these detected particles was similar in all samples as shown in Figure 4.

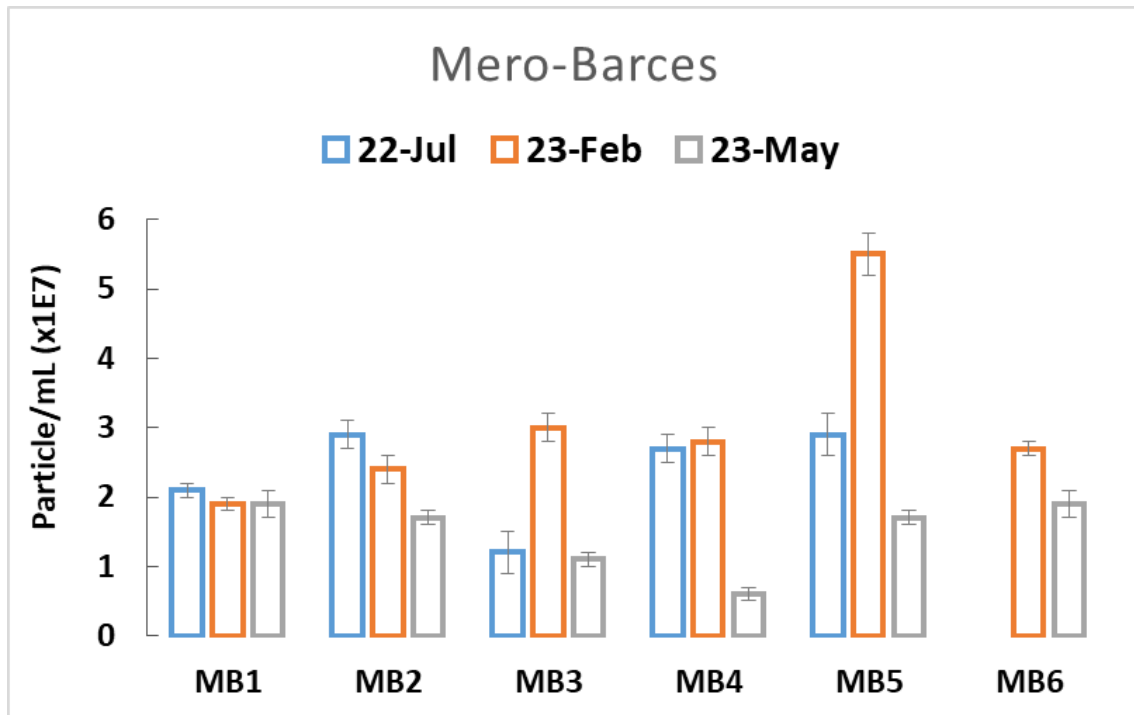


Figure 3. Concentration of particles below 1 µm per millilitre for all water samples from Mero-Barcés received. For the NTA measurements, samples were filtered.

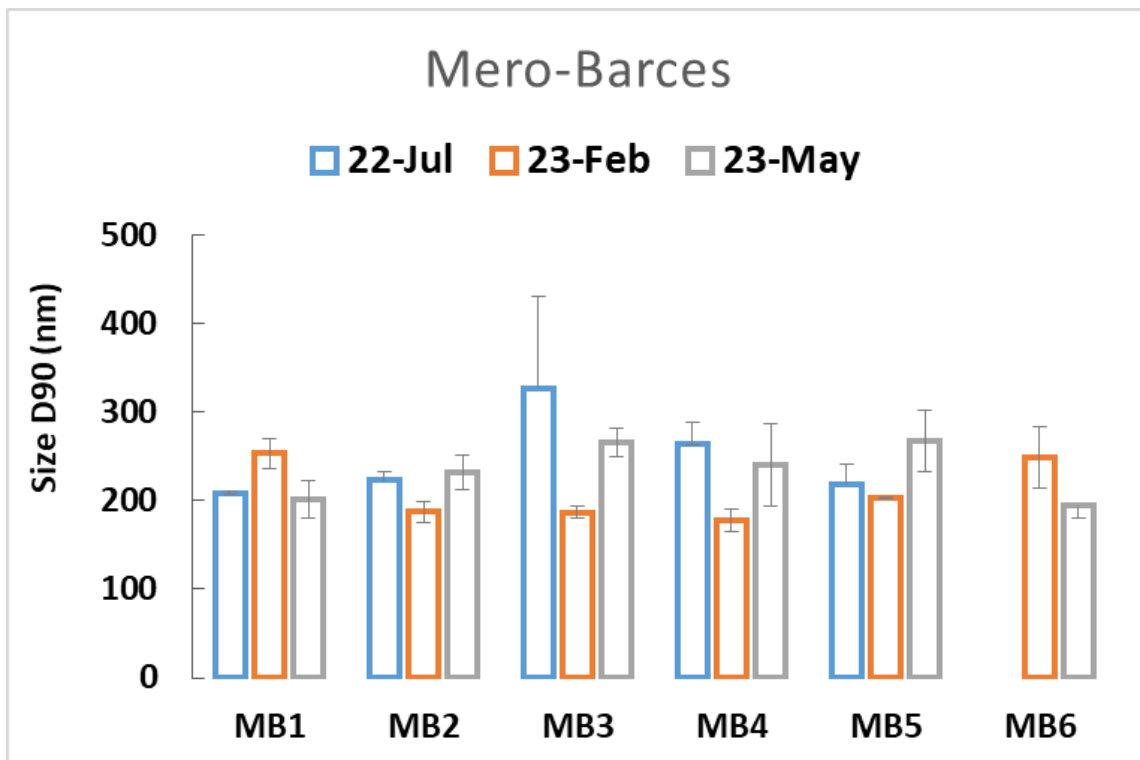


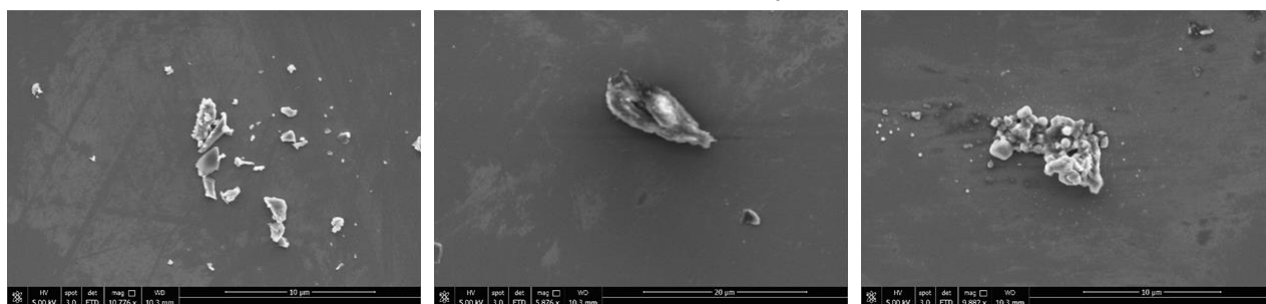
Figure 4. Size d90 of the particles obtained by NTA for all water samples from Mero-Barcés received. For the NTA measurements, samples were filtered.

The contents of this document are the copyright of the **LABPLAS** consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

2.1.1.2 SEM analysis

SEM analysis was performed by using high vacuum conditions and Everhart Thornley secondary electron detectors (ETD). All samples were analyzed using an accelerating voltage of 3, 5 kV, or 15 kV and a spot size of 3 or 4 depending on the stability of the sample. Samples were prepared by dropping casting 10 μ L of treated water samples (see Figure 2) on a silicon wafer. The sample was dried at room temperature under dark conditions inside a closed box to minimize contamination (see more details in D4.1). The size range that is analyzed by SEM is from $\leq 1 \mu\text{m}$ – 100 nm being comparable with NTA analysis.

Procedural Blank – Air deposition



Procedural Blank – Ultrapure water

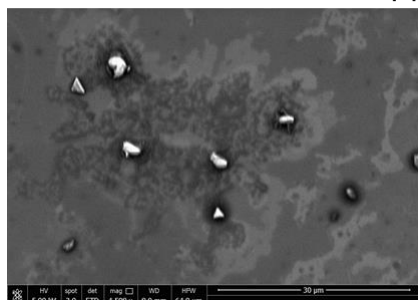


Figure 5. SEM images for relevant control data for blanks measured to ensure study quality. Two procedural blanks (without and with 10 μ L of ultrapure water) were performed to check the presence or contamination of particles by experimental procedure steps.

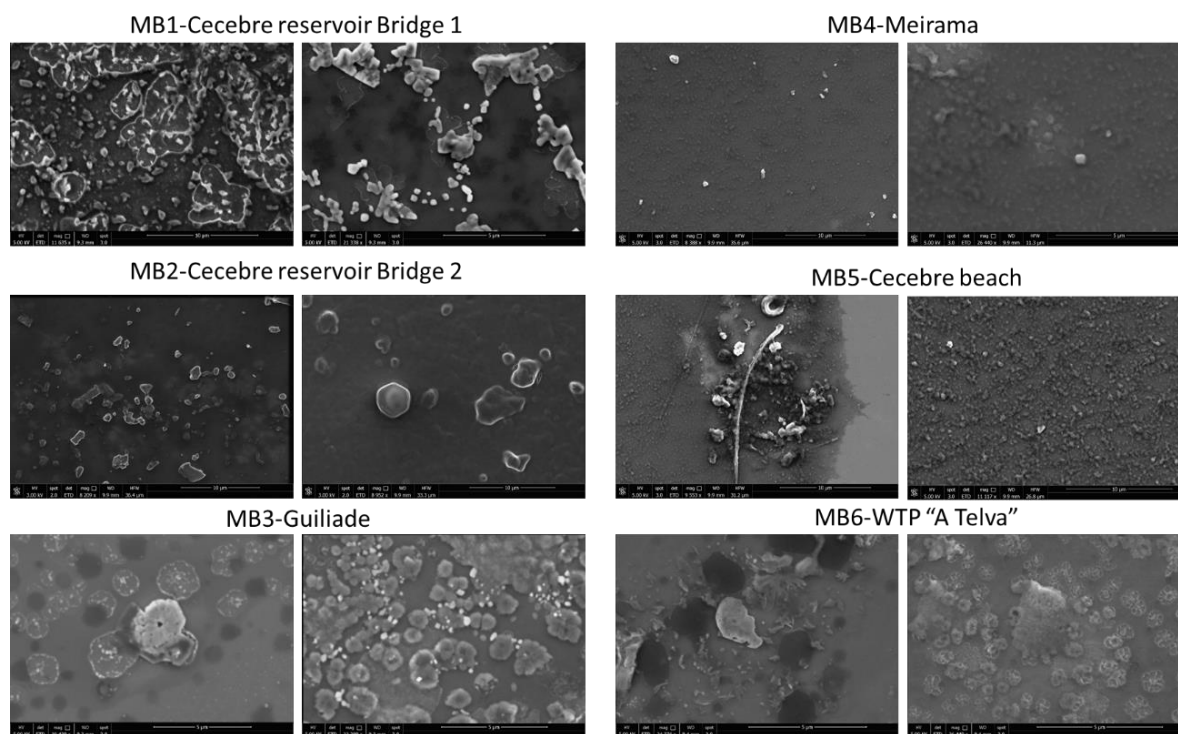


Figure 6. Representative SEM images for all water samples from Mero-Barcés collected in the second campaign (Winter season). The biggest particles observed in these SEM images were identified as salts, probably calcium carbonate and sodium chloride, crystallized during the drying process.^{5,6}

Samples from the Winter campaign were selected for SEM analysis because almost all the stations presented higher concentrations of particles estimated by NTA (Figure 3). Particles were visualized by SEM for all the samples from the Winter campaign of Mero-Barcés (Figure 6). These particles were different in quantity, size, and shape in comparison with the particles observed in the controls prepared simultaneously with the water samples (Figure 5). MB5 – Cecebre beach and M4-Meirama presented a higher number of small particles in comparison with the rest of the samples. SEM analysis of the other two campaigns is being performed and will be included in the D4.4.

2.1.1.3 Raman analysis

The limit of detection (LoD) established in Deliverable 4.2 for Raman analysis of nanoplastics in freshwater was 5 mg/L for both 161 nm PS and 450 nm PET, which correspond to $\sim 10^9$ particles/mL - $\sim 10^8$ particles/mL. Therefore, Raman analysis of any of the samples from Mero-Barcés would be highly hindered even if all particles detected by NTA would be plastics as LoD concentration cannot be attained. We are currently working on improved pre-concentration strategies using cloud-point extraction (CPE) not only for Raman analysis but also for Py-GC-MS analysis.

For the analysis of the smallest small microplastics, the lowest concentration detected by Raman using the sorting and pre-concentration microfluidic device is 1 mg/L for PS and PET, but 10 mg/L for PE and PP. Therefore, we are working on establishing a strategy to improve the limit of detection for samples with low content of particles as the ones obtained in Mero-Barcés.

⁵ Feng, Q. (2011). Principles of Calcium-Based Biomineralization. In: Müller, W. (eds) Molecular Biomineralization. Progress in Molecular and Subcellular Biology(), vol 52. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-21230-7_6

⁶ Katherine E. Hurst, Charfedinne Ayed, Ivan N. Derbenev, Louise Hewson, Ian D. Fisk, Physicochemical design rules for the formulation of novel salt particles with optimised saltiness, Food Chemistry, 2021, **360**

The contents of this document are the copyright of the LABPLAS consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

2.2 Suspended Sediments

Most of the Mero-Barcés samples were clean and free of any suspended solids, except for the MB4/03/B0/06/01 (i.e. M4-Meirama sampled in the Spring campaign). Suspended sediments from this sample were isolated and a density separation procedure was performed following the decision tree shown in Figure 7 (more details in D4.1).

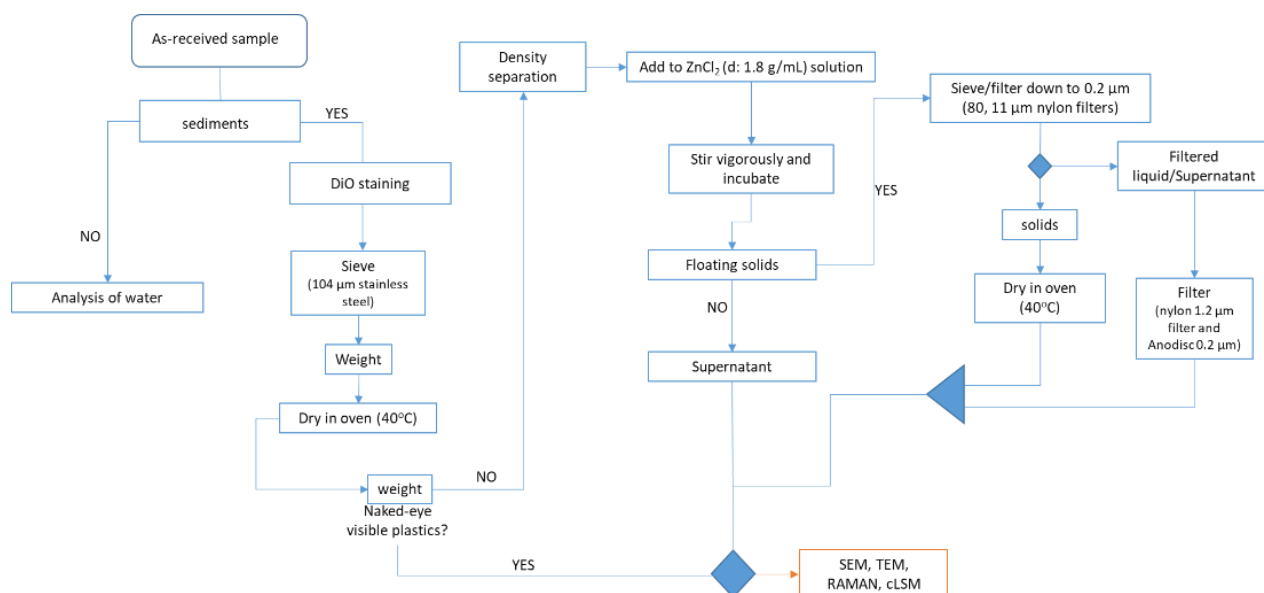


Figure 7. Decision tree for suspended sediments sample preparation.

The final quantity of sediments isolated from the water was very small, so no beakers were weighed.

2.2.1 Detection/Identification/Quantification

2.2.1.1 NTA analysis

After density separation, the viscosity of the dispersion is high enough to technically limit the analysis by NTA.

2.2.1.2 Fluorescence microscopy analysis

Analysis of stained particles was performed using fluorescence microscopy. Nile Red must be avoided in the case of suspended sediments since the particulate matter displays auto-fluorescence in the same spectral range as Nile Red (559 nm excitation, 635 nm emission),^{7, 8} interfering with the fluorescence analysis of the particles. Thus, the suspended sediments were stained from the beginning of the sample preparation using DiO and thus the final nylon filters and anodiscs can be directly observed under the microscope. Samples were imaged using an objective of at least 40× and the DiO imaging optimal conditions (483 nm excitation, 501 nm emission). The results are shown in Figure 8. Few fluorescent particles were detected in the MB4-Meirama sample collected in the spring campaign. These fluorescent particles had spherical shape and a size close to 10 µm.

⁷ Cottrell BA, Timko SA, Devera L, Robinson AK, Gonsior M, Vizenor AE, et al. Photochemistry of excited-state species in natural waters: A role for particulate organic matter. *Water Research* 2013, **47**.

⁸ Cabrera-Brufau M, Marrasé C, Ortega-Retuerta E, Nunes S, Estrada M, Sala MM, et al. Particulate and dissolved fluorescent organic matter fractionation and composition: Abiotic and ecological controls in the Southern Ocean. *Science of the Total Environment* 2022, **844**.

The contents of this document are the copyright of the LABPLAS consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

MB04/03/B0/06/01

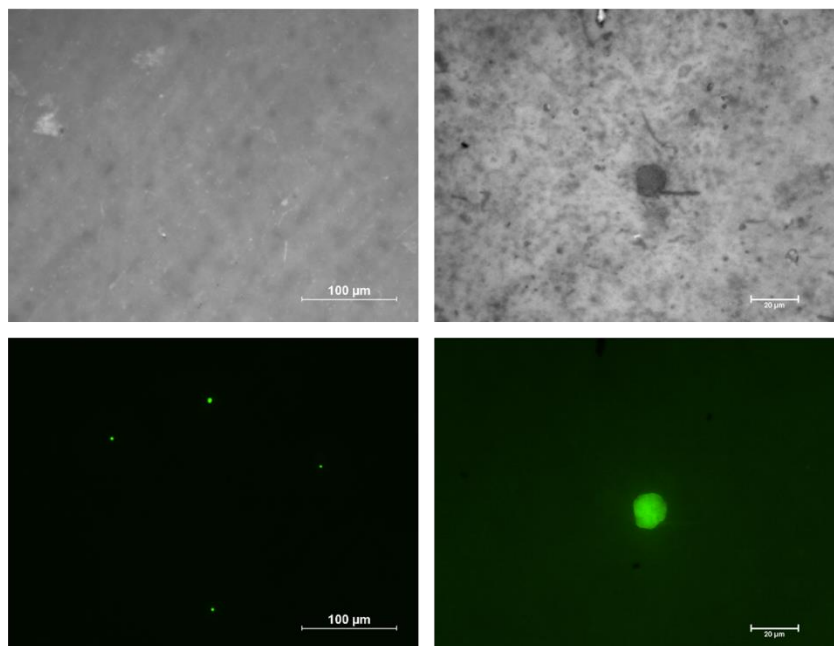


Figure 8. Bright-field and fluorescence images of Anodisc after plastic extraction from suspended sediments of MB4-Meirama sample collected in spring campaign (MB04/03/B0/06/01).

