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Pilot sediment microplastics determination from Sweden's contaminant monitoring stations 2020

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Summary

With the aim to determine if measurement of microplastics in offshore sediments can feasibly be employed in routine national monitoring, offshore sediment samples from Sweden's 16 contaminant monitoring sites were prepared and analysed. A repeatable workflow based on current best practice and harmonisation guidelines is presented and evaluated. The influence of the metric applied for data normalisation is highlighted. The results can be considered a baseline for comparison with future measurements and other European sea areas.

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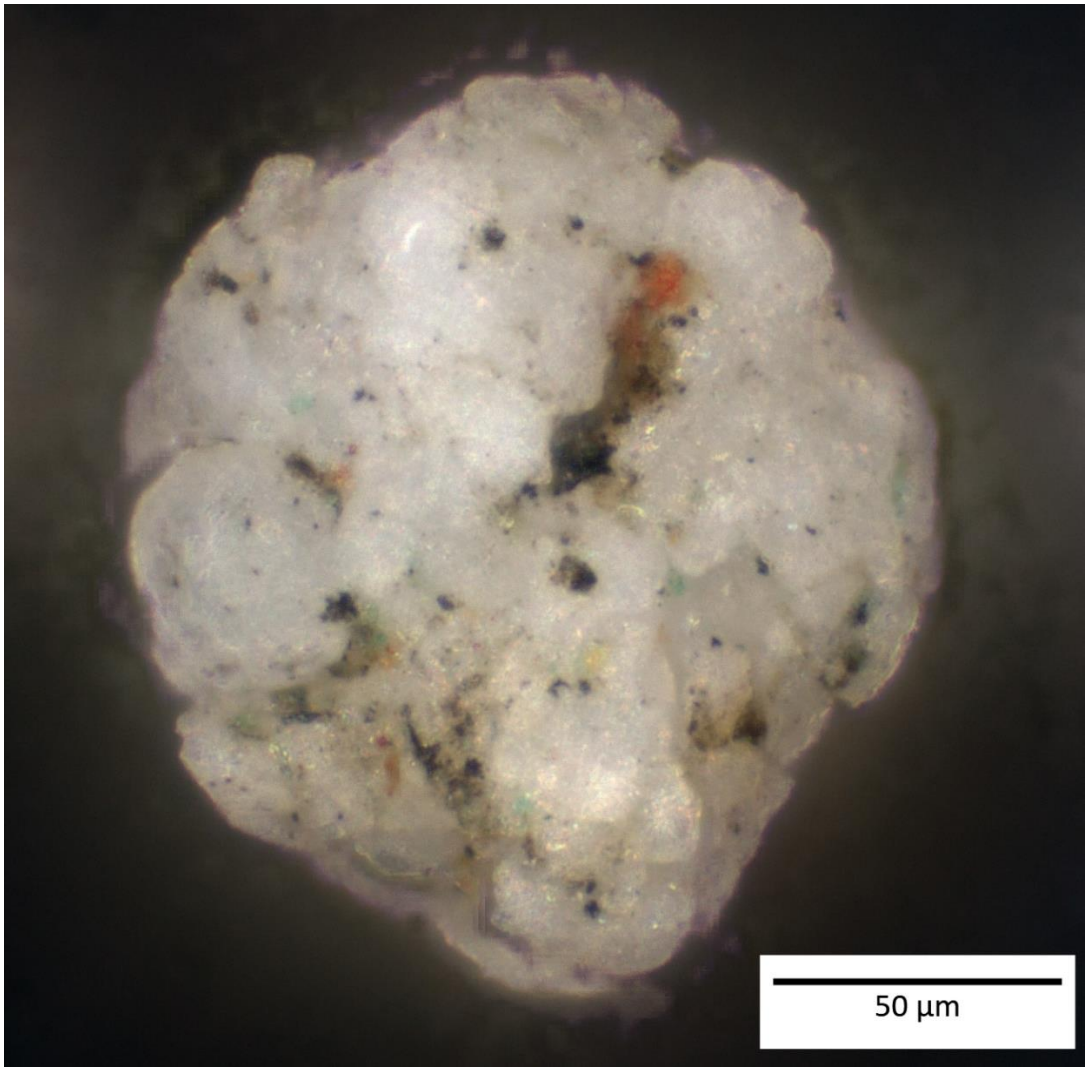

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Background

Since 2003, there has been a national environmental monitoring programme for hazardous substances in offshore sediments around Sweden's coast. Stations are revisited every 5-6 years and samples measured for a suite of prioritized substances. The sampling is done at 16 stations distributed in each of the major basins of the larger Baltic Sea and Eastern North Sea basins in the Swedish EEZ (**Figure 1**). During the 2020 survey, samples were taken for the first time to analyse microplastics. A case study evaluating methodologies and challenges was presented at the December 2024 OSPAR Microplastic Expert Group (MPEG) meeting.

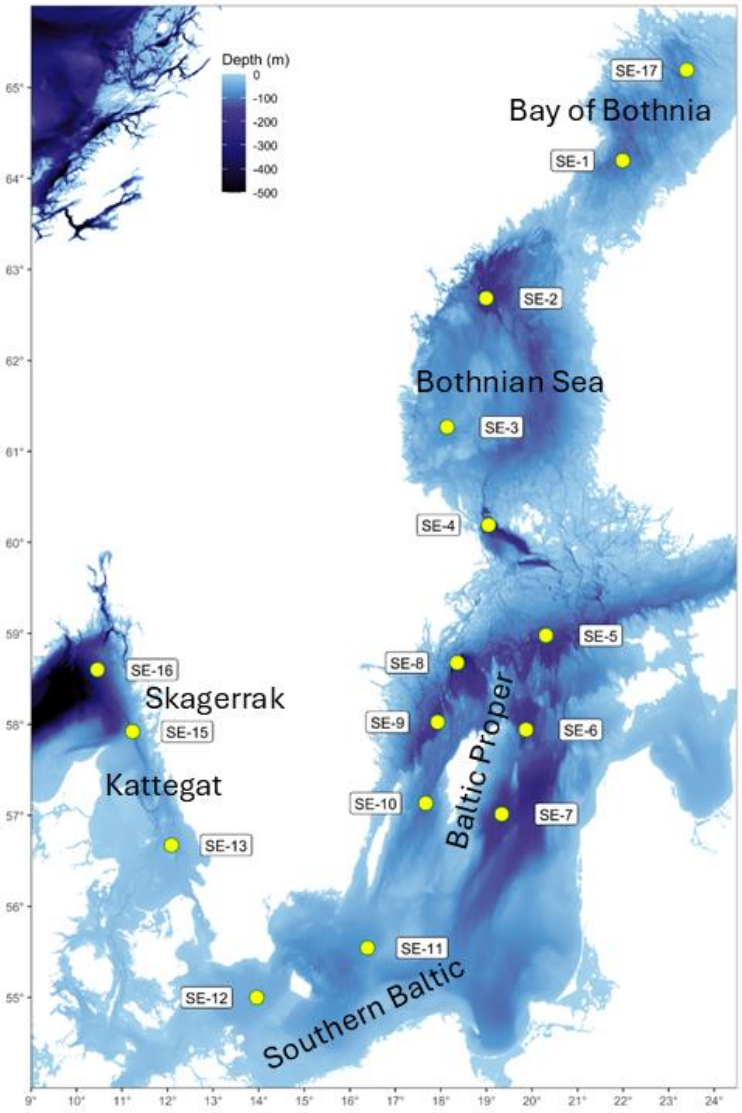

Figure 1. *Sweden's contaminant monitoring stations (with EMODnet bathymetry data).*

Purpose

The overall aim is to evaluate if measurements of microplastics in offshore sediments, which is a formal commitment by Sweden to the EU Marine Strategy Framework Directive (Descriptor 10, Criteria D10C2), is feasible and appropriate to be employed in the routine monitoring program to meet demands according to the national marine environmental legislation. The task within this assignment was to prepare the samples, analyse the microplastics abundance and compile a report.

