Centre for Environment Fisheries & Aquaculture Science

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Monitoring of microplastics in Dutch marine sediments: a pilot study

Author(s): Dr Adil Bakir

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Project Manager:	Isabel Banham
Report compiled by:	Adil Bakir
Quality control by:	Holly Nel
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1. Introduction

Rijkswaterstaat, who organizes the marine litter monitoring for The Netherlands, has to deliver microplastic data for marine sediment and the sea surface to Europe and OSPAR. In this context, Rijkswaterstaat (hereafter referred to as RWS) is looking for an experienced microplastic laboratory with a focus on monitoring quality. Because this is a relatively new and difficult analytical method, RWS is also looking at foreign laboratories such as Cefas UK via a pilot project. In this report, the results of a pilot project with RWS sediment samples and Cefas microplastic and particle size analysis are reported.

Two samples have been tested by Cefas: (i) Bocht van Watum, which is a sandy mud location in the Ems-Dollard estuary and (ii) Terschelling 50 km from the coast, which is a muddy sand location in the Friese front area in the North Sea. These two samples cover the more muddy marine sediments in the Netherlands, which are usually more difficult to analyse due to the higher silt and organic carbon contents. There are also a few sandy Dutch microplastic monitoring locations.

The main aim of this project was to apply and to compare two analytical techniques for the detection and quantification of microplastics in seafloor sediments namely:

- Nile red tagging of polymers coupled with automatic counting and micro-FTIR
- Focal plane array detector-based micro-Fourier-transform infrared imaging

The main objectives were to i) apply both techniques on two seafloor sediments collected from the Netherlands, ii) to produce a data set on the abundance of particles per kg dry weight sediment, iii) to select the best technique for the short to long term monitoring of microplastics in seafloor sediments and iv) to carry out a particle size analysis of the sediments under investigation.

2. Materials and methods

2.1. Sample collection

The subtidal sediment samples were taken using a Rheineck boxcorer (Figure 1). Onboard the sampling vessel, the top layer of the boxcore sample was scraped off and collected in a stainless-steel sampling jar. The samples were stored at the laboratory at 4 ^{0}C. The samples were homogenized in the jar using a metal spoon, and wet subsamples of approx. 150 mL were transferred to prewashed glass bottles covered with aluminium disks which were secured with a plastic cap.

- Sediment 1: + 21004290, TERSLG50 (Terschelling 50 km from the coast), 1979a

- Sediment 2: + 21004294, BOCHTVWTM (Bocht van Watum, in the Ems-Dollard estuary), 1839c

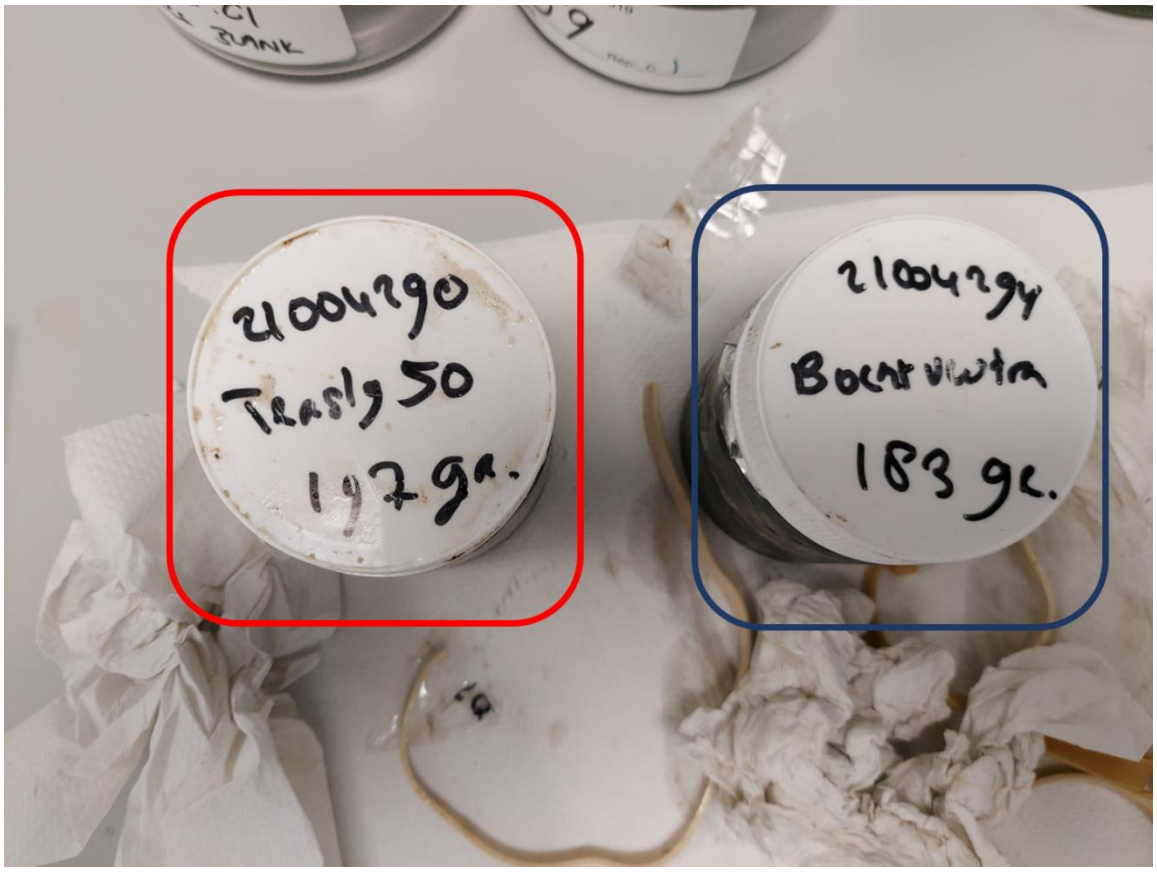

Figure 1 Samples investigated in this study.

2.2. Chemicals

Chemicals used in this study are listed in Table 1.

Table 1 List of Chemicals, manufacturer, and suppliers

Chemicals	Molecular formula	Manufacturer/Supplier	Purity (%)
Potassium hydroxide	KOH	VWR/VWR	-
Sodium hypochlorite	NaClO	VWR/VWR	14% active chlorine
Ethanol	C_2H_6O	Acros organics/ ThermoFisher scientific	95% purity

Chemicals	Molecular formula	Manufacturer/Supplier	Purity (%)
Nile red	$C_{20}H_{18}N_2O_2$	Acros organics/ ThermoFisher scientific	99% purity
Zinc chloride	$ZnCl_2$	VWR/VWR	-
Hydrogen peroxide	H_2O_2	VWR/VWR	30%

2.3. Contamination control procedures

Several contamination control procedures were also implemented while handling samples in the laboratory. Such procedures included:

- Use of glassware as much as possible
- All glassware was rinsed with reverse osmosis (RO) water in triplicate
- Use of 100% cotton lab coats
- All samples were handled in a biological safety cabinet (BSC)
- All surfaces were cleaned with plastic free cloths
- Laboratory floors were vacuumed each day before carrying out any work
- All chemicals added to the samples (including RO water) were previously filtered onto a 0.2 μm regenerated cellulose (RC) filter
- Restricted lab access and air filters fitted in the laboratory

2.4. Extraction, isolation, and characterisation of microplastics from sediments using Nile red tagging of polymers coupled with automatic counting and micro-FTIR

2.4.1. Sample preparation and microplastic extraction

Collected sediment samples were homogenised using a metal spatula in a BSC for at least 5 to 10 min. Aliquots were transferred to previously rinsed 100 mL glass jars and the lids were replaced with 15 cm Whatman 509 filter papers, held into place using small metal wires. Each sample was dried in a drying cabinet below 50°C for three days. Prior to weighing of the samples, each pot was homogenised using a rinsed metal spatula. 5g of the

sediment were weighed into three 50 mL polypropylene centrifuge tubes (n=3) in a BSC with ventilation. Density separation was carried out by using a 1.5 g mL^{-1} solution of zinc chloride ($ZnCl_2$). Approximately 35 mL of $ZnCl_2$ was added to each of the centrifuge tubes and 37 mL was added to an empty tube as a control. The tubes were then well shaken to homogenise the samples. Each tube was centrifugated at 3900xG for 5 minutes. Each supernatant was transferred to a previously cleaned filtration unit and filtered using a 47 mm diameter 0.2 μm porosity Whatman cellulose nitrate membrane. The whole process was repeated two more times and the supernatants combined on the same filter. Previous recovery studies indicated a recovery of above 80% for a range of polymers including UHMW PE, PP, Nylon, uPVC, PA, PS and PEST covering densities from 0.9 to 1.4 g cm^{-3} (Cefas, unpublished).

Residues of $ZnCl_2$ were rinsed with 100 mL of RO water and particles stuck onto the funnels were rinsed using RO water. Each filter was then carefully transferred to previously cleaned 100 mL glass beakers before digestion using a 30% KOH:NaClO v:v solution (Strand and Tairova, 2016; Enders *et al.*, 2017). 40 mL of the digest solution was added to each beaker, in a BSC, containing the extraction funnel on top to rinse the last particles potentially attached to the sides of the apparatus. The beakers and funnels were covered with a glass cover to avoid room contamination. The covered beakers containing samples were placed in a Flowgen Bioscience incubator and incubated for three days at 40°C while shaking using a VWR incubating orbital mini shaker at 120 rpm (Figure 2).