2.2.1.3 Raman analysis

The anodiscs characterized by fluorescence microscopy were also analyzed using Raman confocal microscopy (300 lines mm^{-1} grating and a CCD camera). The measurement conditions for stained suspended sediments have been slightly modified. This modification is related to the spectral interference of DiO⁹ as shown the Figure 9. For stained samples, 50x should be used, and at least 4 mW of laser power. Raman analysis should be performed using both 785 nm and 633 nm laser lines, if possible. Due to the DiO interference, the characteristic peaks for the identification of each plastic were slightly different^{10, 11}:

- PET: The peaks centered at 1615-1620 and 1730 cm^{-1} corresponding to the aromatic bending and carbonyl stretching vibration modes, respectively, are normally used for PET identification. The band centred at 860 cm^{-1} , which corresponds to ring C-C breathing, and ester C(O)-O stretching, was included for DiO-stained samples.
- PS: The characteristic peaks for PS identification kept the same for DiO-stained samples: 1002 and 1032 cm^{-1} corresponding to ring breathing and C-H in-plane deformation, respectively.
- PP: Characteristic peaks selected for the PP identification depended on the excitation laser lines:
 - 785 nm: Three peaks were not shielded by the DiO fingerprint: ca. 808 cm^{-1} (C-C stretching, related to its crystallinity), ca. 840 cm^{-1} (CH_3 rocking), and 1459 cm^{-1} (CH_2 bending).
 - 633 nm: Three extra characteristic peaks could be used for PP identification: 2840 cm^{-1} (CH_2 symmetric stretching), 2883 cm^{-1} (CH_2 asymmetric stretching), and 2952 cm^{-1} (CH_3 asymmetric stretching).

⁹ Feng G, Suzuki N, Zhang Q, Li J, Inose T, Taemaitree F, et al. A light-mediated covalently patterned graphene substrate for graphene-enhanced Raman scattering (GERS). *Chemical Communications* 2023, **59**

¹⁰ Caldwell J, Taladriz-Blanco P, Rodriguez-Lorenzo L, Rothen-Rutishauser B, Petri-Fink A. Submicron- and nanoplastic detection at low micro- to nanogram concentrations using gold nanostar-based surface-enhanced Raman scattering (SERS) substrates. *Environmental Science-Nano* 2024, **11**.

¹¹ Caldwell J, Rodriguez-Lorenzo L, Espiña B, Beck A, Stock F, Voges K, et al. Detection of submicron- and nanoplastics spiked in environmental fresh- and saltwater with Raman spectroscopy. *Marine Pollution Bulletin* 2024, **203**.

The contents of this document are the copyright of the LABPLAS consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

- PE: Characteristic peaks selected for the PE identification depended on the excitation laser lines:
 - 785 nm: The characteristic peaks are ca. 1067 cm^{-1} (asymmetric stretching C-C), ca. 1133 cm^{-1} (symmetric stretching C-C) and ca. 1301 cm^{-1} ($-\text{CH}_2-$ twisting)
 - 633 nm: There are two extra peaks for PE identification: 2850 cm^{-1} (CH_2 asymmetric stretching) and 2883 cm^{-1} (CH_2 asymmetric stretching).

It is worth noting that all PE peaks in the spectral window of 1000 – 2000 cm^{-1} overlapped with the DiO spectra. Therefore, the identification of PE stained with DiO was limited to cases when DiO staining was low and upon 633 nm laser excitation.

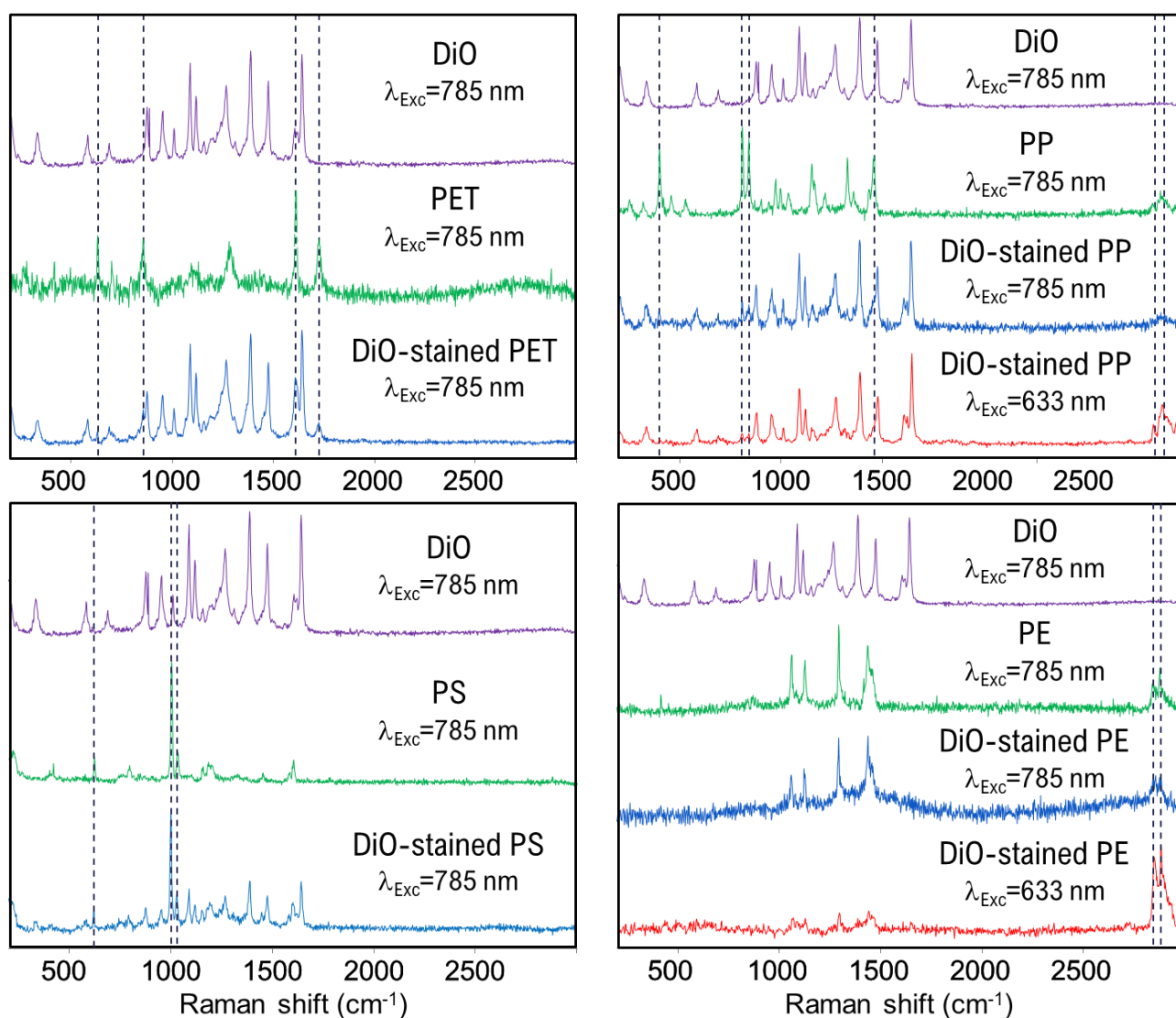


Figure 9. Raman spectra of DiO dye, unstained plastic, and DiO-stained plastic were acquired using both 785 nm and 633 nm laser lines. The representative peaks of each plastic are indicated with black dashed lines.

Once the conditions were established, the Raman analysis of the MB4-Meirama sampled in the spring campaign was performed. Unfortunately, the sample could not be characterized using 633 nm due to the high fluorescence background coming from the rest of the particles present in the matrix (e.g. particulate organic matter^{7,8}), as shown in Figure 10. Therefore, the possible identification of PE in this kind of sample is limited. However, the presence of PS particles was detected using a 785 nm laser line (Figure 10).

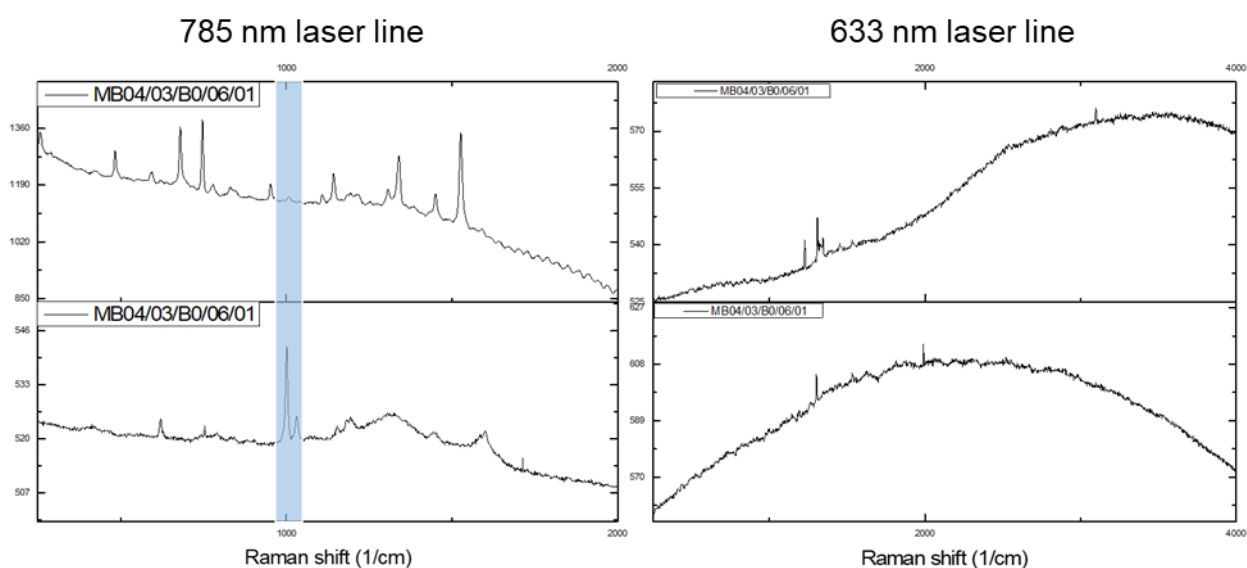


Figure 10 - Raman spectra of anodisc after plastic extraction procedure from Mero-Barcés sample (MB04/03/B0/06/01), acquired using both 785 nm and 633 nm laser lines.

2.3 Conclusions

The concentration of particles below 1 μm estimated by NTA was low, in the range of 5.5×10^7 – 0.6×10^7 particles/mL. The size of these particles was similar, between 200 – 300 nm, in all the sampling locations and seasons. The highest and lowest concentrations of particles below 1 μm were obtained in Cecebre beach (MB5) in Winter and Meirama (MB4, reservoir or “Cleaned Spot”) in Spring, respectively. Samples from the Winter campaign were also characterized by SEM. The highest amount of smaller particles was detected in MB5-Cecebre beach, which was in agreement with NTA results. Samples from the other two campaigns are being analyzed to complete SEM characterization. The low concentrations in all samples limited their analysis by Raman, and therefore, the presence of SMNPs could not be confirmed. Only one MB4-Meirama sample from the spring campaign contained enough suspended sediment to be analyzed. PS particles were detected using Raman spectroscopy and a 785 nm laser line. The 633 nm laser line could not be used due to the auto-fluorescence from other particles in the matrix (i.e. particulate organic matter).

3 THAMES RIVER

3.1 Water

The water samples were collected in six locations (more details D2.2 and D2.3):

1. Location 1: Lechlade (T01 - rural upper reaches)
2. Location 2: Dorchester on Thames (T02 - rural with urban influence)
3. Location 3: Upstream of tidal limit (T03 - Windsor)
4. Location 4: Downstream of tidal limit (T04 - Richmond)
5. Location 5: Central London (T05 - urban)
6. Location 6: Thames estuary (T06 - Chapman buoy)

However, water samples for SMNPs analysis were collected only in: Location 3 (Upstream of tidal limit, T03 - Windsor) at two different depths (at a few centimeters from the water surface and 1-meter depth) in July 2022; and Location 5 (Central London, T05 - urban) and Location 6 (Thames estuary, T06 - Chapman buoy) in April 2023.

Freshwater samples were prepared for the analysis following the decision tree established in deliverable 4.1, as shown in Figure 12. Thus, suspended sediments were isolated from water samples, and subsequently, water samples were filtered as requested by the analytical technique for the analysis.

3.1.1 Detection/Identification/Quantification

3.1.1.1 NTA analysis

The analysis was carried out as described above in section 2.1.1.1.

NTA results obtained from all water samples are shown in Table 2. Only the water sampled in Location 5 Central London (T05 - urban) displayed a particles/frame higher than 10 (i.e. 27.3 particles per frame); while the rest of the samples presented particles per frame lower than 5. The urban character of this location, situated in a central zone of London city with an important level of touristic/anthropogenic activities, could be a potential explanation for these results.

Table 2. NTA results for all the water samples from Thames River received. D90: 90% of the total particles are smaller than this size; D50: 50% of the total particles are smaller than this size; D10: 10% of the total particles are smaller than this size. Control samples and Milli-Q water as procedural blank were also analyzed.

Sample	particle/mL (xE7)	SD (xE7)	particle/frame	SD	Mean size (nm)	SD	Mode size (nm)	SD	size D10 (nm)	SD	size D50 (nm)	SD	size D90 (nm)	SD
MilliQ water_1umFilter	0.2	0.0	0.1	0.0	180	39	179	40	135	46	179	40	234	36
T3-01-BO-06-01-1m	3.9	0.4	2.6	0.1	104	5	82	4	67	4	89	3	161	16
T3-01-BO-06-01-surface	3.8	0.4	3.0	0.3	98	5	75	3	64	2	86	4	153	9
T05-05-NP-06-01	26.1	0.9	27.3	0.4	149	0	113	14	82	2	141	1	226	3
T06-05-NP-06-01	2.1	0.2	1.7	0.1	118	3	81	9	65	2	93	7	212	40

Clearly, the highest concentration per mL estimated by NTA was shown in T05 – Central London, which was 20-fold higher than in the rest of the water samples from the Thames River (Figure 11). In addition, the size of particles in T03 for both depths determined by NTA was smaller than for T05 and T06 (Figure 12).

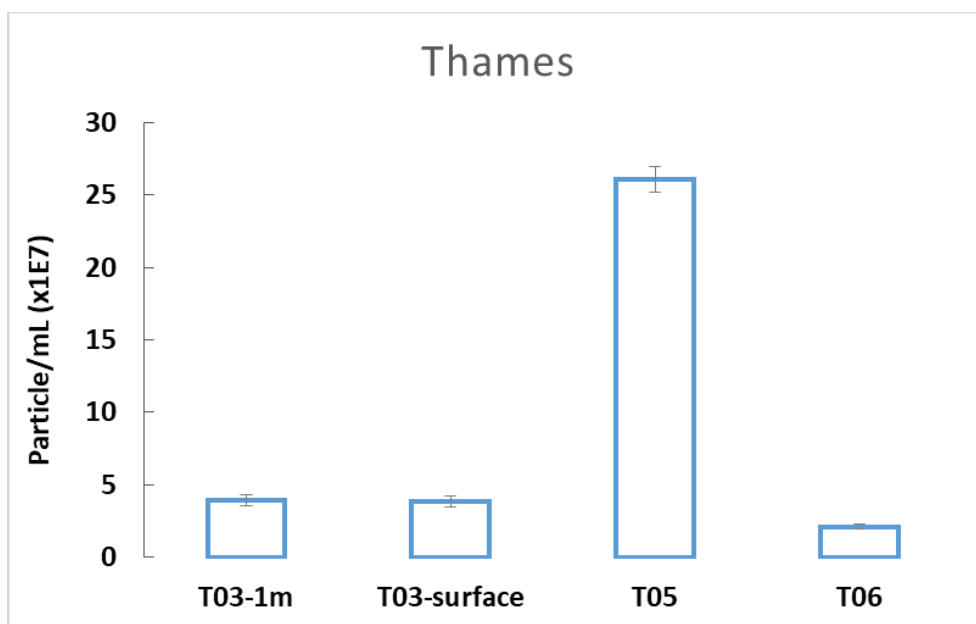


Figure 11. Concentration of particles below 1 μm per millimeter for all water samples from Thames River. For the NTA measurements, samples were filtered.

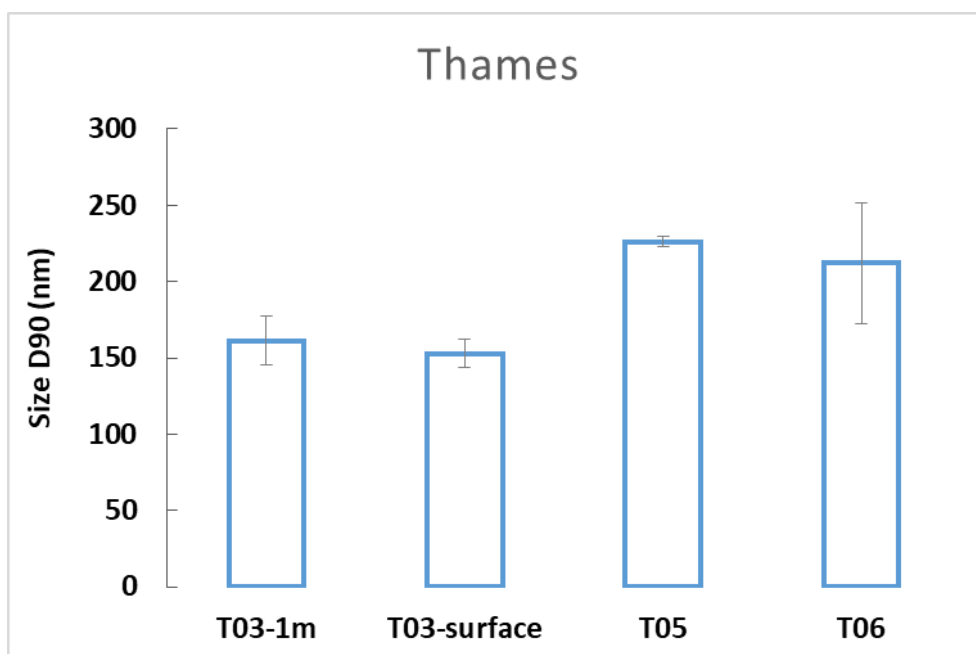


Figure 12. Size d90 of the particles obtained by NTA for all water samples from Thames River. For the NTA measurements, samples were filtered.