1. Introduction

Microplastics are a morphologically and chemically complex form of pollution, whose interaction with natural systems is poorly understood. Micro-litter, which is mainly composed of microplastics, is a criteria for the assessment of environmental status within the Marine Strategy Framework Directive (MSFD). The directive confers responsibility to ensure micro-litter abundance is at levels that do not cause harm.

Seafloor sediment is considered an appropriate matrix for monitoring marine microplastics because it integrates temporal fluctuations that influence concentrations in surface water or biota (Fischer et al., 2022). However, the maturity of agreed methodology for seafloor micro-litter analysis is classified as intermediate by the MSFD technical group on marine litter (TGML) (Galgani et al., 2023), highlighting the need for real-world trials and experience-based development. The current pilot study documents method development, experiences, and baseline results for offshore Baltic and Eastern North Sea sub basin sediments.

Due to their diverse forms and weathering effects, identifying microplastics among natural particles carries analytical challenges. Spectroscopy permits reliable detection and chemical identification of a wide range of plastic particle types, including cryptic forms not repeatably detectable by visual methods. At the same time, it presents new challenges associated with automation, and management and extraction of data from large, information-rich, spectral datasets. Adequate spectral libraires, reflecting the diversity of environmental microplastic spectra, have not yet been compiled, and burdensome data validation is required to understand and detect emerging library-matching limitations and artefacts. Despite development challenges, spectroscopy presents the most promising state of the art for repeatable, reliable and cost-effective quantification and identification of environmental microplastic particles. The

resolving power of this technique is further increased in correlation with light microscopy, enabling additional validation and characterisation of particle form.

2. Materials and methods

2.1 Sediment sample collection

QA/QC measures:

- Field blanks exposed during sample collection.
- Documentation of operator clothing material and colour.
- Glass jars were used for sample storage.

Cores were collected at all 16 Swedish contaminant monitoring sites using a GEMAX sampler. Sediment samples comprised of the upper 2 cm layer, from 4 to 5 cores, were stored in 1L capacity jars.

2.2 Sediment sample extraction

QA/QC measures:

- Only cotton outer cloths and a cotton lab coat were worn.
- Laboratory benched were wiped before commencing work with a cotton cloth and laboratory floors mopped weekly with a cotton mop.
- Human traffic into the laboratory was minimised.
- Glassware and other laboratory equipment was prewashed with detergent (1% Helmanex III), before rinsing with 0.2 µm filtered Milli-Q water.
- Sample contact with plastic items was kept to a minimum.
- Because of the volumes involved, zinc chloride solution was filtered through tiered polypropylene water filter cartridges at (10, 5 and 1 µm).
- All other solutions, chemicals and Milli-Q water were filtered at 0.2 µm.

The sample jar was photographed and weighed, and the outside surfaces washed with Milli-Q water. The sample was homogenised by stirring and a 10 to 20 mL aliquot removed for dry-wet weight measurement. Wet-dry weight aliquots were weighed before and after drying at 105 °C for a minimum of 24 hours, and until no further decrease in weight was recorded.

A digestive solution of 3% potassium hydroxide with 5% sodium hypochlorite in Milli-Q water was made up to twice the sample volume. This solution was used to rinse the sample from its jar into a 2 L glass conical flask, which was covered with prewashed aluminium foil and agitated for one hour. The digest was spun in a centrifuge, the supernatant poured through a 26 µm steel mesh mounted in the bottom of a sight chamber, to retain buoyant microplastics, and the pellet resuspended in Milli-Q water. The procedure was repeated twice more to thoroughly rinse the sediment.

The sediment was transferred into the Kristineberg Microplastic Sediment Separator (KMSS), which is a stirred, heavy density liquid buoyancy extraction tower, improved from the MPSS design of Imhof (2012). The KMSS, originally used in the HARMIC study (Setälä et al., 2019), was here improved from the first version (Mattsson et al., 2022), in the following ways: connections were changed from threaded to Tri-Clamp food industry connectors to minimise particle trapping and the upper chamber above the ball valve was replaced with a sight chamber, allowing visual inspection of extracted liquids. The same sight chamber and 26 µm steel mesh through which the supernatant was strained are inverted and clamped above the ball valve. The column is gravity filled to just below the 26 µm steel mesh with 1.8 cm^3 zinc chloride solution. After a 3-hour stirred period, the column is allowed to stand undisturbed for 21-hours.

The upper sight chamber is then isolated using the ball valve. The upper chamber together with ball valve is removed, inverted, and drained through the 26 µm steel mesh. Zinc chloride is rinsed from the mesh with 100% ethanol, then Milli-Q water. Material retained on the 26 µm steel mesh is subject to 30 seconds 7% hydrogen peroxide digestion, before rinsing with Milli-Q water followed by 100% ethanol. The sight chamber is refilled with approximately 200 ml of zinc chloride solution from below (through the steel mesh) and the chamber covered and subjected to agitation for one hour to free particles adhering to the steel mesh. A second sight chamber is mounted above the ball valve forming a new column, and the apparatus filled with zinc chloride solution fed from below. The column is settled for 12 hours. Steel 300 and 91 µm meshes are mounted on top of the column, and two 30 µm platinum coated filters are mounted downstream in parallel. The entire column is inverted to allow zinc chloride and suspended particles to drain through the meshes and filters. Zinc chloride is rinsed away with 100% ethanol followed by Milli-Q water. Loaded meshes and filters were stored covered at room temperature away from direct sunlight.

Before analysis, particles were transferred from the 91 µm meshes using a three-tiered protocol of increasingly aggressive rinsing, to minimise particle damage. Inside a ventilated cabinet, but without airflow, each steel mesh was clamped between two steel sight chambers, with the lower open end capped with a steel plate drained with a steel pipe, forming a semi-closed chamber. The first rinse was with Milli-Q water poured through the apparatus. Particles were collected

on one or multiple (depending on the amount of material) 47 mm diameter, 30 µm pore size, platinum-coated membranes (Mattsson et al, 2024), custom-made in collaboration with Chalmers University of Technology. Membranes were clamped in glass Buckner filtration apparatus with a 47 mm steel washer on top, which later served to weigh down the edges of the membrane during analysis. The inner diameter of the steel washers matched the active filtration region of the filtration apparatus. The second rinse was with Milli-Q water directed from a low-pressure (4 bar) solid stream steel nozzle. For the third step, the steel mesh was placed in a glass beaker with Milli-Q water for 20 seconds sonication, and the water passed through the platinum-coated membrane. Just before filtration was completed, particles were washed down from the inner wall of the filter funnel with 100% ethanol. This created microturbulence, which aided even distribution of particles over the filter surface. After rewetting via the membrane edges, the membrane together with the steel washer was slid onto a glass microscope slide and allowed to dry inside a closed glass petri dish.

