Supplementary Information

A spatial and temporal assessment of microplastics in seafloor sediments: A case study for the UK.

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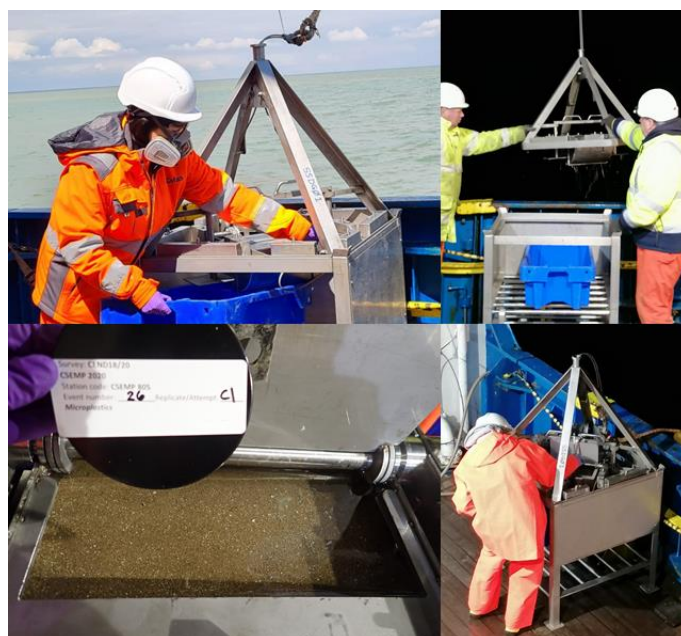
1. Sample collection

Figure S1. Collection of seafloor sediment samples on Cefas Endeavour research vessel.

A list of CSEMP sediment stations and corresponding locations can be found in Table S.1.

Table S1. CSEMP stations and corresponding locations

Station	Location	UK regional seas	OSPAR region	Sampling years
CSEMP 245	Off Tyne	Northern North Sea	Greater North Sea	2013, 2015*, 2017. 2019, 2021
CSEMP 285	Off Tyne/Tees	Northern North Sea	Greater North Sea	2013, 2015, 2017, 2019, 2021
CSEMP 295	Off Tees	Northern North Sea	Greater North Sea	2013, 2015*, 2017, 2019, 2021
CSEMP 345	Off Humber/Wash	Northern North Sea	Greater North Sea	2013, 2015, 2017, 2019, 2021
CSEMP 376	Off Wash	Southern North Sea	Greater North Sea	2013, 2015, 2017, 2019, 2021
CSEMP 386	Wash	Southern North Sea	Greater North Sea	2015, 2017, 2019, 2021
CSEMP 466	Thames	Southern North Sea	Greater North Sea	2013, 2017, 2019, 2021
CSEMP 475	Outer Gabbard	Southern North Sea	Greater North Sea	2013, 2015, 2021
CSEMP 484	Dungeness	English Channel	Greater North Sea	2013, 2015, 2017, 2019, 2021
CSEMP 536	Lyme Bay	Western English Channel	Greater North Sea	2014, 2016, 2018, 2020
CSEMP 575	Off Tamar	Celtic Sea	Greater North Sea	2014, 2016, 2018, 2020
CSEMP 605	Celtic Deep	Western English Channel & Celtic Sea	Celtic Sea	2014, 2016, 2018
CSEMP 655	Cardigan Bay	Irish Sea	Celtic Sea	2014, 2016, 2018, 2020
CSEMP 715	Liverpool Bay	Irish Sea	Celtic Sea	2014, 2016, 2018, 2020
CSEMP 805	SE Isle of Man	Irish Sea	Celtic Sea	2014, 2016, 2018, 2020

2. 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
- 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 were previously filtered onto a 0.2 μm regenerated cellulose (RC) filter
- Restricted laboratory access

3. Sample processing

Table S2. List of chemicals, manufacturers and suppliers

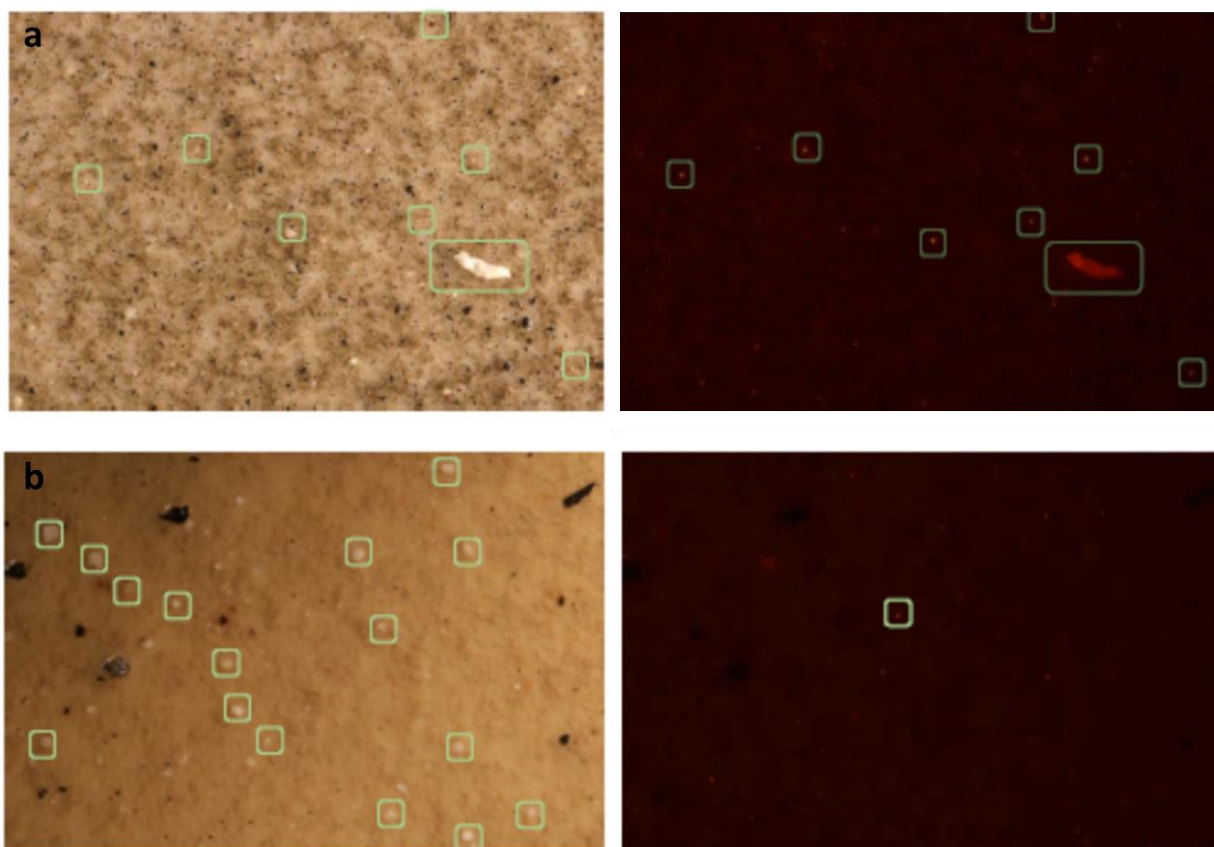| Chemicals	Molecular formula	Manufacturer/Supplier	Purity (%)
Potassium hydroxide	KOH	VWR/VWR	-
Sodium hypochlorite	NaClO	VWR/VWR	14% active chlorine
Hydrogen peroxide	H_2O_2	VWR/VWR	30%
Ethanol	C2H6O	Acros organics/ThermoFisher scientific	95% purity
Nile Red	C20H18N2O2	Acros organics/ThermoFisher scientific	99% purity
Zinc chloride	ZnCl2	VWR/VWR	-