3.1.1.2 SEM analysis

The analysis was carried out as described above in section 2.1.1.2.

Differences were observed between water samples collected in different stations when they were analyzed by SEM as shown in Figure 13. However, samples from the same location, i.e. T03 – Windsor, but collected at different depths did not show a significant difference. The station T06 – Chapman presented few particles by SEM. High shape and size variability were observed, which can be due to the crystallization of salts and potential aggregation of the particles. Higher concentrations of smaller irregular-shaped particles were visualized in both T03-Windsor samples, even though the NTA analysis detected low particle concentrations smaller than 1 μm .

The contents of this document are the copyright of the LABPLAS consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

This could be because these particles may present low scattering properties, and consequently, they were not detected by NTA. The size limit detection by NTA is strongly dependent on the refractive index and light scattering properties of the particle under study, varying between 30 – 600 nm.¹² Analysis of chemical composition by Raman and Py-GC-MS is needed to identify the nature of particles.

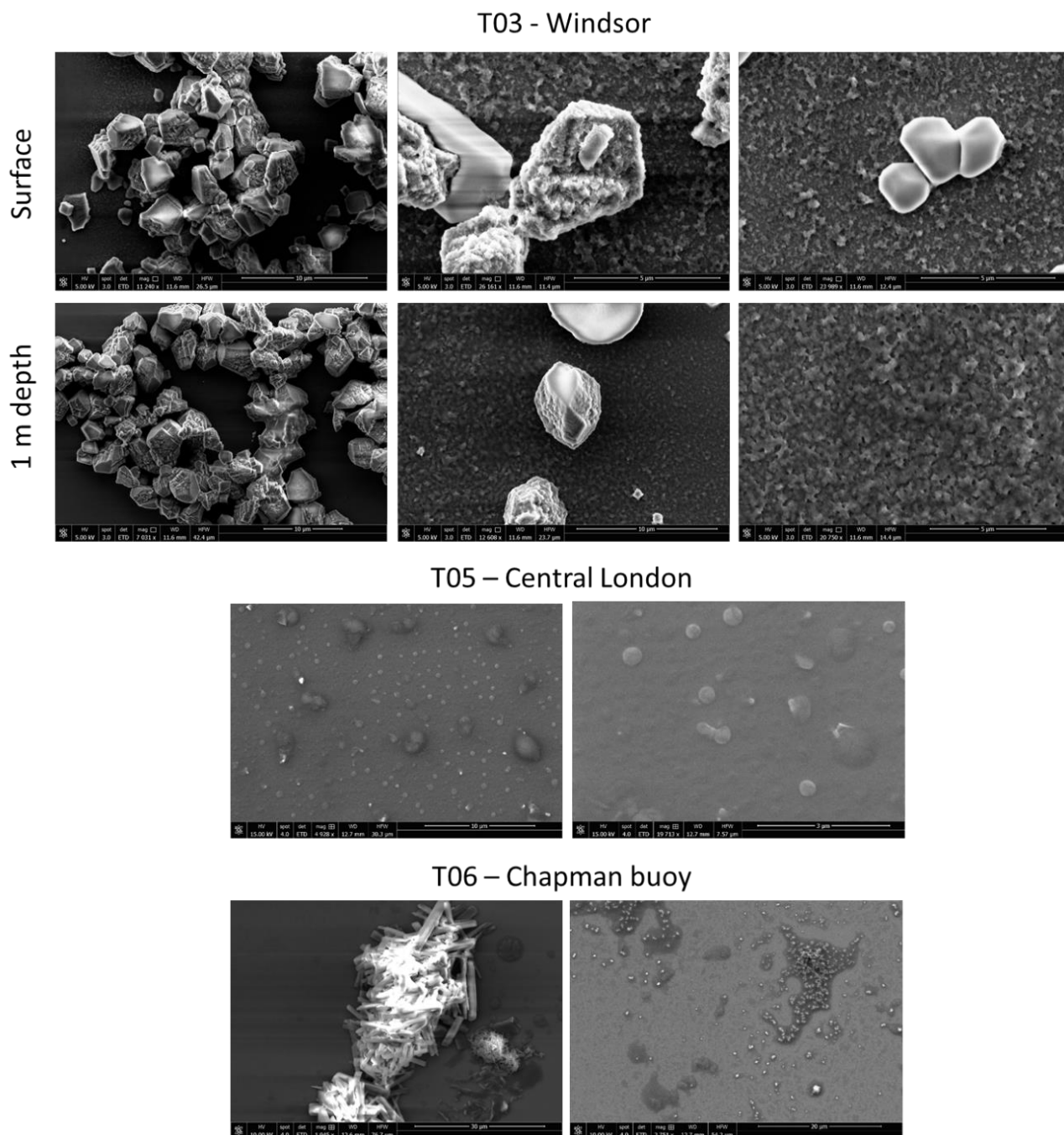


Figure 13. Representative SEM images for all Thames water samples at high vacuum conditions. Samples were not sputtered with a conductive layer. The biggest particles observed in these SEM images were identified as salts, probably calcium carbonate and sodium chloride, crystallized during the drying process.^{5,6}

¹² Maguire, C.M., et al., Benchmark of Nanoparticle Tracking Analysis on Measuring Nanoparticle Sizing and Concentration. Journal of Micro and Nano-Manufacturing, 2017. 5

3.1.1.3 Raman analysis

In the case of the Thames River, station T05 (Central London) displayed a concentration of 26×10^7 particles/mL estimated by NTA, which is a concentration that allows performing the Raman analysis. The LoD established in Deliverable 4.2 for Raman analysis of nanoplastics in freshwater was 5 mg/L for both 161 nm PS and 450 nm PET, which correspond to $\sim 10^9$ particles/mL - $\sim 10^8$ particles/mL.

Figure 14 shows the Raman analysis of these samples after filtration. The sample was filtered by 11 and 5 μm nylon membranes and 200 nm Anodisc. Autofluorescence from the matrix was observed in the analysis and only at some points, it was possible to acquire Raman spectra from sediments.

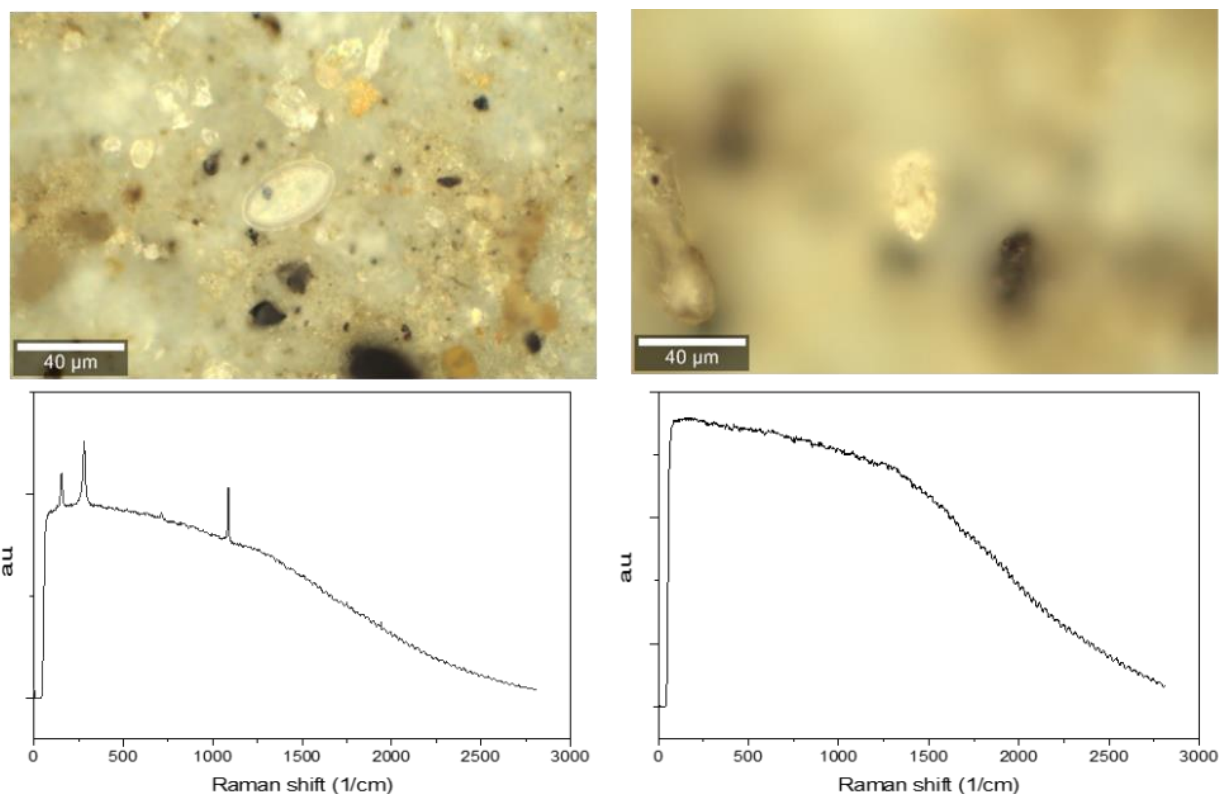


Figure 14. Raman analysis of T05 water sample after triggering a filtration procedure. The particles collected on a 200 nm Anodisc were analyzed using a 785 nm laser line.

To reduce the matrix interference, samples were subjected to cloud-point extraction (CPE) to pre-concentrate and eliminate part of the undesirable particulate organic matter and suspended sediments (more details in D4.1). Briefly, 40 mL of the water sample was dispersed in 4 mM Triton-X114 and incubated in pre-boiled water for 1h. Then, the supernatant (surfactant-poor phase) was removed using glass Pasteur pipettes, and the pellet (surfactant-rich phase) was re-dispersed in 1 mL of ultrapure water. Finally, this surfactant-rich phase was filtered by 0.2 μm anodisc and washed using 4 mL ethanol:water (1:1) mixture. This last step removes the excess of Triton, allowing the Raman analysis. Raman analysis is being performed using both 633 and 785 nm laser lines and 20x and 50x objectives. The laser power at the sample should be ≤ 7 mW.

3.2 Suspended Sediments

All Thames samples contain suspended sediments as shown in Figure 15.



Figure 15. Illustrative photographs for Thames River samples

The samples were treated according to the flow diagram shown in Figure 7 in Section 2.2. The final quantity of sediments isolated from the water was very small and for this reason, no beakers were weighed during the procedure.

3.2.1 Detection/Identification/Quantification

3.2.1.1 NTA analysis

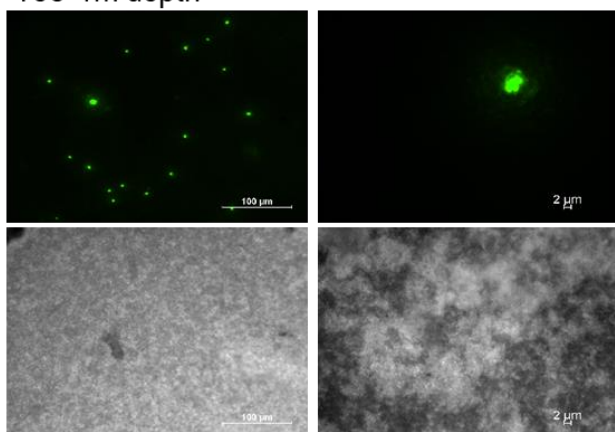
After density separation, the viscosity of the dispersion is high enough to technically limit the analysis by NTA.

3.2.1.2 Fluorescence microscopy analysis

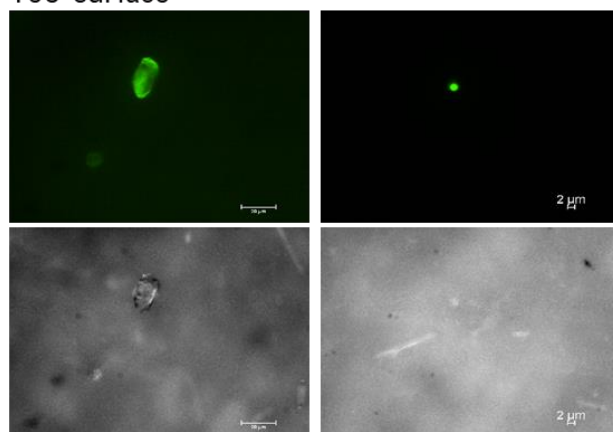
The conditions of the analysis are described in 2.2.1.2.

The results are shown in Figure 16. Several fluorescent fragments were observed. From here, the explanation of the observations is referred to fluorescent particles and not the total amount of particles in the sample. In sample T03-Winsor sampled at 1m depth many particles with irregular shapes were observed. In contrast, some small and roundish particles were observed in T03-Winsor sampled on the surface. Both in T05-Central London and T06-Chapman buoy samples bigger particles were observed, although in smaller quantity in comparison to T03 samples.

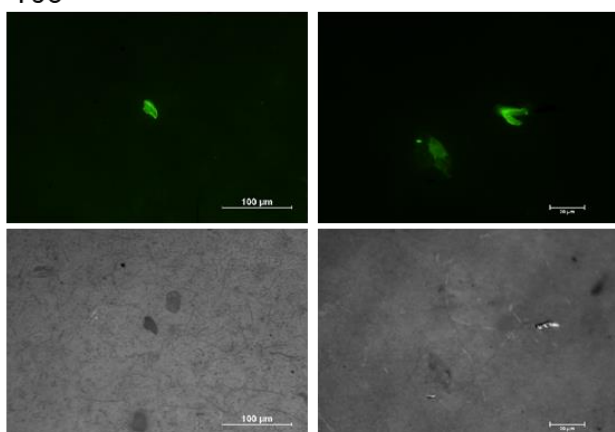
T03-1m depth



T03-surface



T05



T06

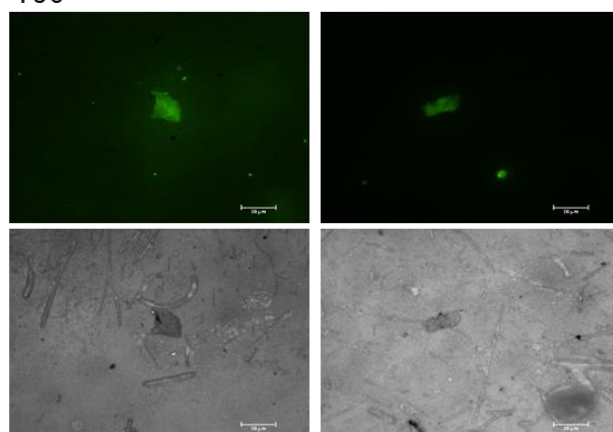


Figure 16. Images (Fluorescent and Bright Field) of Anodisc after plastic extraction from suspended sediments of Thames samples.

3.2.1.3 Raman analysis

The conditions of the analysis are described in 2.2.1.3.

The stained samples on anodiscs were also analyzed by confocal Raman microscopy. Like what was observed for Mero-Barcés samples, the 633 nm laser line could not be used due to the auto-fluorescence coming from other particles present in the matrix, as shown in Figure 18. Upon excitation with a 785 nm laser line, a Raman peak centered at 1000 cm^{-1} was observed in several spots in all the samples the Thames samples, except the T03-1m depth, which could be assigned as corresponding to PS. However, further analysis is needed to confirm the presence of PS particles, since only one of the 2 characteristic peaks was identified (see Figure 9).

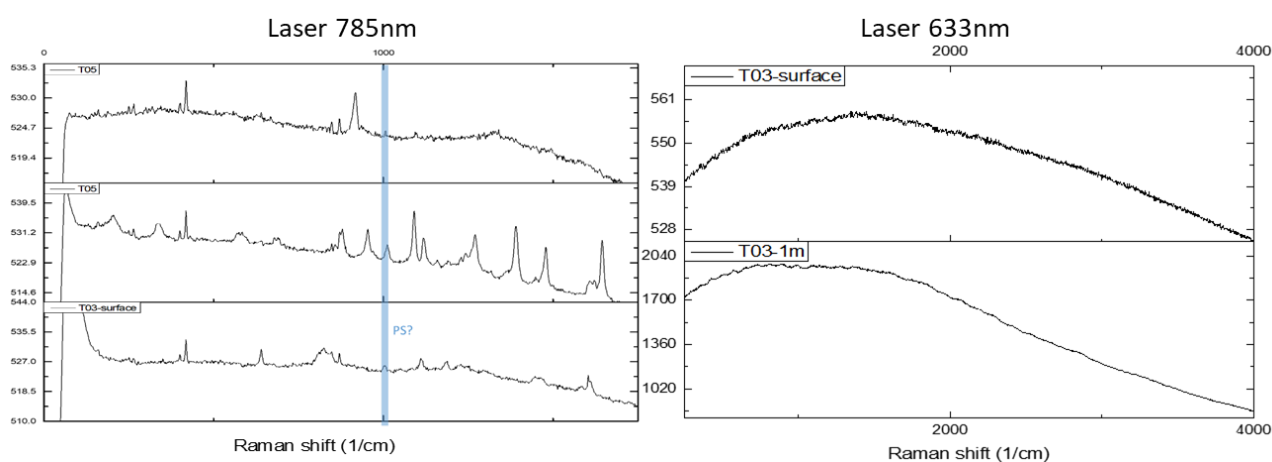


Figure 17. Raman spectra of anodisc after plastic extraction procedure from Thames River, acquired using both 785 nm and 633 nm laser lines.

3.3 Conclusions

The concentration of particles smaller than 1 μm was low, $< 4 \times 10^7$ particles/mL, in the waters sampled in Thames River, except for the sample collected in central London (T05), which presented a 20-fold higher concentration, 26.1×10^7 particles/mL, than the rest of the samples. All samples contained suspended sediments. Thus, the presence of SMNPs in suspended sediments was analyzed. Fluorescent particles were detected in all samples of suspended sediments. The identification of the particles detected in both water and suspended sediments is being carried out by Raman spectroscopy. Matrix interference was observed and consequently, extra steps of the sample preparations and/or data acquisition had to be included.

4 ELBE RIVER

4.1 Water

The water samples were collected in five locations (more details D2.2 and D2.3):

1. Location 1: Estuary (Cuxhaven; E13)
2. Location 2: Downstream of the urbanized area of Hamburg (E14)
3. Location 3: The tidal limit in Geesthacht (E15)
4. Location 4: Industrial and rural influences at Dömitz (E16)
5. Location 5: Industrial and rural influences at Dessau (E17)

Water samples (1 L) for SMNP analysis were collected in Location 2 (E14; Hamburg) in February 2022 and Location 4 (E16; Dömitz) in May 2022, using the pump and the microlayer respectively. In the case of E14, the freshwater samples were collected at three different depths: 10 cm (surface), 50 cm, and 200 cm. Freshwater for SMNPs analysis was also sampled via surface microlayer in Location 1 (E13; Cuxhaven), Location 3 (E15; Geesthacht), and Location 5 (E17; Dessau) in May 2023, January 2023, and April 2023, respectively. Blank samples of Milli-Q water were collected in the Location 4 (E16, Dömitz).