Figure 2 Picture of a VWR incubating orbital mini shaker

The cellulose nitrate membrane dissolved in approximately 10 minutes (showing the correct activity of the digestion solution). Each digested sediment sample was transferred to a previously cleaned filtration unit and filtered using a 47 mm diameter 0.2 μm porosity Whatman RC filter membrane. All filtrations were carried out in the BSC to minimise ambient contamination. Excess KOH:NaClO was washed down using 100 mL RO water. Approximately 5 mL of Nile red dissolved in ethanol was poured onto the filter disc and incubated for 30 minutes. Following incubation, Nile red was filtered through, and excess dye was rinsed with 100 mL previously filtered RO water. Particles stuck to the sides of the funnels (visual observation) were carefully washed down using a glass Pasteur pipette and RO water. Each filter was imaged and the quantification of microplastics was achieved using an automatic counting tool ('Microplastic tool') of the fluorescent items.

2.4.2. Single particle analysis using micro-FTIR

Suspected microlitter were identified for microplastic confirmation and polymer identification. A LUMOS II (Bruker, UK) using micro-ATR and transmission FT-IR with a liquid nitrogen-cooled MCT detector was used. All particles of interest were imaged and sized. For micro-ATR FT-IR, spectra (32 scans) were collected in reflectance mode in the range 4000-500 cm^{-1} at a resolution of 4 cm^{-1}. For particles that couldn't be analysed using micro-ATR FT-IR, transmission mode was used. For transmission mode, particles of interest were transferred to 25 mm Anodisc filters (0.2 μm porosity, Whatman, VWR, UK). Spectra (32 scans) were collected in transmission mode in the range 4000-1250 cm^{-1} at a resolution of 4 cm^{-1}. For all cases, polymer identification was verified based on the % match against provided polymer libraries (ATR-FTIR-Library complete, Vol.1-4; Bruker Optics ATR-Polymer Library; IR-Spectra of Polymers, Diamond -ATR, IR-Spectra of Polymers, Geranium-AT & IR-Spectra of Additives, Diamond-ATR). Only matches above 60% were selected for a positive microplastic validation and polymer identification (Leistenschneider *et al.*, 2021).

2.4.3. Reporting

Units were expressed in number of particles kg^{-1} dry weight sediment. Additional parameters are being reported such as polymer type and sizes of the items analysed using micro-FTIR.

2.5. Extraction, isolation, and characterisation of microplastics from sediments using Focal plane array detector-based micro-Fourier-transform infrared imaging

2.5.1. Sample preparation and microplastic extraction

A spectroscopic based method was also developed in parallel to the Nile red method using a LUMOS II FTIR microscope with focal plane array (FPA) detector (Bruker, UK). 10 g of dried sediment were weighed into a single 50 mL polypropylene (PP) centrifuge tube for each sample. Dried sediments were digested using the dropwise addition of filtered (0.2 μm) H_2O_2 (30%) until bubbling stops. Following the digestion step, the tubes were covered using a RO rinse filter membrane secured into place using a fine metal wire and subsequently placed in a drying cabinet < 50 °C for 48 hours prior to density separation. Density separation was carried out by using a 1.5 g mL^{-1} solution of $ZnCl_2$. Approximately 35 mL of $ZnCl_2$ was added to each of the centrifuge tube and 37 mL was added to an empty tube as a control. The tubes were then well shaken to homogenise the samples. Each tube was centrifugated at 3900xG for 5 minutes. Each supernatant was transferred to a previously cleaned filtration unit and filtered using a 25 mm diameter 0.2 μm porosity Whatman Anodisc (VWR, UK). The whole process was repeated one more time and the supernatants were filtered onto 2 filters (1 extract onto 1 filter), to avoid their saturation due to the high proportion of very fine sediments in each sample. Excess $ZnCl_2$ was rinsed with 15 mL of RO water, along with any particles stuck onto the funnels. Each filter was then carefully transferred to previously cleaned glass petri dish and transported to a drying cabinet for 24 to 48 hours under 50°C prior to analysis using a LUMOS II micro-FTIR with FPA detector (Bruker, UK).

2.5.2. Analysis using Focal plane array (FPA) detector-based micro-Fourier-transform infrared imaging

The whole area (100%) of the 25 mm Anodiscs filters (0.2 μm porosity, Whatman, VWR, UK) was scanned. FTIR spectra were collected using FPA detector in transmission mode using a single scan in the range 4000-1300 cm^{-1} at a resolution of 8 cm^{-1} using a 4x4 binning (LOD of ~ 20 μm). Spectra were converted using a macro in Bruker OPUS (version 8.5) and particle identification was carried out using the siMPle software developed by Aalborg

University (Denmark) and Alfred Wegener Institute (Germany) (https://simple-plastics.eu/) (Primpke *et al.*, 2020). Results were expressed as number of microplastics, corresponding sizes (in μm), and weight (in ng). The software also allowed for the identification and counting of non-microplastic items including natural items (e.g., chitin) and fibres (e.g., cotton) decreasing the inclusion of non-plastic items in reported data.

2.6. Particle size analysis (PSA)

Particle Size Analysis (PSA) using the NMBAQC Method and including a visual description. The sediment sample is received at the laboratory, usually frozen, and defrosted before it is thoroughly homogenised with a clean metal spatula to ensure a representative subsample. A visual description is carried out, this will provide information on sediment type in addition to the particle size, and will include colour, viscosity, and smell (if notable) as well as identifying any unusual or manmade material. A representative subsample is then taken to allow laser diffraction on the <2mm sediment using a Beckman Coulter LS13320 laser sizer. The remainder of the sample is wet sieved over a 1mm Endecotts certificated stainless steel sieve to split the sample at 1mm. The <1mm sediment passes through the sieve into a bucket, this is then allowed to settle for 24 – 48 hrs before excess water is siphoned off. Both fractions (<1mm and >1mm) are then oven dried. The <1mm fraction is weighed and used to calculate the proportion of <1mm:>1mm sediment. The >1mm fraction is dry sieved through a series of Endecotts certificated sieves at 0.5φ intervals within a HEPA filtered dust cabinet. Finally, the sieve and laser data are merged to produce a complete Particle Size (PS) distribution at 0.5φ intervals.

3. Results

3.1. Extraction, isolation, and characterisation of microplastics from sediments using Nile red tagging of polymers coupled with automatic counting and micro-FTIR

3.1.1. Negative controls

No items larger than 100 μm were detected in the laboratory negative control while 4 items down to ~ 20 μm were recorded for sediment 1. No microplastics were detected for the negative control for sediment 2. Raw data are presented in **Appendix Tables A1 & A2.**

3.1.2. Sediment 1: + 21004290, TERSLG50 (Terschelling 50 km from the coast), 1979a

The mean abundance of microplastics in sediment 1 was 1133 ± 416 (range: 800 – 1600) particles per kg dry weight sediment for items above ~100 μm in size and a mean concentration of 1400 ± 800 (range 600 – 2200) particles per kg dry weight sediment for items above ~20 μm in size. Raw data are presented in **Appendix Table A1**.

Micro-FTIR analysis detected the presence of a range of polymers including acrylic fibres and rayon. FTIR spectra and related library matches are shown in **Figure A3**.

3.1.3. Sediment 2: + 21004294, BOCHTVWTM (Bocht van Watum, in the Ems-Dollard estuary), 1839c

The mean abundance of microplastics in sediment 2 was 1267 ± 416 (range: 800 – 1600) particles per kg dry weight sediment for items above ~100 μm in size and a mean concentration of 3200 ± 1058 (range 2400 – 4400) particles per kg dry weight sediment for items above ~20 μm in size. Raw data are presented in **Appendix Table A2**.

Micro-FTIR analysis detected the presence of a range of polymers including PE, black rubber (car tyre) and paint particles. FTIR spectra and related library matches are shown in **Figure A6**.

3.2. Analysis using Focal plane array (FPA) detector-based micro-Fourier-transform infrared imaging

3.2.1. Negative control

No microplastics above the limit of detection of the technique (20 μm) were detected on the negative laboratory control. 8 natural items were however detected consisting of items corresponding to the animal furs/natural polyamides category and 1 corresponding to the cellulose/plant fibres category. The size of the natural items ranged from 27.90 to 160.50 μm with an average mass of 93.40 ± 85.85 ng (mean ± SD). Raw data are presented in **Appendix Table A3.**

3.2.2. Sediment 1: + 21004290, TERSLG50 (Terschelling 50 km from the coast), 1979a

Analysis using Focal plane array (FPA) detector-based micro-Fourier-transform infrared imaging detected 21 microplastics in the size range 20 – 5000 μm while 10 microplastics were detected in the size range 100 – 5000 μm. This corresponded to concentrations of 1000 particles per kg dry weight sediment for particles in the size range 100 – 5000 μm and 2100 particles per kg dry weight sediment for particles in the size range 20 – 5000 μm. Raw data are presented in **Appendix Table A4**.

Microplastics extracted from sediment were also categorised according to their polymer type (Figure 3). Main polymer detected was PP (48%), followed by Polystyrene (PS) (19%), Polyethylene (PE) chlorinated (14%) and PE (9%), Acrylates/polyurethanes/varnish (5%) and Polyamide (PA) (5%).