Figure S2. Chemical resistant biological items after staining using NR following (a) a 10% KOH digestion process at 40°C for 3 days and (b) a 30% v:v KOH:NaClO digestion process at 40°C for 3 days.

3.1 Density separation and microplastics recovery

Table S3. Polymeric materials used for density separation recovery studies.

Supplier	Polymer type	Characteristics	Particle type	Size (μm)	Density (g cm^{-3})	Spiked amount (number of items)
Goodfellows	PA	Opaque	Spheres	1590	1.16	10 x 2
Goodfellows	uPVC	Opaque	Fragments, spherical	Max. 250	1.3-1.45	50 x 2

| Supplier	Polymer type	Characteristics	Particle type	Size (μm)	Density (g cm⁻³)	Spiked amount (number of items)
Goodfellows	U.H.M.W. PE	Opaque	Fragments, spherical	Max. 150	0.95	50 x 2
Goodfellows	PS	Opaque	Fragments, spherical	900	1.05	50 x 2
Goodfellows	PP	Transparent	Sheet, 1 mm thick, shavings	~ 500	0.90	50 x 2
Amazon	PET	White	Fibre	~ 2700 length 300 μm diameter	1.39	20 x 2
Amazon	Nylon	White	Fibre	~ 3951 length, 20 μm diameter	1.14	20 x 2
Amazon	Nylon	Yellow	Fishing line	~ 536	1.14	50 x 2
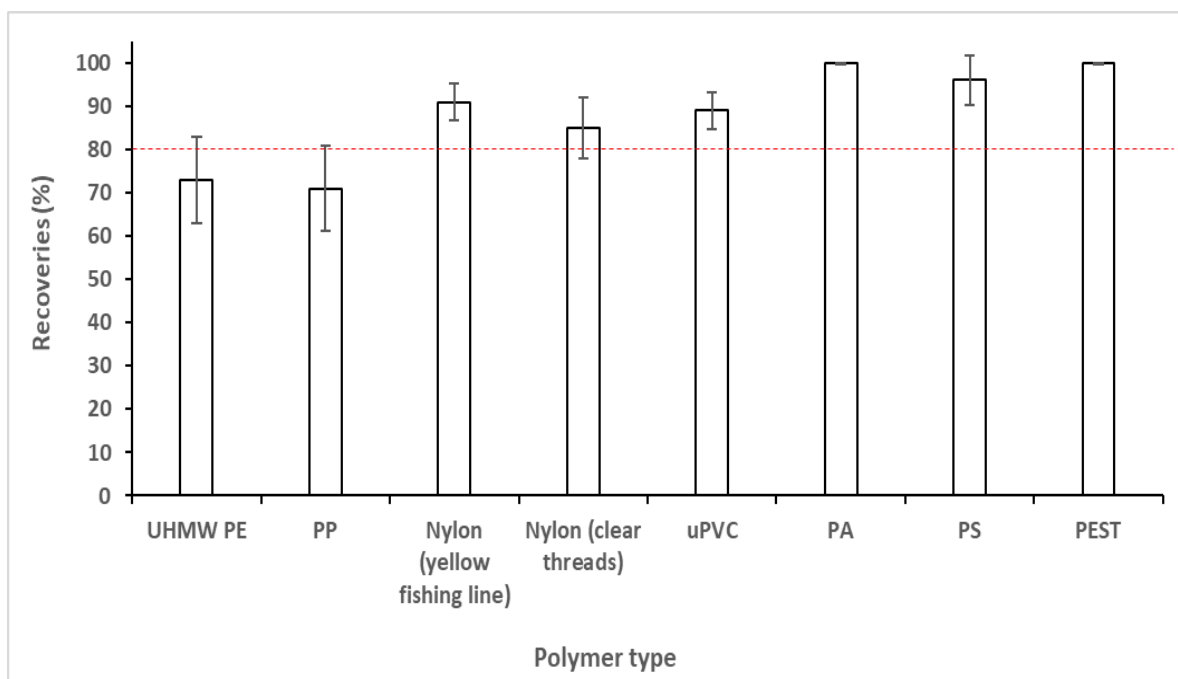
Figure S3. Polymer recoveries (%) from spiked clean sand using zinc chloride (1.5 g cm^{-3}).

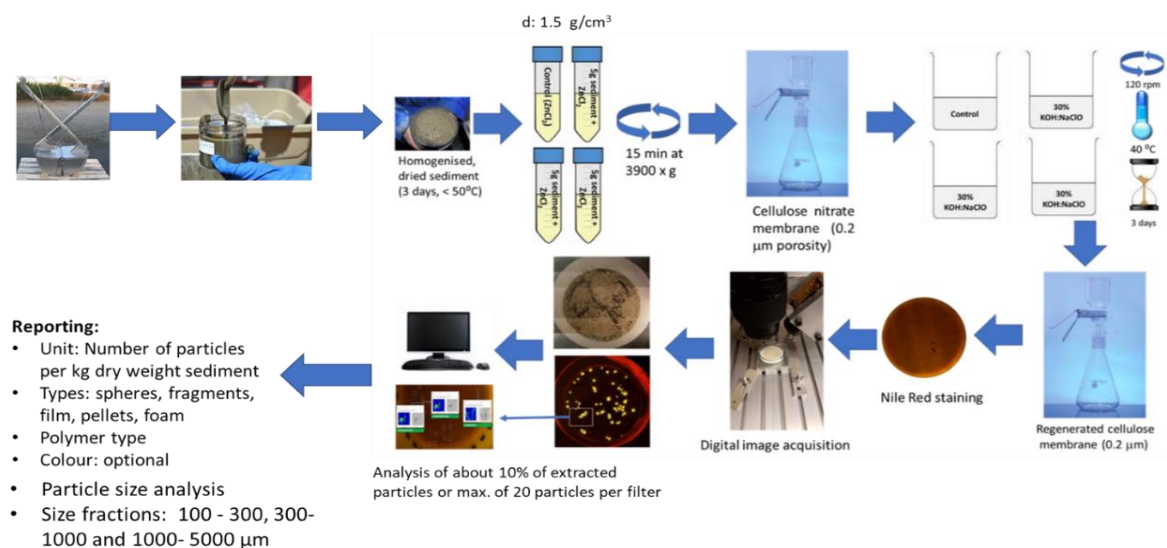4. Quantification of microplastics

Figure S4. Schematic diagram of the protocol for the detection and quantification of microplastics in sediment samples.