The freshwater samples were prepared for the analysis following the decision tree established in deliverable 4.1 as shown the Figure 2. Thus, suspended sediments were isolated from water samples, and subsequently, water samples were filtered as requested by the analytical technique for the analysis.

4.1.1 Detection/Identification/Quantification

4.1.1.1 NTA analysis

The conditions of the analysis are described in 2.1.1.1.

Table 3 summarizes the NTA results obtained for water samples from the Elbe River. All water samples presented particles per frame higher than 5 and specifically E14-Hamburg at depths of 50 cm and 200 cm, E15-Geesthacht and E17-Dessau displayed particles per frame higher than 10. Different blanks (e.g. ultra-pure water, surface microlayer procedure, and washing) analyzed did not show any significant value of particles (particle per frame smaller than 1).

Table 3. NTA results for all the water samples from Elbe River received. D90: 90% of the total particles are smaller than this size; D50: 50% of the total particles are smaller than this size; D10: 10% of the total particles are smaller than this size. Control samples and Milli-Q water as procedural blank were also analyzed.

NTA Analysis														
Sample	particle/mL (x $E7$)	SD (x $E7$)	particle/frame	SD	Mean size (nm)	SD	Mode size (nm)	SD	size D10 (nm)	SD	size D50 (nm)	SD	size D90 (nm)	SD
MilliQ water_1umFilter	0.2	0.0	0.1	0.0	180	39	179	40	135	46	179	40	234	36
E14-01-PF-06-01-10cm	7.5	0.3	5.1	0.2	107	1	140	11	91	1	155	2	251	4
E14-01-PF-06-02-10cm	7.4	0.3	6.0	0.3	143	3	99	23	69	4	129	6	223	11
E14-01-PF-06-01-50cm	15.8	0.4	13.5	0.8	89	3	89	3	83	2	147	3	257	5
E14-01-PF-06-02-50cm	12.5	0.2	11.0	0.1	163	1	86	7	76	2	146	2	273	6
E14-01-PF-06-01-200cm	12.8	0.6	10.4	0.3	162	0	91	6	77	2	148	4	283	8
E16-01-SM-06-Blank	0.4	0.0	0.3	0.0	94	5	94	9	68	3	92	7	135	14
E16-01-SM-06-Washing	0.5	0.0	0.4	0.0	121	7	99	9	84	8	110	7	206	28
E16-01-SM-06-01	9.0	0.3	6.9	0.2	121	3	87	10	73	2	113	3	192	8
E16-01-SM-06-02	8.6	0.5	6.5	0.3	120	4	84	5	73	2	110	4	182	8
E16-01-SM-06-03	8.5	0.5	6.3	0.3	122	4	88	11	69	2	113	2	187	6
E15-01-SM-06	10.4	0.6	8.1	0.4	120	4	79	3	70	3	92	2	214	20
E13-01-SM-06-01	6.0	0.4	4.9	0.1	127	4	93	11	68	3	122	5	197	3
E13-01-SM-06-02	6.6	0.2	6.0	1.6	135	2	107	34	71	2	119	2	205	13
E17-01-SM-06-01	12.9	1.0	10.8	0.5	122	4	84	2	66	3	98	2	202	10
E17-01-SM-06-02	10.6	1.4	13.0	1.7	128	5	108	2	81	5	115	5	192	18

In the case of sampling at different depths (E14; Downstream of an urbanized area of Hamburg), it is important to highlight the increase in particle concentration at higher depths of sampling as shown in Figure 18.

For surface microlayer samples (E13-Cuxhaven, E15-Geesthacht, E16-Dömitz, and E17-Dessau) results were very similar, being the lowest values of particle concentration for samples at the Elbe River estuary, i.e. E13-Cuxhaven (Figure 18). This could be explained by the higher water mass presented in this station, which may dilute the particle concentration.

Figure 19 shows the size of particles estimated by NTA. Slightly bigger particles were detected in E14-Hamburg than in the rest of the water samples analyzed from Elbe River.

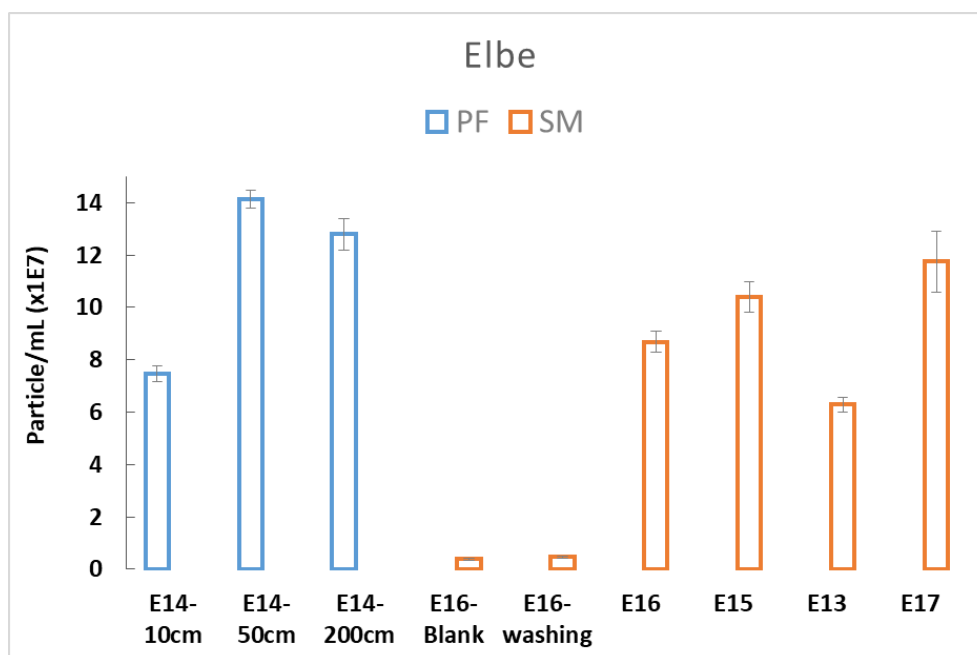


Figure 18. Concentration of particles below 1 μ m per milliliter for all water samples from Elbe River. For the NTA measurements, samples were filtered. PF and SM indicate the sampling method used: the pump and the surface microlayer, respectively.

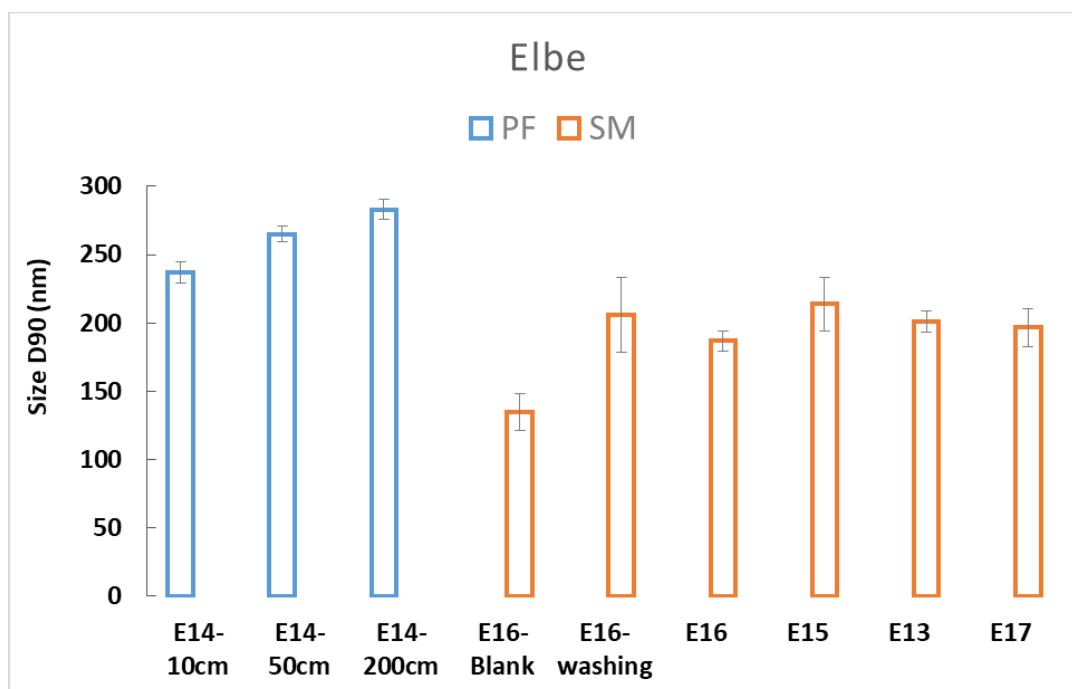


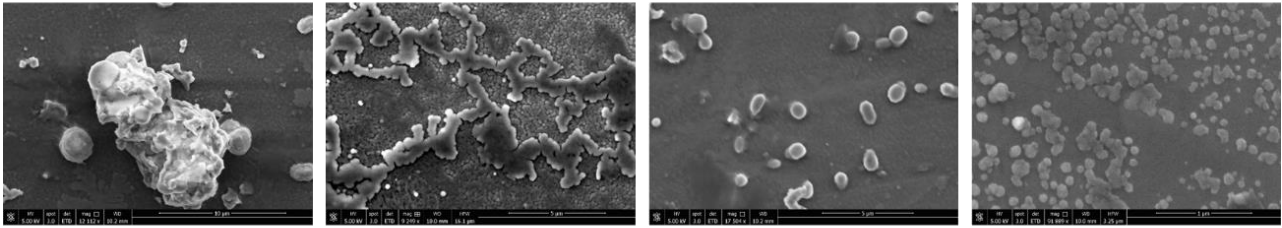
Figure 19. Size d90 of the particles obtained by NTA for all water samples from Elbe River. For the NTA measurements, samples were filtered. PF and SM indicate the sampling method used: the pump and the surface microlayer, respectively.

4.1.1.2 SEM analysis

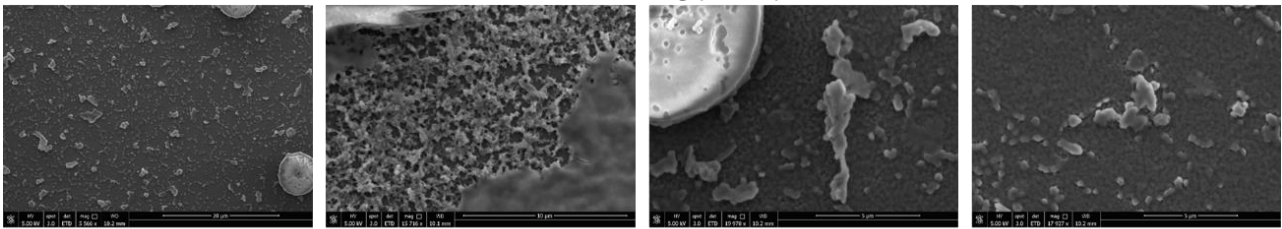
The conditions of the analysis are described in 2.1.1.2.

Figure 20 shows SEM images of particles with different sizes and shapes present in water samples collected in E14 – Hamburg. In all samples, particles with more or less regular shape and irregular shape particles were observed independently of the sampling depth. Most particles displayed sizes smaller than 250 nm. Some particles observed were bigger than 1 μm , which could be a consequence of the formation of aggregates/agglomerates during the drying process.

E14 – Hamburg (10 cm)



E14 – Hamburg (50 cm)



E14 – Hamburg (200 cm)

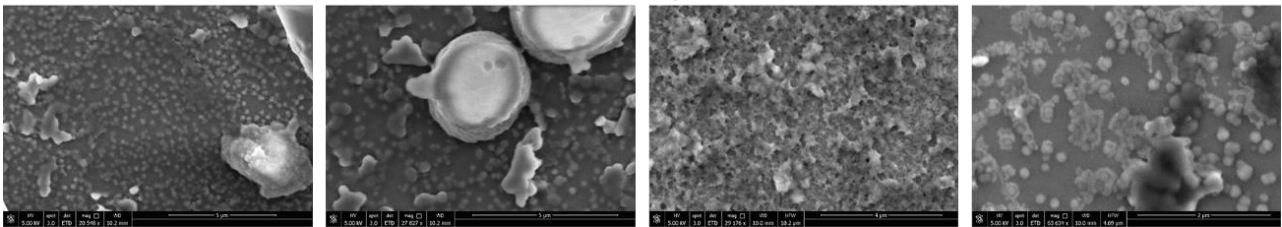
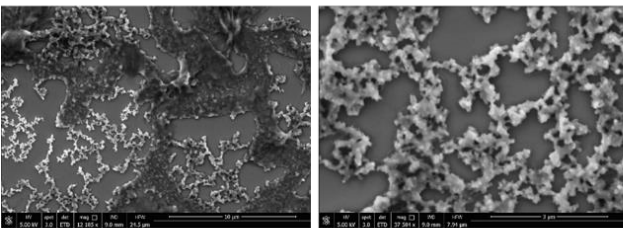


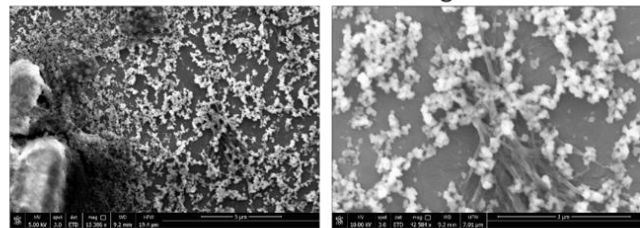
Figure 20. Representative SEM images for water samples collected from the Elbe station “E14” (Hamburg) at different depths: 10, 50, and 200 cm. The biggest particles observed in these SEM images were identified as salts, probably calcium carbonate and sodium chloride, crystallized during the drying process.^{5,6}

SEM analysis of controls included during the sampling of water from E16-Dömitz using the surface microlayer method showed that particles detected in these controls had irregular shapes and tended to interact among others (Figure 21). The particles observed in the water samples collected from E16-Dömitz differed from those detected in the controls (Figure 21). However, the size and shape of these particles were like those from E14 – Hamburg.

E16 - Dömitz Blank



E16 - Dömitz Washing



E16 - Dömitz

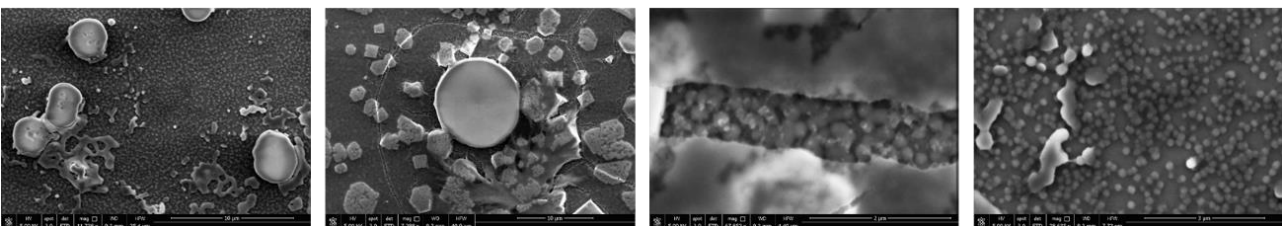


Figure 21. Representative SEM images for water samples collected from the Elbe station “E16” (Dömitz) and controls for the sampling method (i.e. surface microlayer): ultrapure water used for the washing (i.e. Blank) and the ultrapure water collected after washing the surface microlayer machine (i.e. Washing). The biggest particles observed in these SEM images were identified as salts, probably calcium carbonate and sodium chloride, crystallized during the drying process.^{5,6}

The contents of this document are the copyright of the **LABPLAS** consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

Bigger differences in the particles observed by SEM were detected in the water samples collected from E13-Cuxhaven, E15-Geesthacht, and E17-Dessau as shown the Figure 22. However, some interference from salt precipitation in the E13-Cuxhaven sample and some sample destabilization in the SEM were observed and therefore, extra analysis is needed.

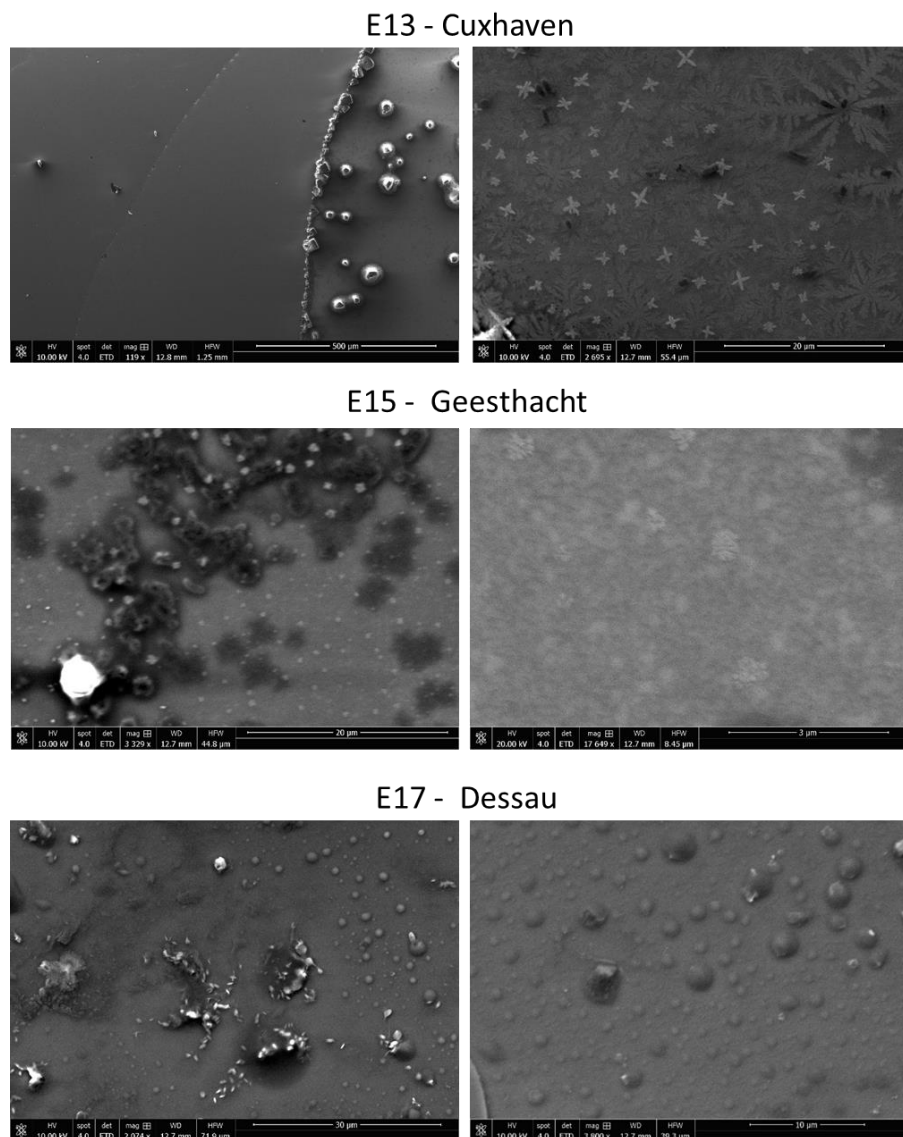


Figure 22. Representative SEM images for water sampled from E13 – Cuxhaven, E15 – Geesthacht and E17 – Dessau. The biggest particles observed in these SEM images were identified as salts, probably calcium carbonate and sodium chloride, crystallized during the drying process.^{5,6}

4.1.1.3 Raman analysis

The conditions of the analysis are described in 3.1.1.3.

