R&D project regarding development of methods for sampling and analysis of microplastics in Danish waters

F&U projekt vedrørende udvikling af metoder til prøvetagning og analyse af mikroplast i danske farvande

Den Europæiske Union
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i.1 Baggrund og formål

Formålet med at undersøge udbredelsen af mikroplast (plaststykker mellem 1 µm og 5 mm) i havmiljøet er at forbedre den viden, der er nødvendig for at vurdere miljøtilstanden mht. hertil, og herigennem skabe grundlag for at kunne arbejde hen imod en god miljøtilstand for havområder. God miljøtilstand, med hensyn til mikroplast, er defineret således, at sammensætning, mængde og rumlig fordeling af mikroplast i vandsøjlen og i havbundens sediment er på niveauer, der ikke skader kyst- og havmiljøet. Der mangler dog tilstrækkelig viden på området, som dette forskningsprojekt vil bidrage til at opnå. Der mangler blandt andet viden om det metodiske grundlag for at opnå repræsentativ prøveudtagning i havmiljøet og viden om sammenligneligheden af metoder til oprensning, analyse og fortolkning af udtagne prøver. Projektet adresserer denne mangel på viden og har til formål at belyse analytiske og metodiske problemstillinger vedrørende mikroplast i sediment og at sammenligne prøveudtagningsmetoder vedrørende mikroplast i vandsøjlen.

i.2 AP 1 – Repræsentativ prøvetagning til statistisk pålidelig vurdering af mikroplast i marine sedimenter

Med afsæt i ovenstående er det et formål med Miljøstyrelsens overvågning af marint mikroaffald (microlitter) at skabe viden om variationen af mikroplast i dansk havsediment, både hvad angår koncentration, partikelstørrelser og polymertyper. Herved er intentionen at skabe grundlag for at vurdere, hvordan og hvor store prøver der skal udtages, for med en given statistisk sikkerhed at kunne oplyse om tilstedeværelsen af mikroplast i forskellige størrelsesfraktioner og polymertypefraktioner. En sådan vurdering vil afhænge af den faktiske sammensætning og koncentration i sedimentet, den faktiske mikroplast inhomogenitet i en sedimentprøve, samt analyseteknikken anvendt til kvantificering. Formålet med AP1 er at vurdere dette eksperimentelt på et sæt af marine sedimentprøver.

i.2.1 Udfordringer

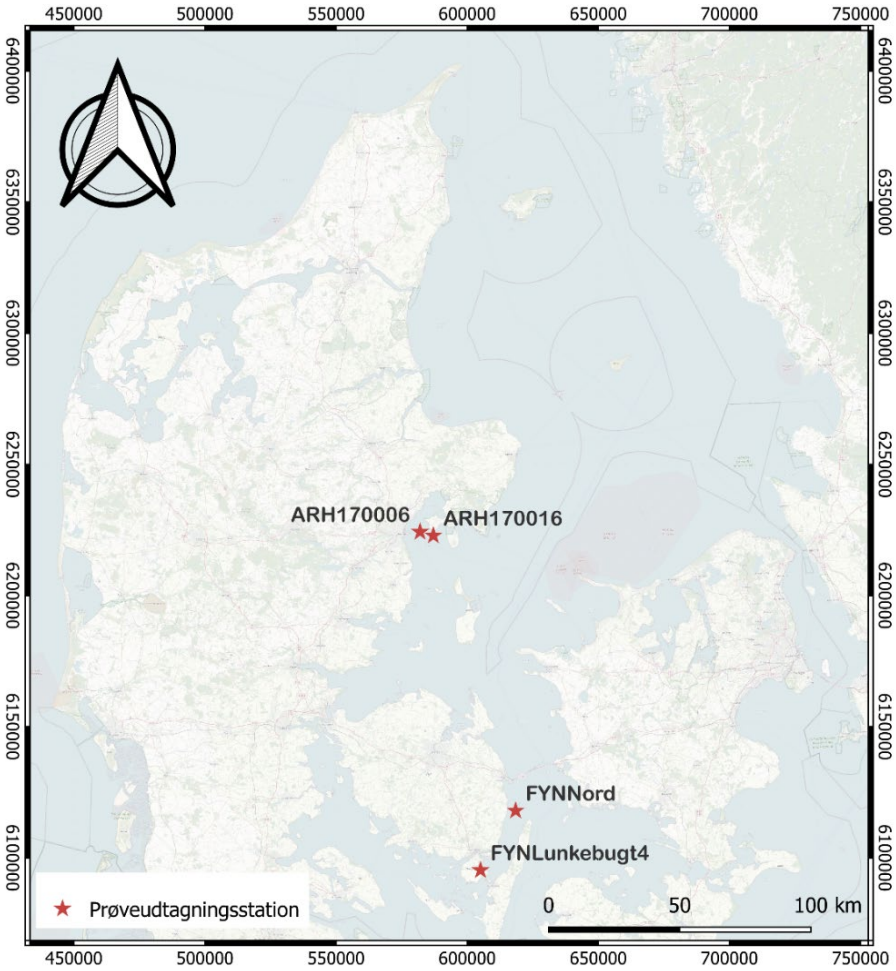
Et af formålene med dansk overvågning af mikroplast er at skabe viden om niveauet af mikroplast i dansk havsediment og dets udbredelse. I den forbindelse er det væsentligt at kende usikkerhederne, der er forbundet hermed. Den samlede usikkerhed på et datapunkt i felten bliver den kombinerede usikkerhed relateret til flere problemstillinger:

- prøveudtagning i felten
- delprøvetagning af den indsamlede prøve i laboratoriet
- ekstraktion af mikroplast
- analysere mikroplast.

Spørgsmålet opstår nu, hvor store disse individuelle usikkerheder er, og hvilke der dominerer den samlede usikkerhed. Usikkerheden relateret til repræsentativ lokal prøvetagning i felten er adresseret i Liu et al. (2022; in prep), mens de tre sidstnævnte adresseres i denne arbejdspakke.

i.2.2 Metode

Der blev indsamlet ca. 20 kg vådt sediment fra fire stationer på en strækning af 50-100 m (Figur 1). Sedimentet blev opsamlet i hver sin metalspand og derefter analyseret på Aalborg Universitet. Mikroplastet kan forventes at have været inhomogent fordelt i de 20 kg prøve i metalspanden. Den bestemte koncentration vil derfor variere afhængigt af f.eks. hvor meget delprøve der udtages og hvor meget der analyseres. Variabilitet blev vurderet ved at udtage delprøver med et rør af rustfrit stål i hele spandens dybde og derpå analysere de enkelte delprøver. Der blev analyseret seks delprøver på hver ca. 500 g vådvægt fra hver af spandene. Tørvægten af delprøverne varierede mellem 94 og 232 g (Tabel 3).



Figur 1. Lokaliteter for udtagning af sediment til laboratorieforsøg

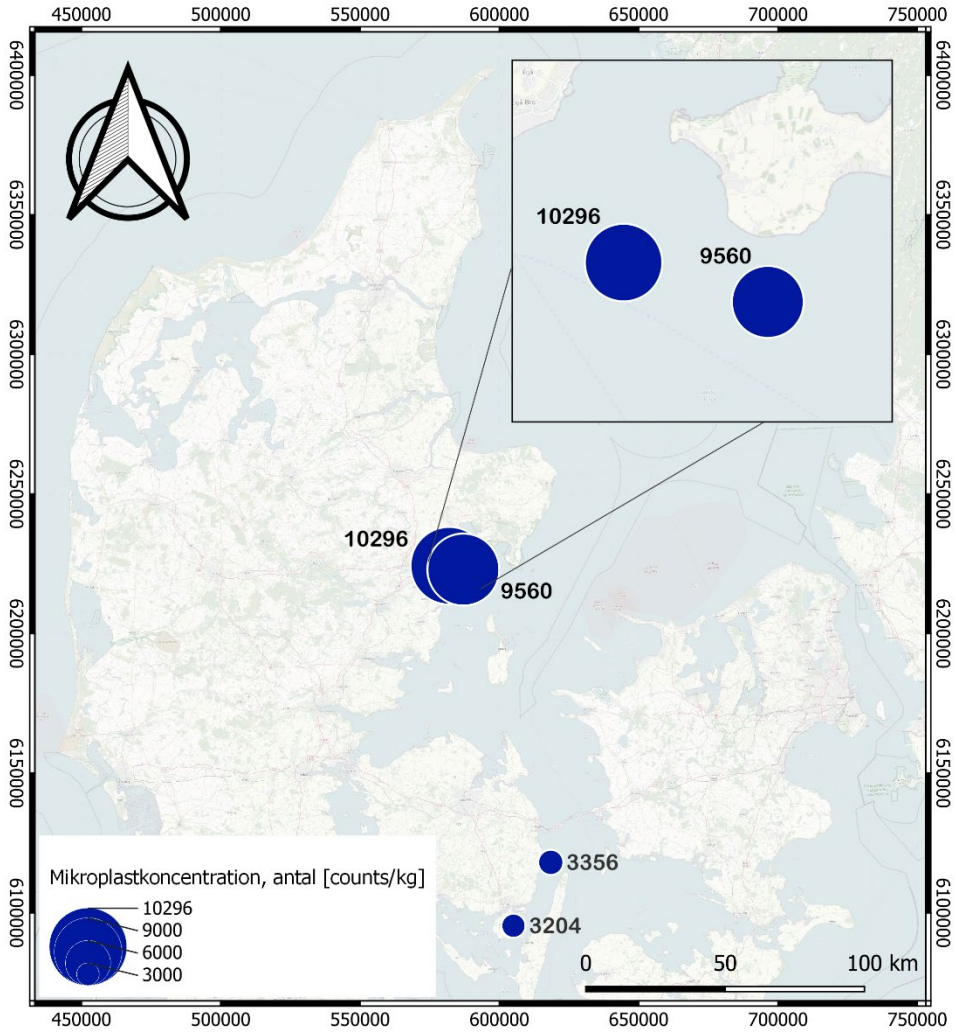
Figur 2. Udtagning af delprøver med stålrør

Prøveforberedelse, dvs. koncentrering af mikroplast i en væske (et koncentrat) der kan analyseres kemisk, såvel som den efterfølgende kemiske analyse blev gennemført som beskrevet i Liu et al. (2022).