Figure S5. Experimental set-up of the digital imaging acquisition system using white light and blue light (420-470 nm).
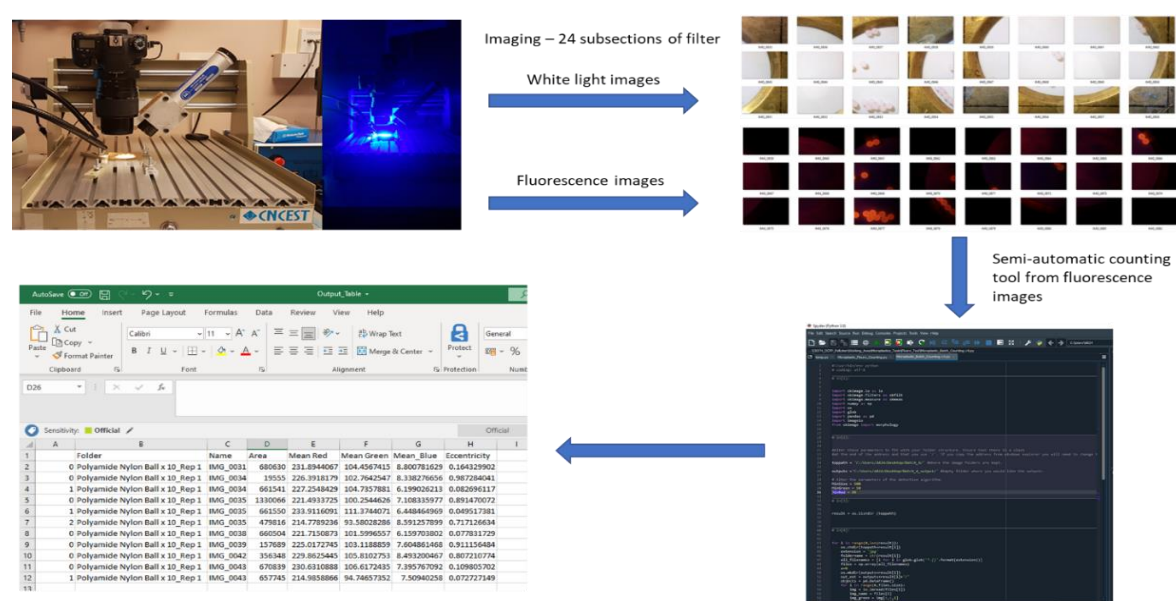