2.3 Spectral library compilation

For this project, to ensure adequate matching of reference spectra against those of potentially weathered and fouled marine environmental microplastics, a new spectral library (Nordic micro-litter) was compiled based on marine microplastic spectra from Finnish, Danish and Swedish microplastics surveys, and with a proportion of spectra obtained from the same instrument/mode used to conduct the analyses (**Table 1**). Reference spectra were primarily compiled from averages of small groups (2-14 spectra) of alike spectral variants, selected within each polymer identity, as well as distinctive individual spectra. Spectra from natural marine sediment particles, pristine plastics, and laboratory sample contamination were also included. The Nordic micro-litter library was tested and corrected iteratively (section 2.6).

Contributing Institute	**Materials characterised**	**Polymers included**	**Instrument/method**	**Code in Nordic Micro-litter library**
GU Sweden	Micro-particles abraded from identifiable weathered marine macro plastics.	PE, PET, PP, PS	Nicolet iN10 Infrared Microscope Mode: Reflectance	W
GU Sweden	Beach micro-litter	PE (5 variants from fluff, irregular, and flat brittle black particles), PMMA, PP (course filaments and flat brittle particles), PS (non-expanded and expanded), PVC, Suspected paraffin (semisolid particles). Also glass (suspected road paint beads).	Nicolet iN10 Infrared Microscope Mode: Reflectance	S

GU Sweden	Natural marine materials and pristine reference materials	Ref. ABS, ref. EPS, ref. HDPE, ref. LDPE, LDPE slice, PET, PLA, PMMA, PHB biopolymer, PLA biopolymer, POM, PP copolymer, PP slice, candle, multiwell and petri dish PS, road paint (white), cockle shell, amphipod exoskeleton, glass cover slip, glass bead from road paint.	Nicolet iN10 Infrared Microscope Mode: Reflectance	K
GU Sweden	Spectra from marine sediment samples under analysis: blank contamination and specific natural spectra found to capture synthetic spectra during library matching.	PS, PP, diatom frustules, a PE-like spectra associated with cellular plant remains. An experimental addition. Their effectiveness at distinguishing contamination and natural particles is under assessment.	Nicolet iN10 Infrared Microscope Mode: Reflectance	C
AU Denmark, kindly provided by Jacob Strand	Marine micro-litter	Cellulose, PA, PC, PE, PET, PMMA, Protein, PS, PU, PVC.	Mode: Reflectance and transmittance	D
Syke Finland, kindly provided by Markus Sillanpää	Marine micro-litter	PP, PS and PE	Perkin Elmer Spotlight 200i Mode: Reflectance	F
Syke Finland, kindly provided by Markus Sillanpää	Marine micro-litter	PP, PE, PS	Mode: Transmittance	T

Table 1. *Composition of the Nordic Micro-litter spectral library, compiled within this study to aid adequate library matching of environmentally weathered spectra and common natural marine sediment particles.*

2.4 Automated FT-IR analysis

QA/QC measures:

- The laboratory floor was wet mopped monthly. Bench surfaces and screens were wiped with a damp cotton cloth weekly.
- FT-IR stage enclosed with foil to reduce ambient contamination.
- Before analysis of each filter membrane, the microscope stage was wiped with a damp cotton cloth to remove dust.
- Full procedural blank filters were analysed under the same conditions as sediment filters.

Sample analysis was conducted using a Nicolet iN10MX infrared microscope controlled by the open-source software, GEPARD Enabled PARticle Detection (GEPARD), developed by Brandt et al., (2020)

(https://gitlab.ipfdd.de/GEPARD/gepard/-/tree/Gepard_GU). GEPARD incorporates both microscope control and spectra evaluation and validation and allows harmonised acquisition between several different Raman and FT-IR microscope brands. For this project, it was further developed to interface with the Nicolet iN10MX. During the analysis stage, GEPARD enables acquisition of a stacked focal plane, optical mosaic of the filter membrane, then detects particle outlines (**Figure 2**), and uses this information to steer the spectroscopic measurement. The Nicolet iN10MX was modified by enclosure of the stage alcove with aluminium foil to reduce airborne contamination.

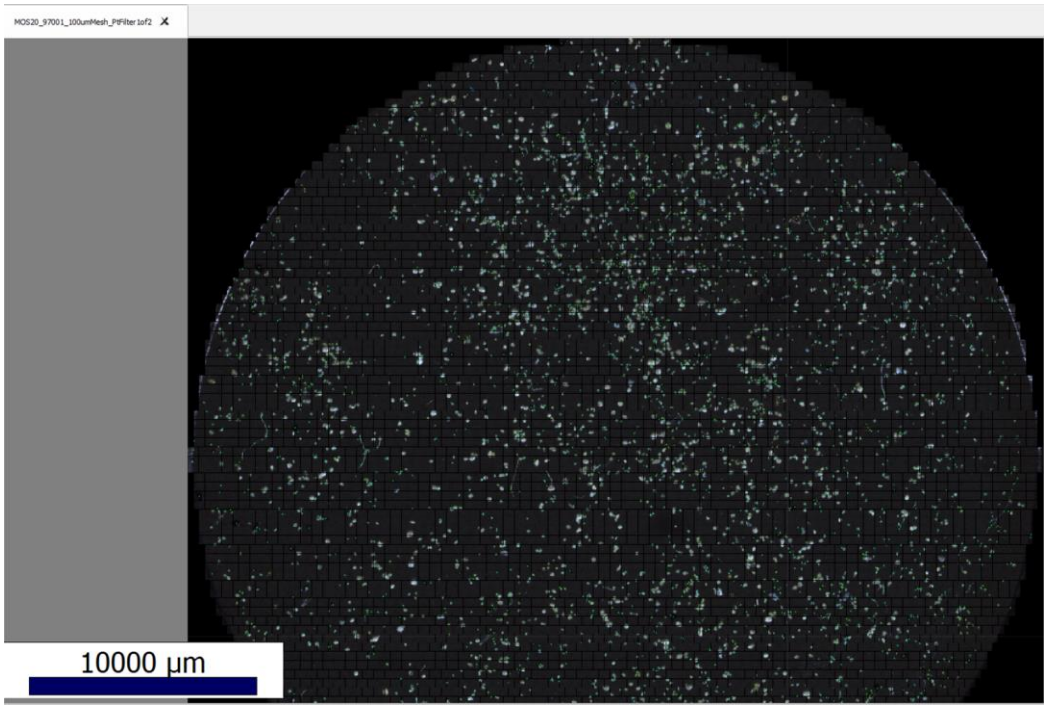

Figure 2. *Optical mosaic of a 47 mm-diameter, platinum-coated filter membrane, used to guide automated FT-IR analysis.*

The condenser was optimised, and the interferometer aligned. Membranes were transferred to the Nicolet iN10MX stage for analysis within glass petri dishes. After removing the petri dish lid, the membrane surface was mapped by measurement of seventy x, y, z coordinates. The optical mosaic (**Figure 2**) was obtained using darkfield illumination. For each tile, a focal depth of around 110 µm was used (up to 140 µm when taller particles called for a greater height range), combining 6 to 10 focal planes. Fibres and dark coloured or black particles were manually marked on the mosaic, to ensure their full outline was detected by the by the neural net algorithm, before running the particle detection. Mapped particles from each membrane were analysed in batches of around three thousand to avoid the need to top up the microscope liquid nitrogen reservoir during analysis. Spectra were acquired at high resolution, over 5 seconds, in reflectance mode, with a cooled mercury cadmium telluride (MCT) detector,

enabling absorbance to be measured over a wavenumber range of 4000 to 675 cm^{-1}.