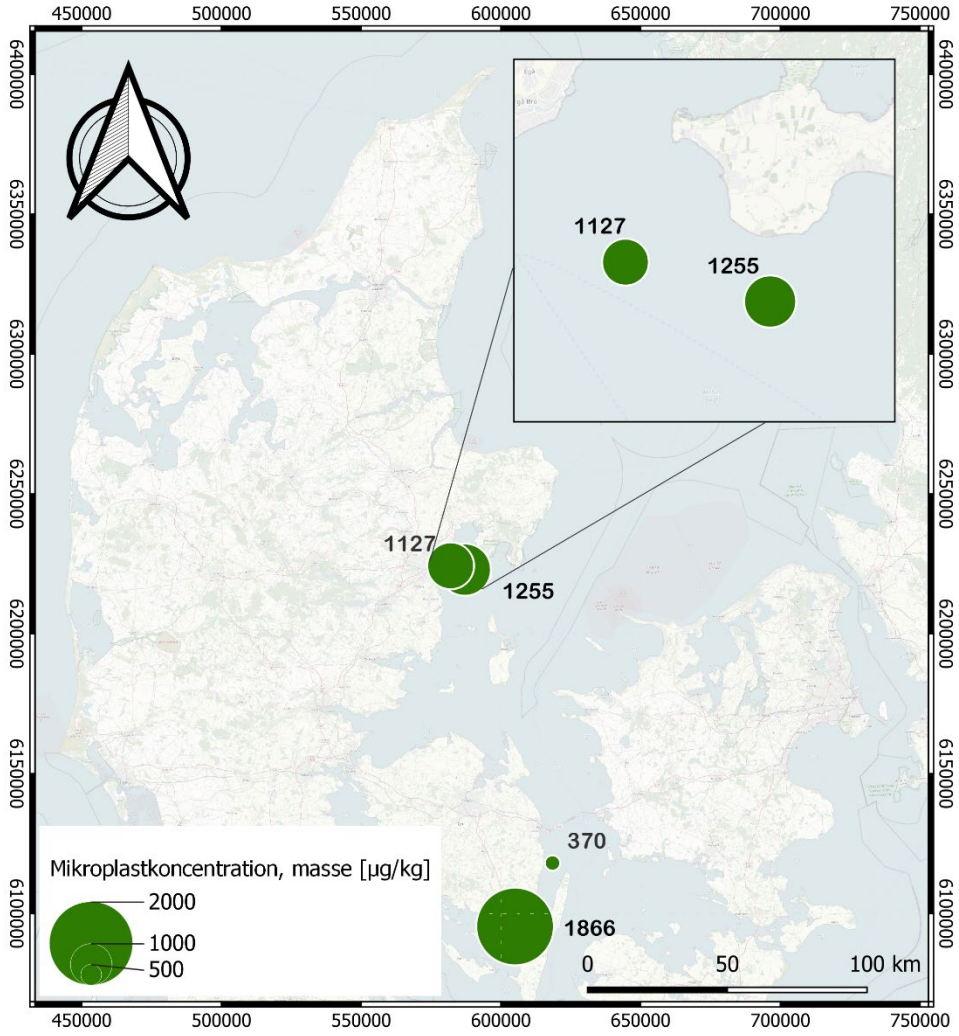
Effektiviteten af ekstraktionen blev vurderet individuelt for hver af de fire gange seks delprøver. Dette blev gjort ved at tilsætte et kendt antal let identificerbare mikrokugler af plast til delprøven før ekstraktion og kontrollere, hvor mange af dem der kunne findes efter den fulde ekstraktion.

i.2.3 Resultater

Uden at korrigere resultaterne for genfinding inden for de enkelte delprøver, og uden at korrigere dem for blindværdier, varierede koncentrationerne i de fire prøver fra 3204 til 10296 partikler kg^{-1}, svarende til 370 til 1866 µg kg^{-1} (Figur 3, Figur 4). I en anden undersøgelse har Liu et al. (in prep) kvantificeret mikroplast i 19 sedimentprøver fra forskellige dele af det danske havmiljø og fundet 119 til 23340 partikler kg^{-1}, svarende til 5 til 6958 µg kg^{-1}. Prøverne indsamlet til nærværende undersøgelse lå derfor indenfor, hvad der tidligere er blevet fundet for andre danske marine sedimenter.

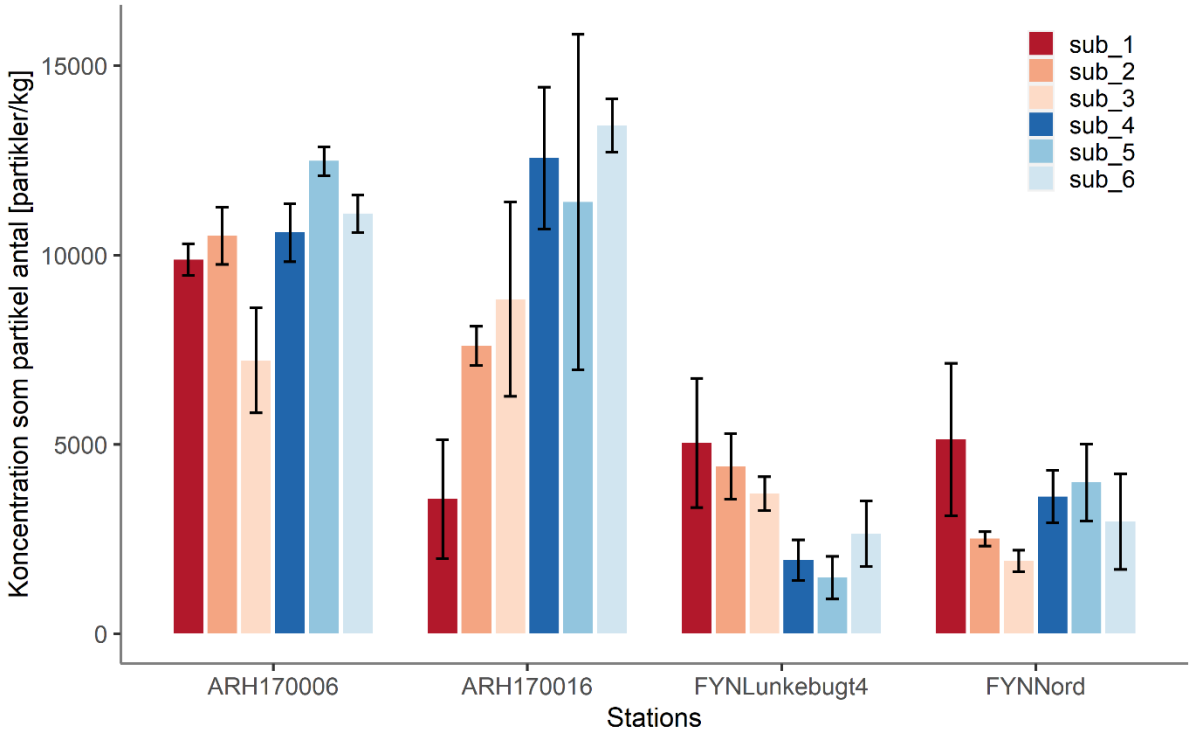


Figur 3. Mikroplastkoncentrationer som partikler pr. kg tørt sediment i de fire undersøgte prøver

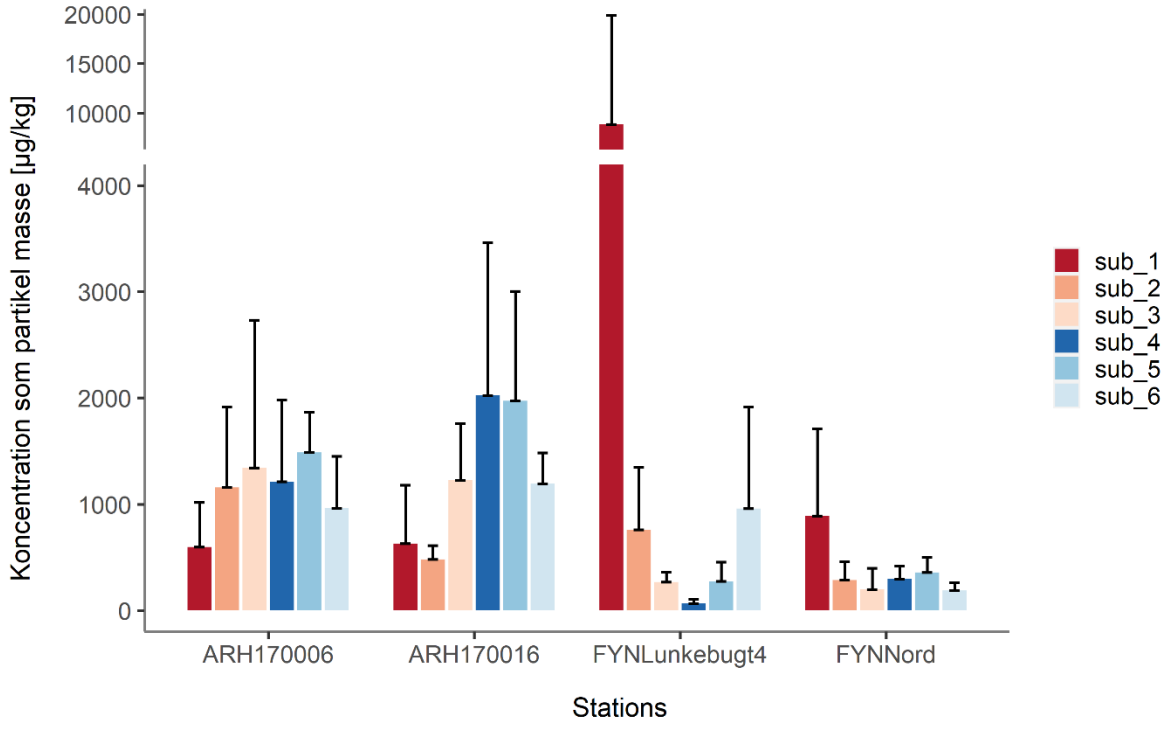


Figur 4. Mikroplastkoncentrationer som masse pr. kg tørt sediment i de fire undersøgte prøver

Hver af de 24 delprøver blev analyseret med mindst 3 scanninger. Koncentration pr. delprøve blev beregnet ved at tage et gennemsnit af værdien fra alle scanninger af denne delprøve. Variationer mellem delprøver var ret udtalte både med hensyn til antal (Figur 5) og massekoncentrationer (Figur 6). For flere af prøverne var den indbyrdes afvigelse mellem de mindst tre scanninger pr. delprøve sammenlignelig med variabiliteten mellem de enkelte delprøver udtaget fra spanden. Forskellen i variabilitet delprøverne imellem og scanninger imellem var mest udtalt ved måling af mikroplast som masse sammenlignet med tællinger. Gennemsnitsværdien pr. delprøve varierede ca. en faktor 0,5-3 for koncentrationer målt som antal mikroplastpartikler (Figur 5), og endnu mere når de blev målt som masse (Figur 6). For sidstnævnte viste FYNLunkebugt4 langt den største variation mellem middelværdier.



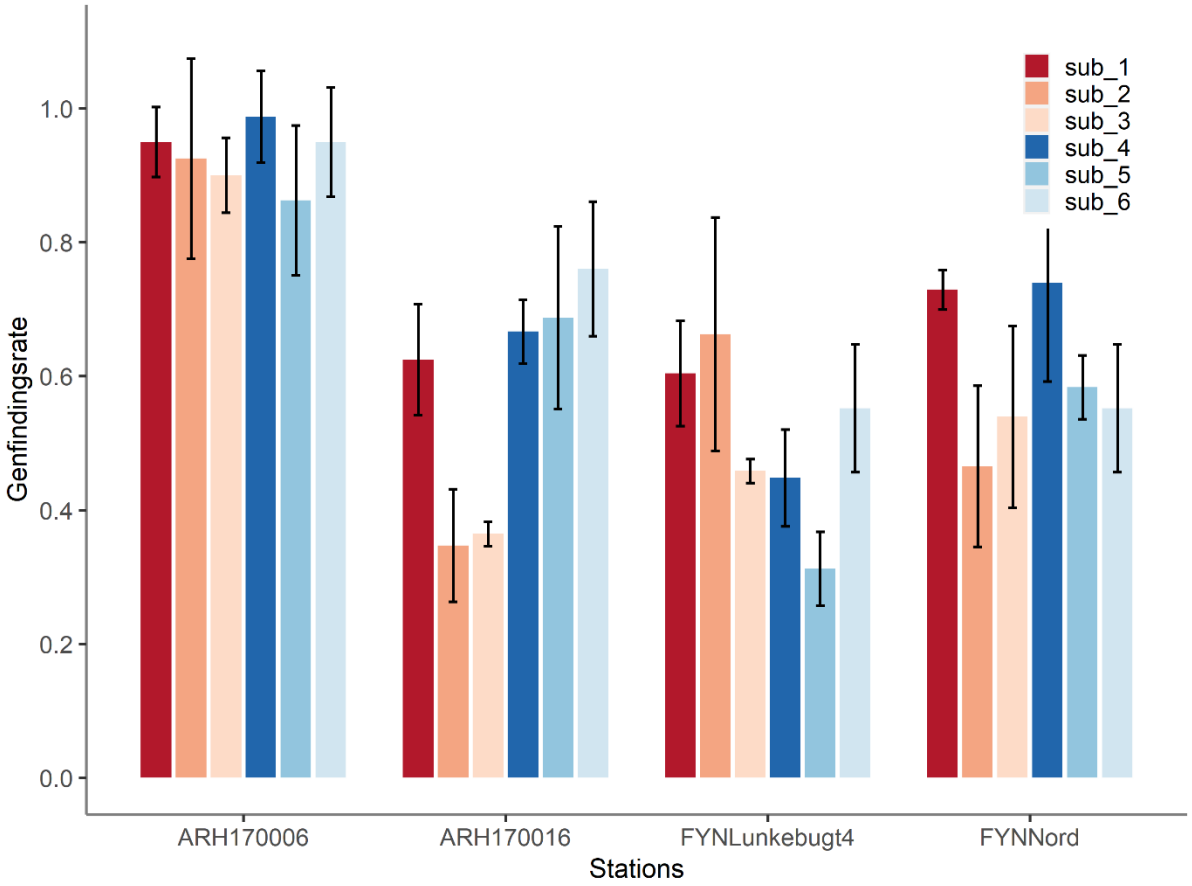
Figur 5. Variation på mikroplast antal koncentration mellem delprøverne. Kolonnerne viser gennemsnitsværdien mellem scanninger. Fejlbjælkerne viser standardafvigelsen mellem scanninger.