Figure S6. Schematic diagram of the protocol for the imaging and semi-automatic counting of fluorescent particles onto filters.
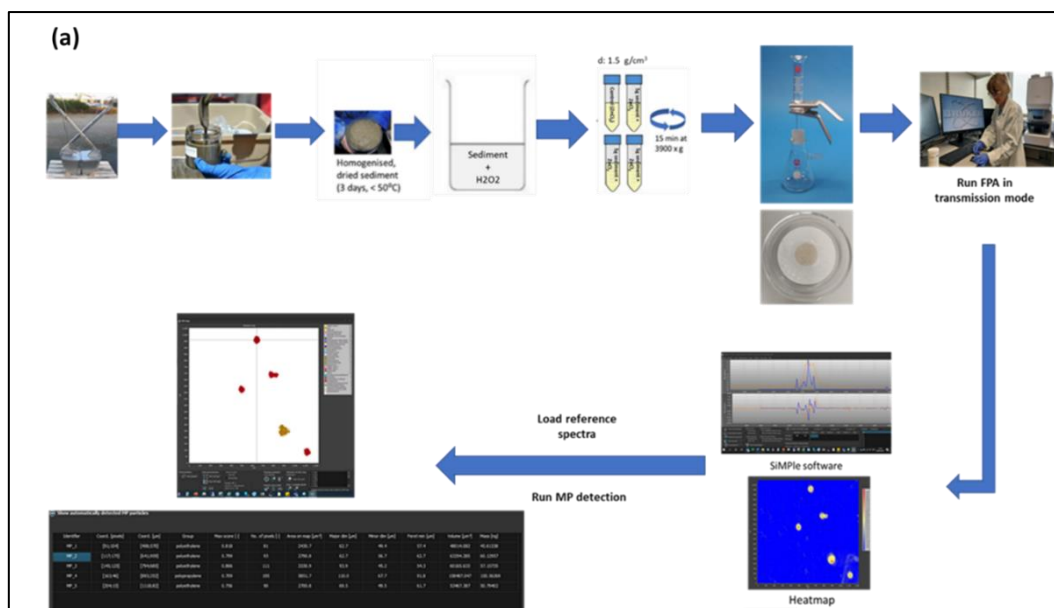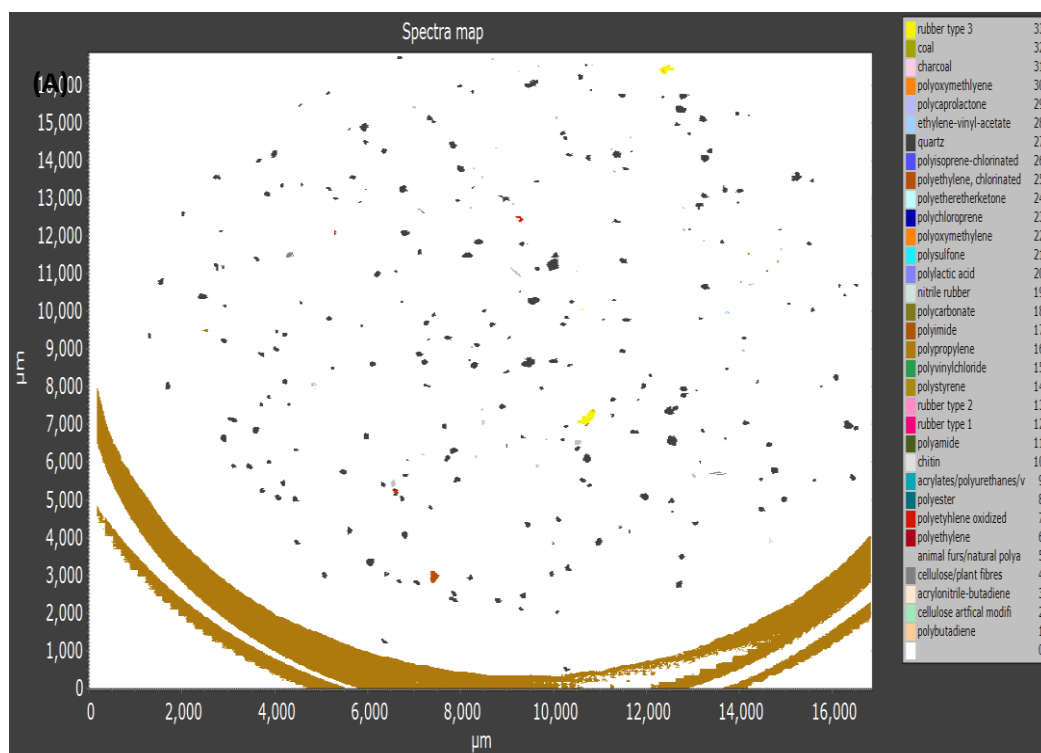
Figure S7. Schematic diagram of the protocol for the analysis of microplastics from sediment samples using micro-FTIR with focal plane array detector (FPA) following oxidative digestion on dry sediment.
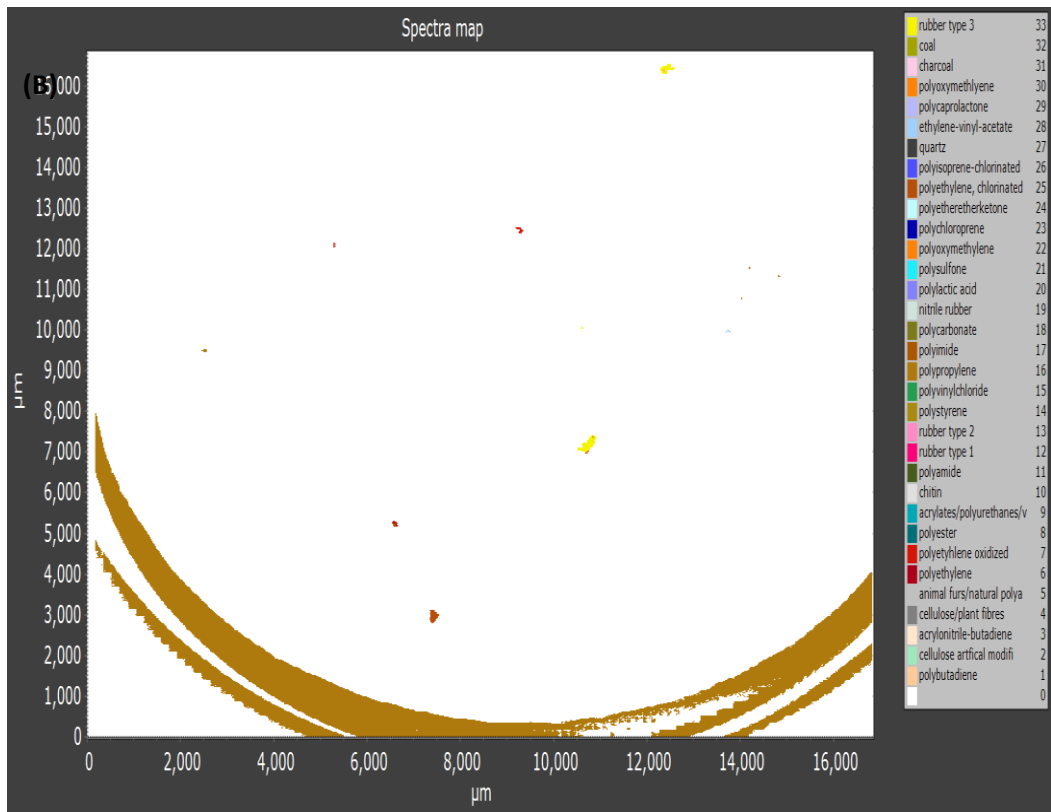
Figure S8. Example of particle mapping onto filter for (A) all particles and (B) microplastics (including rubbers) only.
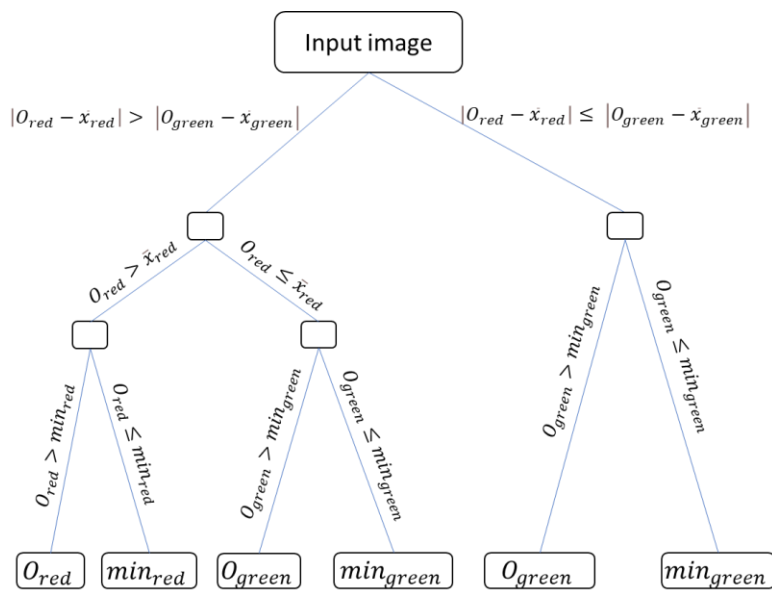
Figure S9. Decision tree used within the tool to pick which threshold to use to create the two classes. Where Ored is the Otsu threshold for the red band, Ogreen is the

Otsu threshold for the green band, $\bar{x}$red is the mean value of the red band for the whole image, $\bar{x}$green is the mean value of the green band for the whole image, minred is the user defined minimum red threshold and mingreen is the user defined minimum greed threshold.