The best LoD established in Deliverable 4.2 for Raman analysis of nanoplastics in freshwater was 5 mg/L for both 161 nm PS and 450 nm PET, which correspond to 109 particles/mL - 108 particles/mL. E14-Hamburg at depths of 50 cm and 200 cm, E15-Geesthacht, and E17-Dessau displayed concentrations in the range of 10^8 particles per mL. Therefore, these samples underwent pre-concentration techniques using cloud-point extraction (CPE) for Raman and Py-GC-MS analysis. Both analyses are being conducted.

4.2 Suspended Sediments

All water samples collected from Elbe River contained suspended sediments (Figure 23). The suspended sediments were isolated from the water samples following the decision tree shown in Figure 12.



Figure 23. Photograph of water samples collected from Elbe River. All of them contain suspended sediments.

The final quantity of sediments isolated from the water was very small and for this reason, no beakers were weighed during the procedure.

4.2.1 Detection/Identification/Quantification

4.2.1.1 NTA analysis

After density separation, the viscosity of the dispersion is high enough to limit technically the analysis by NTA.

4.2.1.2 Fluorescence microscopy analysis

Conditions of the analysis were described in 2.2.1.2.

The filters were observed under the fluorescent microscope and results are represented in Figure 24. In general, all the samples presented a low quantity of fluorescent particles in contrast with the concentration estimated by NTA, which was in the range of 10^8 particles/mL. Specifically, E13-Cuxhaven showed smaller particles, and some were lower than $1\ \mu\text{m}$ in size. Very few fluorescent samples were observed in the E15-Geesthacht, E16-Dömitz, and E17-Dessau samples. Most were small and with irregular shapes.

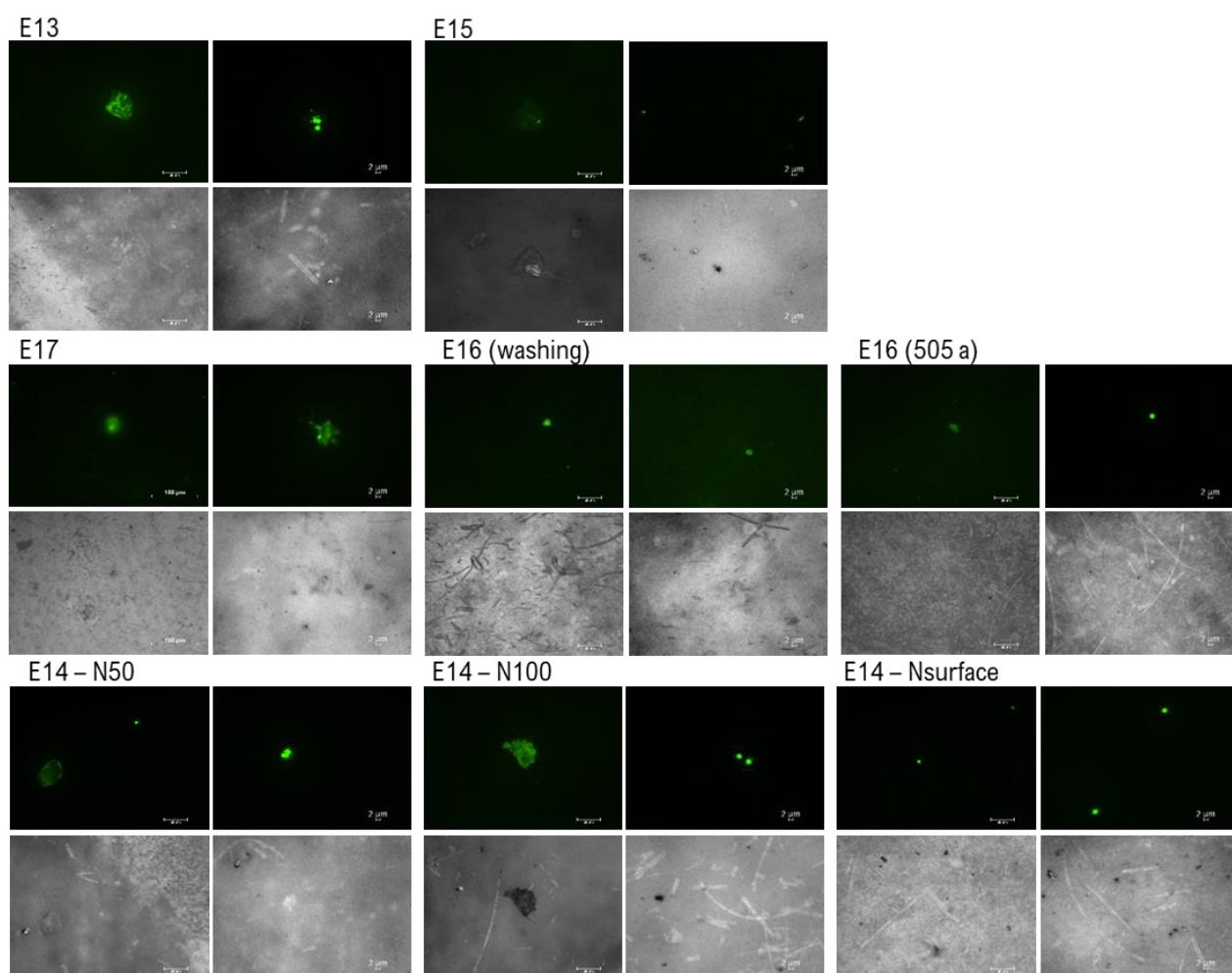


Figure 24. Images (Fluorescent and Bright field) of Anodisc after plastic extraction from suspended sediments of Elbe samples.

4.2.1.3 Raman analysis

The conditions of the analysis are described in 2.2.1.3.

The anodiscs were also analyzed by confocal Raman and the results are shown in Figure 25.

After analyzing the anodiscs using both 785nm and 633nm laser lines, no plastics have been detected in the sediments of the Elbe River up to the moment. Further analyses are ongoing.

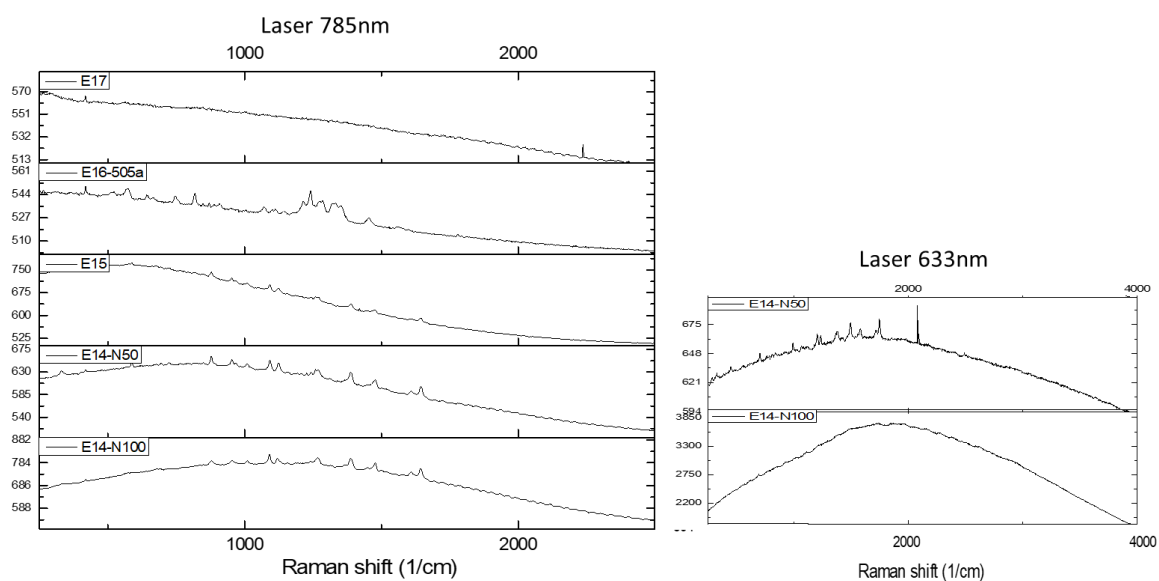


Figure 25. Raman spectra of anodisc after plastic extraction procedure from Elbe River, acquired using both 785 nm and 633 nm laser lines.

4.3 Conclusions

The concentration of particles smaller than 1 μm was relatively high in the water sampled from the Elbe River. The lowest concentrations were detected in E14-Hamburg at 10 cm depth (7.5×10^7 particles/mL) and E13-Cuxhaven (6.0×10^7 particles/mL). All samples contained suspended sediments. Thus, the presence of SMNPs in suspended sediments was analyzed. Fluorescent particles were detected in all samples of suspended sediments. However, the number of fluorescent particles was very low in comparison with the concentration estimated by NTA. The identification of the particles detected in both water and suspended sediments is being carried out by Raman spectroscopy. However, no confirmed plastic particles were detected up to the moment. Matrix interference was observed, and consequently, extra steps of the sample preparations and/or data acquisition had to be included.

5. THE NORTH SEA

5.1 Water

The water samples were collected in winter and summer sampling campaigns carried out in February 2023 and July 2023. Six locations were selected (more details D2.2 and D2.3):

1. Station 1: Thames estuary (NS7)
2. Station 2: Southern Bight (NS8)
3. Station 3: Broad Fourteens (NS9)
4. Station 4: West Frisian Is. (NS10)
5. Station 5: East Frisian Is. (NS11)
6. Station 6: Elbe estuary (NS12)

Due to weather constraints in the winter campaign, only four of the planned six stations, NS7, NS9, NS10, and NS12, were sampled, but these spanned the full study region. NS11 was not sampled in the summer campaign. The sampling was performed via CTD, Garrett screen (surface microlayer), and snow catcher.

5.1.1 Sampling methods

Conductivity, Temperature, and Depth (CTD)

The predominant sampling method involves the collection of 2 L of water from three different sampling depths (i.e. above the thermocline (~ 1 m), below the thermocline (~ 50 m), and center of the water column (~ 25 m)) using a CTD sampler. The position of the thermocline is measured automatically as the CTD is lowered through the water column, and then the samples are collected in polyvinyl chloride (PVC) bottles at the desired locations during ascent. Upon the return of the instrument to the deck, samples were collected from the PVC sampling bottles into pre-rinsed (with Milli-Q water) glass bottles. Each bottle was rinsed twice more with sampled water before the final 2 L were filled and stored at 4 °C for the remainder of the cruise. Blank samples of Milli-Q water before and after passing through a PVC CTD sampling bottle were also collected and stored. Here the sampling bottles are assumed to be “rinsed” as they lower (in the open position) through the water column, so only a quick rinse of the instrument with freshwater is done on deck after sampling is complete. All blanks and true samples were sealed and stored at 4 °C for the remainder of the cruise.

Surface Microlayer with Garrett Screens

At every station, Garrett screen samples of 2 L were collected from the surface microlayer and filtered through 10 µm stainless steel filters into a pre-rinsed glass Erlenmeyer flask. From this filtered water, 1 L was collected into a pre-rinsed (with Milli-Q water) glass jar. The jar was sealed and stored at 4 °C for the remainder of the cruise.

Here it is important to note that any filtered sample has the (very slight) risk of containing trace amounts of sodium dodecyl sulfate (SDS) or Triton-X due to the utilization of the same filtration unit for all samples. However, between each sample all equipment is thoroughly rinsed and additional water is filtered to clean the system, thus, the risk is minimal.

Residual Water and Aggregates from the Marine Snow Catcher

At every station, marine snow catcher samples of 80 L were collected from a depth of 5 m and allowed to settle for at least 2 hours before separating the water column into a fraction containing organic aggregates and the remaining water above. The water above was subsampled and filtered through 10 µm stainless steel filters into a pre-rinsed glass Erlenmeyer flask.

In the winter sampling campaign, 20 mL of this filtered water was collected into a pre-rinsed (with Milli-Q water) glass vial. Two total vials were collected for the filtered water above the aggregate samples. Then one vial of water was collected from the organic aggregates water after large aggregates had been removed. Two final glass vials were collected from the organic aggregates containing the water layer after it had been filtered, as described above. All blanks and true samples were sealed and stored at 4 °C for the remainder of the cruise.

Here there is a similar contamination warning for SDS and Triton-x as was described above. As a control, 1 L of Milli-Q water was passed through the filtration system at the end of the last sampling day and stored in a glass jar.

In the summer sampling campaign, 2 L from the filtered water was collected into a pre-rinsed (with Milli-Q water) glass bottle. In station 1 (NS7), three vials with ~2mL were collected from bottom organic aggregates containing water layers after they had been filtered as described above. All samples were sealed and stored at 4 °C for the remainder of the cruise.

5.1.2 Detection/Identification/Quantification

The seawater samples were prepared for the analysis following the decision tree established in deliverable 4.1 as shown in Figure 2. Thus, these samples were first dialyzed and then filtered as required for the analytical technique.

5.1.2.1 NTA analysis

The conditions of the analysis are described in 2.1.1.1.

The following results are presented considering the different sampling methods used to collect the samples. However, samples collected during the winter campaign displayed, in general, a higher concentration of particles smaller than 1 µm than samples collected during the summer campaign independently of the sampling method used (see Table 4 and Figure 26 for CTD samples, Table 5 and Figure 28 for surface microlayer samples and Table 6 and Figure 30 for snow catcher samples).

Comparing between different stations and sampling methods, we can conclude the following:

- **Water samples collected using CTD.** Almost all water samples collected using CTD presented particles/frame estimated by NTA smaller than 5 (Table 4), except samples collected above thermocline (1 m depth) from NS7 station –Thames estuary in any of the campaigns and samples collected above thermocline from NS12 station – Elbe estuary. In fact, some of the samples presented a lower number of particles than the ultrapure water (i.e. MilliQ) used for washing steps.

Figure 26 clearly shows that the highest concentration of particles detected was in the sample collected above thermocline from NS7 – Thames estuary in the Winter campaign (16.2×10^7 particles/mL). Although the concentration was lower in summer, the NS7 station presented the highest concentration also in this campaign. Additionally, the size estimated by NTA for particles detected was similar in all the samples collected using the CTD system as shown in Figure 27.

Table 4. NTA results for water samples from the North Sea collected using CTD. D90: 90% of the total particles are smaller than this size; D50: 50% of the total particles are smaller than this size; D10: 10% of the total particles are smaller than this size. Control samples and Milli-Q water as procedural blank were also analyzed.

NTA Analysis														
Sample	particle/mL (xE7)	SD (xE7)	particle/frame	SD	Mean size (nm)	SD	Mode size (nm)	SD	size D10 (nm)	SD	size D50 (nm)	SD	size D90 (nm)	SD
Winter sampling campaign														
MilliQ Only Control (unfiltered)	0.1	0.0	0.0	0.0	80	41	86	43	57	36	86	43	91	46
MilliQ Only Control (unfiltered) (cruise)	2.0	0.1	2.1	0.1	120	7	97	11	75	3	116	6	174	12
CTD Procedural Blanck MilliQ (unfiltered)	2.3	0.2	2.0	0.1	95	4	60	5	54	2	83	2	164	15
NS7 - CTD Top	16.2	1.7	10.7	1.1	133	3	94	10	80	3	123	3	200	6
NS7 - CTD Middle	1.5	0.0	1.3	0.1	110	7	82	6	73	2	99	7	155	15
NS7 - CTD Bottom	0.4	0.1	0.4	0.1	146	16	114	3	101	6	122	5	241	40
NS12 - CTD Top	5.1	0.1	3.7	0.0	124	3	96	5	74	3	111	4	200	7
NS12 - CTD Middle	4.2	0.1	3.3	0.1	124	2	103	4	79	5	112	4	176	4
NS12 - CTD Bottom	1.4	0.2	1.1	0.1	112	6	88	5	74	5	101	6	169	12
Summer sampling campaign														
NS7 - CTD Top	9.2	6.3	6.2	4.2	123	15	110	23	64	8	119	18	191	30
NS7 - CTD Top	2.7	0.2	2.1	0.1	119	3	82	8	66	3	104	5	174	3
NS7 - CTD Bottom	1.9	0.1	1.5	0.1	136	9	80	6	68	6	98	11	265	20
NS7 - CTD Bottom	1.7	0.2	1.0	0.1	156	10	95	6	79	3	111	13	318	40
NS8 - CTD Top	0.9	0.1	0.6	0.1	138	8	135	24	86	6	119	8	202	9
NS8 - CTD Bottom	1.7	0.4	1.1	0.2	123	12	88	12	67	2	99	11	200	18
NS9 - CTD Top	0.9	0.1	0.7	0.1	120	9	89	10	74	8	103	6	226	50
NS9 - CTD Bottom	0.5	0.2	0.4	0.1	127	22	111	8	80	18	113	11	168	38
NS10 - CTD Top	1.2	0.1	0.8	0.0	111	6	99	10	70	6	98	6	180	17
NS10 - CTD Bottom	1.3	0.2	1.0	0.1	123	6	98	15	74	3	108	11	180	28
NS12 - CTD Top	1.1	0.1	1.0	0.1	130	9	116	30	83	4	118	13	199	14
NS12 - CTD Bottom	0.8	0.1	0.6	0.1	130	13	116	15	84	4	117	14	207	44
NS9 - CTD Filtered by 10 um	4.4	0.3	2.7	0.1	169	5	92	6	82	4	161	7	274	17
NS9 - CTD Filtered by 10 um	4.0	0.3	2.4	0.2	190	12	94	6	93	4	161	16	308	22

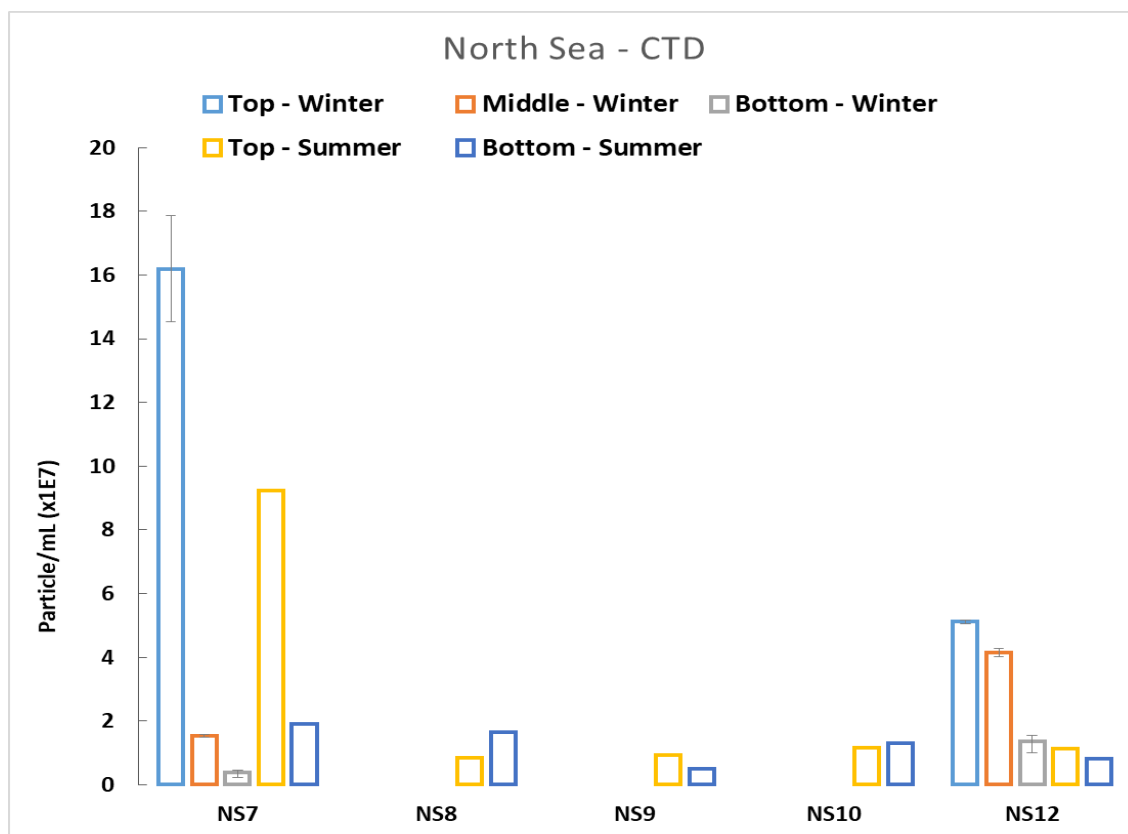


Figure 26. Concentration of particles below 1 µm per milliliter for all water samples from the North Sea collected using the CTD method. For the NTA measurements, samples were filtered.