Figur 6. Variation på mikroplast masse koncentration mellem delprøverne. Kolonnerne viser gennemsnitsværdien mellem scanninger. Fejlbjælkerne viser standardafvigelsen mellem scanninger.

Den kemiske analyse af en prøve indebærer flere usikkerheder delprøvetagning og antallet af scanninger af ekstraktet. En væsentlig usikkerhed relaterer sig til effektiviteten af prøvebehandlingsprotokollen. For at vurdere størrelsen heraf blev genfindingen bestemt. Genfindingen varierede mellem stationer såvel som

mellem delprøver fra samme station (Figur 7). Den højeste genfinding blev fundet i station ARH170006 med et gennemsnit på 93%.



Figur 7. Genfindingsrate for de fire stationer per delprøve

Der blev endvidere analyseret for blindværdier (blankprøver), der dog viste en ganske beskeden forurening af prøverne i forhold til de målte koncentrationer.

i.2.4 Diskussion

Variabiliteten blev målt ved den relative standardafvigelse i procent i forhold til gennemsnittet, således at variabiliteten er sammenlignelig på tværs af prøver med divergerende resultater. Den relative variabilitet mellem delprøver og den relative variabilitet mellem individuelle scanninger var af samme størrelsesorden. Absolutte koncentrationer varierede dog mærkbart mellem delprøverne. Forøgelse af antallet af scanninger pr. delprøve vil give en mere sikker analyse, men ikke nødvendigvis ændre gennemsnitsværdierne meget. Derfor er det **ikke** sandsynligt, at analyse af blot én delprøve, sammen med at øge antallet af scanninger, vil flytte den bestemte koncentration tættere på sandheden, her defineret som gennemsnitsværdien af alle analyserede delprøver.

Dette leder til, at antallet af analyserede delprøver har betydning for at opnå et mere sikkert estimat for mikroplastkoncentrationen. Samtidig skal en væsentlig del af ekstraktet scannes for at forbedre præcisionen af delprøveanalysen. Desværre fører dette til en tilgang, hvor indsatsen for at analysere en enkelt prøve bliver ganske betydelig. Nuværende praksis hos Aalborg Universitet er at analysere én delprøve ved at anvende op til tre scanninger. At øge dette til at analysere for eksempel seks delprøver ville seksdoble analyseomkostningerne. Det er billigere at øge antallet af scanninger, selvom der også er grænser for dette.

Det ville i princippet være ideelt at scanne hele ekstrakten fra en delprøve. En fuldstændig oprensning af marine sedimentprøver er imidlertid ikke mulig, og forstyrrende materiale (partikler) vil være til stede selv efter omfattende prøveforberedelse. Dette betyder, at der kun kan deponeres små portioner af ekstrakt pr. scanning, hvilket så betyder, at mange scanninger ville være nødvendige for at kemisk analysere hele ekstraktet. Der er i praksis grænser for, hvor mange scanninger der kan laves, da hver scanning kræver en del maskintid og dermed ressourcer.

Konfidensen på bestemmelsen af mikroplastindholdet ved analysen af de store sammensatte prøver i nærværende undersøgelse var væsentligt mindre end usikkerheden på prøveudtagning inden for et område som rapporteret af Liu et al. (2022; in prep), der undersøgte variationer i to områder, der hver dækkede ca. 1 km^2. Set i dette lys vil det ikke give mening at øge den analytiske nøjagtighed og præcision uden også at øge repræsentativiteten af prøveudtagningen.

Forudsat de relative usikkerheder i denne undersøgelse og undersøgelserne af Liu et al. (2022; in prep) holder, er den største usikkerhed prøvetagning i felten, der synes at være den langt største potentielle kilde til usikkerhed, idet der er stor lokal variation over korte afstande. En repræsentativ prøve kræver derfor mange nedstik over det areal, der ønskes repræsenteret. Delprøvetagning af den indsamlede prøve i laboratoriet kan medføre en del usikkerhed, da komplekse matricer som sedimenter er svære at homogenisere, og ekstraktion af mikroplastet, kemisk analyse herfor og blankforurening kan alle være vigtige kilder til usikkerhed, men synes mindre tilbøjelige til at dominere sammenlignet med prøvetagningsusikkerheder.

Dette leder til en diskussion om, hvordan den totale usikkerhed for mikroplastkvantificering bedst minimeres med henblik på overvågning i marine sedimenter. Naturligvis skal der lægges vægt på prøvetagning, men også det, der sker i laboratoriet, når prøven er indsamlet, spiller en stor rolle. Samtidig skal der tages hensyn til de hertil forbundne omkostninger, idet prøvetagnings- og analysesikkerhed i høj grad er et spørgsmål om hvor mange ressourcer, der lægges i opgaven.

Selvom skibstiden er dyr, er det umagen værd at investere ressourcer i at tage en sammensat prøve, der på systematisk vis dækker det areal der ønskes repræsenteret ved prøvetagningen. Et bud på en pragmatisk tilgang for marin overvågning kunne være at udføre nedstik på 5 steder jævnt fordelt indenfor et 1×1 km kvadrat. Prøverne bør udtages med uforstyrret overflade. På hvert sted kunne der udtages cirka 1 L sediment fra de øverste 2 cm af sedimentlaget. Disse 5×1 L sediment samles til én prøve, der repræsenterer den givne lokalitet.

Desuden er det umagen værd at optimere delprøvetagningen i laboratoriet. I princippet ville det være at foretrække at analysere mange individuelle delprøver, men dette øger omkostningerne proportionalt med det antal delprøver, der skal analyseres. Derfor kan det være mere hensigtsmæssigt at tage en pragmatisk tilgang, for eksempel at tage mange små kerner fra en større prøve, blande dem og derefter lade én prøve gå til analyse. Et bud på en sådan tilgang kunne være: Bland de 5 L prøve grundigt i en passende beholder; Udtag jævnt fordelte delprøver i hele sedimentets tykkelse med fx et 30 mm rør; Udtag tilstrækkeligt mange delprøver til der er taget cirka 0,5 L vådt sediment.

Med hensyn til den kemiske analyse vil det sandsynligvis være umagen værd at øge antallet af scanninger så en større del af prøven bliver scannet, selvom dette vil øge omkostningerne lidt. Et pragmatisk bud kunne være at scanne 3 delprøver, og addere resultaterne fra disse til ét resultat. Mængden der kan analyseres i ét scan, afhænger af hvor godt sedimentet kunne oprenses, og det tilstræbes at scanne 10% af den samlede prøve, dog ikke flere end maksimalt 5 scanninger.

For at imødegå problemer ved bestemmelse af de mindste og største stykker mikroplast, kunne data afrapporteres som antal og masse koncentration i størrelsesintervallerne 10-20, 20-50, 50-100, 100-300, 300-1000 og 1000-5000 µm.

i.3 AP 2 – Evaluering af analysemetoder for mikroplast i sedimenter

Formålet med arbejdspakke 2 er at undersøge præcision og nøjagtighed af kvantificering ved forskellige analysemetoder anbefalet af OSPAR/HELCOM og JRC/EU TGML til marin overvågning af mikroplast i f.eks. sedimenter. Disse spænder fra relativt simple metoder til de mere præcise metoder, der er tilgængelige for forskning. Dette løses eksperimentelt ved at sammenligne en af de mest avancerede analysemetoder, der muliggør kemisk identifikation af partikler i en prøve, med en 'simplere' tilgang, der udelukkende er rettet mod identifikation af, om en partikel tilhører materialegruppen mikroplast.

i.3.1 Screeningsanalyse baseret på Nile Red-farvning for store mikroplastpartikler

i.3.1.1 Baggrund

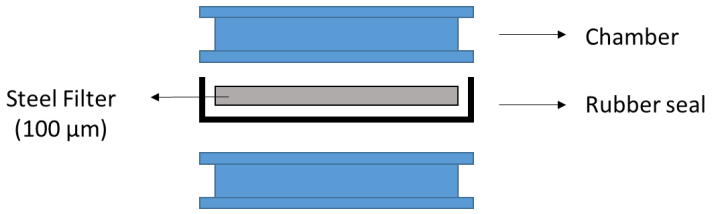
Forskellige analyseteknikker er i spil til måling af mikroplast. Ofte identificeres de større fraktioner (> 300 µm) visuelt ved optisk mikroskopi, hvilket er en besværlig og ret subjektiv metode med høj risiko for falsk identifikation. Det er blevet foreslået, at hurtigere screeningsmetoder der bruger farvningsteknikker og fluorescensmikroskopi kan forbedre den visuelle identifikation af plastpartikler, som efterfølgende kan suppleres med kemisk bestemmelse af plasttype (f.eks. FTIR-analyse). Sådan screeningsstrategi er blevet foreslået som en potentiel overvågningsmetode af OSPAR og HELCOM havkonventionerne. Nile Red (NR) er et farvestof der kan bruges i denne sammenhæng, og dens anvendelse har været demonstreret med succes. NR adsorberes på polymeroverfladen og kan fluorescere under visse lysforhold, hvilket hjælpe med at skelne plastpartikler fra naturligt materiale.

NR-farvningsmetoder kræver stadig et element af visuel identifikation under et mikroskop, idet farvestoffet hjælper med at identificere plastpartikler, men også markerer naturligt organisk materiale. Signalet fra naturligt organisk materiale adskiller sig lidt fra plast, men der er stadig en risiko for fejlidentifikation. Det betyder, at der er behov for yderligere metodeudvikling til denne farvningsteknik, såsom udvikling af en automatiseret protokol til sortering af plast fra organisk materiale ved hjælp af det fluorescerende signal; samt brug af computerbaserede løsninger til polymersortering og deres antal og størrelsesfordeling.