5. Contamination control procedures
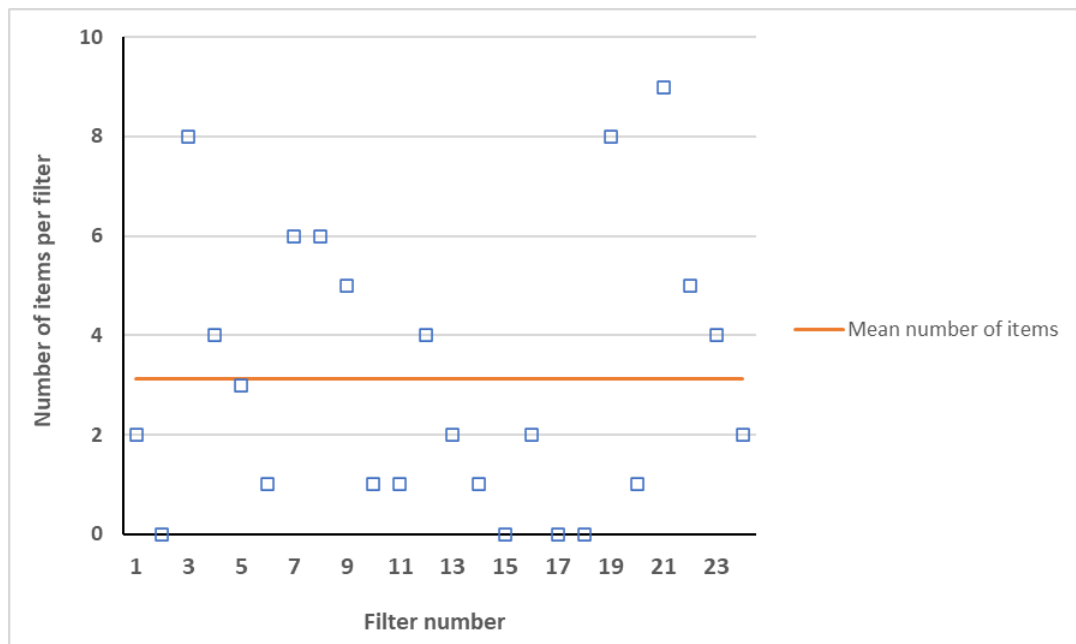
Figure S10. Number of items per filter for laboratory negative controls (n=24).
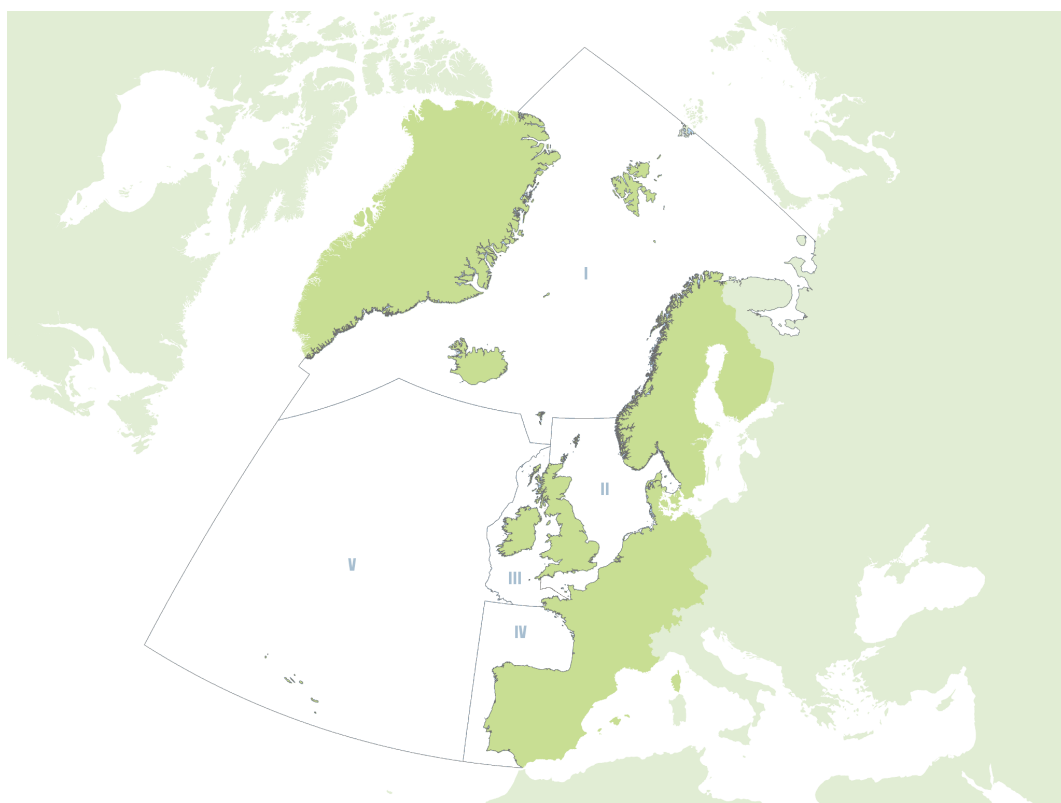
Figure S11. OSPAR regions. Region I: Arctic waters, II: Greater North Sea, III: Celtic Seas, IV: Bay of Biscay and Iberian Coast, V: Wider Atlantic.

Coastal vs offshore stations
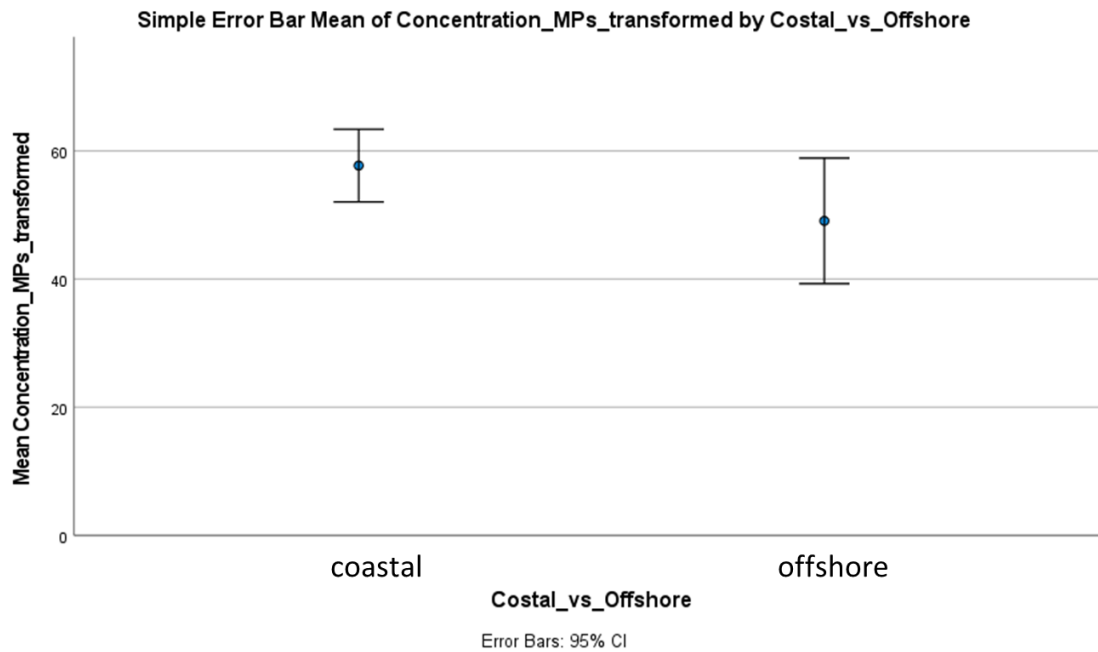
Figure S12. Plot of mean concentration of microplastics (data transformed) from coastal and offshore locations.