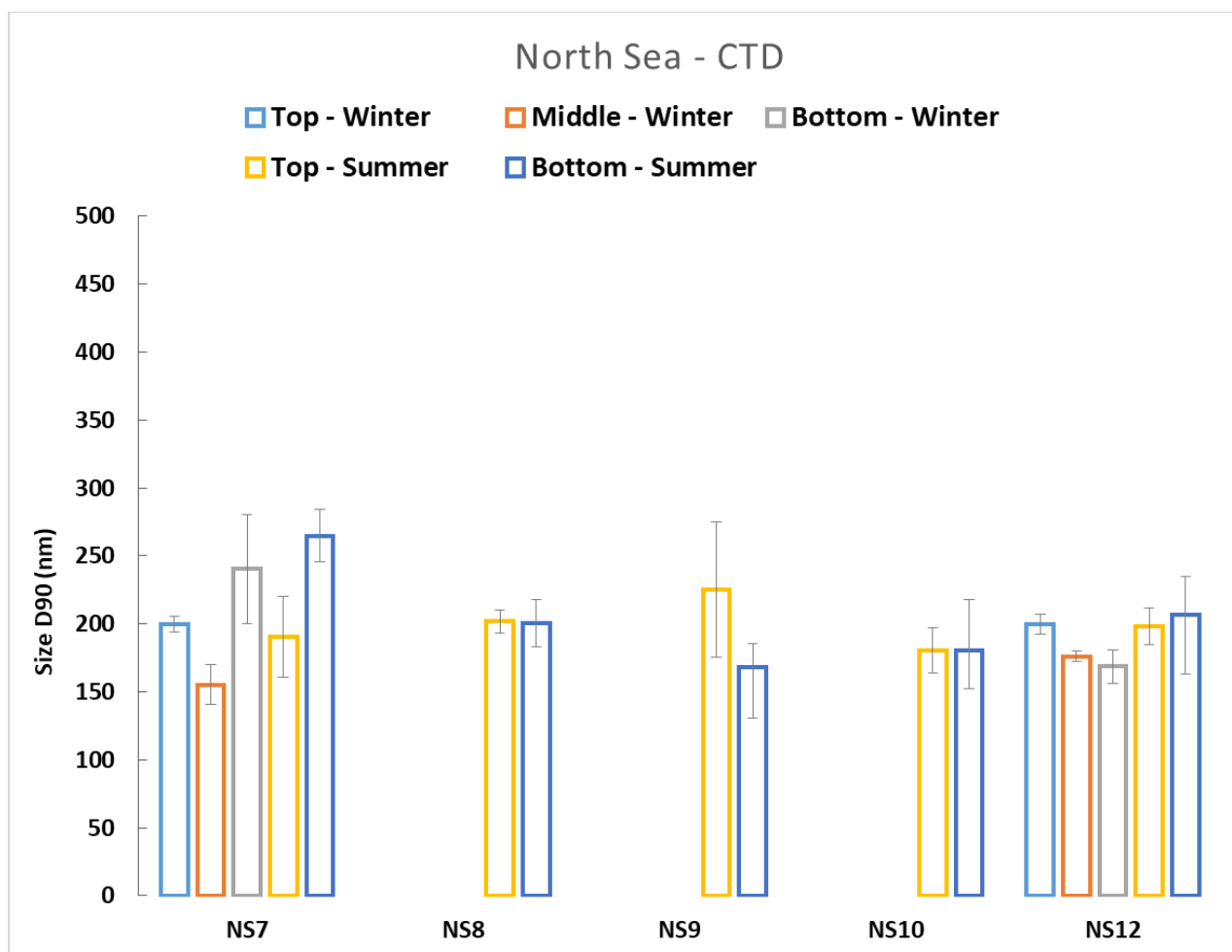


Figure 27. Size d90 of the particles obtained by NTA for all water samples from the North Sea collected using the CTD method. For the NTA measurements, samples were filtered.

- **Surface microlayer samples collected using the Garrett screen system.** Table 5 summarizes the NTA results. NTA analysis revealed that all surface microlayers sampled in the Winter campaign presented particle/frame higher than 5. In contrast, all surface microlayers sampled in Summer displayed lower particle/frame than 3 and in general, the content of particles detected was smaller than in the ultrapure water used during the cruise.

In this case, the highest concentrations of particles smaller than 1 µm were detected in NS10 - West Frisian Island and NS12 - Elbe estuary when the winter campaign was made. The particle concentration dropped around 10 times when samples were collected in the summer (Figure 28). The lowest concentration of particles was detected in NS8- Southern Bight (< 10⁷ particles/mL). Figure 29 shows the particle size estimated by NTA for all sea surface microlayer samples. The size estimated in samples collected in summer was slightly smaller than for the microlayer sampled in winter: 320 nm in winter versus 205 nm in summer for NS7-Thames estuary station and 301 nm in winter versus 207 nm in summer for NS12-Elbe estuary station. The difference in size was less evident in the case of NS10-West Frisian Is. station: 277 nm in winter version 272 nm in summer.

Table 5. NTA results for surface microlayer samples from the North Sea collected using the Garrett screen system. D90: 90% of the total particles are smaller than this size; D50: 50% of the total particles are smaller than this size; D10: 10% of the total particles are smaller than this size. Control samples and Milli-Q water as procedural blank were also analyzed.

NTA Analysis														
Sample	particle/mL (xE7)	SD (xE7)	particle/frame	SD	Mean size (nm)	SD	Mode size (nm)	SD	size D10 (nm)	SD	size D50 (nm)	SD	size D90 (nm)	SD
Winter sampling campaign														
MilliQ Only Control (unfiltered)	0.1	0.0	0.0	0.0	80	41	86	43	57	36	86	43	91	46
MilliQ Only Control (unfiltered) (cruise)	2.0	0.1	2.1	0.1	120	7	97	11	75	3	116	6	174	12
NS7 - Surface microlayer	10.3	0.5	6.6	0.3	210	6	130	11	101	2	207	8	320	13
NS10 - Surface microlayer	14.5	0.3	10.6	0.1	178	1	139	14	96	2	165	2	277	4
NS12 - Surface microlayer	14.4	1.3	11.3	1.0	192	4	141	11	111	4	170	5	301	10
Summer sampling campaign														
NS7 - Surface microlayer	2.8	0.2	2.3	0.1	129	4	94	16	75	4	116	2	205	16
NS8 - Surface microlayer	0.7	0.1	0.5	0.0	123	6	107	13	72	3	137	16	169	17
NS10 - Surface microlayer	2.6	0.2	1.8	0.1	154	2	99	17	78	2	133	6	272	21
NS12 - Surface microlayer	2.1	0.2	1.6	0.1	131	8	86	2	79	3	111	10	207	13

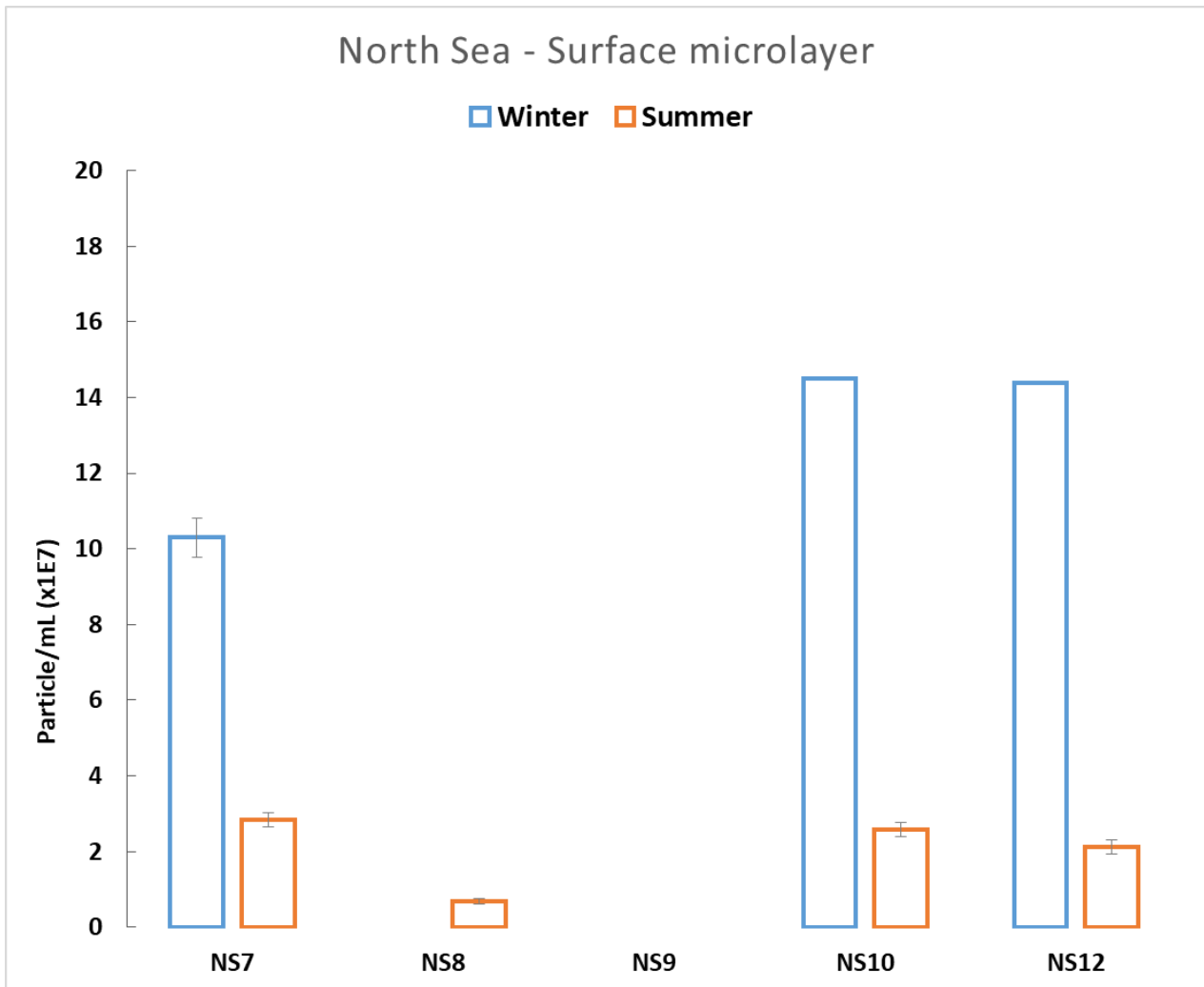


Figure 28. Concentration of particles below 1 µm per milliliter for surface microlayer samples from the North Sea collected using the Garrett screen system. For the NTA measurements, samples were filtered.

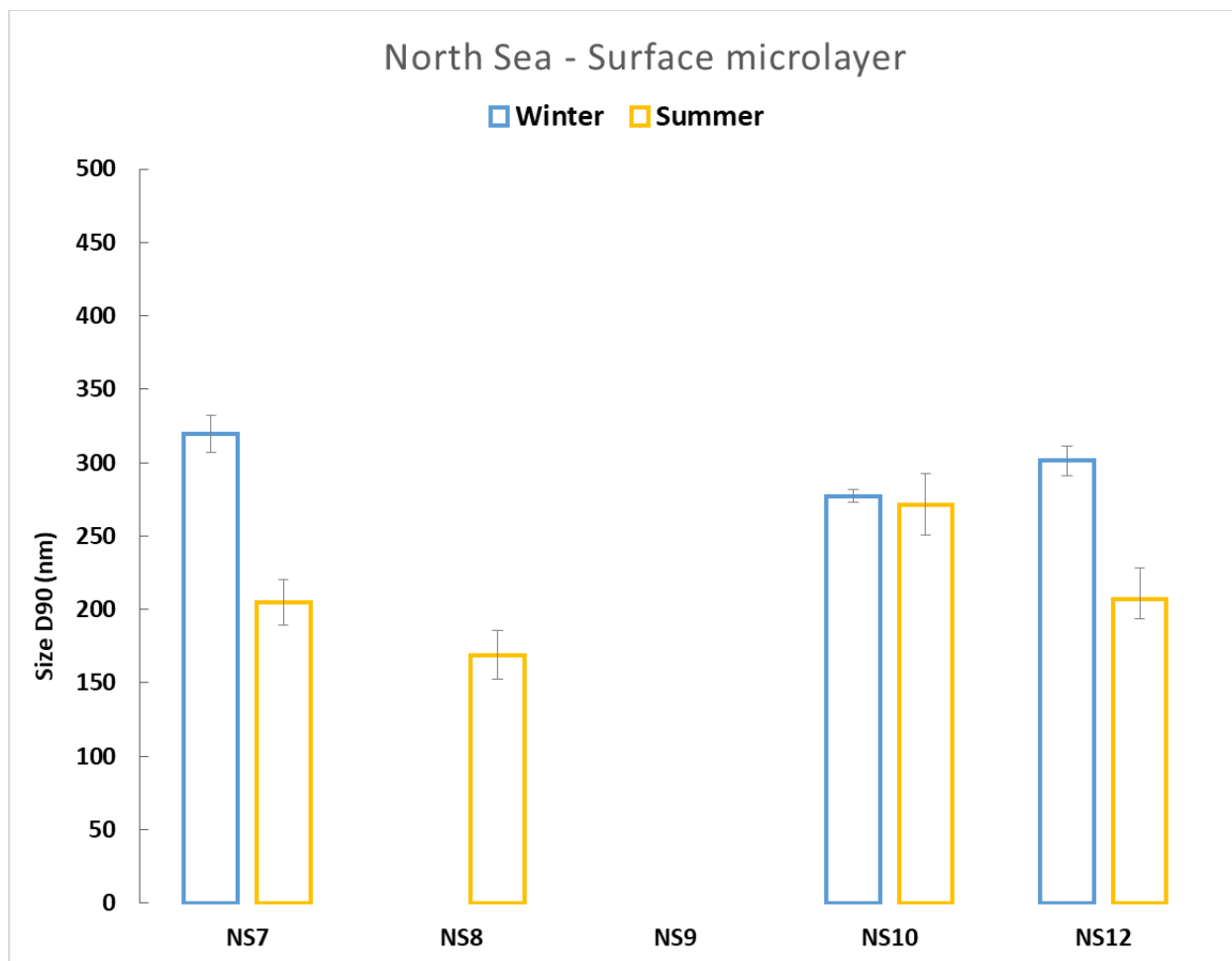


Figure 29. Size d90 of the particles obtained by NTA for surface microlayer samples from the North Sea collected using the Garrett screen system. For the NTA measurements, samples were filtered.

- **Residual Water from the Marine Snow Catcher.** Water from above the aggregate layer was sampled in both campaigns. Clearly, NTA analysis showed that samples collected in winter presented higher particle/frame (> 5 particles/frame) than samples from summer sampling (Table 6). Figure 30 shows that the concentration of particles in winter was more than 10-fold higher than the concentrations estimated in summer for water samples from above the aggregate layer. Interestingly, the size estimated by NTA for all these samples was similar in both campaigns (Figure 31).

Unfiltered and filtered residual water from the aggregate layer was sampled also in the winter campaign. Unfiltered water from the aggregate layer contained a higher concentration of particles as shown in Figure 30. Additionally, samples from NS10-West Frisian Is. and NS12-Elbe estuary stations presented a higher concentration of particles than samples collected above the aggregate layer. In contrast, the concentration in NS7-Thames estuary and NS9-Broad Fourteens was lower than in water sampled above the aggregate layer. The size of particles detected both above and from the aggregate layer was similar as shown in Figure 31. Interestingly, filtered water from the aggregated layer presented a very low concentration of particles smaller than 1 µm although samples were filtered by 10 µm stainless steel membrane (Figure 30). This variation of the concentration in this range of particles could be attributed to the adsorption of ≤1 µm particles on the bigger particulate matter trapped on the filter.

Table 6. NTA results for water samples from the North Sea collected using a marine snow catcher. D90: 90% of the total particles are smaller than this size; D50: 50% of the total particles are smaller than this size; D10: 10% of the total particles are smaller than this size. Control samples and Milli-Q water as procedural blank were also analyzed.