2.5 Library matching and spectral evaluation in GEPARD

Within the scope of this project, substantial software development was undertaken to enable the spectral evaluation part of GEPARD (GEPARD EVALUATION) to efficiently manage very large datasets associated with sediment particle analysis.

Spectra are temporarily processed to improve their quality during library matching. Savitzky-Golay filtering, and baseline subtraction are applied, and the C02 region removed. Library matching for the current suite of samples was performed against three libraries: 1) Nordic micro-litter (134 spectra), 2) Standard polymers (a small library of 16 in-house reference polymers), and 3) a reduced version (146 spectra) of the SiMPle database (Primpke et al., 2020).

GEPARD enables reference spectra from multiple libraries, including the diverse spectral variants of each polymer species, to be compiled into user-defined groups by polymer affinity (polymer-group), e.g., polyethylene (PE), polypropylene (PP) or polyethylene terephthalate (PET).

For validation, spectra can be visually evaluated by polymer-group, by viewing multiple sample spectra per screen in tiled view, overlayed with one or more of the most closely matching reference spectra. The quality of the match to each library reference is scored from 1 to 100 in the hit quality index (HQI).

2.6 Manual spectral data validation

To identify an optimal HQI lower cutoff that would minimise under or overestimation of valid records, preliminary visual validation of spectra was performed. The HQI at which suggestions became unreliable, i.e. where false positives outweighed valid records, differed between polymer groups. Based on this assessment, polymer-specific HQI cutoffs of 50 for polystyrene (PS) and PET, and 70 for PE and PP were applied. These cutoffs were further assessed by visual validation of PE and PP suggestions within the HQI ranges 70-74.9 and 75-79.9, and PS and PET suggestions within the HQI range 50-79.9 (**Table 2**). For PP, PS and PET, this was performed for 25 % of filters, and 75 % of filters for PE. PE suggestions were additionally evaluated between HQI ranges 80-89.9 and 90-100 for 25% of filters. For all other (scarcer) polymer types, 100 % of library suggestions with a HQI >50 were visually validated. During ground-truthing of spectra against library suggestions, artefacts resulting from capture of incorrect spectral groups by particular library reference spectra, or from natural

materials with spectral forms resembling synthetic polymers, were corrected (**Appendix 4**), either by: 1) simple record-reassignment, 2) removal of problem reference spectra, 3)addition of problem natural marine spectra, or 4) re-averaging of poor or noisy environmental spectra within the Nordic Micro-litter library. Corrective measures were followed by iterative re-matching and visual validation of spectra. At this stage, paraffin was distinguished from PE, based on validated matching to multiple averages of environmental semisolid micro-particle spectra within the Nordic micro-litter library. Paraffin records were excluded, as the category of pollution to which they belong is under discussion.

Polymer	HQI range	% of samples for which library suggestions were ground-truthed	Mean proportion of valid suggestions
PE	70-74.9	75	66
	75-79.9	75	75
	80-90	25	83
	90-100	25	100
PP	70-74.9	25	92
	75-79.9	25	100
PS	50-79.9	25	100
PET	50-79.9	25	100
Other	50-100	100	-

Table 2. *Summary of the mean proportion of valid records as a percentage of all library suggestions within defined HQI ranges.*

2.7 Manual correction of particle selections

Once a set of valid microplastic particles was established, library suggestions were checked against the optical mosaic and occasional multiple particle selections of single particles (**Figure 3**) were corrected by deleting subordinate selections.

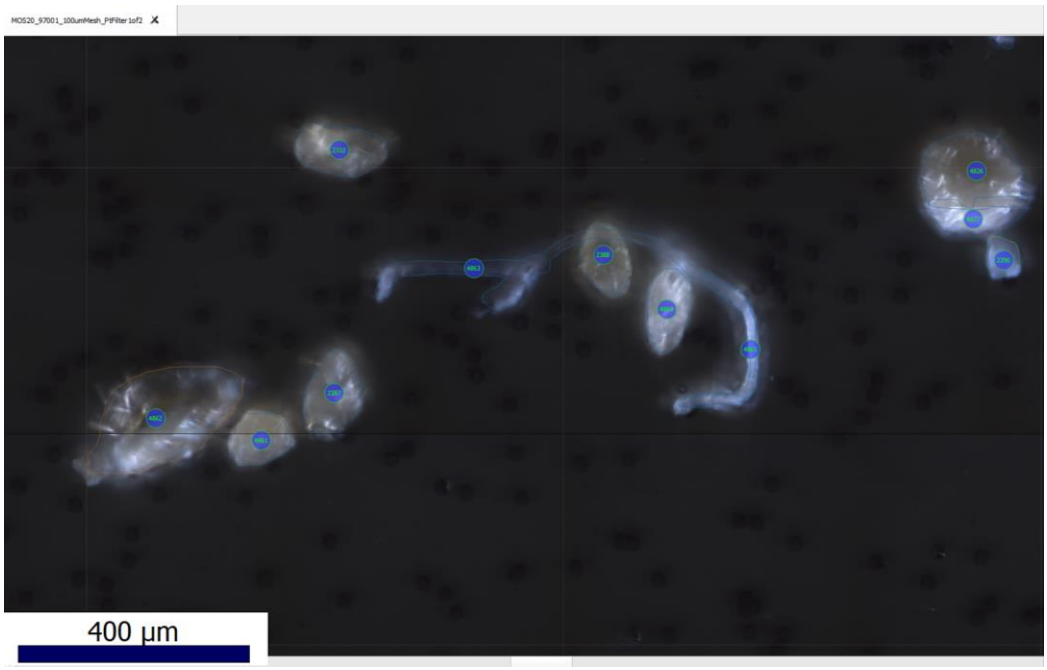

Figure 3. *Particle outlines detected by the neural net algorithm in GEPARD including examples of single particles selected multiple times (the central fibre and larger particle at the right side of the screen), requiring manual correction. Black spots in the background are 30 µm diameter filter pores.*

2.8 Imaging of individual microplastics

To provide parallel ground-truthing, and document particle form and colour, a Zeiss Axio Imager M2M light microscope and Zeiss Zen software were used to take stacked-focal-plane images of >20% of detected MPs, including examples from each polymer group.

2.9 Blanks and normalisation of sample results

The average counts from the four full procedural blanks are presented here alongside the raw sample results (**Table 3**). Within this study, blank counts have not been subtracted from sample counts. Sample results are also presented after normalisation with two different metrics: 1) microplastics per kilogram of dry sediment (**Table 4**), and 2) accumulation rate (microplastics per square metre per year) (**Table 5**).