Table S4. One-Way ANOVA output for the investigation of differences for the abundance of microplastics from seafloor sediments from coastal to offshore locations.

ANOVA

Concentration_MPs_transformed

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3052.468	1	3052.468	2.591	.109
Within Groups	220270.089	187	1177.915		
Total	223322.557	188			
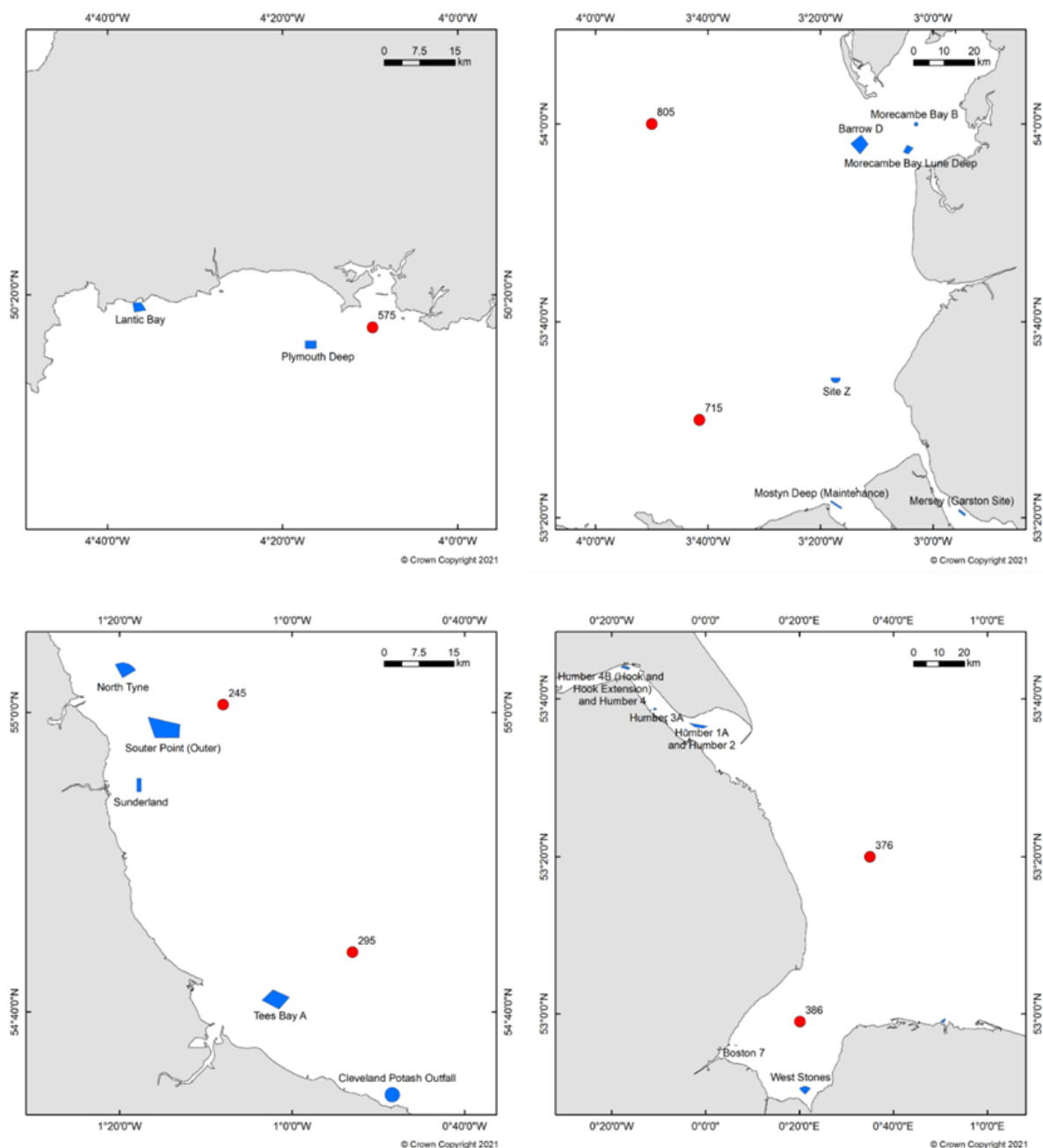
Figure S13. Location of near-shore CSEMP sediment monitoring stations to dredge disposal sites.
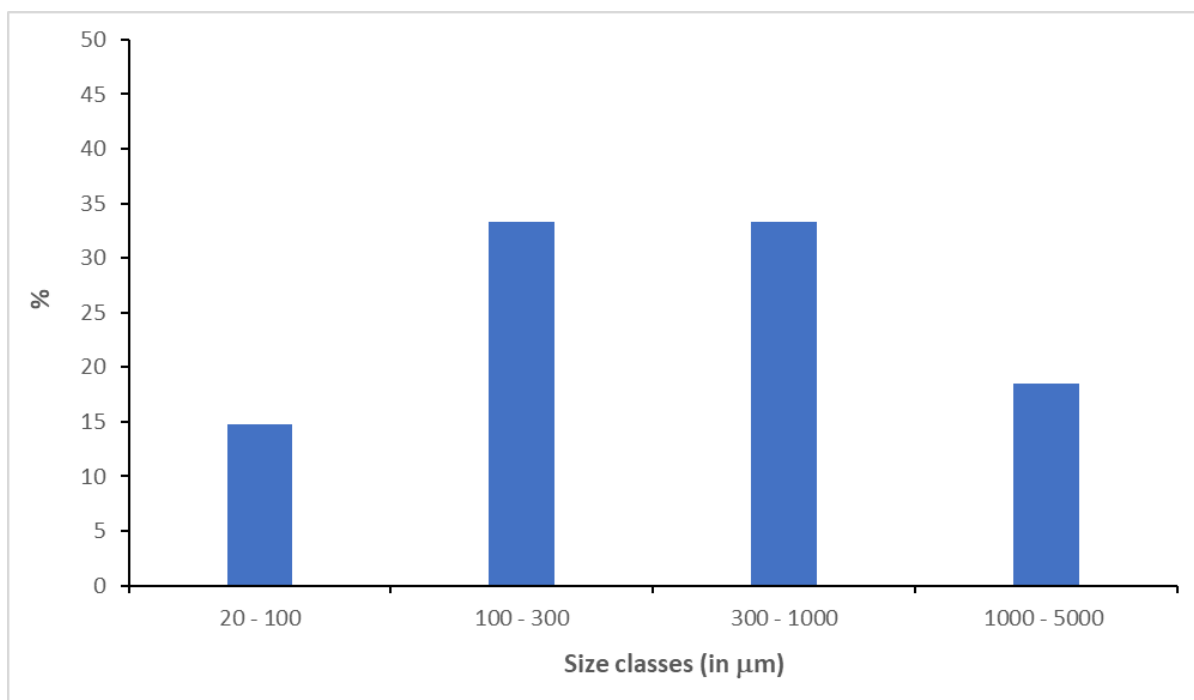
Figure S14. Microplastics analysed using μ-FTIR categorised per size classes (n=27).
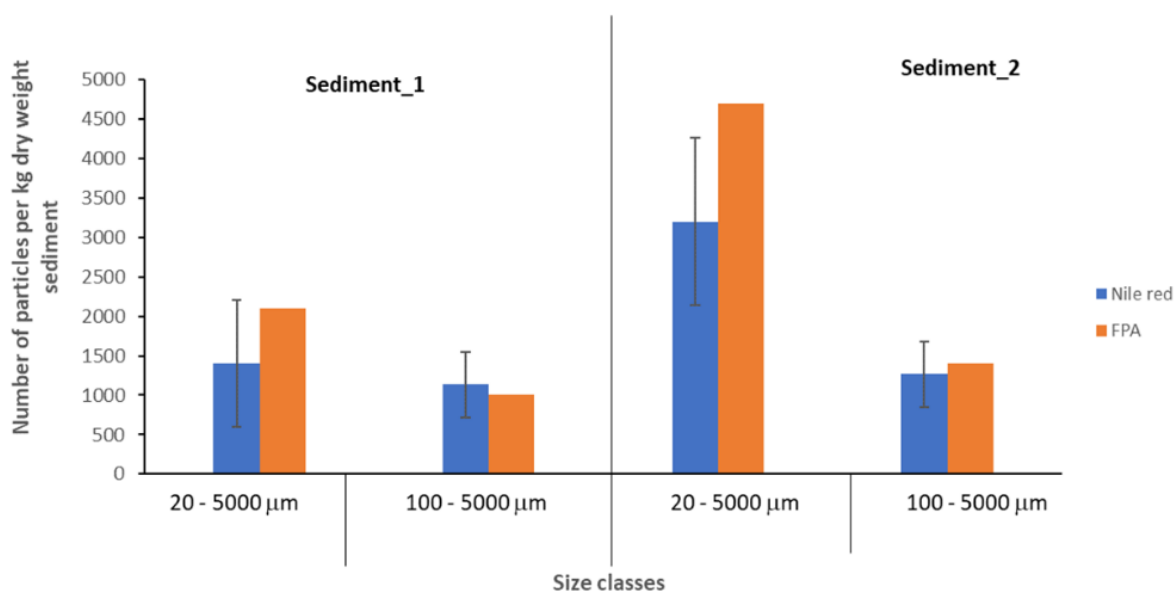
Figure S15. Number of particles per kg dry weight sediment for 2 marine Dutch sediments using two analytical techniques: i) Nile red tagging of polymers coupled with automatic counting and micro-FTIR and ii) Focal plane array detector-based micro-Fourier-transform infrared imaging (Bakir, 2022).