En omkostningseffektiv metode kunne benytte et digitalkamera til at tage det fluorescerende billede og automatisk billedbehandling til at bestemme, hvilke partikler der er mikroplast. Denne tilgang blev undersøgt i arbejdspakken og kombinerer digitalkamera og billedbehandling til en screeningsanalyse af mikroplast ved hjælp af Nile Red-farvning. Metoden giver betydelige fordele for en mere automatiseret metode, øger analysehastigheden og forbedrer kvaliteten af visuel identifikation af mikroplast. En protokol og en fotoboks blev udviklet, og det blev vurderet, at denne tilgang i høj grad kan gavne overvågningsaktiviteter på storskala prøvepuljer. Resultaterne fra studiet blev brugt til at starte udviklingen af en automatisk metode til identifikation af større mikroplastpartikler ved differentiering af plastpartikler fra naturlige partikler. Her fokuserede vi hovedsageligt på partikler > 1 mm, selvom det er muligt for mindre partikler.

i.1.1.1 Metode

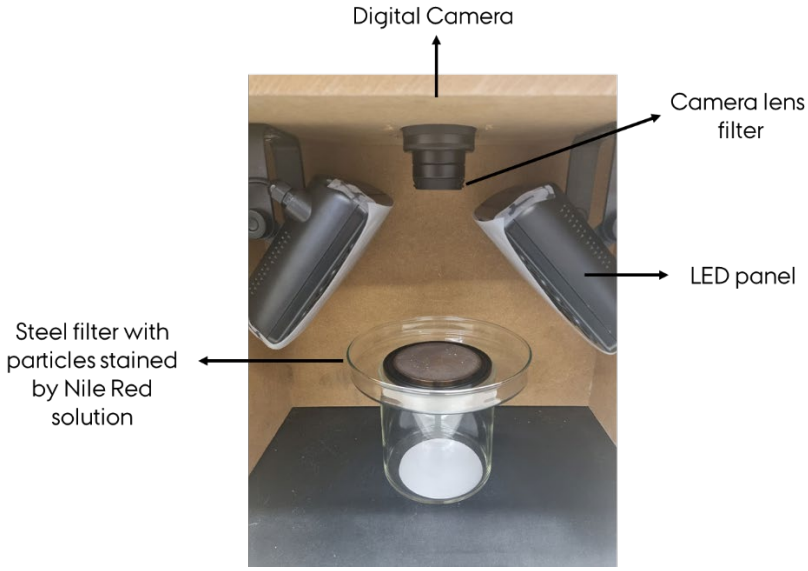
I denne undersøgelse blev 0,01 mg mL^{-1} NR i etanol forberedt og påført et stålfiltersystem indeholdende mikroplastpartikler > 1 mm (PE, PP, PET og PA) med en blanding af tilsatte organiske materialer (havplanter, træ, proteiner, osv.), der virkede som interfererende partikler. Filtersystemet (Figur 8) blev anbragt i en petriskål, og inkuberet i NR-opløsningen.



Figur 8. Stålfiltersystem brugt til Nile Red-farvning. Filteret er placeret i midten af to metalkamre med en gummi O-ring tætning

Indledende tests blev udført for at bestemme forholdene hvor polymeren blev fremhævet mest muligt på en mørk baggrund. Der blev bygget en fotoboks af træ til billedoptagelse (Figur 9). Blåt og UV-lys blev evalueret for excitation, og både orange og røde filtre blev brugt til at tælle NR-emissionssignalet.

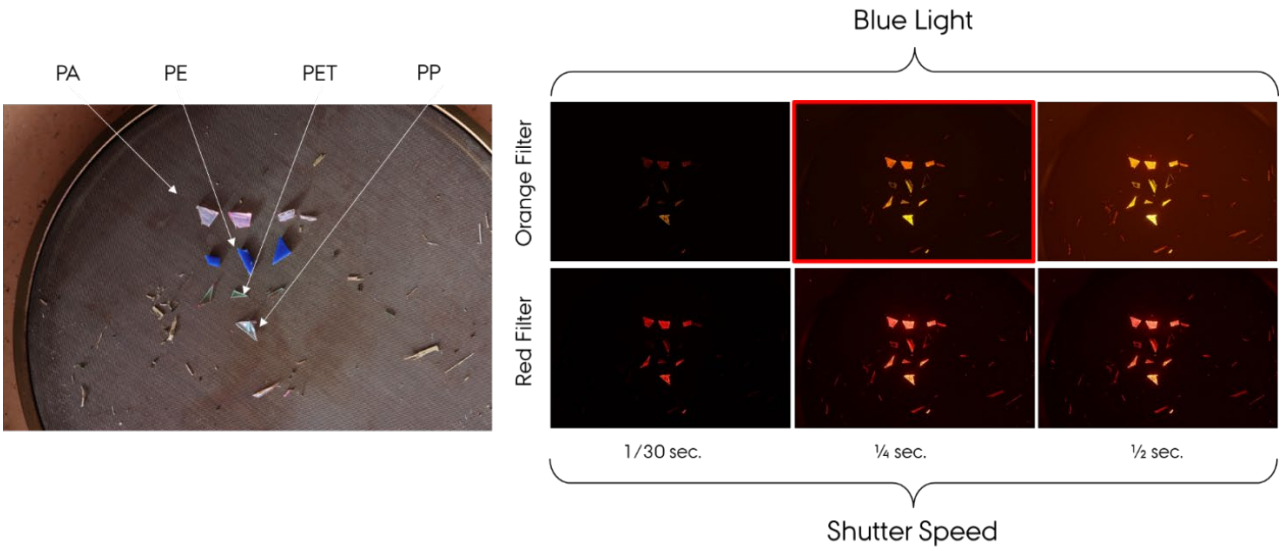
Herefter blev en automatisk metode til screening evalueret, der kombinerede billedbehandling med billedsegmentering og objektmåling. Forskellige farvemodeller blev evalueret til billedbehandling for at skelne plasten fra interfererende partikler. Farvemodeller er matematiske beskrivelser af, hvordan farver kan repræsenteres ved tal, typisk som tre værdier. Strategien blev valideret på en marin sedimentprøve taget øst for Skagen, Danmark.



Figur 9. Fotoboks-prototype (indvendigt) til optagelse af Nile Red-farvet mikroplastbilleder

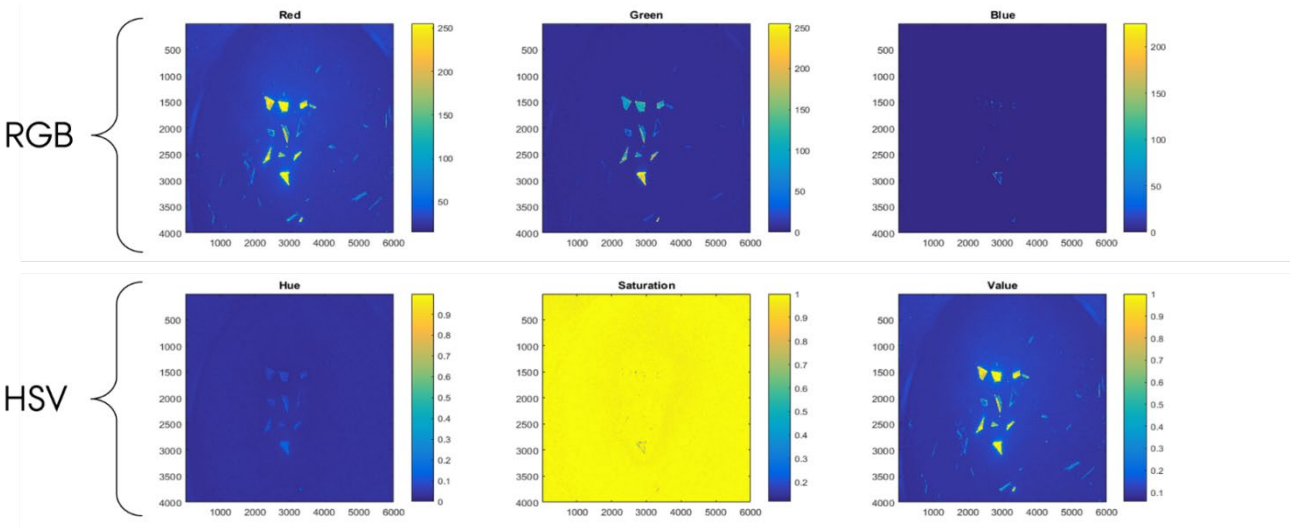
i.1.1.2 Resultater og diskussion

Indledningsvis blev betingelserne under hvilke billederne skulle tages undersøgt. Flere kriterier blev evalueret, og tre hovedfaktorer identificeret for at opnå optimalt fluorescenssignal: Lyskilde, filter og lukkerhastighed. I en første vurdering viste blåt lys sig bedst til at fremhæve polymerpartikler, mens det orange filter var bedst til at differentiere dem fra organisk materiale. Desuden gav lukkerhastighed på 0,5 sekunder passende kontrast mellem partiklerne og baggrunden (Figur 10).



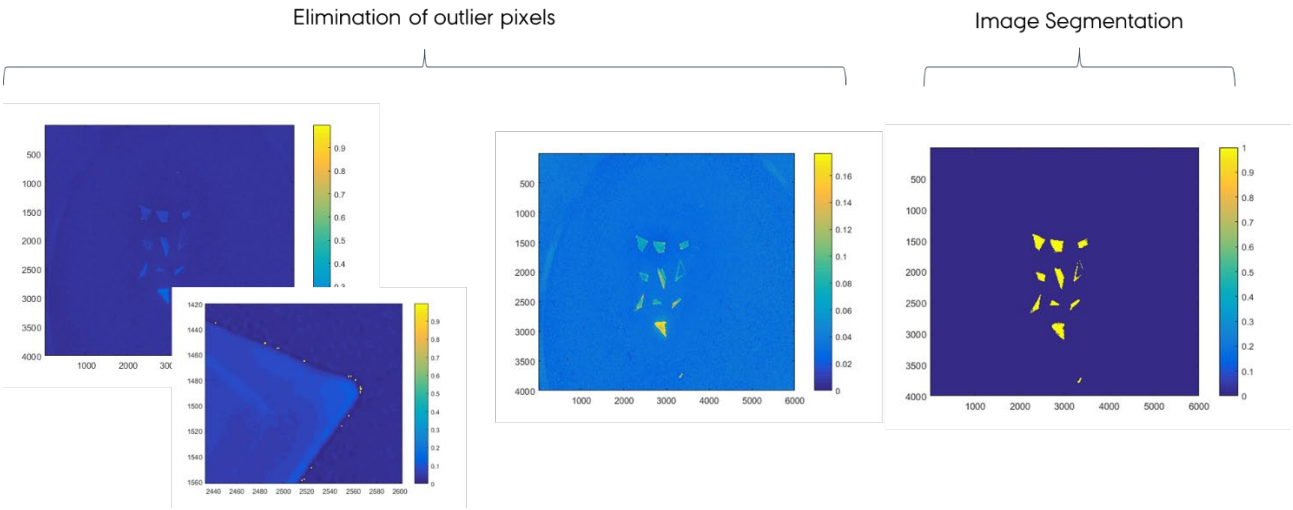
Figur 10. Visuelle og Nile Red-farvede billeder af PA, PE, PET og PP-partikler. De fluorescerende billeder blev opnået med blåt lys ved hjælp af røde og orange filtre og anvendelse af forskellige lukkerhastigheder. Billedet fremhævet med rødt blev valgt til yderligere billedbehandling

To forskellige farvemodeller blev undersøgt: RGB og HSV. Sidstnævnte er et alternativ til den almindelige RGB-model. Figur 11 giver et eksempel på farvekanalerne for RGB- og HSV-farvemodellerne.