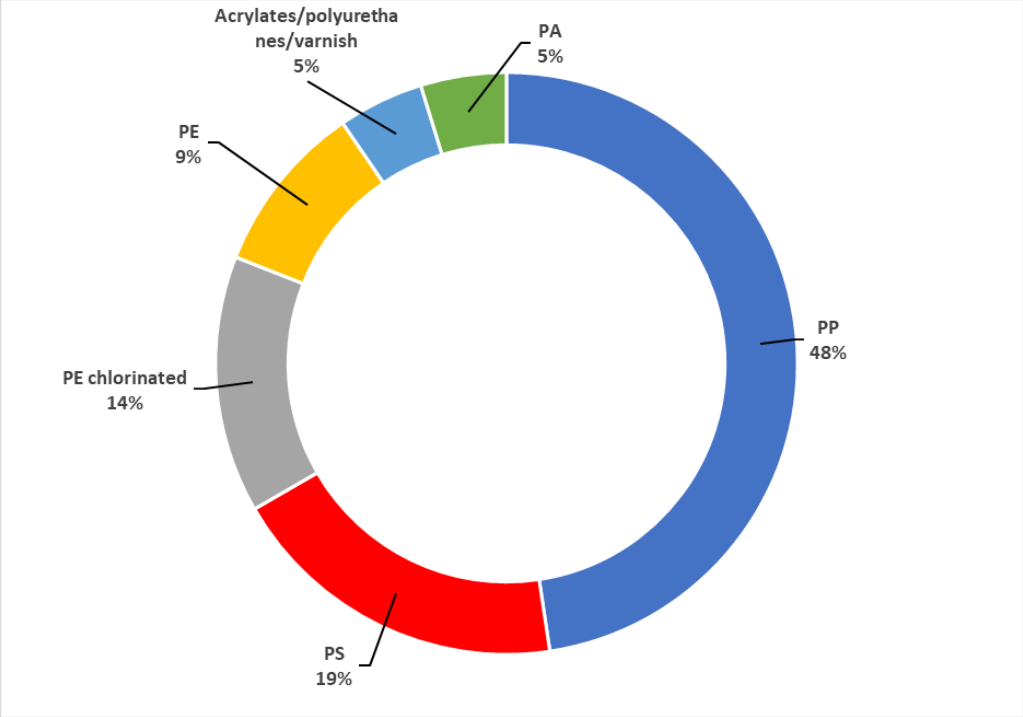

Figure 3 Microplastics extracted from sediment from the location Terschelling50 and categorised per polymer type (n=21).

3.2.3. Sediment 2: + 21004294, BOCHTVWTM (Bocht van Watum, in the Ems-Dollard estuary), 1839c

Analysis using Focal plane array (FPA) detector-based micro-Fourier-transform infrared imaging detected 34 microplastics in the size range 20 – 5000 μm while 13 microplastics were detected in the size range 100 – 5000 μm. This corresponded to concentrations of 1400 particles per kg dry weight sediment for particles in the size range 100 – 5000 μm and 4700 particles per kg dry weight sediment for particles in the size range 20 – 5000 μm. Raw data are presented in **Appendix Table A5**.

Microplastics extracted from sediment were also categorised according to their polymer type (**Figure 4**). Main polymer detected was PP (70%), followed by PE chlorinated (17%), Acrylates/polyurethanes/varnish (5%), PE oxidized (4%), PE (2%) and Rubber type 3 (2%).

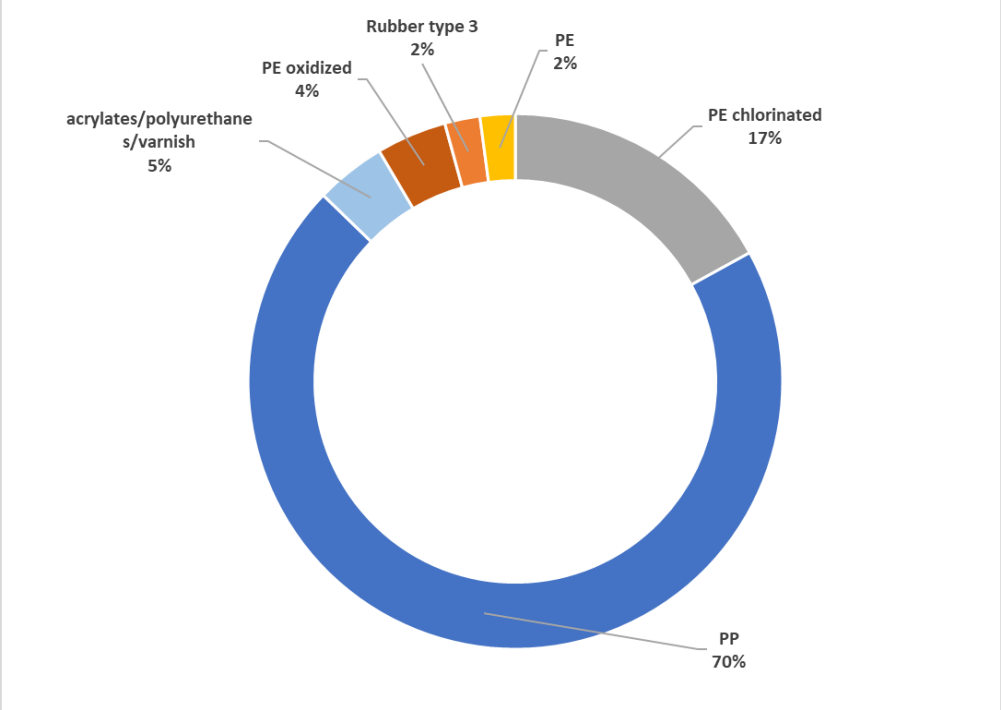

Figure 4. Microplastics extracted from sediment from the location Bocht van Watum and categorised per polymer type (n=47).

4. Discussion

4.1. Extraction, isolation, and characterisation of microplastics from sediments using Nile red tagging of polymers coupled with automatic counting and micro-FTIR

Nile red was developed as a low cost and fast approach for the detection and quantification of microplastics in environmental samples (Maes *et al.*, 2017). Since its development, the application of Nile red in relation to microplastic research has increased substantially. Shruti *et al.* (2021) recently published a review on the application of Nile red for the analysis of microplastics in environmental samples including food products. While the need for standardised protocols for Nile red use was highlighted in the review, the authors concluded that Nile red tagging of microplastics was a promising approach for a low-cost and fast screening of microplastics from environmental samples, especially for laboratories lacking more advanced and often costly infrastructure (e.g., pyrolysis GC-MS or μ-FTIR, μ-Raman facilities). Nile red has also previously been used for the large-scale mapping of microplastics from sediment indicating its suitability in a monitoring context (Wang *et al.*,

2018; Bakir, Desender, *et al.*, 2020; Preston-Whyte *et al.*, 2021). Nile red was also applied to the detection and quantification of microplastics in biota (Catarino *et al.*, 2018; Bakir *et al.*, 2020; Bakir *et al.*, 2020; Coc *et al.*, 2021; Nalbone *et al.*, 2021) and water (Bakir, Desender, *et al.*, 2020; Preston-Whyte *et al.*, 2021).

4.2. Extraction, isolation, and characterisation of microplastics from sediments using Focal plane array detector-based micro-Fourier-transform infrared imaging

FPA detector-based micro-Fourier-transform infrared imaging has been proposed as an effective tool for the accurate assessment of microplastics in environmental samples (Löder and Gerdts, 2015; Löder *et al.*, 2015; Mintenig *et al.*, 2017; Primpke *et al.*, 2017). While most laboratories are already equipped with micro-FTIR facilities, the cost of FPAs is often a limiting factor for many laboratories which currently limits its use in routine monitoring programmes. Focal plane array detector-based micro-Fourier-transform infrared imaging was suitable for seafloor sediments with high sand and silt/clay composition (**see PSA report**).

FPA analysis allowed the identification of polymers not identified using the Nile red technique such as rubber type 3 and PP. In addition, the techniques allowed the identification of items too small for manual transfer from stained filters to Anodiscs, for micro-FTIR analysis, with identification of particles down to ~ 20 μm. The main polymer type detected for both sediments consisted of PP, followed by PS and PE for sediment 1 and PE and acrylates/polyurethane/varnish for sediment 2. Rubber particles were also detected for sediment 2. The presence of rubber particles was also consistent with the single particle characterisation from the Nile red section with the identification of black rubber items from car tyre (**Figure A6**). Parker-Jurd *et al.* (2020) demonstrated the importance of land-based sources of tyre particles to the marine environment, detecting these microplastics in treated wastewater effluent, in storm water drains adjacent to roads and deposited from urban dusts near roadsides (Parker-Jurd *et al.*, 2020). Land-based sources have been generally assumed to be the main contributors for the entry of plastic waste to the marine environment (Gilardi *et al.*, 2020; Meijer *et al.*, 2021). Tyre wear and (macro) litter were identified as the

largest land-based sources of microplastics in the OSPAR regions, with estimated amounts of around 100,000 tons year^{-1} entering the marine environment (OSPAR, 2017).

Focal plane array–based μ-FTIR imaging is therefore proposed as a more sensitive and precise technique for the monitoring of microplastics in seafloor sediments. It is also proposed that sediment samples will be prepared in duplicates for data validation and to investigate for reproducibility and related errors for repeated measurements for the same sample.

5. Summary and recommendations

- Analysis of two seafloor sediments from the Netherlands was carried out using two analytical techniques namely using Nile red tagging of polymers coupled with automatic counting and micro-FTIR and using Focal plane array (FPA) detector-based micro-Fourier-transform infrared imaging.
- Both techniques gave comparable results in relation to the abundance of microplastics in seafloor sediments expressed as number of particles per kg dry weight sediment.
- Focal plane array (FPA) detector-based micro-Fourier-transform infrared imaging allowed for a more precise and detailed assessment of microplastics from sediments and was recommended for the short to long term monitoring of microplastics from seafloor sediments.

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Appendices

A.1 Raw data - Extraction, isolation, and characterisation of microplastics from sediments using Nile red tagging of polymers coupled with automatic counting and micro-FTIR

A.1.1 Sediment 1: + 21004290, TERSLG50 (Terschelling 50 km from the coast), 1979a

Table A1. Raw data for the analysis of microplastics from the laboratory negative control using Nile red tagging of polymers coupled with automatic counting and micro-FTIR.