Table S5. Microplastics analysis from micro-FTIR-FPA and comparison to NR output.

Sampling year	Station number	Sediment type	Number of microplastics on filters from FPA	Polymer type	Size fraction (µm) [min – max]	Mean number of microplastics on filters from NR technique* (mean ± SD; n=3)
2019	386	High % sand	300	PP, PE	84 - 1920	200 ± 200
2019	484	High % sand	1,500	PP, PA	44 - 284	2,200 ± 721
2019	466	High % sand	700	PP, PA	67 - 112	800 ± 1,058
2020	805	High % sand	1,400	CA (modified), PP, PA	22 – 140	2,067 ± 945
2021	475	High % sand	400	PP	37 - 88	67 ± 115
2021	285	High % sand	900	PP, PE	30 - 1920	533 ± 924
2021	295	High % sand	1,500	Rubber type3, PE, PP	23 – 214	733 ± 306
2021	484	High % silt/clay	2,100	PE, PP, EVA, Rubber	24 - 503	2,200 ± 0.01
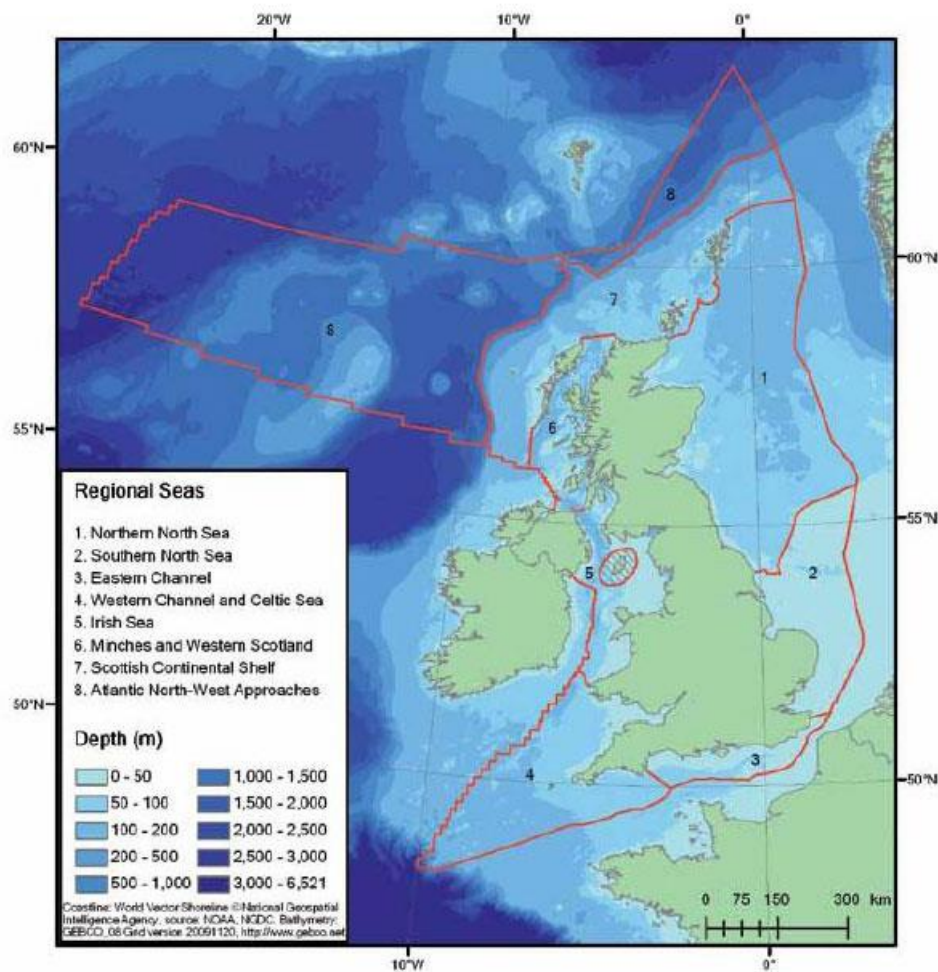
Figure S16. Charting Progress 2 Regional Sea boundaries (UKMMAS, 2010).