2.10 Data storage and accessibility

Once finalised, it is planned that data will be uploaded as one of several test cases of the harmonised ICES DOME - EMODnet Chemistry dataflow, initiated following the workshop on revision of the DOME litter data format (WKLIDA) (ICES, 2023). As previously for data from commissioned Swedish screening studies, utilising the DomeConverter script developed by GU for SNV.

2.11 Limit of detection

Quantifying the limit of detection (LOD) as the lowest level that can be determined to be statistically different from an analytical blank, has been adopted as standard by the International Union of Pure and Applied Chemistry (IUPAC) for sediment contaminant analysis. In general, this is expressed as the blank mean plus 3x SD of a series of blanks (Wright et al., 2021). While sometimes applied to microplastics analysis, its validity depends on whether the particle counting distribution, characterized by Poisson statistics, can be approximated by a normal (Gaussian) distribution (Karlsson et al., 2020; Strand, 2023). As such, quantification of LOD for typical microplastics analysis is currently under discussion, e.g. by the OSPAR marine litter expert group (MPEG). Therefore, raw sample counts are presented here beside associated blank counts (Table 3).

2.12 Particle size limits

Wright et al. (2021) define the method limit of detection (MLD) as either the smallest mesh size used for sample collection, or the smallest size resolvable by an instrument. The OSPAR MPEG notes that mesh fractions cannot be directly compared to absolute instrument resolution, as meshes typically retain particles smaller than their nominal size while losing some larger particles (Strand, 2023).

This study analysed particles collected on two grades of square-weave steel mesh, the finer with 91 µm open gaps (measured in a horizontal plane) between the edges of woven steel strands of 50 µm diameter, and the larger with 300 µm gaps between 180 µm diameter steel strands. To measure particle length and width, GEPARD uses the OpenCV function Rotated Rectangle (which rotates and fits a rectangle of minimum area to the selection). Therefore, most particles with a longest dimension >91 µm should have been retained, along with some <91 µm, while irregularly shaped particles are likely to lead to appreciable deviation from the mesh capture efficiency. To include smaller particle selections, the absolute lower particle selection size limit in GEPARD was set at 50 µm to ensure inclusion of the dwindling number of particles below 91

microns, but also of larger particles, incompletely or inaccurately selected due to optical artifacts (e.g., shadows or light-flare from transparent materials). Such artifacts posed challenges for the neural net outline selection algorithm, presumably leading to relatively high particle size measurement error (compared to manual size measurement under a light microscope), and a higher proportion of <91 µm records than expected for a 91 µm mesh fraction. To determine the effective lower size limit, Bakir et al. (2024) proposed a lower length reporting limit (LLL), identified by fitting a power function to a histogram of size bins and determining the point where the function deviates from the data. In the current study, to avoid loss of incompletely selected particles, a LLL has not been applied to data presented in Tables 3 and 4, which can be considered a 91 µm mesh fraction. Particle counts in the 50–91 µm range should be considered underestimates of the true numbers in this size range.

The upper particle size limit was set at 5 mm (included polymer-groups between 1 and 5 mm, comprised just 13 microfibres).

3. Results

3.1 Raw sample and blank counts

Blank contamination associated with laboratory contamination was high for two polymers, PP and PS. In consequence, it was necessary to exclude PP from the presented results (**Tables 3** and **4**) (Appendix # shows PP and full PS blank counts). Based on visual analysis of PP particles from a single sample, environmental PP (that was weathered or visually distinct from PP in the blank samples) was estimated to comprise <10% of PE abundance for the same sample. Additionally, polyurethane (PU) fragments of a specific form and colour (flat, blue) were excluded from PU (fragment) results.

Pending additional spectral validation, modified cellulose records, including viscose, were also excluded, although they comprise a potentially important group and have been reported at high levels in wastewater and marine sediments (Zhang et al., 2019; Back, et al., 2024). Extraction of data for modified cellulose is complex, due in part to the abundance of suggested modified cellulose records, but also to spectral overlap with abundant natural cellulose, and currently inadequate spectral library resources relating to this group.

Comparison of sample and blank counts (**Table 3**) shows that two samples for PE (SE-2 and SE-9), and two samples for PET (SE-2 and SE-8), would fall below the limit of detection (LOD) (if calculated as blank mean + 3x blank SD).

However, such clear cut-offs are inappropriate for the low number counts of PET. As such, PET results for several samples should be considered close to the LOD and treated with appropriate caution. No LOD could be calculated for PU (fragments) or polyamide (PA), but their counts are close to blank levels in most samples. For PU (fragments), the colour and form of the contaminating particle (flat, black) in Blank 1 is distinct from those in the samples.

3.2 Results normalisation - the impact of metric choice

Expression of microplastic results per kg of dry sediment, is accepted as a standard metric for reporting microplastic contamination (Galgani et al., 2023), enabling comparison between surveys. However, as discussed in Josefsson (2022), the suitability of a normalisation method may depend on prevailing sediment conditions. In the Baltic Proper, where sediment water content is exceptionally high, influenced by reducing conditions and associated high total organic carbon, results normalised per kg of dry sediment appear disproportionately inflated (**Table 4**). For sediments with widely differing water content, alternative normalisation metrics can provide a more balanced inter-sample comparison. Microplastics per litre of wet sediment might more readily relate to ecotoxicological concentrations, experienced by an organism living in the sediment. Microplastics accumulation rate (microplastics per m^2/year[1]) (**Table 5**) might better highlight areas subject to polluting inputs. Contrasting results are obtained by these metrics. Expressed as accumulation rate, SE-12 to SE-16 (Southern Baltic, Kattegat-Skagerrak) and SE-4 to SE-5 (Baltic Proper-Gulf of Bothnia) appear subject to a higher rate of microplastic pollution, in this case influenced by sediment accumulation rate (**Table 5**).

The range of total sediment microplastic levels of 81-2425 per kg dry sediment measured in the current study, is comparable to ranges (all in this paragraph expressed per kg dry sediment) measured using similar methodologies from offshore sediments in the wider North Sea of 106-1189 by Lorenz et al. (2019), 617-2830 by Bakir et al. (2022), and Mediterranean of 62-931 by Saliu et al. (2023), but are lower than those measured from coastal North Sea sediments of 133-6933 by Bakir et al. (2023) or coastal Baltic sediments of 103-10179 by Chubarenko et al. (2022).