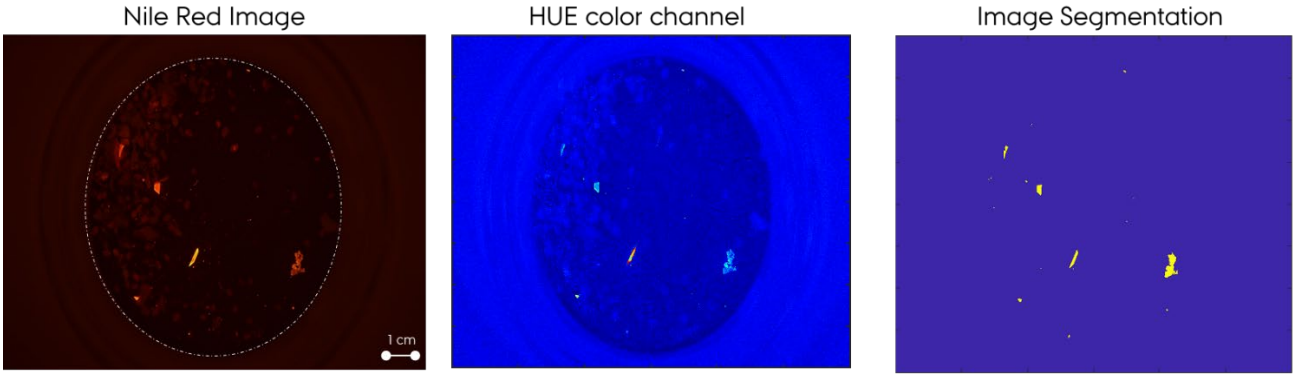
Figur 11. Nile Red-farvet billede dekomponeret i tre farvekanaler til RGB- og HSV-farvemodeller

Figur 11 viser, at hver farvekanal har forskellig information fra det visuelle billede, hvor farvelinjen (til højre for hvert billede) refererer til farveintensiteten. Nogle farvekanaler, f.eks. Red og Value er ens med organisk materiale klart synligt. På den anden side demonstrerede Hue, at polymerinformationen er fremherskende, i modsætning til Blue og Saturation. Ses nærmere på Hue-kanalen for yderpunkter, kan polymerpartiklerne endda fremhæves ved blot at fjerne dem. Dette kan følges ved at vælge en pixelværditærskel for billedsegmentering, som vist i Figur 12. Dette fremhæver vigtigheden af at undersøge ekstremværdier, når man har at gøre med digitale billeder, hvilket er relateret til kamerasensoren og farvemodeltransformationen.



Figur 12. Visualisering af afvigende pixels og billedsegmentering på Hue-farvekanalen af et Nile Red-farvet billede

Et par billedbehandlingstrin tillader billedsegmentering, adskillelse af målpartikler fra baggrunden og interfererende elementer. Dette kan forbedre den visuelle analyse af mikroplast væsentligt ved at udpege partikler, der bør valideres kemisk. Denne automatiserede strategi blev anvendt på en sedimentprøve (Figur 13). De udvalgte partikler blev manuelt frasorteret og valideret kemisk ved hjælp af FTIR. Partiklerne blev positivt identificeret som plastik, og de var PE, PVC og malingsflager. For mørkfarvede polymerer var det udfordrende at blive opdaget ved brug af Nile Red. Samme for mindre partikler uden høje forstørrelsesopsætninger. Dette demonstrerer metodens anvendelse til at sortere plastikpartiklerne fra interfererende materialer.



Figur 13. Automatiseret billedbehandling ved hjælp af Nile Red påført en sedimentprøve (Skagen, DK)

Den automatiserede billedprocestilgang forbedrer den visuelle analyse af store mikroplastpartikler (> 1 mm), som stadig er den mest almindelige procedure til partikelidentifikation. Screeningsstrategien reducerer bias og falsk positive rater ved udvælgelsen af partikler. Derudover reducerer den tiden der skal bruges til analyse ved at præsortere hvilke partikler, der skal håndplukkes og analyseres kemisk hvis polymertypen skal bestemmes.

Den foreslåede strategi kan effektivt udnyttes til mikroplastikanalyse i forskellige miljøer, herunder sediment, vand (via mantratrawl) og biota, forudsat at der udføres passende prøvebehandling. Nile Red, som foreslået af både OPAM og HELCOM, tilbyder en lovende metode til overvågning af aktiviteter på grund af dens enkelhed og omkostningseffektivitet, som det fremgår af resultaterne af denne undersøgelse. Mens undersøgelsens fokus var på partikler, så længe de kan vælges manuelt til yderligere analyse uden at kræve udstyr med højere forstørrelse. Desuden er der potentiale for at bruge denne teknik til karakterisering af polymerer, men yderligere undersøgelser er nødvendige.

i.1.2 Maskinlæringsstrategi for mikroplastkarakterisering og kvantificering for små mikroplastpartikler ved µFTIR billeddannelse

i.1.2.1 Baggrund

Metoden beskrevet ovenfor er rettet mod de større mikroplastpartikler. De mindre bestemmes ofte med µFTIR hyperspektral billeddannelse, en teknik der i dag er den mest almindelige og nyeste til identifikation af små mikroplastik. Denne teknik indsamler kemisk og rumlig information om mange partikler på samme tid ved automatiseret kortlægning af en prøve, hvilket muliggør analyse for små mikroplastpartikler uden manuel sortering og som tillader estimering af partikelegenskaber såsom deres areal og diametre.

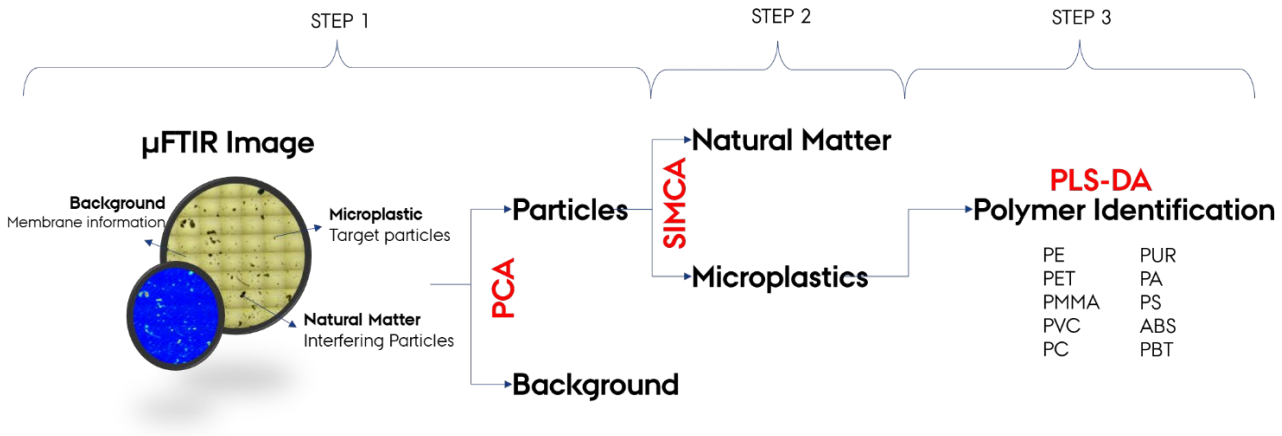
µFTIR hyperspektral billeddannelse skaber komplekse og store mængder information (millioner af spektre), hvilket fører til et behov for automatisk dataanalyse. Der findes forskellige tilgange til, hvordan man håndterer et sådant datasæt, lige fra biblioteksøgningstilgange (korrelation til et referencebibliotek) til mere avancerede maskinlæringsstrategier. Sidstnævnte anvender ofte flere dataforbehandlingsstrategier, eksplorativ analyse og multiklassemodeller, der dækker de plasttyper der findes i miljøet. Dette arbejdes der med i denne arbejdspakke med henblik på at skabe ny vide, der kan benyttes ved overvågningsaktiviteter.

i.1.1.1 Metode

Forskellige multivariant teknikker (PCA, SIMCA og PLS-DA) blev evalueret for at bestemme små mikroplastik (< 300 µm) fra µFTIR hyperspektral billeddannelse.

Mikroplast blev fremstillet af de mest almindelige plastmaterialer (PE, PET, PMMA, PVC, PC, PUR, PA, PS, ABS og PBT) og brugt som reference til identifikation af plastik fra miljøet. Disse materialer blev formalet i en metalkværn og sigtet. Partikler fra 10 til 300 µm blev placeret på et silicium (Si) filter og et filter for hver polymer, samt en blanding af al mikroplastik tilsat naturligt stof, blev fremstillet og analyseret med et µFTIR hyperspektralt billeddannelsessystem. En tilgang til maskinlæring ved anvendelse af hierarkisk analyse (HA) blev evalueret for at trække mikroplastinformation ud af de hyperspektrale billeder. Figur 14 viser arbejdsgangen udviklet til karakterisering af mikroplast.

Først blev Principal Component Analysis (PCA) anvendt til udvælgelse af området af interesse, dvs. partikelinformation. Soft Independent Modeling Class Analysis (SIMCA) blev efterfølgende anvendt til at sortere det naturlige stof og mikroplastinformation (TRIN 2, Figur 14), hvor sidstnævnte blev yderligere brugt til polymerdiskrimination ved anvendelse af Partial Least Squares-Discriminate Analysis (PLS-DA - TRIN 3).

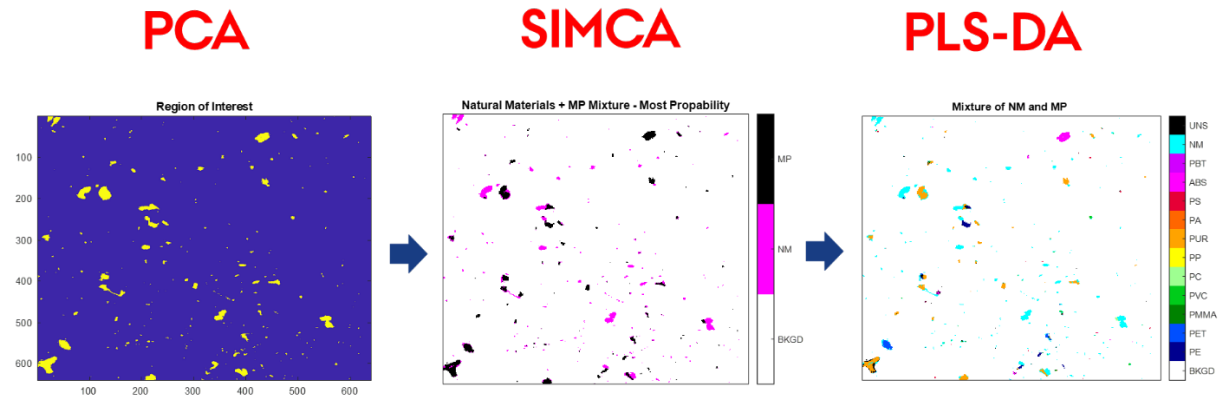


Figur 14. Workflow af FTIR-databehandlingen og multivariantteknik anvendt i hvert trin af den hierarkiske analyse

Detaljeret information om partiklerne blev dannet ved at bruge billedets rumlige information til partikeltælling og størrelsesfordeling. Sidstnævnte blev yderligere undersøgt for at vurdere deres variabilitet i forhold til de to almindelige måder at rapportere partikelstørrelsen på: (1) Længde (maksimum Feret diameter) og (2) Filter porestørrelse (minimum Feret diameter). Feret diametre blev beregnet for hver partikel.