Sample ID	Output from automated count	
	Smaller setting (min ~ 20 µm)	Large settings (> 100 µm)
Negative control_1	4	0 (< LOD)
R_1	11	5
R_2	7	4
R_3	15	8
R_1_control corrected	7	5
R_2_control corrected	3	4
R_3_control corrected	11	8
Mean	7.0	5.7
SD	4.0	2.1
Mean number of particles per kg dry weight sediment	1400	1133
SD	800	416
Min	600	800
Max	2200	1600

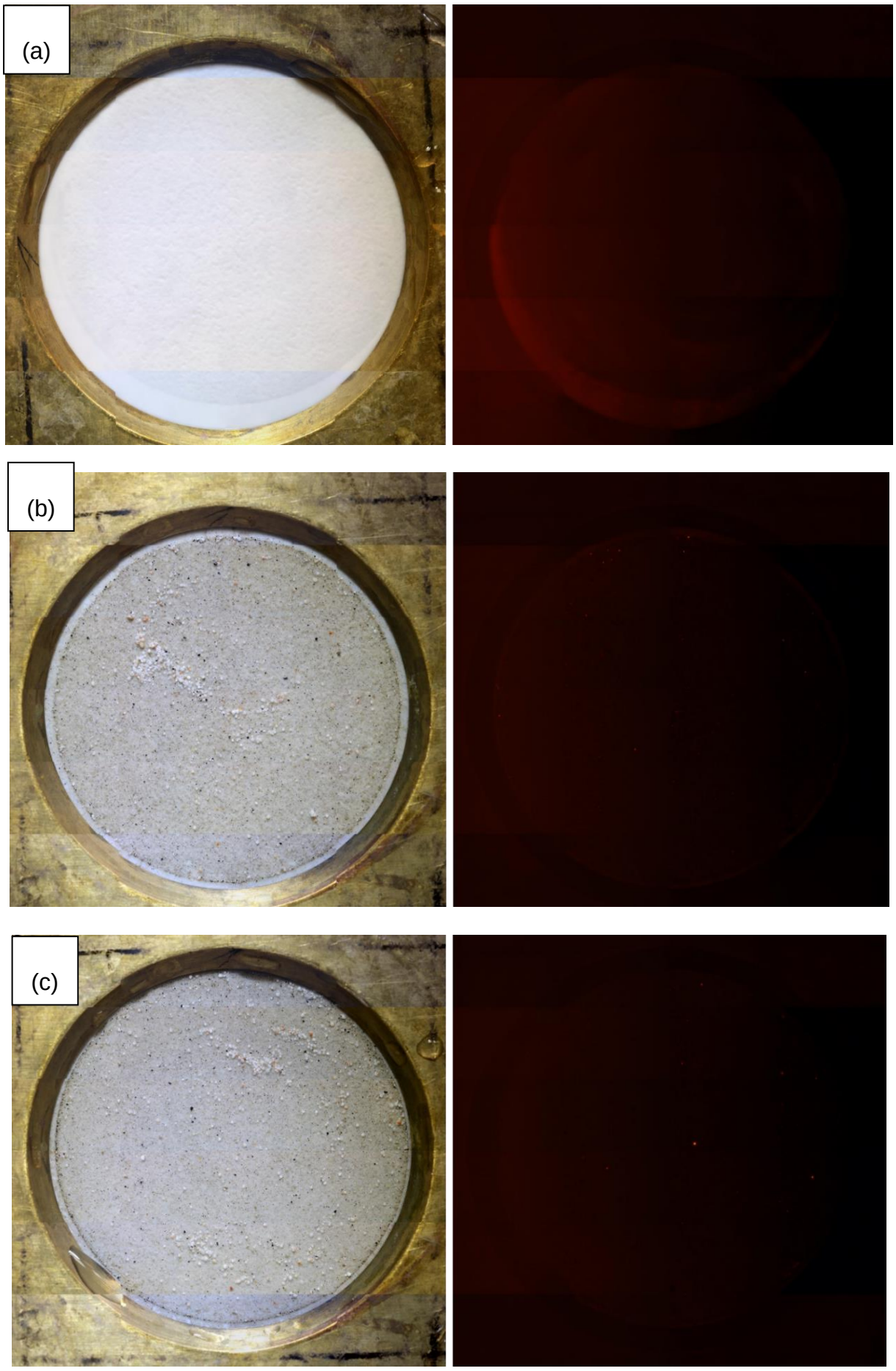

(a)
(b)
(c)

Figure A1. White and fluorescence imaging of filters for sediment 1 (a: negative control, b-c: replicates 1-3).

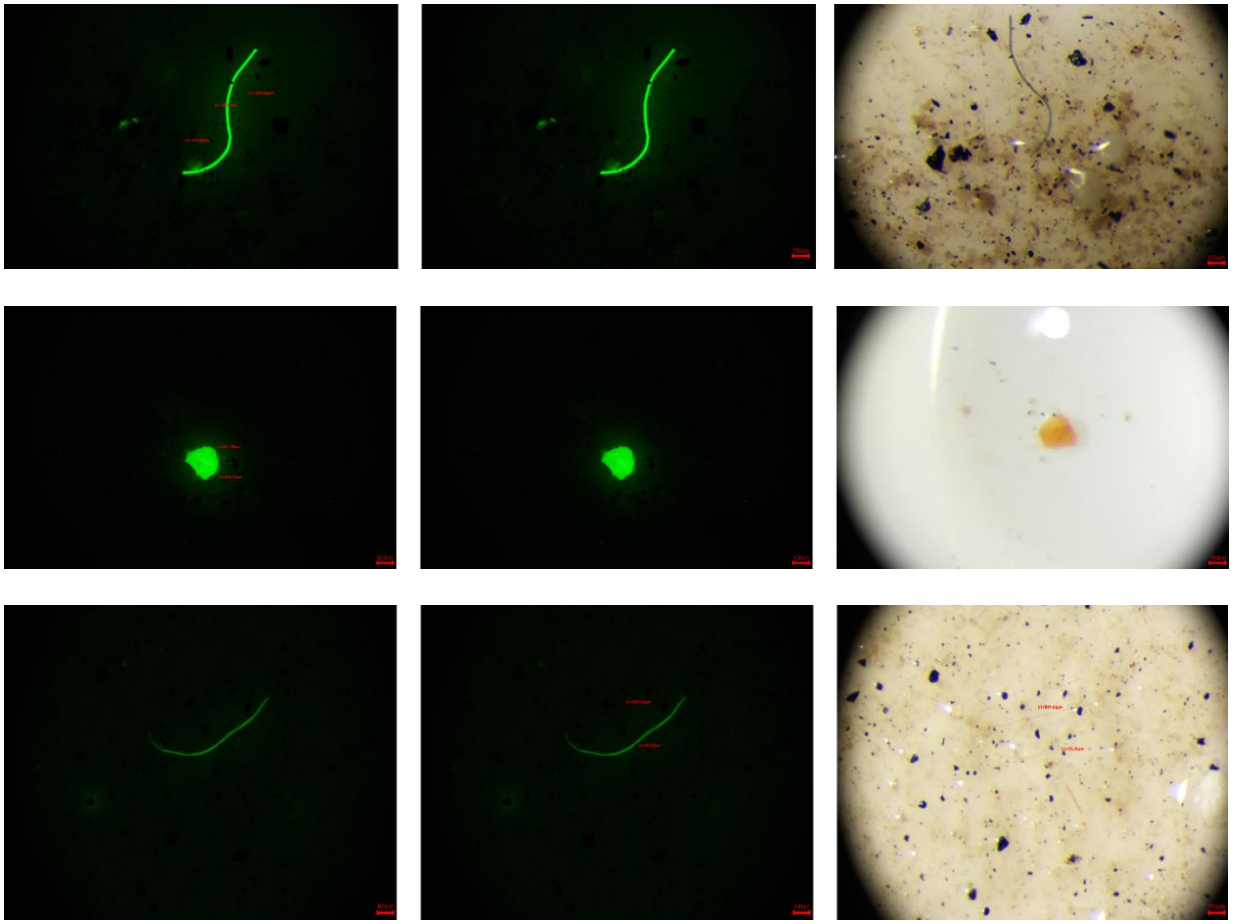
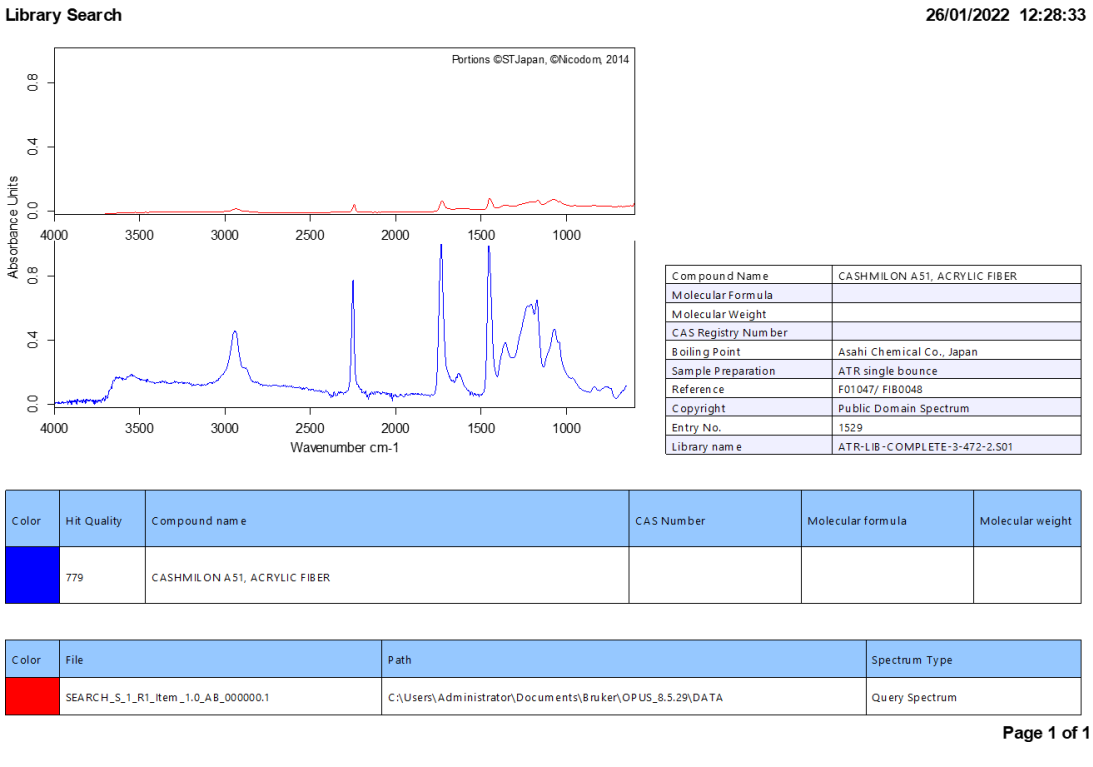


Figure A2. Example of Manual picking and visual characterisation of items onto filters using microscopy.