	Blank 1	Blank 2	Blank 3	Blank 4	Blank mean	SE 17	SE 1	SE 2	SE 3	SE 4	SE 5	SE 6	SE 8	SE 7	SE 9	SE 10	SE 11	SE 12	SE 13	SE 15	SE 16
ABS (fragments)							1														
ABS (pellet form)																				2	2
PA				1	0.25		2				1	3	1				2		1	8	
PE	3	9	2	7	5.25	43	25	4	72	49	31	17	26	46	8	23	24	25	29	69	43
PET		4		5	2.25	7	10	4	9	12	6	9	3	6	8	6	10	11	24	24	12
BPA-based PES						1				2											
PHB (fragments)									1		1							1	3		
Phenoxy resin									1									2			
PLA (fragments)										1											
PMMA (fragments)							1	2	1	1	4			14							
POM																1				4	
PS (fragments distinct from blank PS form)							1			1								1			
PS (solid <200 µm sphere. Not expanded)																		6	2	3	6
PU (fragments)	1				0.25		1		1	1	2	1				1	1	1		2	
PU (foamed open-cell)							1		2		5							2	2	1	
PVC (fragments)										1				2						4	
PVC (pellet form)																		4		2	1
Sum of microplastics counted	**4**	**13**	**2**	**13**		**51**	**42**	**10**	**87**	**68**	**50**	**30**	**30**	**68**	**16**	**31**	**37**	**53**	**61**	**119**	**64**
						Bothnian Bay		Bothnian Sea			Baltic Proper						Southern Baltic & Kattegat			Skagerrak	

Table 3. *Raw microplastic particle counts per blank and sediment sample. Cell shading from light to dark reflects low to high values within each row. BPA-based (bisphenol A-based) polyester records matched a poly(4 4' dipropoxy 2 2' diphenyl propane fumarate) reference. PP particles and transparent PS fragments are excluded from the results due to high blank counts resulting from laboratory contamination.*

3.3 Distinct morphologies within polymer-groups

As a second step, conducted after primary FT-IR screening for microplastics, optical stacked-focal-plane imaging of >20 % of identified particles, not only provided further validation, by detection of false-positive chemical identifications, but added substantial information about particle form, colour and surface texture. This enabled distinction of primary versus secondary microplastics, and solid versus foamed fragments; characteristics that can suggest sources of pollution.

Off Sweden's south and west coasts, SE-12 to SE-16 (Southern Baltic, Kattegat and Skagerrak) appear subject to pollution by visually distinct, compact polyvinyl chloride (PVC) and PE forms, and smooth, spherical (non-expanded) PS particles, while SE-15 and SE-16 are additionally subject to accumulation of pellet-like acrylonitrile butadiene styrene (ABS). PVC preproduction powders have previously been detected close to industrial point sources on Sweden's west coast (Karlsson et al., 2019), but were not expected in offshore sediments, especially given PVC's relatively high density. The origin of the distinctive transparent PS microspheres is uncertain and warrants further investigation.

In the Baltic, SE-1 to SE-7 (Gulf of Bothnia and Baltic Proper) appear subject to polymethyl methacrylate (PMMA) fragment accumulation. Green polyamide/nylon fibres, commonly attributed to shipping or fishing industries, were rare and only detected in the Baltic Proper.

Foamed PU fragments were detected at geographically distant sub-basins off both the west and east coasts of Sweden.

	Site name	SE 17	SE 1	SE 2	SE 3	SE 4	SE 5	SE 6	SE 8	SE 7	SE 9	SE 10	SE 11	SE 12	SE 13	SE 15	SE 16
	Sediment water content (%)	85	90	82	82	84	97	95	96	95	96	96	88	87	75	65	70
MPs per kg of dry sediment	ABS (fragments)		14														
	ABS (pellet form)															6	9
	PA		29				46	103	42				28		6	25	
	PE	383	357	32	485	526	1415	583	1092	1641	268	1127	341	367	179	216	200
	PET	62	143	32	61	129	274	309	126	214	268	294	142	161	148	75	56
	BPA-based PES	9				21											
	PHB (fragments)				7		46							15	18		
	Phenoxy resin				7									29			
	PLA (fragments)					11											
	PMMA (fragments)		14	16	7	11	183			499							
	POM											49				13	
	PS (fragments distinct from blank PS form)		14			11								15			
	PS (solid <200 µm sphere. Not expanded)													73	12	9	28
	PU (fragments)		14		7	11	91	34				49	14	15		6	
	PU (foamed open-cell)		14		13		228							29	12	3	
	PVC (fragments)					11				71						13	
	PVC (pellet form)													59		6	5
	Sum	**454**	**600**	**81**	**586**	**731**	**2282**	**1029**	**1260**	**2425**	**536**	**1519**	**526**	**762**	**376**	**373**	**297**
	Sea area	Bothnian Bay		Bothnian Sea			Baltic Proper						Southern Baltic & Kattegat			Skagerrak	

Table 4. *Results normalised as microplastics per kg of dry sediment. Cell shading from light to dark reflects low to high values within each row. Sediment water content is shown near the top of the table to highlight its influence.*

Site name		SE 17	SE 1	SE 2	SE 3	SE 4	SE 5	SE 6	SE 8	SE 7	SE 9	SE 10	SE 11	SE 12	SE 13	SE 15	SE 16
Sediment accumulation rate (years/cm)		5.6	6.3	1.5	2.7	2.4	1.0	1.1	1.0	13.0	22.0	21.0	2.8	1.8	1.6	1.6	0.7
MP accumulation rate m^{-2} $year^{-1}$	ABS (fragments)		1														
	ABS (pellet form)															8	20
	PA		2				8	23	7				6		5	33	
	PE	54	29	21	158	196	258	130	179	27	2	11	72	164	154	282	438
	PET	9	12	21	20	48	50	69	21	4	2	3	30	72	128	98	122
	BPA-based PES	1				8											
	PHB (fragments)				2		8							7	16		
	Phenoxy resin				2									13			
	PLA (fragments)					4											
	PMMA (fragments)		1	11	2	4	33			8							
	POM											0				16	
	PS (fragments distinct from blank PS form)		1			4								7			
	PS (solid <200 µm sphere. Not expanded)													33	11	12	61
	PU (fragments)		1		2	4	17	8				0	3	7		8	
	PU (foamed open-cell)		1		4		42							13	11	4	
	PVC (fragments)					4				1						16	
	PVC (pellet form)													26		8	10
	Sum	**64**	**49**	**53**	**190**	**273**	**417**	**229**	**207**	**40**	**5**	**14**	**111**	**341**	**324**	**486**	**652**
Sea area		Bothnian Bay		Bothnian Sea			Baltic Proper						Southern Baltic & Kattegat			Skagerrak	

Table 5. *Results normalised as microplastics accumulation rate (microplastics per m^2 per year). Cell shading from light to dark reflects low to high values within each row. Sediment accumulation rate is shown near the top of the table to highlight its influence.*

5. Method evaluation and conclusions

5.1 Weaknesses and corrective measures

- To avoid possible harm to fragile environmental microplastics, a cautious approach was taken to chemical digestion of the sediment. In practice, the high abundance of natural biological particles at the analysis (FT-IR) stage greatly hindered analysis, since most samples had to be spread over multiple (2-4) 47-mm PT coated filters, while every particle was selected and measured automatically. Consequently, the resultant spectral dataset was unnecessarily large, presenting data handling challenges. The balance of harm to fragile microplastics versus effectiveness at removing biological material for different methods has since been carefully demonstrated (Fischer et al., 2022, Olsen et al., 2020) for different methods, aiding future selection of a sufficiently aggressive digestion protocol, with minimal impact on polymers. Improving the effectiveness of digestion has potential to speed up and simplify sample analysis and data extraction.