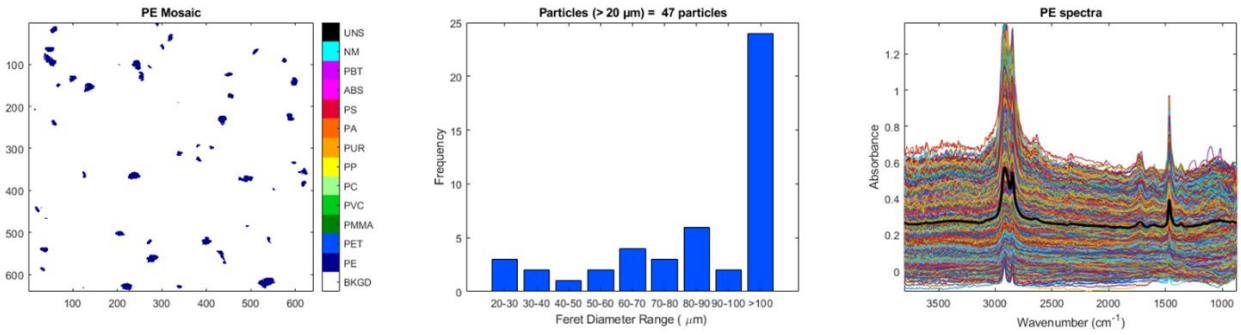
i.1.1.2 Resultater og diskussion

PCA var i stand til at udvælge området af interesse ved at fjerne eventuelle pixels, der ikke var relateret til partiklerne i billedet, hvilket reducerede behandlingstiden og risikoen for falsk positiv identifikation. Både SIMCA (TRIN 2) og PLS-DA (TRIN 3) modellerne viste stor gennemsnitlig følsomhed og specificitet til at frasortere naturligt stof og skelne mellem polymertyperne. Hvad angår klassificeringsfejl, blev der opnået et gennemsnit på 3% og 0,2% på henholdsvis TRIN 2 og TRIN 3. Disse evalueringsparametre bruges til at estimere sandsynligheden for, at pixels tilhører den korrekte målkategori og er gode eksempler på en robusthedsevalueringsmetode, der kan anvendes ved rapportering og sammenligning af mikroplastdata fra forskellige analytiske teknikker data. Det referer til både True Positive Rate og True Negative Rate, det bruges til at vurdere modellens ydeevne ved korrekt klassificering af plastikpartiklerne. Figur 15 viser resultatet af en prøveafbildning indeholdende en blanding af plast og naturligt stof i hvert trin af den hierarkiske analyse.



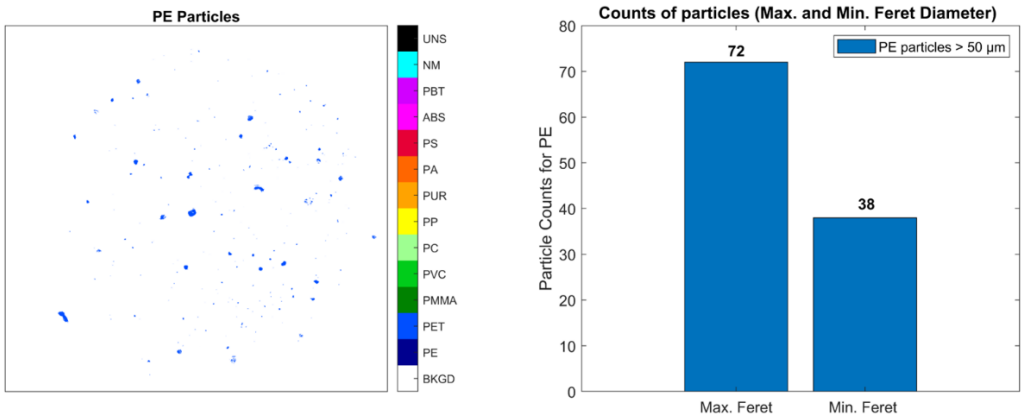
Figur 15. Resultater af en billedprøve indeholdende en blanding af plast og naturligt stof i hvert trin af den hierarkiske analyse

Den udviklede metode kan anvendes til forskellige prøvematricer, så længe prøven er oprenset med henblik på mikroplastanalyse. Metode kan øge hastigheden af dataanalyse, forbedre kvalitet og reproducerbarhed i polymerbestemmelse, og demonstrerer hvordan potentialet ved µ-FTIR hyperspektral billeddannelse til bestemmelse af mikroplast kan udnyttes fuldt ud. Morfologisk information om prøverne findes ud fra de identificerede billeder, for eksempel partikelstørrelse og deres frekvens (Figur 16).



Figur 16. PE forudsagt billede med partikelantal og størrelsesfordeling. Infrarøde spektre af alle PE-partikler vises

En sammenligning af partikelantal som maksimum og minimum Feret diameter (mikroplastik > 50 µm) blev udført på et billede, der kun indeholdt PE-partikler, Figur 17. Resultatet viser, at antallet af partikler blev halveret, da filterafskæringen blev brugt til at beregne partikelfrekvensen. Dette peger på nødvendigheden af standarddefinitioner for, hvordan partiklernes størrelse og frekvens skal beregnes og/eller rapporteres i forsknings- og overvågningsaktiviteter for at kunne sammenligne resultater, da det kan variere betydeligt med den anvendte strategi. Der findes ingen standardprocedurer eller retningslinjer for hvordan dette skal gøres, og viser vigtigheden af i det mindste at rapportere den valgte beregning frem for kun at angive antallet af identificerede partikler.



Figur 17. Forudsagt billede for PE og deres partikelantal (> 50 µm). Beregning af partikelmængde baseret på maksimal og minimum Feret diameter

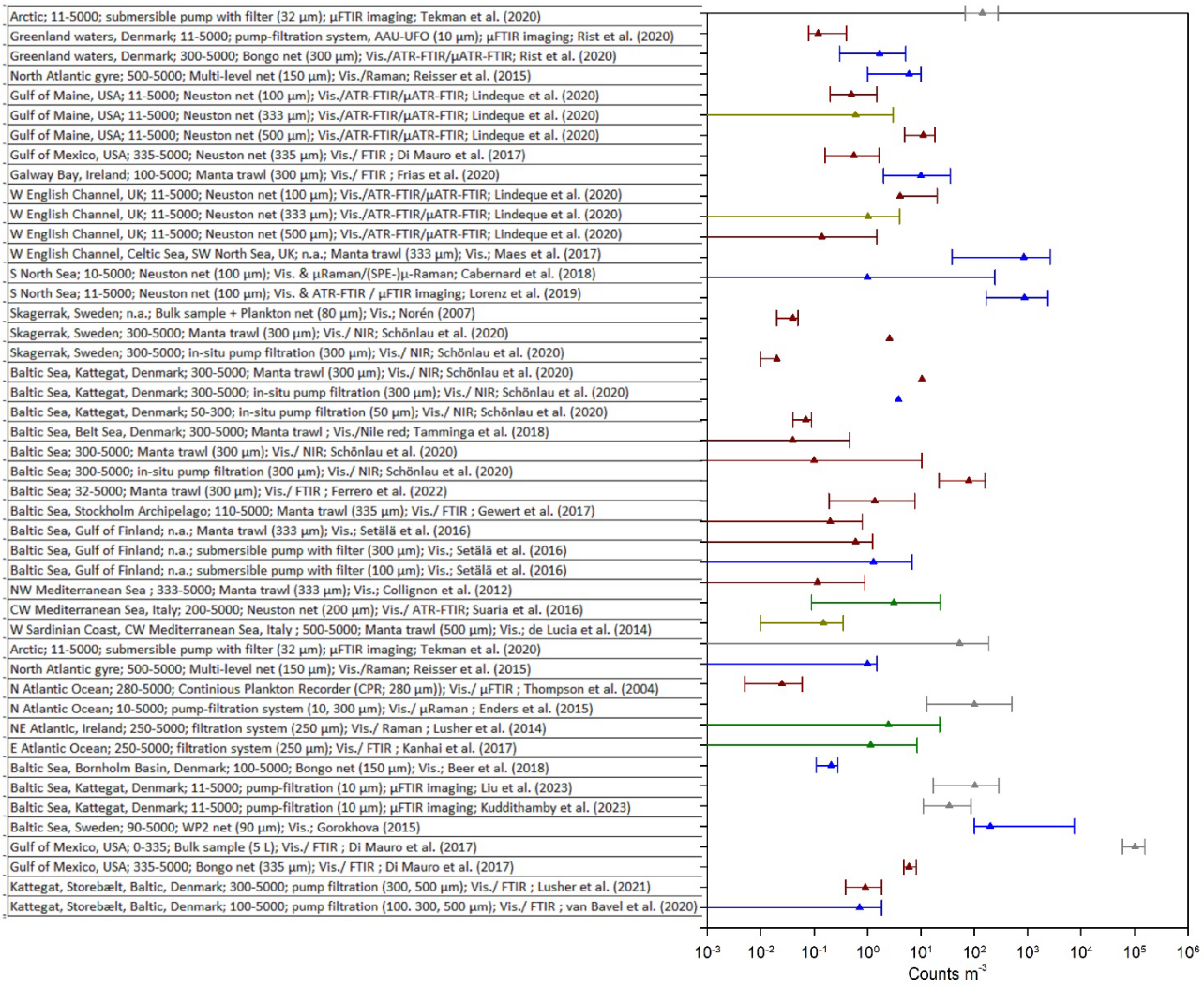
Den tilgang, der præsenteres i denne undersøgelse, tilbyder et væld af information, der kan hjælpe med karakterisering og sporing af mikroplast fra forskellige kilder i miljøprøver. Det er dog vigtigt at behandle og udtrække mikroplastik korrekt fra den undersøgte matrice for at opnå nøjagtige resultater. Denne strategi kan effektivt implementeres til overvågning af aktiviteter ved hjælp af de her præsenterede parametre. Hvis der anvendes data fra et andet instrument eller en anden kilde, skal der etableres en kalibreringsoverførsel, før der udføres nogen analyse. Ikke desto mindre kan metoden replikeres ved hjælp af alle de angivne oplysninger i et hvilket som helst andet µFTIR-instrument. Desuden kan tilgangen opdateres til at omfatte andre polymerer eller klasser, afhængigt af forskningsbehovene

i.2 AP 3 – Evaluering af prøveudtagningsmetoder for mikroplast i overfladevand og vandsøjle

Formålet med arbejdspakke 3 er at skabe viden om forskelle mellem forskellige prøveudtagningsmetoder for mikroplast i overfladevand og vandsøjlen. Her sammenlignes tilgængelige data indsamlet med net trukket af et skib, fx manta-trawl, og pumpefiltrerende prøvetagning på metalfiltre, som kan bruges til prøvetagning af den finere plastfraktion, og som kan bruges i fx FerryBoxes.