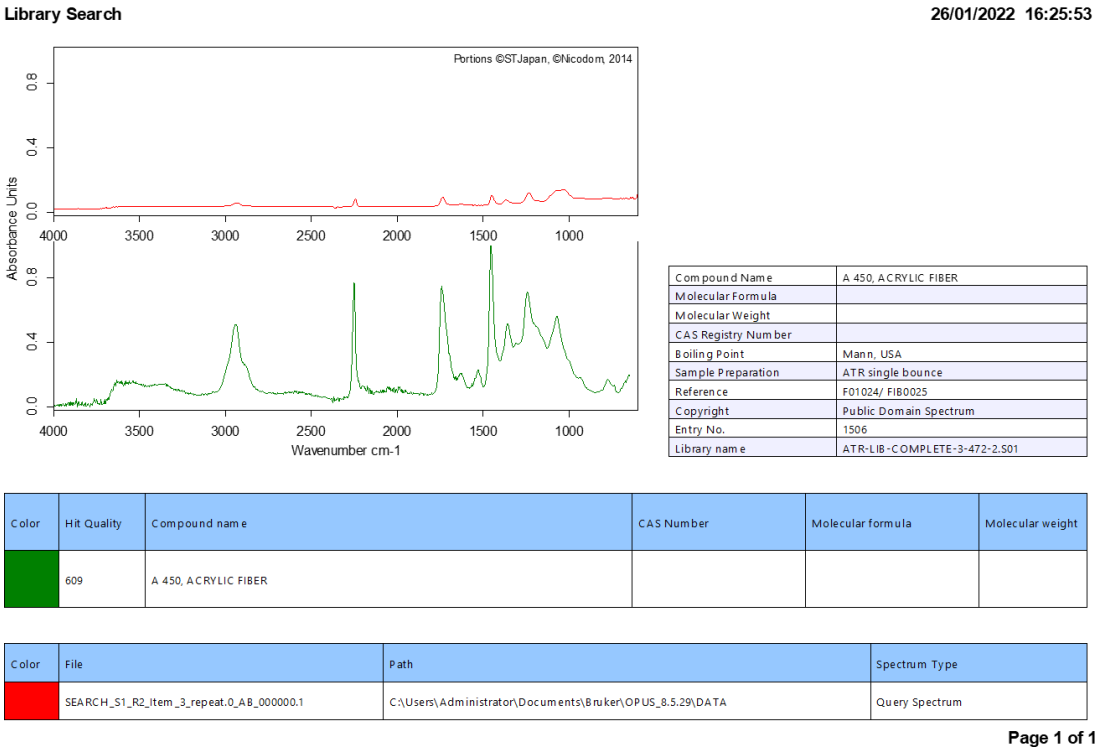
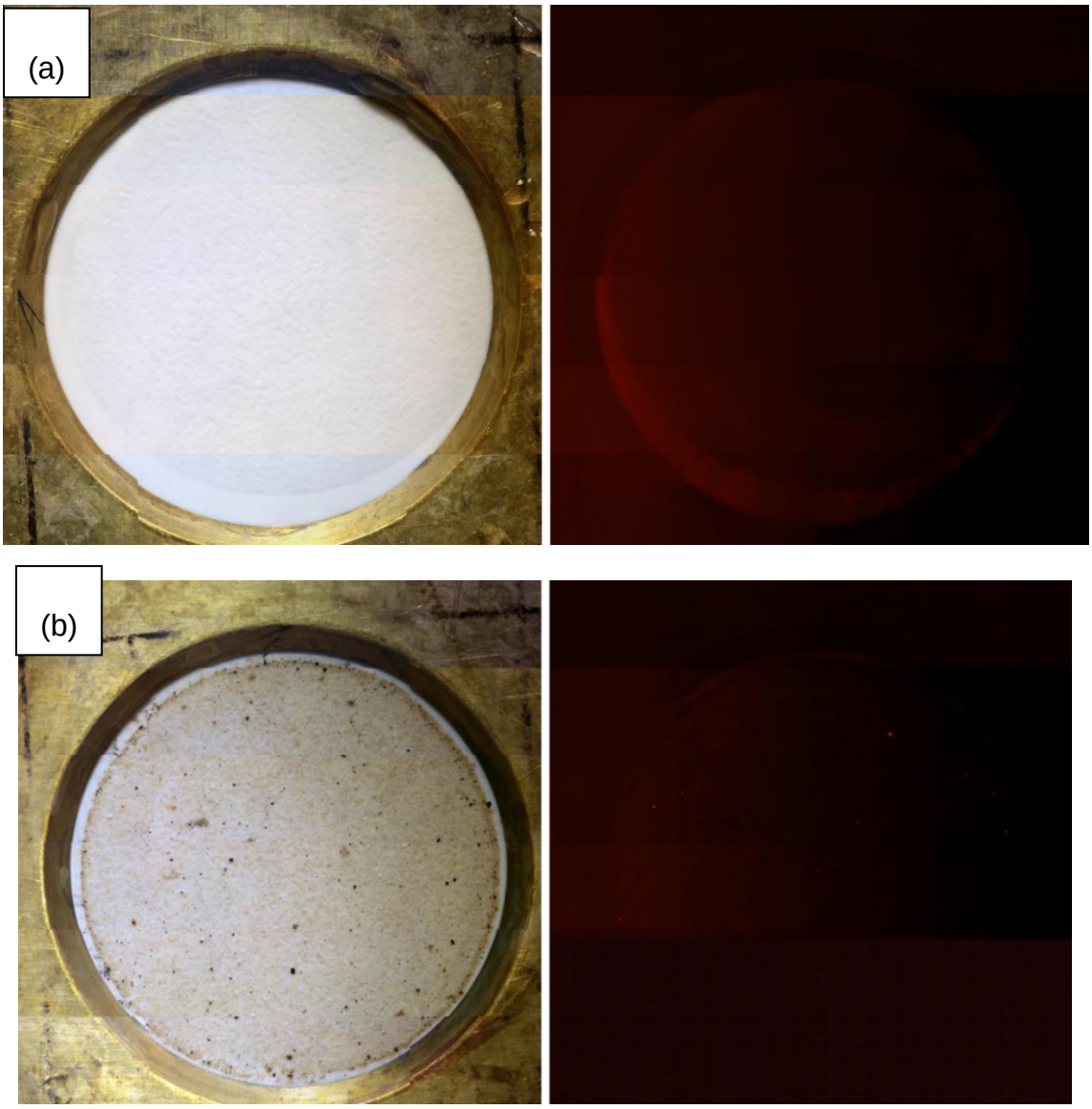


Figure A3. Example of micro-FTIR spectra for items extracted from sediment 1.

A1.2 Sediment 2: + 21004294, BOCHTVWTM (Bocht van Watum, in the Ems-Dollard estuary), 1839c