- For offshore sediments, associated with relatively low microplastic pollution, maintaining sufficiently clean working practices during multi-step procedures is challenging. Despite the rigorous QA procedures applied during this study, blank contamination was higher than expected. PP and PS contamination of the blanks was particularly high. PP contamination primarily took the form of grey-white fibrous matts, suspected to be derived from polypropylene filters used to remove particles from the large volumes of zinc chloride required for density separation. PS contamination consisted of irregular 3D transparent fragments, suspected to be derived from storage of steel meshes in PS petri dishes. Replacement of plastic high-volume filters with a non-plastic material, and polystyrene petri dishes with glass is expected bring PP and PS contamination down to acceptable levels. Modest blank contamination from PET and PE may be associated with procedural complexity, that is, the number of steps applied and items of equipment which contact the sample. Therefore, method adjustment towards simplification and streamlining, for example, completing digestion within single step, is likely to improve cost effectiveness and data quality.

- Microplastics recovery with the full method, including GEPARD controlled FT-IR analysis, has not yet been measured. Therefore, the extent to which the results underestimate levels of sediment microplastics

is uncertain. Full analysis of samples, spiked with realistic microplastics, is needed to quantify percentage recovery. This undertaking is complicated by observations that laboratory generated microplastics tend not to behave like environmental microplastics. Advice should be sought from expert groups.

- Multiple sediment cores were subsampled and subsamples pooled in each sample. Pooled samples were prepared analysed as one unit, meaning that sample standard deviation (SD) could not be calculated. If desired, to improve statistical robustness, consideration could be given to different ways to collect or prepare all, or a proportion of, samples, although this would likely increase analysis time.

- Compilation of an optical mosaic within the GEPARD software, utilising the FT-IR instrument optics, formed an effective basis for substrate mapping and particle selection. This avoids moving the filter between instruments and potentially disturbing the position of loose particles on the filter. Post-analysis and library matching, GEPARD links particle records (spectrum, library suggestion, HQI, unique particle identifier and particle dimensions) to the associated particle image on the optical mosaic of the filter, so that particles can be visually checked by polymer group. However, after realising the resolving power of FT-IR in correlation with high resolution images obtained later under a Zeiss Axio Imager M2M light microscope, the potential of instead providing GEPARD with a higher quality optical mosaic, acquired under a light microscope, became apparent. If implemented, the risk of particles moving on transfer between instruments would need to be managed (for example by locating FT-IR and LM close together), but the benefits, in terms of rapid cross validation of records, and additional information on form, colour and texture that permits identification of visually apparent sub-groups within polymer identities, providing clues to particle origin, would substantially raise the resolving power of the method.

- A limitation of FT-IR is that vehicle tire particles are unlikely to be detected (without compressing particles as thin layers) due to light absorption. Pyrolysis GC-MS has potential to measure tire wear mass, e.g., Wilkinson et al. (2023). Quantification of individual tire particles in environmental matrices is challenging, partly due to their dark colour, e.g., Kovochich et al. (2020), Mattsson et al. (2023).

- External intercalibration exercises for microplastics, for example QUASIMEME (van Mourik et al., 2021), or (Cadiou et al., 2020), are under development and participation is recommended for national monitoring. Although not possible within the scope of this pilot study,

participation should be factored into future endeavours and is likely to accelerate harmonisation.

5.2 Strengths and proposed developments

- The developer-driven open-source software Gepard Enabled Particle Detection (GEPARD) enables mapping and selection of thousands of particles, automated microscope control during FT-IR analysis, management of large spectral datasets, matching against multiple libraries, and linking of particle records to images on an optical mosaic of the whole filter.

- Matching of weathered and fouled sampled microplastics against a tailor-made spectral library (Nordic Micro-litter) compiled from a high proportion of distinct individual, and averaged, environmental microplastic spectra from three institutes. The weakness of this library is that, while it has multiple environmentally relevant variants, it is limited in its range of polymer types. The influence of library quality on the results is substantial. Pan-European collaboration is needed to compile and validate adequate spectral libraries for comparable statutory microplastics monitoring.

- FT-IR in reflectance mode, with detector cooled, on a platinum vaporization-deposition filter substrate (Mattsson et al., 2024) enabled acquisition of full spectra from 4000-675 cm^{-1}.

- Collection, and preparation using the high-volume Kristineberg Microplastic Sediment Separator (KMSS) of relatively large sediment sample volumes, enabled detection of a variety of polymers typically present at low concentrations in offshore sediments.

- Automated screening and identification of 100% of particles with FT-IR, provided objective, reliable and repeatable chemical identification of polymers, while stacked-focal-plane imaging of >20% of detected microplastics documented additional form, texture, and colour information for particles of interest, and examples from each polymer/particle form group. This order of analysis, in these proportions, provided substantial resolving power.

5.3 Concluding remarks

The goal of the Marine Strategy Framework Directive MSFD is to assess environmental status and support coordinated actions at the national and regional level.

This pilot study demonstrates the feasibility of measuring microplastics in offshore sediments as part of routine national monitoring. Offshore sediment samples collected from Sweden's 16 contaminant monitoring sites were successfully prepared and analysed, providing baseline data on microplastic levels in offshore sediments. These baseline values serve as an essential reference for evaluating future changes in environmental status and guiding targeted remedial actions.

Experience gained during this study highlights specific areas for method improvement, as detailed in sections 5.1 and 5.2. Addressing these needs is crucial for establishing quality-assured methodologies, robust analytical tools, and efficient dataflows to support a long-term, reliable monitoring framework for microplastics.

By integrating microplastic measurements into Sweden's routine national monitoring program, it will be possible to track trends over time, evaluate the effectiveness of mitigation strategies, and contribute to the broader regional understanding of microplastic pollution under the MSFD. Establishing such a program will be a significant step forward in advancing Sweden's commitment to protecting marine environments and achieving GES.

6. Appendices

Appendix 1.

Article preprint: *Microplastics accumulation on sub basin scales in Baltic Sea sediments. Tim Wilkinson, Karin Mattsson, Josef Brandt, Martin Hassellöv. 2025. Scientific manuscript in preparation*

Appendix 2.

Modified from Josefsson and Apler (2019). Sampling station coordinates and depths.

Station	Sub basin	Sea area	N SWEREF 99TM	E SWEREF 99TM	Depth (m)
SE-17	N Bothnian Bay	Bothnian Bay	7255864	892219	87
SE-1	S Bothnian Bay	Bothnian Bay	7137579	839033	113
SE-2	Härnösand Deep	Bothnian Sea	6956651	704410	200
SE-3	S Bothnian Sea	Bothnian Sea	6796706	668233	79
SE-4	Åland Deep	Bothnian Sea	6679643	724318	230
SE-5	NE Gotska Sandön	Baltic Proper	6549635	804899	175
SE-6	Fårö Deep	Baltic Proper	6432633	788137	195
SE-7	SE Gotland Basin	Baltic Proper	6327222	763118	173
SE-8	Landsort Deep	Baltic Proper	6509084	694601	403
SE-9	Norrköping Deep	Baltic Proper	6435238	672991	178
SE-10	Karlsö Deep	Baltic Proper	6335390	661492	111
SE-11	N Bornholm Basin	Southern Baltic	6156130	587700	70
SE-12	Arkona Basin	Southern Baltic	6095142	433358	47
SE-13	S Rödebank (Kattegat)	North Sea	6284719	321401	47
SE-15	Deep Trench (Skagerrak)	North Sea	6426119	276793	94
SE-16	E Skagerrak	North Sea	6504578	236241	197

Appendix 3.