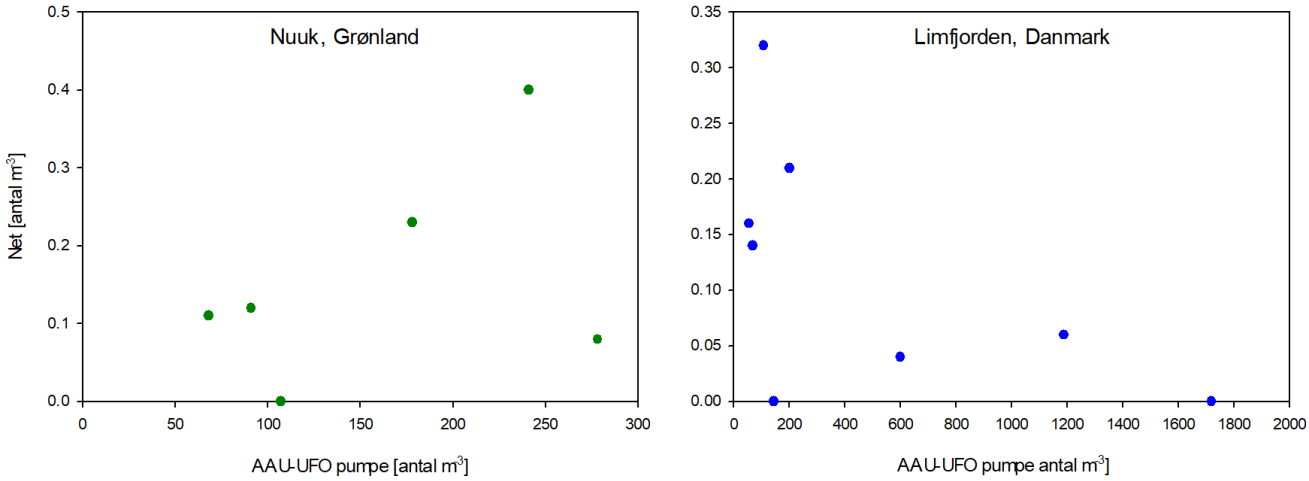
Den mest hensigtsmæssige prøvetagningsteknik for mikroplast defineres ud fra hvilket system, der skal prøvetages (f.eks. strande, sublitorale sedimenter, havoverfladen, vandsøjle) samt de efterfølgende behandlings- og analysekapaciteter. Sidstnævnte sætter barren for, hvad der kan opnås med hensyn til analytisk output, såsom hvordan små mikroplaster kan identificeres pålideligt, hvorvidt polymertyper kan identificeres hvorvidt partikelstørrelse og form kan identificeres, osv. Eksisterende data fra litteraturen er derfor ganske diverse, og rapporterede resultater for forskellige vandområder varierer indenfor et

koncentrationsinterval på 8-9 dekader (Figur 18). Dette interval afspejler næppe 'virkelige' koncentrationsforskelle, men snarere forskelle i prøvetagnings- og analysemetoder i kombination med 'virkelige' forskelle i koncentration i de pågældende havområder. Hvor meget der kan tilskrives hvilket forhold, er dog vanskeligt at sige.



Figur 18. Et udvalg af undersøgelser, der har analyseret koncentrationen af mikroplast i havvand. Linjerne viser det registrerede område af mikroplastikkoncentrationer, og prikkerne markerer middelkoncentrationerne i partikler pr. m^3. Undersøgelserne er grupperet efter oceanografisk region. Linjernes farver refererer til den anvendte maskestørrelse fra 10-50 µm (grå), over 50-150 µm (blå), 150-250 µm (grøn), 250-350 µm (rød) til 450-550 µm (gul). Bemærk, at undersøgelserne brugte forskellige analysemetoder, hvoraf nogle er bedre end andre til at identificere små mikroplastik.

Til konkret sammenligning af data fra prøvetagning med net, der typisk har en maskevidde på cirka 300 µm, med prøvetagning med pumpefiltrering, der typisk har porestørrelse på filtre på 10 µm, foreligger der kun to datasæt, der har udført begge metoder på samme vandområde og samme tidspunkt. Det ene blev udført i Grønland, omkring Nuuk, det andet blev udført i Limfjorden (Figur 19). Disse undersøgelser viste, at der var cirka tre til fire størrelsesordener forskel på resultaterne fra de to metoder (målt som antal partikler per vand volumen). Der var endvidere ingen korrelation mellem hvad der blev målt med den ene versus den anden teknik.



Figur 19. Sammenligning af koncentrationer fundet af prøver indsamlet med AAU-UFO pumpesystem og med net

I 2022 blev der gennemført en undersøgelse med henblik på at etablere en overvågningsstrategi til vurdering af mængden af flydende mikroaffald i overfladelaget af de danske kystnære farvande. Undersøgelsen anvendte de seneste internationale anbefalinger til rutinemæssig prøveudtagning, analyse og datarapportering. Mikroaffald > 300 µm blev indsamlet på syv kystnære lokaliteter omkring Sjælland, Danmark, ved hjælp af et Manta-trawl. De opsamlede partikler blev sigtet i størrelsesfraktioner > 5 mm, 1-5 mm og 0,3-1 mm, og derpå vurderet visuelt for at manuelt at udtage mikroplastlignende partikler og fibre. Antallet og koncentrationerne af identificeret mikroaffald ved hver prøvetagningsstation ses i Tabel 1. Medianmikroplastkoncentrationen var 0,057 partikler m^{-3} og en maksimal koncentration på 0,213 partikler m^{-3}, hvilket indikerer lave forureningsniveauer af de undersøgte havoverfladevande. Dette niveau er også sammenligneligt med resultater i andre publicerede undersøgelser fra danske farvande.

I lighed med andre internationalt publicerede undersøgelser finder denne undersøgelse, at inter-prøvevariabiliteten kan være høj. Prøvetagning og analyse af mindst 2-3 replikater fra hvert sted bør derfor bruges i overvågningsøjemed. Derudover bør mere end én prøveudtagning pr. år pr. sted overvejes. Det blev også konkluderet, at der kræves en prøvetagning på mindst 100 m^3 overfladevand for at indsamle en repræsentativ prøve i overensstemmelse med anbefalingerne i de internationale retningslinjer for overvågning med Manta-trawl.

Tabel 1. Koncentrationen af visuelt identificerede mikroplastiske fibre og partikler og det beregnede prøvevolumen af hver analyseret prøve

Prøve	**Sample volume [m^3]**	**Koncentration [antal m^{-3}]**		
		Fibre	**Partikler**	**Total**
Køge Bugt, Brøndby st T1	193	0.047	0.010	0.057
Køge Bugt, Brøndby st T2	173	0	0.006	0.006
Sejerøbugten, Gudmindrup	222	0.036	0	0.036
Køge Bugt, Kofoeds enge T1	183	0.005	0.005	0.011
Køge Bugt, Kofoeds enge T2	185	0.005	0.07	0.076
Østfalster, Pomlenakke	172	0.035	0.017	0.052
Roskilde Bredning, Risø	184	0.011	0.005	0.016
Roskilde Vig East I T1	184	0.027	0.016	0.043
Roskilde Vig East I T2	126	0.095	0.095	0.191
Roskilde Vig West I T3	162	0.056	0.037	0.093

Roskilde Vig West I T1	135	0.081	0.030	0.111
Roskilde Vig East II T1	149	0.047	0.134	0.181
Roskilde Vig East II T2	85	0.047	0.166	0.213
Roskilde Vig West II T1	140	0.057	0.021	0.078
Roskilde Vig West II T2	130	0.031	0	0.031

1 Background

In consequence of the EU Marine Strategy Framework Directive, the environmental status of the marine waters must be assessed with respect to marine litter, here among the composition, quantity, and spatial distribution of micro-litter. In Denmark's Marine Strategy II, Part 1, a supplementary environmental goal is set that the Ministry of the Environment shall work towards developing indicators and measurement methods for microplastics in seabed sediment and water column.

The Danish Environmental Protection Agency's monitoring of Danish marine waters is partly coordinated through the regional collaborations OSPAR and HELCOM. In these organizations, there is a demand for knowledge about the methodological basis for achieving representative sampling of microplastics, and knowledge of comparability of methods for microplastic extraction, analysis, and interpretation of taken samples.

This project contributes to reaching the supplementary environmental goal set in Denmark's Marine Strategy II, Part 1, and thus contributes to the creation and expansion of monitoring and action programs according to the Marine Strategy Directive. It addresses analytical and methodological issues regarding microplastics in sediment and analysis methods in general as well as sampling methods regarding microplastics in the water column. The project's results intend to bring Denmark and the regional collaborations closer to a coordinated and harmonized monitoring program for microplastics in the marine environment.

2 Objective

The purpose of investigating the distribution of microplastics (pieces of plastic between 1 µm and 5 mm) in the marine environment is to improve the knowledge needed to assess the environmental status with respect here to, and hereby creating a basis for being able to work towards a good environmental status of marine areas with respect to microplastics. Good environmental status with respect to microplastics is defined such that the composition, amount, and spatial distribution of microplastics in the water column and in seabed sediment are at levels that do not harm the coastal and marine environment. However, there is a lack of sufficient knowledge in the area, which this research project will contribute to obtaining. There are, among other things, missing knowledge of the methodological basis for achieving representative sampling in the marine environment, and knowledge of the comparability of methods for purification, analysis, and interpretation of taken samples. The project addresses this lack of knowledge and is intended to shed light on analytical and methodological issues regarding microplastics in sediment and to compare sampling methods regarding microplastics in the water column.

2.1 WP1 – Representative sampling for statistically reliable assessment of microplastics in marine sediments

It is an objective of Danish and European micro-litter monitoring to create knowledge about the variation of microplastics in Danish marine sediment, both regarding concentration, particle sizes, and polymer types. In doing so, a basis must be created for assessing how and how large samples must be taken, to be able to state with a given statistical certainty about the presence of microplastics in different size fractions and polymer type fractions. Such assessment will depend on the actual composition and concentration in the sediment, the actual microplastic inhomogeneity in a sediment sample, and the analytical method applied. The objective of WP1 is to assess this experimentally on a set of marine sediment samples.

2.2 WP2 – Evaluation of analytical methods for microplastics in sediments

The objective of WP2 is to investigate precision and accuracy of quantification by different analysis methods recommended by OSPAR / HELCOM and JRC / EU TGML for marine monitoring of microplastics in, e.g.,

sediments. These range from relatively simple methods to the most precise methods available to research. This is addressed experimentally by comparing one of the most advanced analytical methods allowing chemical identification of particles in a sample to a 'simpler' approach targeting solely identification of whether a particle belongs to the material group of microplastics.

2.3 WP3 – Evaluation of sampling methods for microplastics in surface water and water column

The objective of WP3 is to create knowledge about differences between different sampling methods for microplastics in surface water and the water column. Here, available data collected with nets pulled by a ship, e.g., manta trawl, and pump-filtering sampling on metal filters, which can be used for sampling the finer plastic fraction and which can be used in, e.g., FerryBoxes, are compared.

3 Content

The project consisted of three work packages:

3.1 WP 1 – Representative sampling for statistically reliable assessment of microplastics in marine sediments

Based on experimental studies on real sediments, it was assessed how much sample is required to yield a solid picture of the microplastic content in Danish marine sediments. For this purpose, four sediment samples of approx. 20 kg wet weight each were collected from the top 2 cm of the seabed and examined for microplastics from 10 to 5000 µm in size. The samples were analysed with a state-of-the-art technique: FPA based µFTIR imaging. Based on the identified microplastics, it was assessed how much sample should be analysed per station. The stations were in areas with different degrees of pollution and distance to sources, as transport and deposition of microplastics can be expected to affect the results. General sediment characteristics, e.g., water content, and organic matter content were determined, and it was investigated whether connections between these parameters and microplastic content could be identified.

In addition, the inhomogeneity of the collected samples was investigated in terms of water content and organic matter content to assess how best to subsample such large sample and to get an understanding of the uncertainty that insufficient subsampling introduces. The uncertainty that sample preparation introduced was addressed, as was the uncertainty introduced by the chemical analysis.