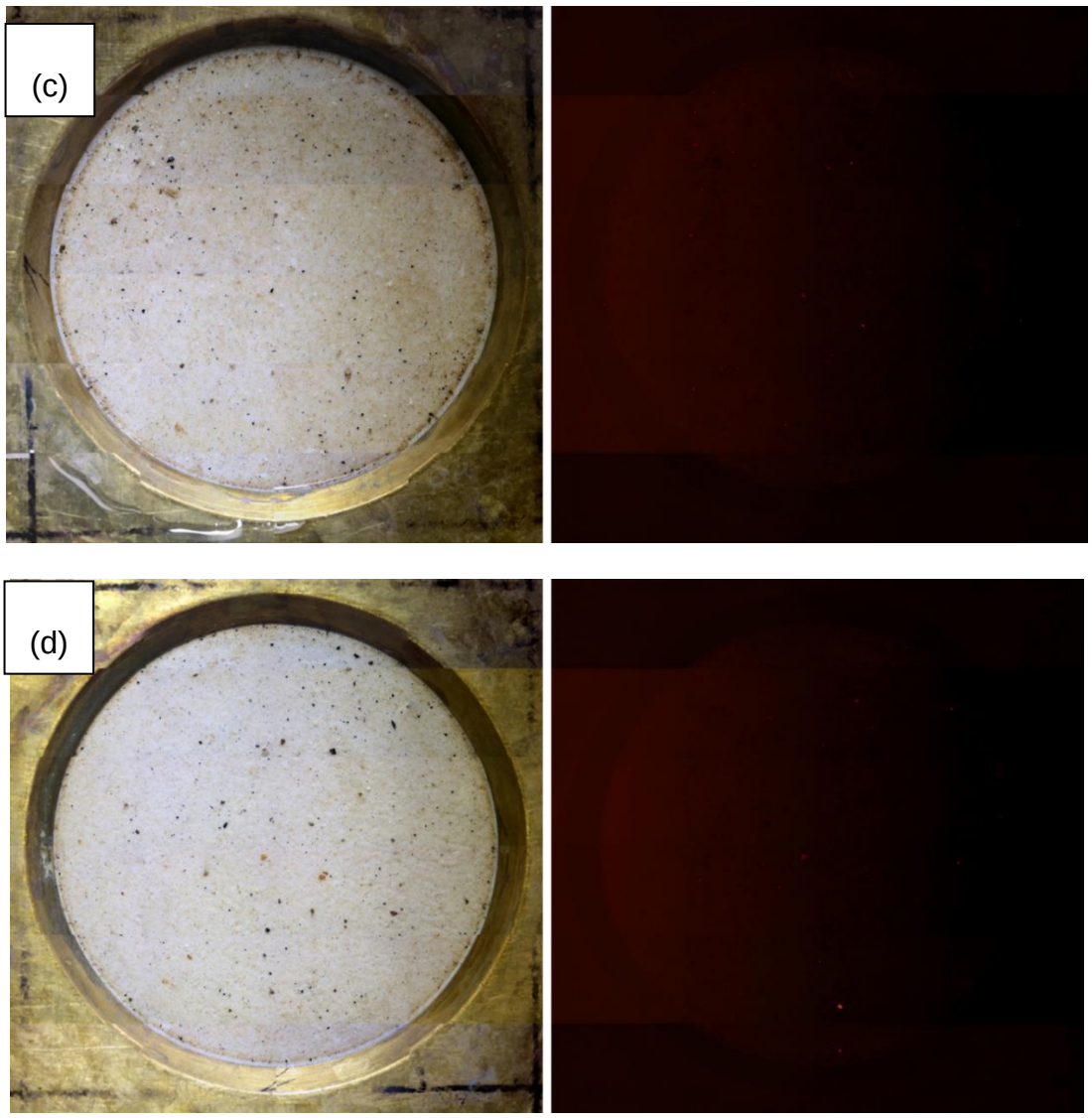
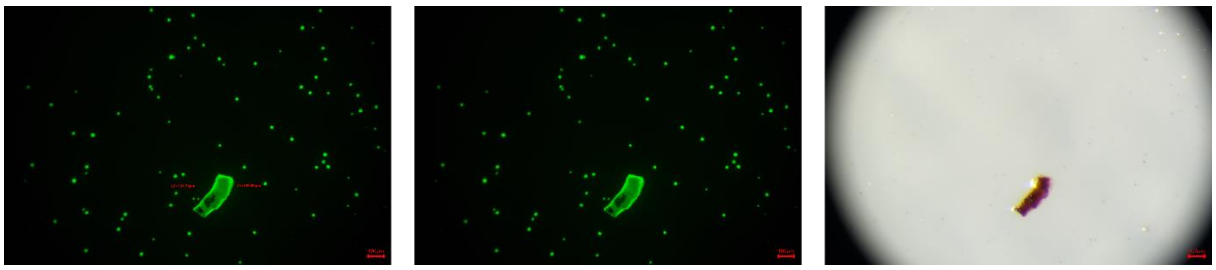


Figure A4. White and fluorescence imaging of filters for sediment 2 (a: negative control, b-d: replicates 1-3).



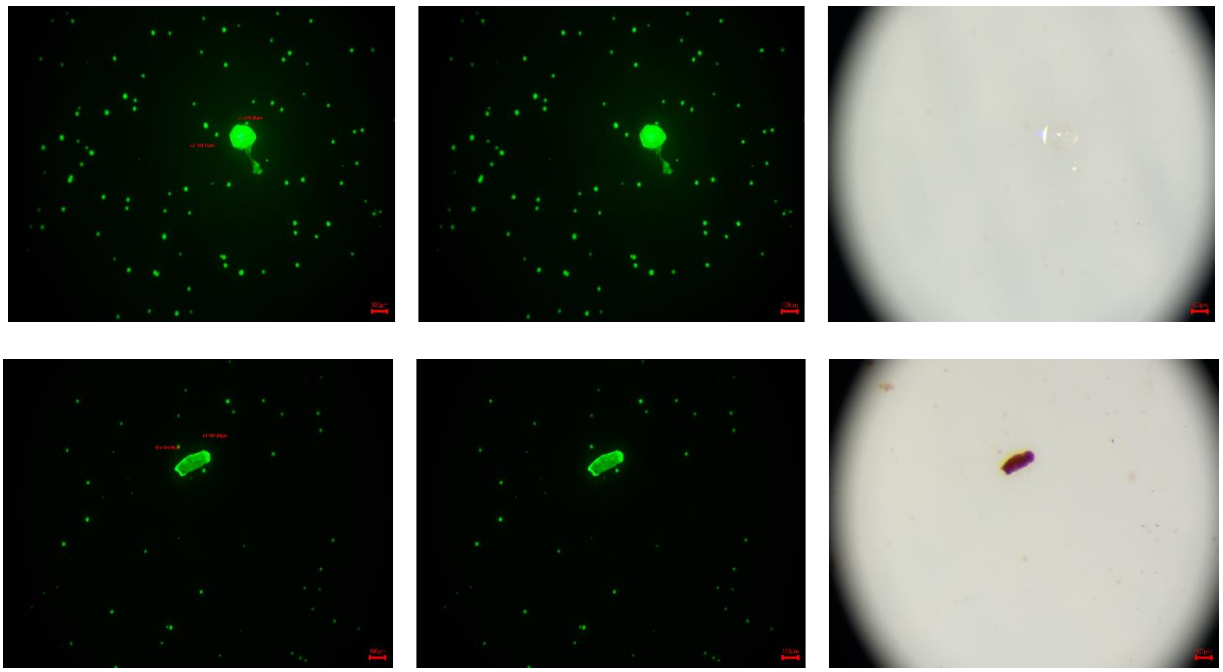


Figure A5. Example of manual picking and visual characterisation of items onto filters using microscopy.

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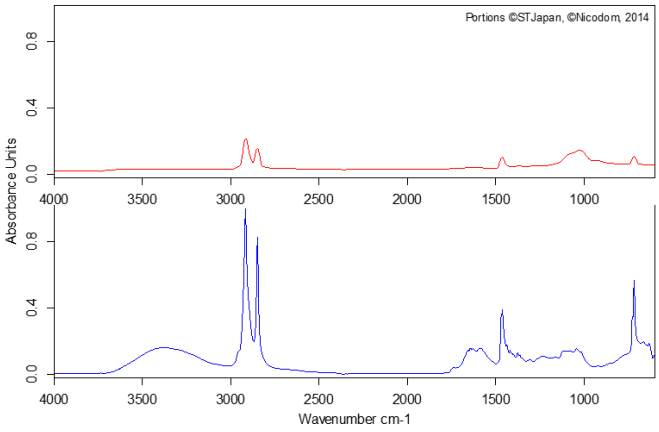

Compound Name	POLYETHYLENE PLASTICIZED #2
Molecular Formula	(C2H4)n
Molecular Weight	
CAS Registry Number	9002-88-4
Sample Preparation	ATR single bounce
Comment	polyethylene
Reference	D848/ MP0145
Copyright	(c) 2014 Nicodom
Entry No.	1277
Library name	ATR-LIB-COMPLETE-2-472-2.S01

Color	Hit Quality	Compound name	CAS Number	Molecular formula	Molecular weight
	844	POLYETHYLENE PLASTICIZED #2	9002-88-4	(C2H4)n	

Color	File	Path	Spectrum Type
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Page 1 of 1