6. ANOVAs statistical outputs
- 2021

ANOVA

SQRT_conc

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18675.797	8	2334.475	20.454	<.001
Within Groups	2054.404	18	114.134		
Total	20730.201	26			

SQRT_conc

Tukey HSD[a]

CSEMP_station	N	Subset for alpha = 0.05			
		1	2	3	4
475	3	.0000			
285	3	13.3333			
295	3	26.6357	26.6357		
484	3		46.9042	46.9042	
466	3			60.3227	60.3227
386	3			62.2904	62.2904
245	3			65.0522	65.0522
376	3			67.4254	67.4254
345	3				83.2531
Sig.		.117	.378	.364	.241

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

- 2020

ANOVA

SQRT_conc_2020

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9811.032	4	2452.758	18.843	<.001
Within Groups	1301.693	10	130.169		
Total	11112.725	14			

SQRT_conc_2020

Tukey HSD[a]

CSEMP_station_2020	N	Subset for alpha = 0.05		
		1	2	3
715	3	6.6667		
575	3		43.5117	
805	3		44.5092	
536	3		73.2696	73.2696
655	3			77.9673
Sig.		1.000	.058	.985

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

- 2019

ANOVA

SQRT_conc_2019

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14889.557	7	2127.080	17.082	<.001
Within Groups	1992.355	16	124.522		
Total	16881.913	23			

SQRT_conc_2019

Tukey HSD[a]

		Subset for alpha = 0.05			
CSEMP_station_2019	N	1	2	3	4
285	3	11.3807			
386	3	11.3807			
466	3	21.5738	21.5738		
345	3	31.7352	31.7352		
484	3		46.4405	46.4405	
295	3		48.2338	48.2338	
376	3			70.5586	70.5586
245	3				83.3220
Sig.		.383	.132	.209	.844

Means for groups in homogeneous subsets are displayed.
   a. Uses Harmonic Mean Sample Size = 3.000.

- 2018

ANOVA

SQRT_conc_2018

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	16985.802	5	3397.160	20.363	<.001
Within Groups	2002.002	12	166.834		
Total	18987.804	17			

SQRT_conc_2018

Tukey HSD[a]

| CSEMP_station_2018 | N | Subset for alpha = 0.05 | | |
		1	2	3
715	3	18.8562		
805	3	27.7272	27.7272	
605	3	40.7324	40.7324	
575	3	43.4439	43.4439	
536	3		59.5223	
655	3			113.0733
Sig.		.254	.088	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

- 2017

ANOVA

SQRT_conc_2017

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45194.582	7	6456.369	34.886	<.001
Within Groups	2961.115	16	185.070		
Total	48155.697	23			

SQRT_conc_2017

Tukey HSD[a]

| CSEMP_station_2017 | N | Subset for alpha = 0.05 | | | | |
		1	2	3	4	5
285	3	.0000				
386	3	29.4281	29.4281			
466	3		50.1838	50.1838		
376	3		58.5035	58.5035		
295	3		59.6045	59.6045		
345	3			78.4986	78.4986	
484	3				105.6001	
245	3					151.4588
Sig.		.208	.187	.243	.287	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

- 2016

ANOVA

SQRT_conc_2016

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1464.603	5	292.921	.643	.672
Within Groups	5468.821	12	455.735		
Total	6933.424	17			

SQRT_conc_2016

Tukey HSD[a]

		Subset for alpha = 0.05
CSEMP_station_2016	N	1
805	3	11.3807
536	3	25.7580
655	3	29.4689
715	3	30.9835
605	3	37.2361
575	3	38.7617
Sig.		.630

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

- 2015

ANOVA

SQRT_conc_2015

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4038.033	5	807.607	3.399	.038
Within Groups	2851.270	12	237.606		
Total	6889.303	17			

SQRT_conc_2015

Tukey HSD[a]

CSEMP_station_2015	N	Subset for alpha = 0.05 1	2
285	3	49.5973	
484	3	64.5637	64.5637
475	3	69.8225	69.8225
386	3	77.0530	77.0530
376	3	89.1713	89.1713
345	3		94.1135
Sig.		.071	.248

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

- 2014

ANOVA

SQRT_conc_2014

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12418.113	5	2483.623	6.688	.003
Within Groups	4456.325	12	371.360		
Total	16874.439	17			

SQRT_conc_2014

Tukey HSD[a]

CSEMP_station_2014	N	Subset for alpha = 0.05 1	2
715	3	13.3333	
605	3	37.9202	37.9202
536	3		77.4525
805	3		77.6554
655	3		80.5917
575	3		81.0837
Sig.		.635	.137

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

- 2013

ANOVA

SQRT_conc_2013

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	29425.080	7	4203.583	8.782	<.001
Within Groups	7658.915	16	478.682		
Total	37083.995	23			

SQRT_conc_2013

Tukey HSD[a]

CSEMP_station_2013	N	Subset for alpha = 0.05		
		1	2	3
484	3	24.4260		
295	3	46.0508	46.0508	
466	3	50.7878	50.7878	
285	3	62.1498	62.1498	
475	3	63.2317	63.2317	
376	3	78.4372	78.4372	
245	3		95.5109	95.5109
345	3			147.2461
Sig.		.111	.171	.138

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

7. References

Bakir, A. (2022). *Monitoring of microplastics in Dutch marine sediments: a pilot study*. https://puc.overheid.nl/rijkswaterstaat/doc/PUC_707712_31/1/

UKMMAS. (2010). *Charting Progress 2 - An assessment of the state of UK seas*.