NTA Analysis														
Sample	particle/mL (xE7)	SD (xE7)	particle/frame	SD	Mean size (nm)	SD	Mode size (nm)	SD	size D10 (nm)	SD	size D50 (nm)	SD	size D90 (nm)	SD
Winter sampling campaign														
MilliQ Only Control (unfiltered)	0.1	0.0	0.0	0.0	80	41	86	43	57	36	86	43	91	46
MilliQ Only Control (unfiltered) (cruise)	2.0	0.1	2.1	0.1	120	7	97	11	75	3	116	6	174	12
Filtered MilliQ Water Control 9/2/2023 (Snow catcher NS09)	2.7	0.1	2.7	0.1	103	1	81	12	59	5	93	3	160	10
NS7 - water from above the aggregate layer	14.4	0.5	13.5	0.4	210	2	181	116	123	3	197	6	322	6
NS7 - Filtered water from aggregate layer	9.9	0.4	9.7	0.4	183	3	164	14	101	2	174	3	264	9
NS7 - Unfiltered water from the aggregate layer	0.8	0.1	0.7	0.1	187	13	101	8	88	7	136	20	432	28
NS9 - water from above the aggregate layer	16.7	0.3	15.3	0.2	196	2	171	7	122	4	185	2	279	2
NS9 - Filtered water from aggregate layer	6.6	0.4	6.2	0.4	224	2	176	18	124	1	203	6	345	9
NS9 - Unfiltered water from the aggregate layer	1.3	0.1	1.4	0.1	168	5	132	26	83	3	150	6	264	19
NS10 - water from above the aggregate layer	9.6	0.3	8.4	0.2	179	2	127	16	95	3	171	4	282	3
NS10 - Unfiltered water from aggregate layer	12.3	0.7	11.2	0.6	190	3	133	13	97	2	170	3	309	9
NS10-Filtered water from the aggregate layer	2.6	0.1	2.4	0.1	137	2	91	16	77	6	127	6	211	10
NS12 - Water from above the aggregate layer	11.0	0.5	10.4	0.5	190	3	142	8	118	3	172	5	285	13
NS12 - Unfiltered water from aggregate layer	14.5	1.1	9.4	0.6	164	1	120	15	90	3	147	4	264	9
NS12 - Filtered water from the aggregate layer	1.4	0.1	1.2	0.1	131	4	98	6	75	3	109	3	202	16
NS9 - unfiltered snow catcher water from the aggregate layer	2.1	0.2	1.4	0.1	139	6	79	5	72	3	100	2	253	18
Summer sampling campaign														
NS7 - water from above the aggregate layer	3.3	0.3	2.4	0.2	168	5	130	18	92	3	154	6	257	17
NS8 - water from above the aggregate layer	1.4	0.2	1.3	0.2	132	8	89	12	76	6	109	9	207	20
NS9 - water from above the aggregate layer	2.4	0.2	1.6	0.1	174	10	98	10	77	2	141	10	305	23
NS10 - water from above the aggregate layer	3.0	0.2	2.0	0.1	173	7	122	18	84	5	164	7	264	8
NS12 - water from above the aggregate layer	4.8	0.1	3.1	0.1	152	3	121	9	88	3	139	5	228	3
NS12 - water from above the aggregate layer	3.0	0.2	2.2	0.1	149	10	103	28	71	11	129	16	273	16

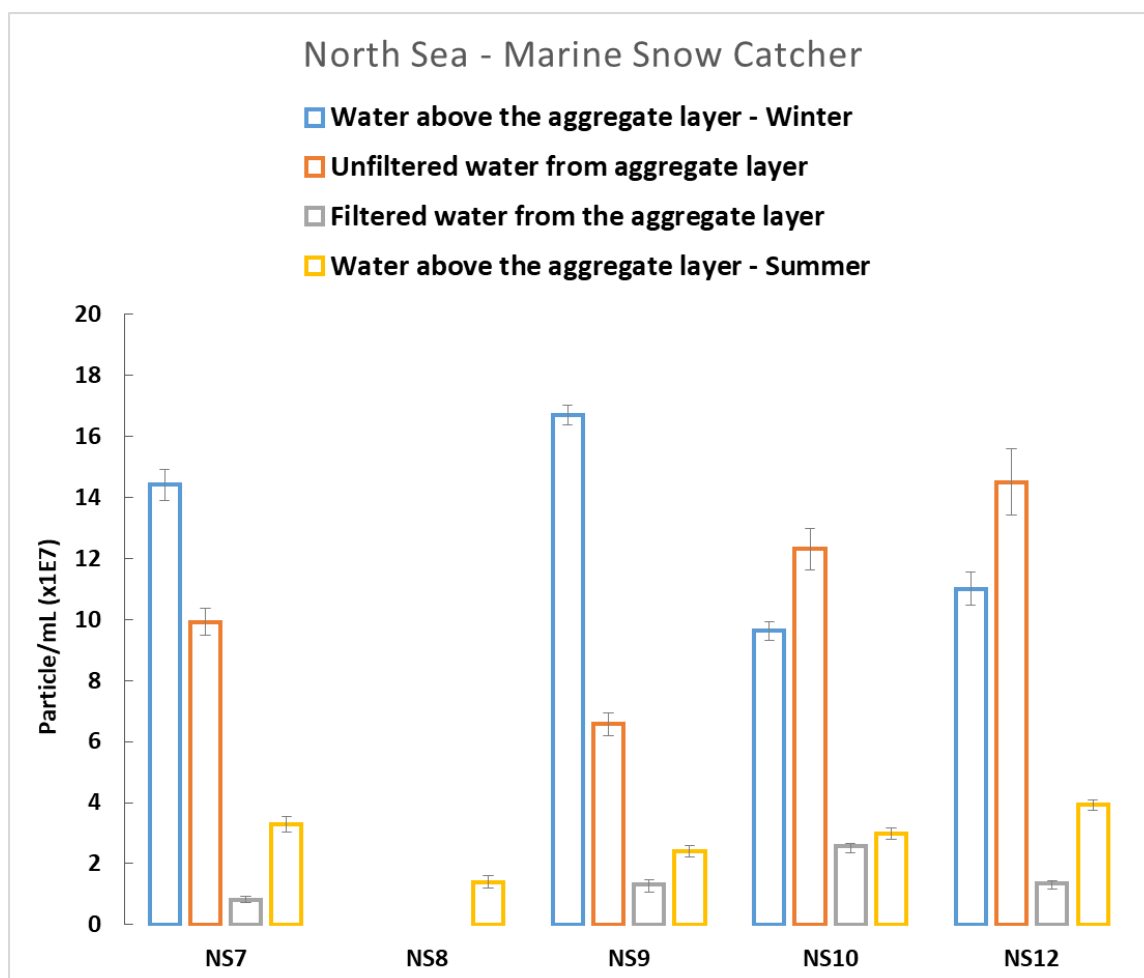


Figure 30. Concentration of particles below 1 µm per milliliter for residual water samples from the North Sea collected using a marine snow catcher. For the NTA measurements, samples were filtered.

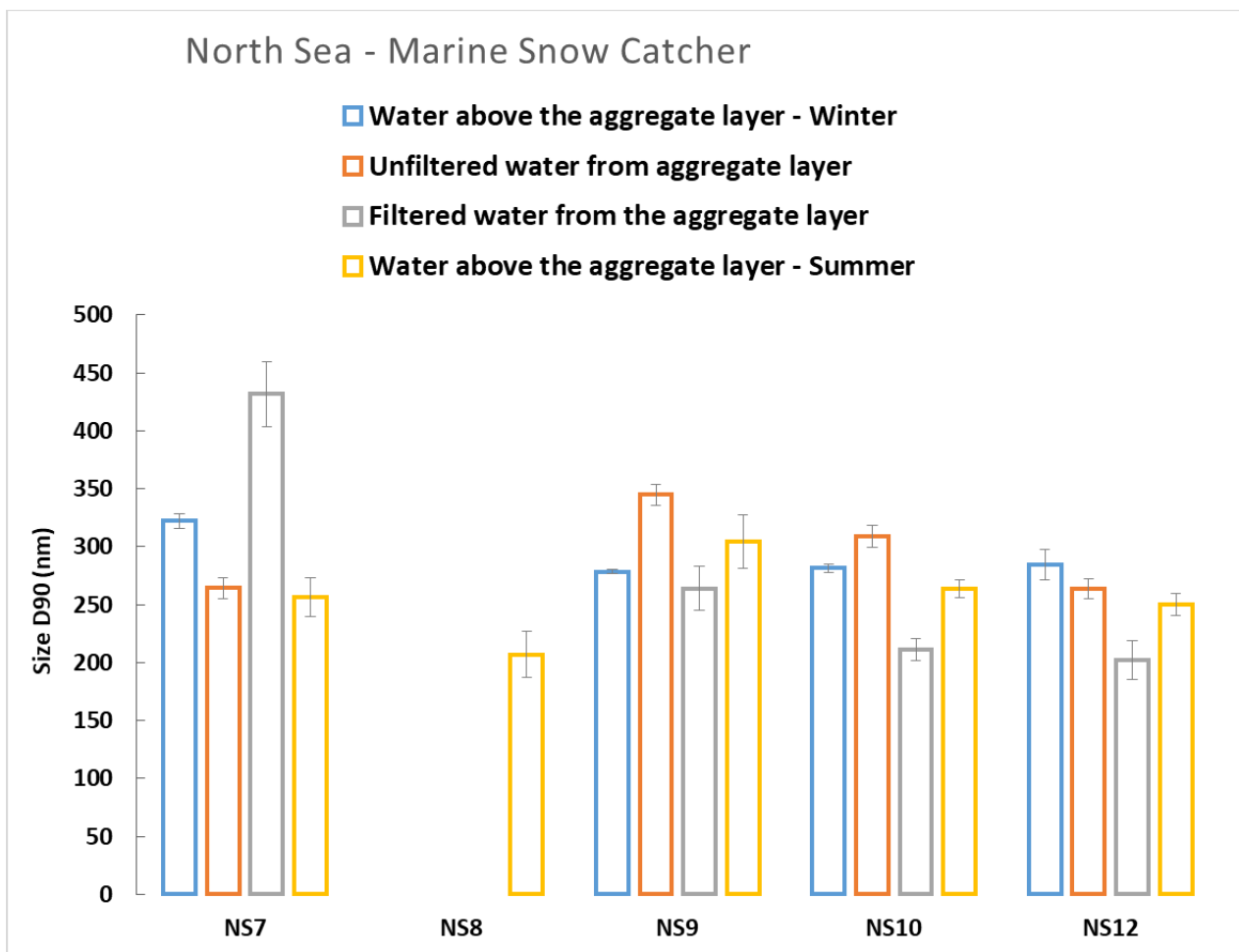


Figure 31. Size d90 of the particles obtained by NTA for residual water samples from the North Sea collected using a marine snow catcher. For the NTA measurements, samples were filtered.

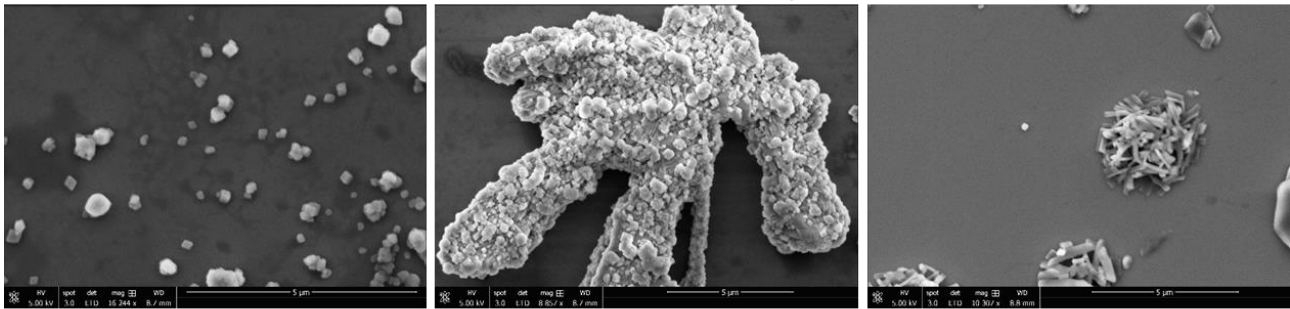
5.1.2.2 SEM analysis

The conditions of the analysis are described in 2.1.1.2.

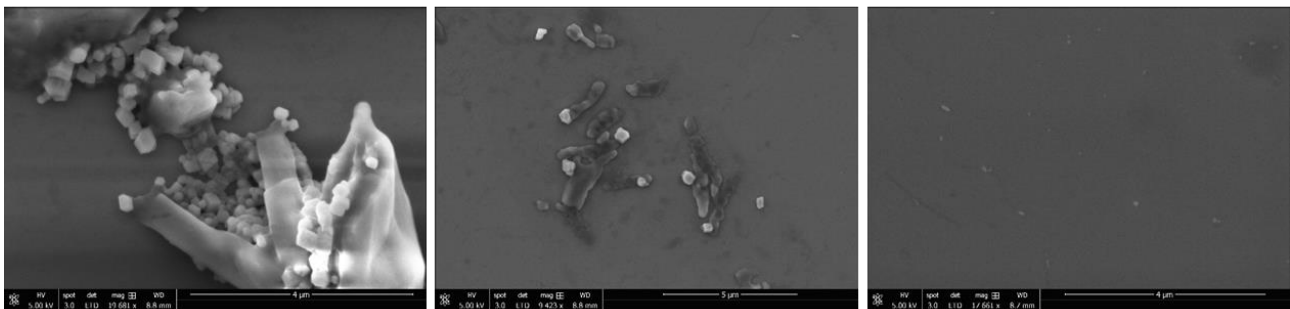
Samples from the Winter campaign were selected for SEM analysis initially since they displayed higher particle concentrations estimated by NTA (Figures 26, 28, and 30):

- **Surface microlayer samples collected using the Garrett screen system.** Figure 32 shows SEM images of NS7-Thames estuary, NS10-West Frisian Is. and NS12-Elbe estuary. Smaller particles were visualized and adsorbed on bigger particles in all samples. This could reduce the localization and visualization of the smallest particles due to the formation of agglomerates/aggregates. Well-dispersed smaller particles were observed in NS10-West Frisian Is. and NS12-Elbe estuary.

NS7-Thames estuary



NS10-West Frisian Is.



NS12-Elbe estuary

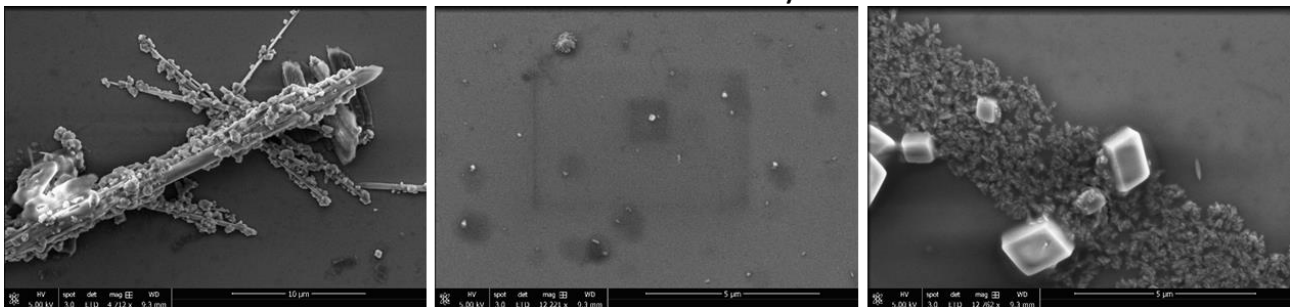


Figure 32. Representative SEM images for surface microlayer samples from NS7-Thames estuary, NS10-West Frisian Is. and NS12-Elbe estuary. The biggest particles observed in these SEM images were identified as salts, probably calcium carbonate and sodium chloride, crystallized during the drying process.^{5,6}

- **Residual Water from the Marine Snow Catcher.** SEM analysis of water from above the aggregate layer and filtered residual water from the aggregate layer sampled in NS7-Thames estuary, NS9-Broad Fourteens, NS10-West Frisian Is and NS12-Elbe estuary during the winter campaign was performed. Interestingly, Figures 33 and 34 show that the number of particles observed by SEM was very low despite the difference in particle concentration estimated by NTA: >10 particles per mL for water above the aggregate layer VS <4 particles per mL for filtered water from the aggregate layer.