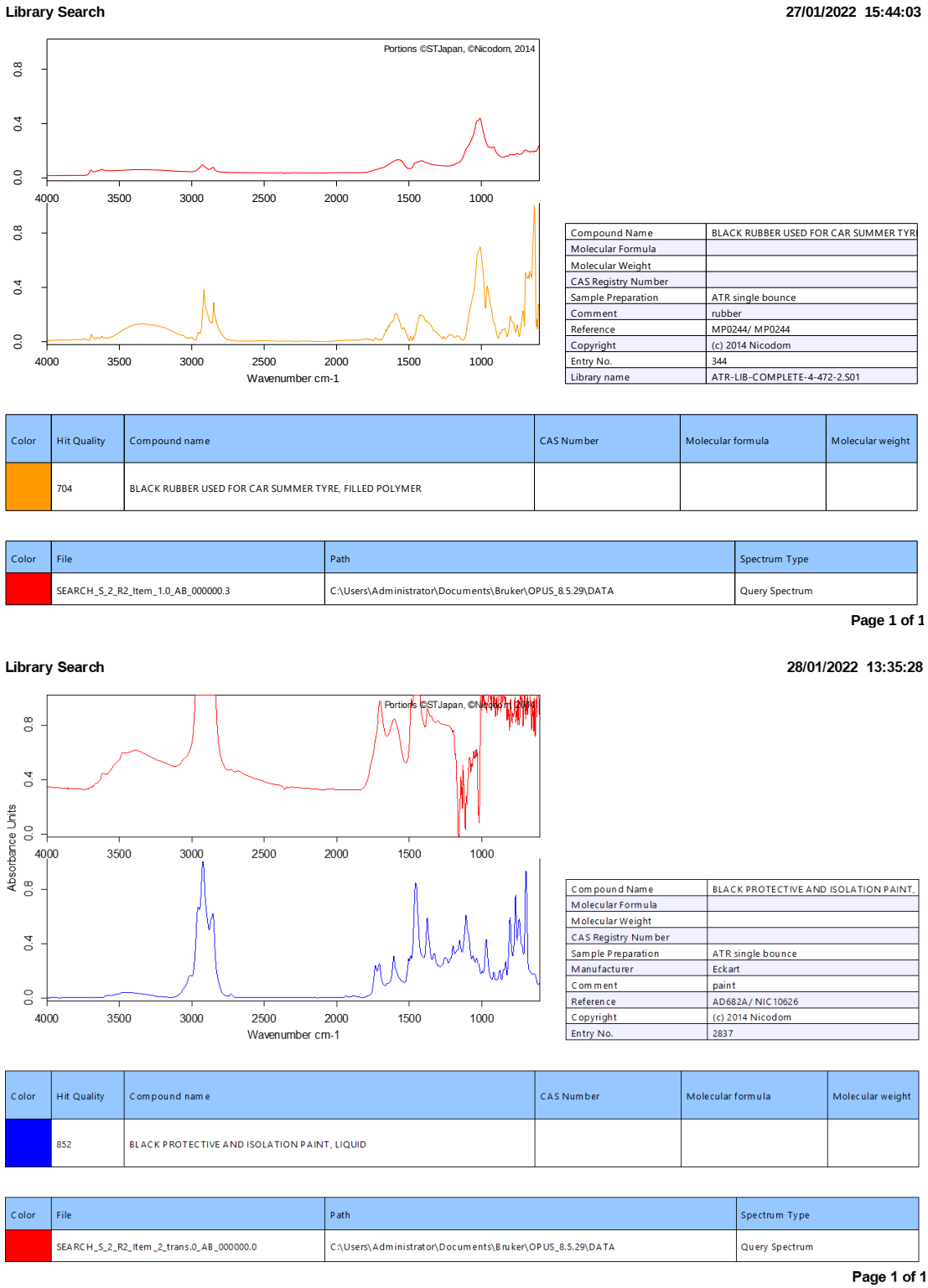


Figure A6. Examples of micro-FTIR spectra for items extracted from sediment 2.

Table A2. Raw data for the analysis of microplastics from the laboratory negative control using Nile red tagging of polymers coupled with automatic counting and micro-FTIR.

Sample ID	Output from automated count	
	Smaller setting (min ~ 20 µm)	Large settings (> 100 µm)
Negative control_1	0 (< LOD)	0 (< LOD)
R_1	22	8
R_2	14	7
R_3	12	4
R_1_control corrected	22	8
R_2_control corrected	14	7
R_3_control corrected	12	4
Mean	16.0	6.3
SD	5.3	2.1
Mean number of particles per kg dry weight sediment	3200	1267
SD	1058	416
Min	2400	800
Max	4400	1600

A.2 Raw data - Analysis using Focal plane array (FPA) detector-based micro-Fourier-transform infrared imaging

A.2.1 Laboratory negative control

Table A3. Raw data for the analysis of microplastics from the laboratory negative control using Focal plane array (FPA) detector-based micro-Fourier-transform infrared imaging.

Sample ID	Number of microplastics	Natural Items	Major dim (mm)	Minor dim (mm)	Mass (ng)
FPA control	0	animal furs/natural polyamides	112.3	81.7	270.62
		animal furs/natural polyamides	65.7	27.9	18.49

Sample ID	Number of microplastics	Natural Items	Major dim (mm)	Minor dim (mm)	Mass (ng)
		animal furs/natural polyamides	91.2	46.9	72.54
		animal furs/natural polyamides	52.9	34.7	22.97
		animal furs/natural polyamides	87.6	41.8	55.46
		animal furs/natural polyamides	83.9	58.3	103.01
		animal furs/natural polyamides	52.9	46.2	40.84
		cellulose/plant fibres	160.5	45.7	163.28

A.1.2 Sediment 1: + 21004290, TERSLG50 (Terschelling 50 km from the coast), 1979a

Table A.4 Raw data for the analysis of microplastics from sediment 1 using Focal plane array (FPA) detector-based micro-Fourier-transform infrared imaging (in green: particles above 100 μm in size).

Sample ID	Filter number	Weight dried sediment (g)	Total number of microplastics [20-5000 μm)	Total number of microplastics [100-5000 μm)	Polymer type	Major dim (mm)	Minor dim (mm)	Mass (ng)
Sediment_1	Filter_1	10	20	10	Polystyrene	83.9	43.7	51.90
					Polystyrene	184.4	109.4	714.34
					Polystyrene	219.7	133.5	1268.05
					Polyamide	296	130.1	1727.72
					Acrylates/polyurethanes/varnish	70.9	60.3	100.60
					Polyethylene chlorinated	91.2	60.3	120.96
					Polyethylene chlorinated	91.2	46.9	73.17
					Polyethylene chlorinated	131.6	177.1	1817.62
					Polyethylene	100.9	66.6	133.73
					Polyethylene	70.9	43.1	39.32
					Polypropylene	52.9	34.7	18.98
					Polypropylene	119.9	61.2	133.95
					Polypropylene	100.9	60.6	110.52
					Polypropylene	100.9	24.2	17.68

Sample ID	Filter number	Weight dried sediment (g)	Total number of microplastics [20-5000 µm)	Total number of microplastics [100-5000 µm)	Polymer type	Major dim (mm)	Minor dim (mm)	Mass (ng)
					Polypropylene	131.5	37.2	54.30
					Polypropylene	445.4	97.5	1262.43
					Polypropylene	176.9	38	76.31
					Polypropylene	70.9	34.5	25.17
					Polypropylene	27.9	21.9	4.00
					Polypropylene	70.9	34.5	25.17
	Filter_2	10	1	0				
Total			21	10				
Number of particles per kg dry weight sediment			**2100**	**1000**				
			Total number of natural items		Particle type	Major dimension (mm)	Minor dimension (mm)	Mass (ng)
			31		Animal furs/natural polyamides	112.3	38.1	58.93548
					Animal furs/natural polyamides	168.9	105	672.2903
					Cellulose/plant fibres			

A.1.3 Sediment 2: + 21004294, BOCHTVWTM (Bocht van Watum, in the Ems-Dollard estuary), 1839c

Table A5. Raw data for the analysis of microplastics from sediment 2 using Focal plane array (FPA) detector-based micro-Fourier-transform infrared imaging (in green: particles above 100 μm in size).

Sample ID	Filter number	Weight dried sediment (g)	Total number of microplastics [20-5000 μm)	Total number of microplastics [100-5000 μm)	Polymer type	Major dim (mm)	Minor dim (mm)	Mass (ng)
Sediment_2	Filter_1	10	34	8	acrylates/polyurethanes/varnish	52.9	34.7	24.77
					acrylates/polyurethanes/varnish	70.9	60.3	100.60
					polyethylene oxidized	176.9	72.6	272.27
					polyethylene	52.9	46.2	33.74
					polyethylene chlorinated	70.9	43.1	48.02
					polyethylene chlorinated	114.9	53.2	118.56
					polyethylene chlorinated	119.9	45.9	92.00
					polyethylene chlorinated	87.6	48.8	76.14
					polyethylene chlorinated	70.9	25.9	17.29
					polyethylene chlorinated	43.8	27.9	12.43
					polyethylene chlorinated	168.9	90.5	503.97
					polyethylene chlorinated	314.8	196.2	4413.94
					polypropylene	91.2	53.6	78.27
					polypropylene	91.2	60.3	99.06
					polypropylene	65.7	27.9	15.27
					polypropylene	52.9	46.2	33.74
					polypropylene	70.9	43.1	39.32441
					polypropylene	70.9	34.5	25.16762
					polypropylene	65.7	27.9	15.27077
					polypropylene	112.3	54.5	99.35885
					polypropylene	100.9	54.5	89.52477
					polypropylene	27.9	21.9	3.99788

Sample ID	Filter number	Weight dried sediment (g)	Total number of microplastics [20-5000 µm)	Total number of microplastics [100-5000 µm)	Polymer type	Major dim (mm)	Minor dim (mm)	Mass (ng)
					polypropylene	70.9	25.9	14.15679
					polypropylene	52.9	46.2	33.73526
					polypropylene	27.9	21.9	3.99788
					polypropylene	83.9	36.4	33.23988
					polypropylene	52.9	34.7	18.97608
					polypropylene	43.8	27.9	10.18052
					polypropylene	52.9	34.7	18.97608
					polypropylene	43.8	27.9	10.18052
					polypropylene	27.9	21.9	3.99788
					polypropylene	83.9	58.3	85.09409
					polypropylene	70.9	25.9	14.15679
					polypropylene	119.9	71.4	182.322
	Filter_2	10	13	6	Rubber type 3	160.5	68.6	260.719
					polyethylene oxidized	83.9	36.4	32.54009
					polypropylene	70.9	43.1	39.32441
					polypropylene	301.6	95.3	816.8831
					polypropylene	100.9	48.5	70.73562
					polypropylene	112.3	65.3	143.0767
					polypropylene	155.2	55.1	140.8618
					polypropylene	52.9	34.7	18.97608
					polypropylene	70.9	25.9	14.15679
					polypropylene	52.9	34.7	18.97608
					polypropylene	43.8	27.9	10.18052
					polypropylene	112.3	49	80.48066
					polypropylene	91.2	46.9	59.92456
Total			47	14				
Number of particles per kg dry			**4700**	**1400**				