Modified from Josefsson and Apler (2019). Sediment accumulation rates (SARs) (± 1 standard deviation) measured by the Swedish Radiation Safety Authority using samples collected in 2014. No SAR could be determined at SE-15 as the core was mixed down to around 25 cm, or due to a very high SAR. O_2 mL/L refers to levels of dissolved oxygen in the bottom water, and if the sediment surface was oxic (Ox) or reduced (Red).

Station	Sea area	SAR, ^{210}Pb (cm/year)	SAR, ^{137}Cs (cm/year)	Years per cm	O_2 (mL/L)	Sediment surface
SE-17	Bothnian Bay	0.18 ± 0.04	0.18 ± 0.05	5.6	8.1, 7.4	Ox
SE-1	Bothnian Bay	0.16 ± 0.01	0.14 ± 0.05	6.3	7.2, 7.2	Ox
SE-2	Bothnian Sea	0.67 ± 0.12	0.66 ± 0.09	1.5	4.2, 3.8	Ox
SE-3	Bothnian Sea	0.37 ± 0.04	0.39 ± 0.09	2.7	4.9, 3.8	Ox
SE-4	Bothnian Sea	0.42 ± 0.05	0.39 ± 0.09	2.4	5.4, 4.4	Ox
SE-5	Baltic Proper	1.03 ± 0.37	0.93 ± 0.09	1.0	0, <0.1	Red
SE-6	Baltic Proper	0.88 ± 0.09	0.75 ± 0.09	1.1	0, <0.1	Red
SE-7	Baltic Proper	0.08 ± 0.01	0.02 ± 0.05	13	0, <0.1	Red
SE-8	Baltic Proper	1.03 ± 0.42	1.02 ± 0.09	1.0	0, <0.1	Red
SE-9	Baltic Proper	0.045 ± 0.003	0.02 ± 0.05	22	0.1, <0.1	Red
SE-10	Baltic Proper	0.047 ± 0.002		21	0, <0.1	Red
SE-11	Southern Baltic	0.36 ± 0.09	0.27 ± 0.04	2.8	0.7, 1.2	Ox/Red[2]
SE-12	Southern Baltic	0.57 ± 0.25	0.56 ± 0.09	1.8	4.4, 4.0	Ox
SE-13	North Sea	0.62 ± 0.15	0.66 ± 0.09	1.6	6.2, 4.4	Ox
SE-15	North Sea				7.4, 4.5	Ox
SE-16	North Sea	1.49 ± 0.95	>1	0.7	7.5, 5.1	Ox

Appendix 4.

Description of corrections applied to artefacts detected during ground-truthing of spectra against library suggestions following library matching.

Problem identified	Correction applied
The greater part of PE (Nordic and SiMPle) suggestions proved associated with biological cell structure. False positive spectra had a distinctive absorption peak 1735 cm-1.	8 x examples from samples copied into Nordic library to capture this group and distinguish them from PE.
One filter, 95001_2of4, generated unusually poor spectra for PE and PP (apparently due to a hump between wavenumbers 800 and 1300). The optical mosaic was unusual with more light flare from particles especially from the diatoms. Many of the invalid PP or PE records seem to be associated with light scatter from abundant diatom frustules. Therefore, it seems likely that they represent false positive suggestions.	Correction applied by manually checking all records above HQI 70 for this filter (95001_2of4) and subtracting invalid records from the final data table for this sample (95001). 23 PE and 353 PP suggestions were subtracted, leaving only 31 PE and 27 PP. 95001_2of4 invalidated PP now deleted from tab11 (combined size frac data) on Feb 28th. As also invalidated 95001_2of4 PE.
PS F4 is associated only with invalid library suggestions, including suggestions between HQI 70 and 50.	Correction: PS F4 suggestions removed from tab 4 (recombined) data. In future, remove PS F4 from Nordic library.
ethylene_ethyl_acrylate also has the 1735 spike and is capturing BioCellstructure spectra. It should be excluded.	Correction: suggestions removed from tab 4 data

ethylene_propylene may potentially be capturing PE. Verify manually if appropriate, i.e.. Multiple records occur. It is only capturing BioCellStructure spectra.	Correction: suggestions removed from tab 4 data
fibre_thermoplastic_elastomere is capturing biolike cellstructure.	Correction: single record removed from tab 4 data
epoxide resin is likely to be capturing PET. Consider moving epoxide resin into the PET group for evaluation as PET.	Correction: The two records in tab 4 data were transferred to PET group.
Polyester epoxide is probably capturing PET. How many records of this exist?	Correction: The single record in tab 4 data assigned to PET.
PVSlikebiocellstructure reference spectra can (rarely) capture valid PP records (one instance observed spec 3065 in sample 94001 captured by BioCellstructurePVSlike21717smthd at HQI 74.8)	Insignificant
Remove polyacrylic acid from PMMA to Other Synthetic. This reference should not have been in PMMA group.	Correction: There are no valid records above HQI 50
Check ABS to assess if HQI 50 cut off is appropriate. Observation that most ABS K1 records appear to be PS spectra.	Correction: Those that appear to be PS have been re-assigned as PS (applied to Tab 11 March 2024). Four valid ABS K1 records remain.
Acrylonitrile_butadiene_styrene (2 suggestions only) reference from SiMPle database does not have 2220 peak and is potentially capturing something else, maybe closest to PET, yet not quite a perfect match.	Correction: by removal of these two uncertain records.
Is windscreen wiper rubber capturing PE? No, only BioCellStructure spectra.	Correction: removal of these 21 suggestions from tab 4 data.
Polyvinyl stearate should be excluded from the Other Synthetic group.	Correction: The 4 records were removed from tab 4 data.
What is resin_dispersion, or what does it capture? It is quite abundant.	The captured spectra do not appear to be derived from a synthetic polymer. Correction: 69 records removed from tab 4 data.
Nylon/polyamide. Poor quality nylon suggestions easily confused with chitin or protein spectra. If applicable, consider a HQI cut off of 75 or 80, or even 90 for this polymer.	Correction: For these samples, all nylon records were manually validated.
POM K1 is successfully capturing a matching spectral form. HOWEVER, the Polyacetal and Polyoxymethylene reference spectra in the SiMPle library are capturing oscillating absorbance signals associated with filter pores. These two reference spectra are candidates for future exclusion.	Correction: records captured by these two problem reference spectra had already been excluded from tab 4 data by the applied HQI cut off at 70. In future, consider removing them from the library.
Check reference spectrum “polyethylene_chlorosulphonated” has not been counted as PE. It is not like PE.	Solved: This was grouped in the Other Synthetic group. The only two records were recorded as invalid.

The in-house reference spectrum PHB K1 and the SiMPle polyhydroxy butyric acid reference have differently aligned peaks and different forms. In one sample, suggestions match to one, while in the other three, suggestions match to the other form.	Suggestions from both references grouped as PHB, although they are different. Could they be different forms, resulting from different production techniques, or derived from different source-bacteria?

8. Literature

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