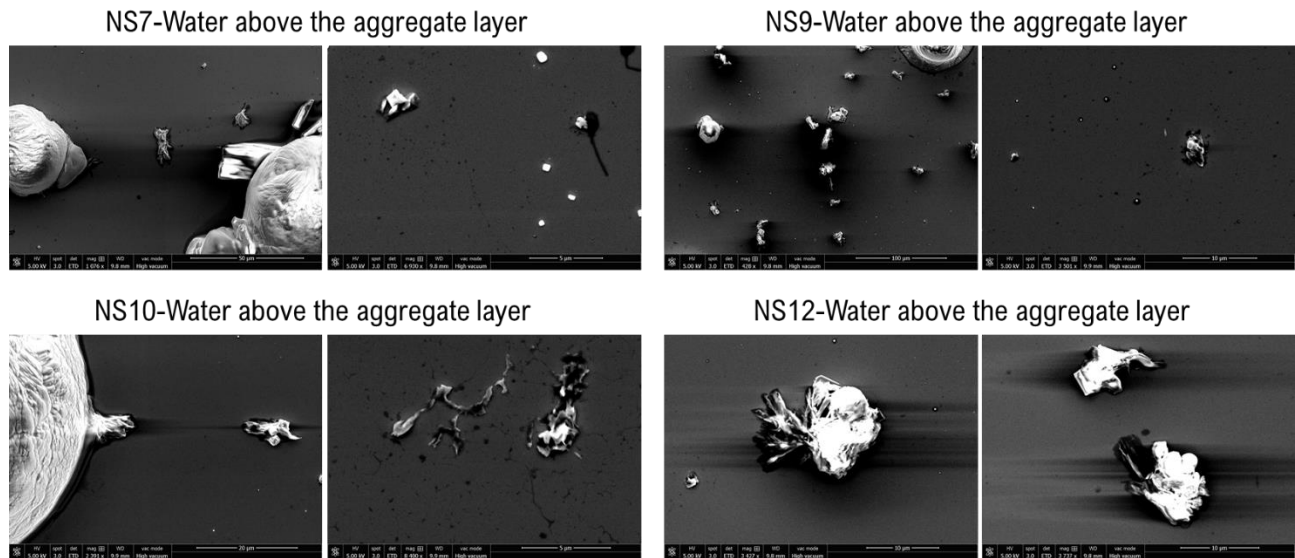


Figure 33. Representative SEM images for residual water from above the aggregate layer sampled in NS7, NS9, NS10, and NS12 using a marine snow catcher system. The biggest particles observed in these SEM images were identified as salts, probably calcium carbonate and sodium chloride, crystallized during the drying process.^{5,6}

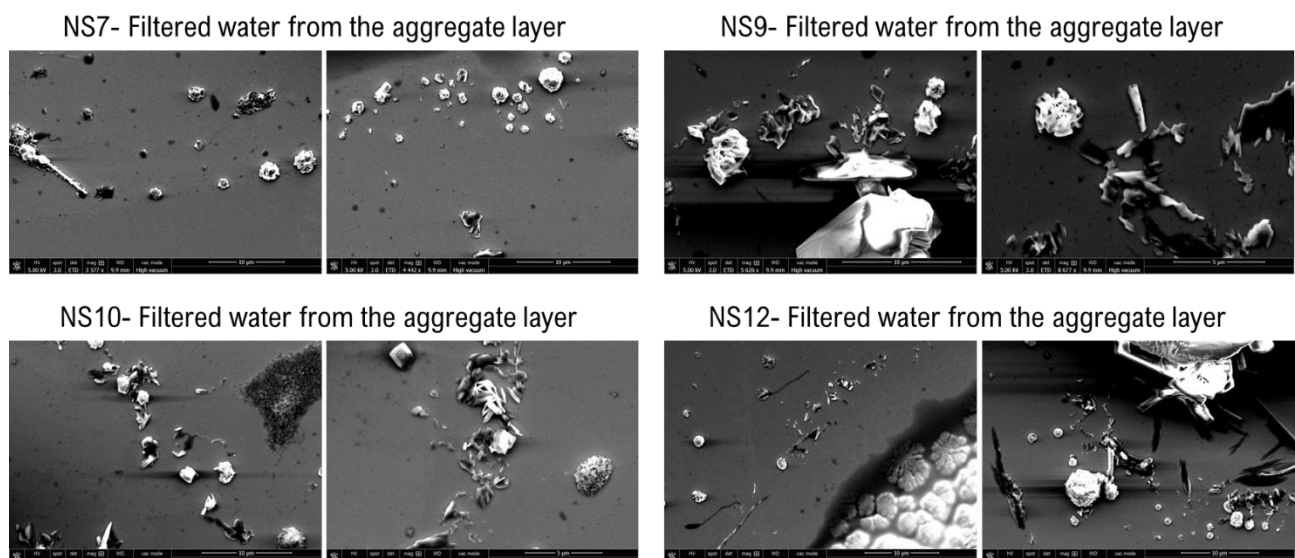


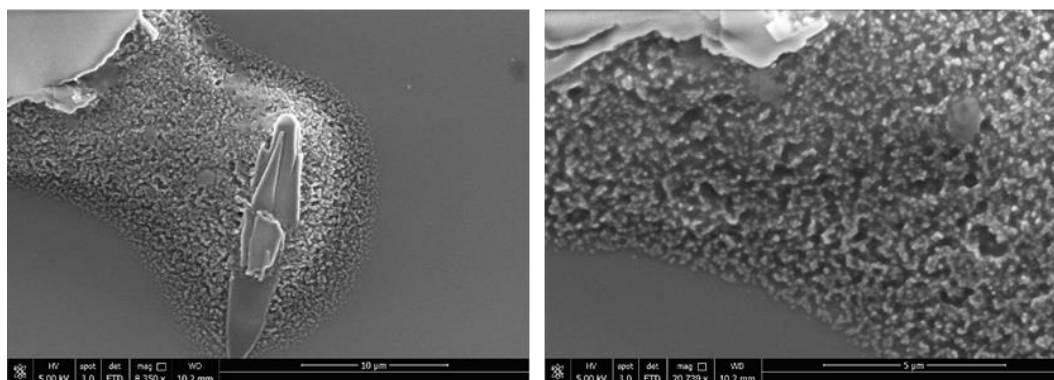
Figure 34. Representative SEM images for residual water from the aggregate layer sampled in NS7, NS9, NS10, and NS12 using a marine snow catcher system. The biggest particles observed in these SEM images were identified as salts, probably calcium carbonate and sodium chloride, crystallized during the drying process.^{5,6}

A plausible hypothesis could be that smaller particles are adsorbed on the surface of the bigger particles, making the visualization of these smaller particles difficult. In addition, these smaller particles could form aggregates during the drying process. Since these particles can be sensible to the electron beam, very low voltages were used on the SEM analysis reducing the size resolution of smaller particles inside of aggregates. This hypothesis was confirmed by analyzing water samples from the North Sea shipped at INL in 2022 due to the delay on the first campaign (see more details D2.1 and D2.2) collected from 1 m depth and 49 m depth using a Limnos water sample fitted with two amber borosilicate bottles (south of Helgoland on cruise L07–22 on FK LITTORINA). Figure 35 shows SEM images of these water samples, which demonstrated the presence of small particles close to or/ under bigger particles. The concentration estimated by NTA for these samples

The contents of this document are the copyright of the **LABPLAS** consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

was lower, 2.6×10^7 particles/mL for North Sea – 1 m depth and 2.3×10^7 particles/mL for North Sea – 49 m depth, than the surface microlayer and snow catcher samples characterized by SEM (see Figure 28 and Figure 30, respectively). It is worth noticing that these smaller particles could be only observed when the SEM instrument was in optimal conditions (e.g. calibration and alignment of apertures for the specific voltage). Therefore, SEM analysis of North Sea samples will be carried out once again to ensure the detection of smaller particles detected by NTA if present.

North Sea 1 m depth



North Sea 49 m depth

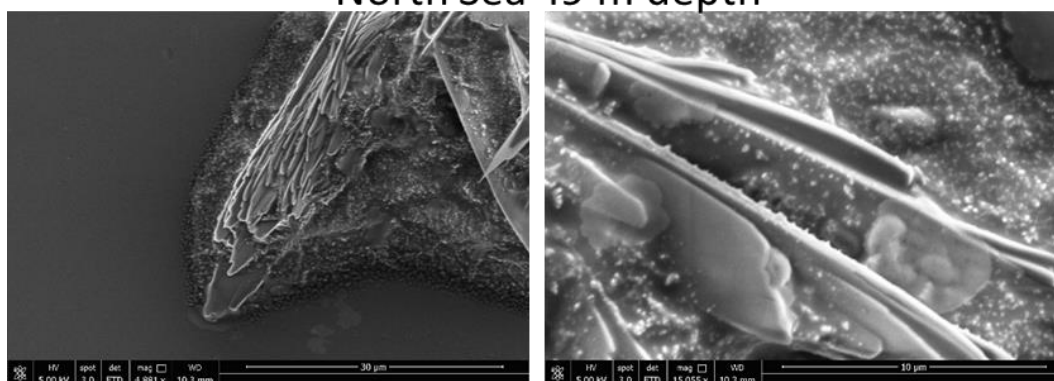


Figure 35. Representative SEM images of water sampled in the North Sea south of Helgoland. These samples were collected as a contingency plan for the delay of the first campaign. Water samples were collected at different depths: 1 and 49 m. The biggest particles observed in these SEM images were identified as salts, probably calcium carbonate, crystalized during the drying process.^{5,6}

5.1.2.3 Raman analysis

Samples that presented a concentration of particles in the range of 10^8 particles/mL were selected for Raman analysis (see Figures 26, 28, and 30). In the case of the North Sea matrix, the best LoD was obtained for 36 nm and 450 nm PET, being $1.25 \text{ mg/L} = 4 \times 10^{10}$ particles/mL and $0.63 \text{ mg/L} = 107$ particles/mL, respectively. However, the best LoD for 133 nm PS was $5 \text{ mg/L} = 4 \times 10^9$ particles/mL. Therefore, to improve the probability of identifying SMNPs, these samples were pre-concentrated using cloud-point extraction (CPE) prior to analysis with Raman, and Py-GC-MS analysis is ongoing. The conditions of the analysis are described in 2.1.1.3.

Raman analysis of treated samples from water above aggregate layer collected in NS7-Thames estuary, NS9-Broad Fourteens and NS10–West Frisian Is. stations are shown in Figure 36, Figure 37, and Figure 38, respectively. It was possible to identify PET and PS particles in the NS7-Thames estuary sample. In contrast, only PS particles were identified in both NS9-Broad Fourteens and NS10 – West Frisian Is. samples. Samples from other stations are being analyzed.

The contents of this document are the copyright of the LABPLAS consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

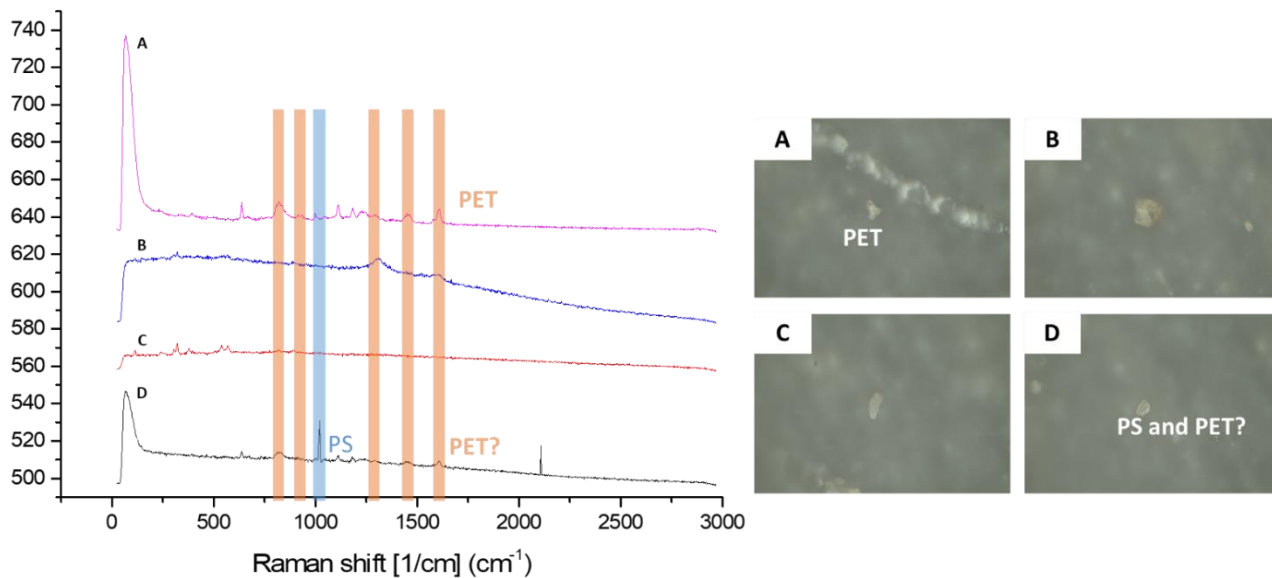


Figure 36. Raman spectra and bright field images of treated samples from water above aggregate layer collected in NS7-Thames estuary using the snow catcher method.

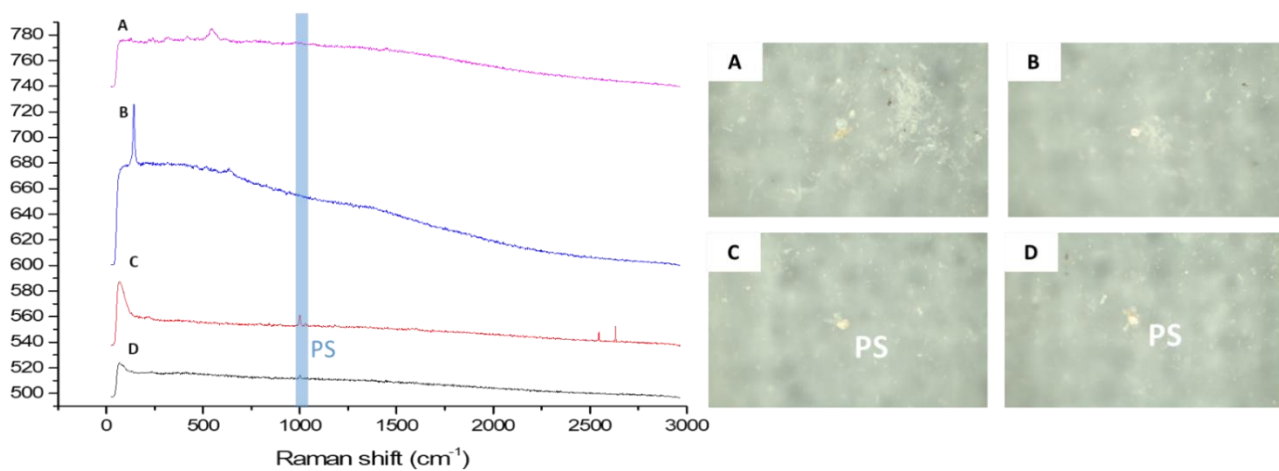


Figure 37. Raman spectra and bright field images of treated samples from water above the aggregate layer collected in NS9-Broad Fourteens using the snow catcher method.

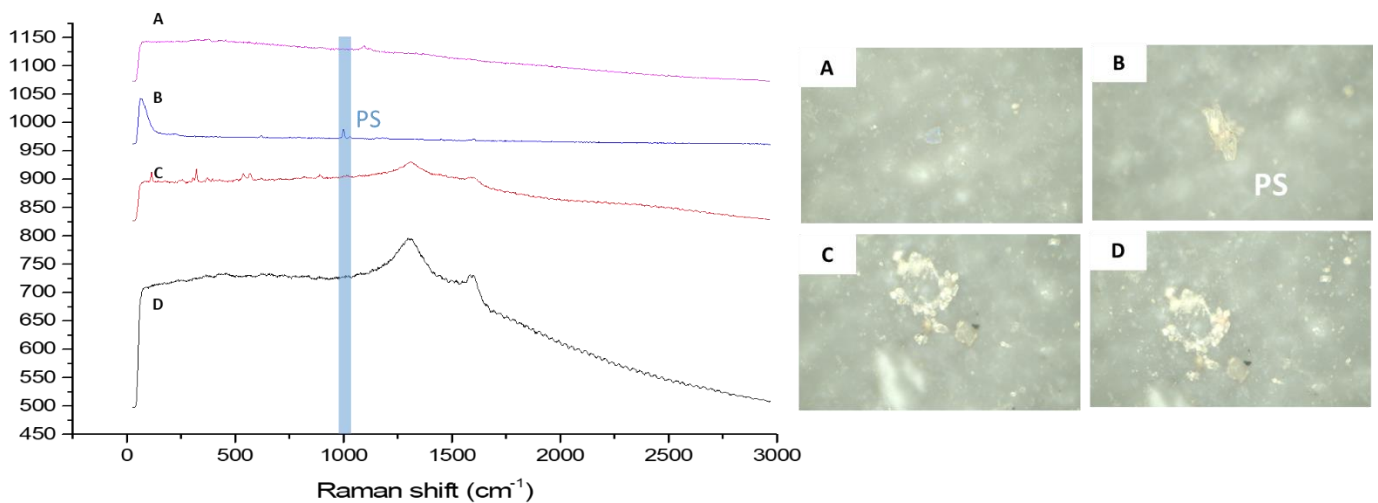


Figure 38. Raman spectra and bright field images of treated samples from water above aggregate layer collected in NS10 – West Frisian Is. using snow catcher method.

5.2 Suspended sediments

No significant number of suspended sediments were isolated from any of the water samples from the North Sea, as the samples were clean and free of any suspended solids as shown in Figure 39.



Figure 39. Representative photograph of water collected from the North Sea.

5.3 Conclusions

Water samples from the winter campaign had a higher concentration of particles smaller than 1 μm than in summer independently of the sampling method used. Specifically, the highest concentration of particles detected by sampling method was: water sampled above thermocline in NS7 – Thames estuary (16.2×10^7 particles/mL) using CTD, surface microlayer samples collected in NS10 – West Frisian Island (14.5×10^7 particles/mL) and NS12 – Elbe estuary (14.4×10^7 particles/mL) and water sampled above the aggregate layer in NS7 – Thames estuary (14.4×10^7 particles/mL) and NS9 – Broad Fourteens (16.7×10^7 particles/mL) for snow catcher. In the case of snow catcher sampling, a big difference in the particle concentration smaller than 1 μm was observed when water from the aggregate layer was filtered although the cut-off of the membrane used was 10 μm . This finding is important to take into account that the sample preparation procedure may affect the size-fraction composition of the sample since particles can adsorb in bigger particulate matter or form aggregates during the sample treatment. Samples with higher concentrations of particles are being analyzed by Raman. PS and PET particles were already identified in water sampled above the aggregate layer using a snow catcher in NS7, NS9, and NS10.

The contents of this document are the copyright of the LABPLAS consortium and shall not be copied in whole, in part, or otherwise reproduced, used, or disclosed to any other third parties without prior written authorisation.

GENERAL CONCLUSIONS

The analysis of field samples allowed for testing the viability of the guidelines established in Deliverable 4.1 for water and suspended sediments. Together with the sample preparation guidelines, the LoD established in Deliverable 4.2 for the use of Raman techniques gave an extra indicator for the selection of the samples with enough particles to be analyzed by this technique reducing the analysis time.

In general, the presence of particles $< 1 \mu\text{m}$ in all the water samples analyzed seems to be in the range of a few 10^7 and 10^8 particles/mL and thermocline seems to be a barrier for them as the number of particles below the thermocline is always lower than above. Additionally, the average size of these particles estimated by NTA was between 150 – 300 nm. Taking into account the size and the particle/mL concentration, we estimated that even assuming that all particles were plastic particles (confirmation by pyr-GC-MS is undergoing), these concentrations were frequently below the LoD described for our Raman-based detection method (details in D4.2). For instance, the LoD calculated for 450 nm PET, 36 nm PET, 161 nm PS, 33 nm PS and 126 nm PE were 0.63 mg/L, 1.25 mg/L, 5 mg/L, 5 mg/L and 0.6 mg/L, respectively, corresponding to 10^7 , 4×10^{10} , 2×10^9 , 3×10^{11} , and 6×10^8 particle per mL, respectively. Although these LoDs seem high, our Raman methodology offers one of the lowest LoD using conventional Raman for the identification of plastic particles smaller than $1 \mu\text{m}$, as reported in our recent publication (<https://doi.org/10.1016/j.marpolbul.2024.116468>).¹¹ Additionally, it is worth noticing that Raman results are influenced by plastic type, water matrix composition and the experimental setup and therefore, the combination of complementary analytical techniques is clearly needed. For all these reasons, water samples required further pre-concentration steps for their Raman analysis, which is currently undergoing. Samples collected during winter present consistently more particles than those collected during summer and there is a strong dependency on the collection point, more than on the depth (except for the samples below the thermocline). In the case of suspended sediments, matrix interferences (i.e. autofluorescence) make difficult the detection of plastics by the Raman method.

The comparison between the Raman-based method, NTA, and Py-GC-MS will allow gaining a better understanding of the nature, ratio plastic:natural particulate matter, and size distribution of the particles that have a size in the range of $\leq 20 \mu\text{m}$ and 100 nm. These results will be presented in the Deliverable 4.4.