Sample ID	Filter number	Weight dried sediment (g)	Total number of microplastics [20-5000 µm)	Total number of microplastics [100-5000 µm)	Polymer type	Major dim (mm)	Minor dim (mm)	Mass (ng)
weight sediment								
			Total number of natural items		Particle type	Major dimension (mm)	Minor dimension (mm)	Mass (ng)
	Filter_1		11		cellulose/plant fibres	3841.1	91.2	127.70
					cellulose/plant fibres	6721.9	155.2	229.83
					quartz	119.9	76.5	482.49
					quartz	114.9	69.2	378.27
					quartz	112.3	70.8	387.09
					quartz	91.2	67	281.92
					quartz	119.9	81.6	548.96
					quartz	112.3	76.2	448.93
					quartz	109.6	55.8	234.69
					quartz	43.8	27.9	23.47
					quartz	114.9	53.2	223.83
	Filter_2		4		coal	360.6	74.6	523.13269
					quartz	197.9	101.9	1414.96675
					quartz	91.2	67	281.92222
					quartz	311.3	100.1	2148.13475

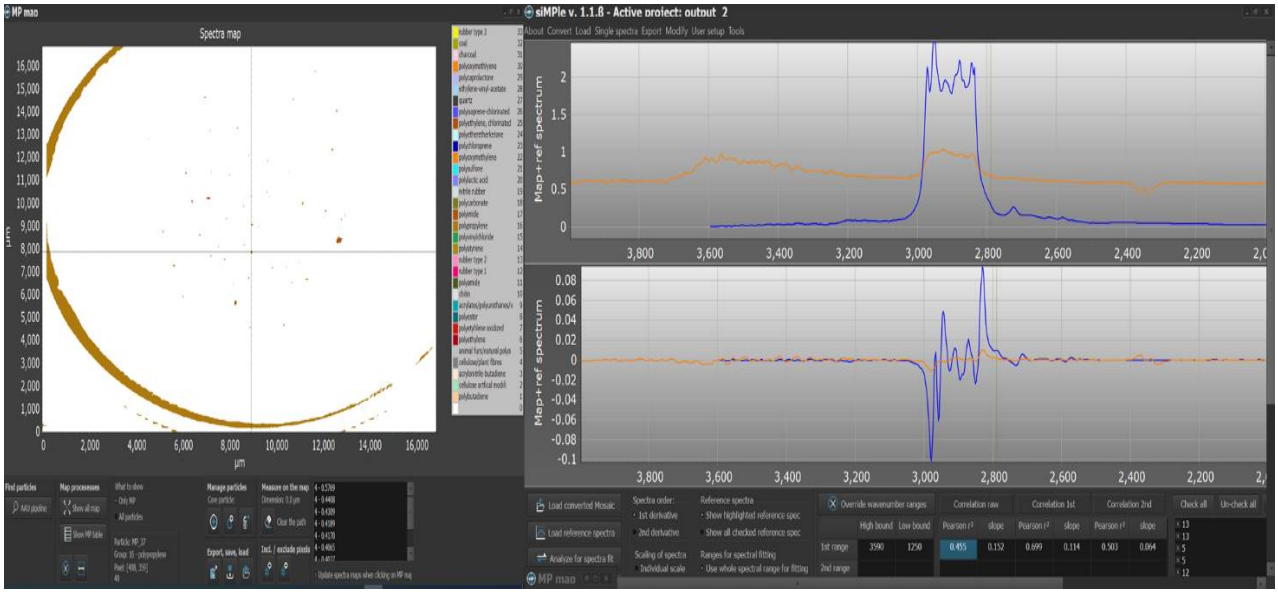


Figure A7. Examples of micro-FTIR FPA spectra for items extracted from sediment 2, in this case, PP.

A.3 Particle Size Analysis

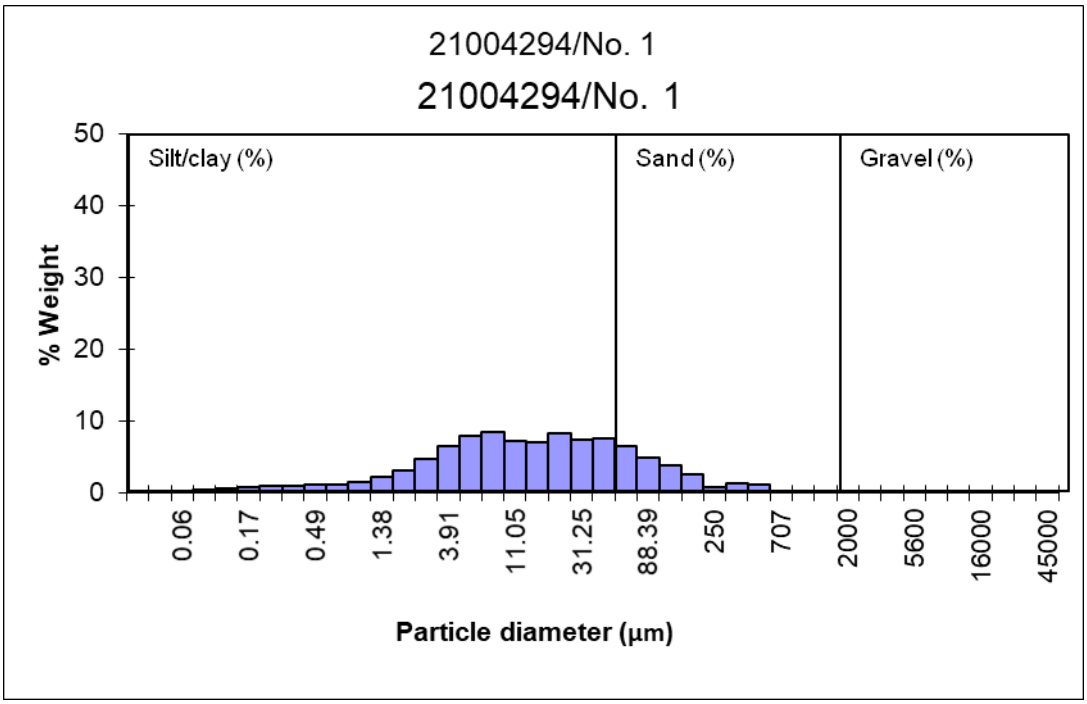
Table A6. Visual description of the samples investigated (n=2).

Sample ID			
21004294	No.1	BOCHTUWTDA	Pale brown, sandy (micaeous) mud.
21004290	No.2	TEASLG50	Pale orangey brown, muddy sand (micaeous) containing broken shell fragments.

Table A8. PSA statistics of the sediments investigated (n=2).

Sample ID	Gravel (%)	Sand (%)	Silt/Clay (%)	Very coarse and coarse sand (%)	Medium sand (%)	Fine sand and very fine sand (%)	Folk symbol	EUNIS group
21004294/ No.1	0.05	21.63	78.32	1.41	2.20	18.03	sM	mud and sandy mud
21004290/ No.2	0.99	62.89	36.11	2.16	3.66	57.07	mS	mud and sandy mud

Folk symbol and EUNIS classification (Long, D. ,2006. BGS detailed explanation of seabed sediment modified Folk classification)
http://www.searchmesh.net/PDF/GMHM3_Detailed_explanation_of_seabed_sediment_classification.pdf



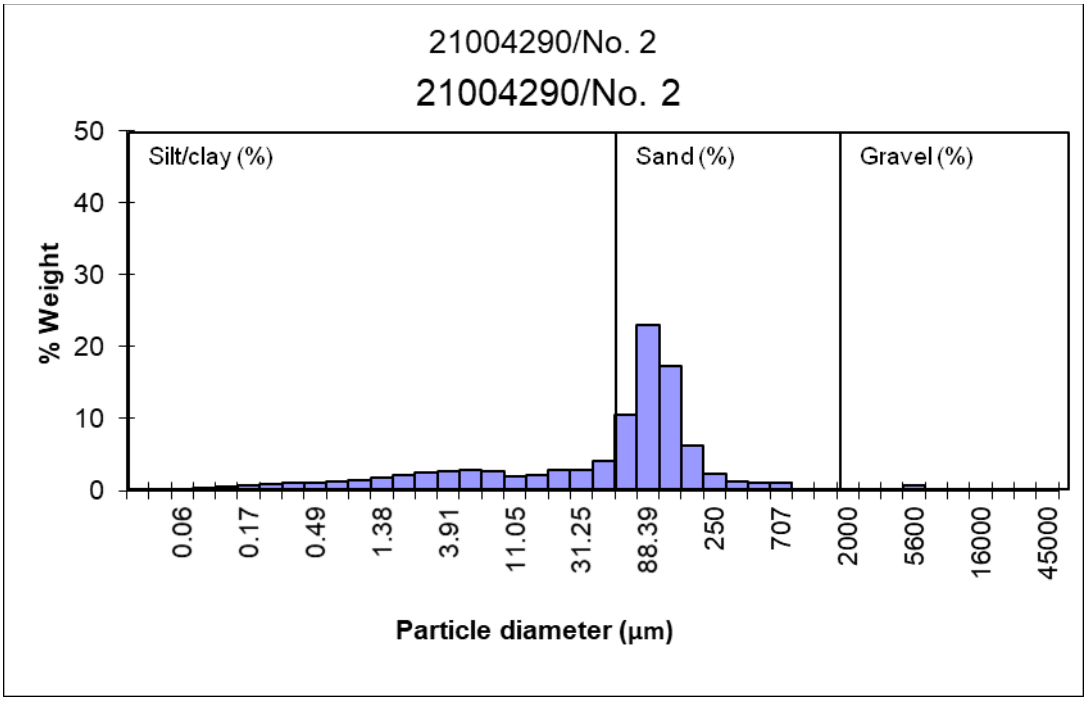


Figure A9. Particle Size (PS) histograms for sediments 1 & 2.

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Pakefield Road, Lowestoft, Suffolk, NILE RED33 0HT

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