3.2 WP 2 – Evaluation of analytical methods for microplastics in sediments

Based on experimental studies, the feasibility and applicability of analysis methods recommended by OSPAR / HELCOM and JRC / EU TGML for marine monitoring of microplastics in, e.g., sediment samples is investigated. Various analytical techniques are currently being considered for microplastic identification and quantification in different size fractions. For larger fractions (>100 or >300 µm) it has been claimed that, e.g., faster screening methods such as staining techniques can act as a first level microplastic indicator based on fluorescence microscopy supplemented with FTIR spectroscopic identification. For microplastics in the size range 10–100 µm, µFTIR imaging is currently state-of-art for the identification of microplastics and is also recommended for marine monitoring as level 2. However, analytical and spectral factors can affect the characterization and quantification of microplastics for both methods used in level 1 and level 2. In this work package it is among other assessed how pre-processing of the spectral data, including reduction of scattering/scattering and other variability in the spectra as well as removal of background can lead to a higher quality of analysis when characterizing and quantifying microplastic analyses. The results of these studies are expected to lead to

improved data processing protocols and the development of more robust analytical identification methods, also with a focus on data quality assurance and quality control, including validation processes to reduce bias and sources of error.

3.3 WP 3 – Evaluation of sampling methods for microplastics in surface water and water column

A comparative analysis was prepared regarding the sampling of microplastics in the water surface/water column in relation to recommendations from HELCOM and EU/TGML. This is to highlight the difference between pump filtering systems and collection with nets such as manta trawls. The analysis was based on existing data collected by Aalborg University and Aarhus University and addressed the advantages and disadvantages of the sampling methods regarding coverage of temporal and spatial variance of microplastic concentrations in the water column.

4 WP1

Representative sampling for statistically reliable assessment of microplastics in marine sediments.

4.1 Challenges

4.1.1 Sample amount

An objective of Danish micro-litter monitoring is to create knowledge about the level of microplastics in Danish marine sediment and its distribution. To obtain a reasonably precise quantification of microplastics in sediment, a reasonable number of plastic particles must be detected. Assume for simplicity that 1 g of sediment was analysed in full and revealed 1 microplastic particle. Concluding that the concentration of microplastics in that sediment is 1 counts g^{-1} is rather uncertain as another sample of 1 g sediment could have contained 0, 2 or more particles, affecting the relative concentration substantially. However, if 100 microplastic particles were detected, one can have more confidence in that number.

The issue is further complicated by the fact that microplastics come in many sizes. Microplastics are created by the breakdown of larger particles and items. Particles of smaller size will hence be more abundant than particles of larger size. This means that less sample is needed to find enough small particles than to find enough large particles.

Microplastic concentration has traditionally been reported as counts per volume or mass, which becomes problematic when addressing particles within a size continuum. For example, finding one particle of 5 mm and 1 particle of 1 µm in 1 g of sediment would yield a microplastic concentration of 2 counts g^{-1}. However, two particles of 5 mm would yield the same concentration as would two particles of 1 µm. Together with the fact that small microplastics are more abundant than large ones, this means that the size distribution of particles is needed to interpret how much sample is enough to quantify the concentration up to a certain particle size.

Another way to quantify the concentration of microplastics is by its mass. Here the main issue lies in the fact that particle mass comes in the third power of particle size (assuming identical shape of the particles). Assume for example that 1 g of sediment was analysed and 1 particle of 5 mm and 1000 particles of 1 µm were found. In terms of counts, the one big particle means little, however, in terms of mass it dominates the concentration. In this extreme example, the mass concentration including the 5 mm particle would be eight orders of magnitude higher than the mass concentration without it. Whether or not to exclude such ‘outlier’ from the dataset is not clearcut, as it obviously was in the sediment, and excluding it would introduce as much uncertainty as including it. Again, this means that a mass concentration must be accompanied by a size distribution to be able to reliably interpret the data.

Furthermore, microplastics come in many polymer types, and when the objective includes quantifying polymer types, the requirement of sufficient particles extends to finding sufficient particles within each polymer type.

4.1.2 Sampling uncertainty

Sampling at sea involves several issues, of which the homogeneity of the seabed sediment with respect to its microplastic content is a main one. The basis for any sampling for monitoring is that the collected sample is representative of the location from where it is collected. How much seafloor is understood by ‘location’ is seldom clear-cut, but typically the intention is to cover a larger area. If the microplastic concentration varies randomly over the area, as exemplified in Figure 20A by a fictive variation along a transect, a representative sample can be obtained within a small area as the variation between two points close to each other is the same as the variation between two points far from each other. To achieve it, several grabs must be collected and mixed, after which the sample can be assumed representative. However, if the variation over the area is large compared to the variation between two points close to each other (Figure 20B), the area must be sampled by several points distributed over the area, but less grabs per point are needed. If the variation is a combination

of the two (Figure 20C), several points distributed over the area must be sampled by several grabs. Alternatively, more points must be sampled. How to organize a sampling hence depends on how the microplastics are distributed over the seabed. Unfortunately, this is seldom known, and an educated choice must be made.

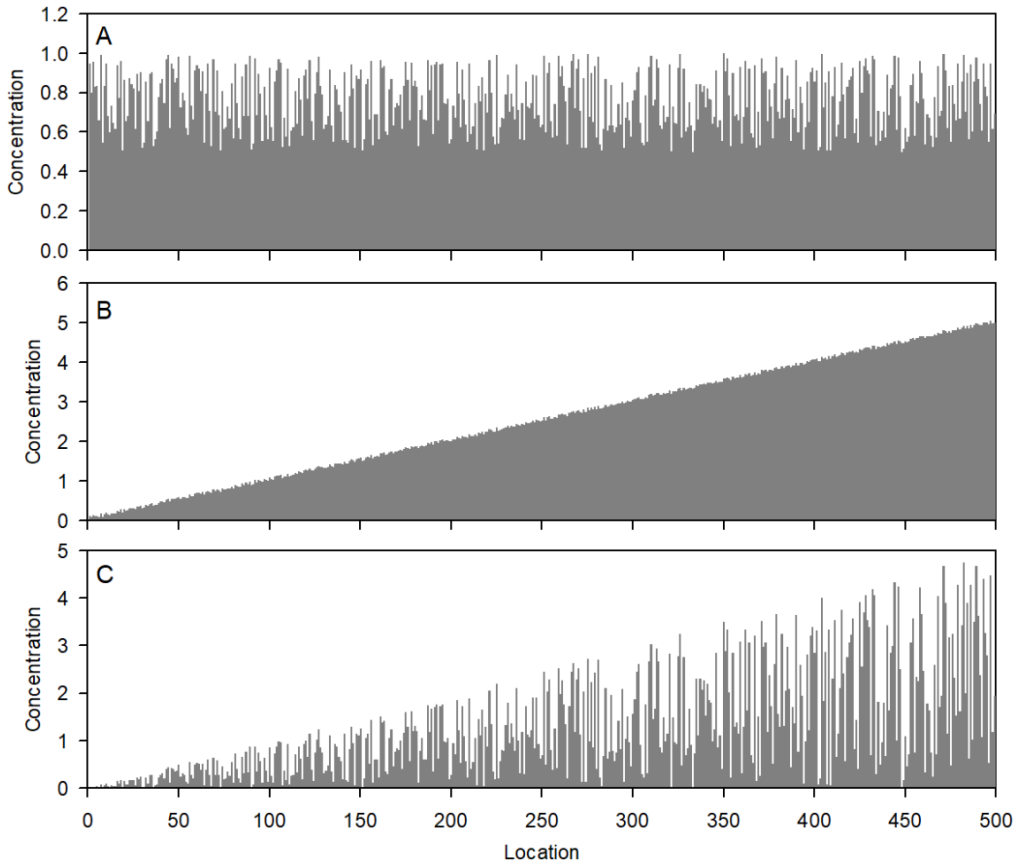


Figure 20. A fictive example of microplastic concentration along a transect

The above example illustrates the risk of taking a single grab and then assume it is representative for the sampled location. It also leads to considering what the spatial scale of variation is. Pragmatically speaking, a good approach for sampling an area would be to divide it into many cells / transects which then are sampled repeatedly, and the collected sample mixed into one (Figure 21). Mixing all the samples will then represent the sediment in the field. How many points and how many grabs per point will depend on the variation of microplastics in the seafloor as illustrated in Figure 20, and on the effort that can be put into the sampling. Sampling is costly and sampling a large grid takes much ship-time. The final decision must hence be made balancing the need for a representative sample and the effort put into the sampling.

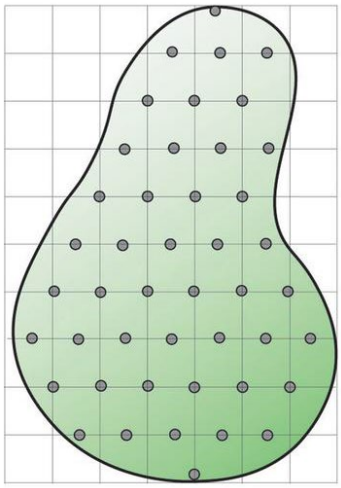


Figure 21. Subsampling from a field

4.1.3 Analysis uncertainty

Another aspect complicates the issue of obtaining a precise quantification of microplastics, namely that it can be impractical or impossible to analyse the whole sample, leading to a need of subsampling the collected sediment for chemical analysis. This is challenging, as matrices like sediment and soil by nature are rather heterogenic (Holland & Elmore, 2008). If the degree of heterogeneity is high, the variation of particulate pollutants like microplastics can also be expected high. Taking a subsample from a heterogenic matrix such as marine sediment sample hence introduces uncertainty on the quantification of microplastics in that sample.

Then there is uncertainty in the analysis itself, partly related to the recovery of the extraction (sample preparation) and partly related to the chemical analysis. The recovery is seldom 100% and not necessarily constant between samples. Especially the latter introduces some error into the analysis. Finally, the chemical analysis requires subsampling of the concentrate extracted from the sample, as the number of extracted particles in most cases by far exceeds what can be analysed in one go. However, subsampling a particle concentrate is also inherently uncertain, as particles tend not to be evenly distributed in the concentrate even though effort is put into homogenizing it.

The analysis hence involves two subsampling's: First a subsampling of the collected sample to be taken into sample preparation; then a subsampling of the concentrate created by the sample preparation to be chemically analysed. The question arises what is the better approach? Take a large subsample into preparation and chemically analyse a small part of the extract here from; or take a small subsample into sample preparation and chemically analyse a large part or all the extract here from. The answer to this lies in where the largest uncertainty is. Whether it is in the subsampling of the collected sample, or in the subsampling of the extract for chemical analysis. All else being equal, one would expect the uncertainty of subsampling an extract to be less than subsampling a raw sample, as it seems reasonable to assume that the extract is a less complex matrix and hence more homogeneous.

4.1.4 Overall uncertainty

The total uncertainty on a datapoint in the field becomes the combined uncertainty related to the above discussed issues. Total uncertainty is a combination of the uncertainty of:

- sampling in the field
- subsampling the collected sample in the laboratory
- extracting the microplastics, and

- analysing the microplastics.

The question now arises how large these individual uncertainties are, and which dominates the overall uncertainty. As an example of a worst case, it could turn out that the variability in subsampling a sediment sample in the laboratory is larger than the variability in the field. This would mean that an observed difference between two monitoring locations are not real differences but an artifact of the analytical protocol. Or it could turn out that the variability over an area like the one illustrated in Figure 21 is huge compared to all other variabilities and uncertainties, and that the sample collected at one point hence does not represent the area it is supposed to.

How to overcome these issues is not straightforward. It is basically a case of 'Known, Unknown, and Unknowable Uncertainties', which is common for many real-life issues. A known uncertainty is where a probability can be precisely specified, an unknown uncertainty is where somebody knows it (where the probability can be obtained at a reasonable effort), while an unknowable uncertainty is missing information unavailable to all (for which it often is impractical or impossible to obtain the actual probability). The approach towards increasing confidence in microplastic concentrations is hence a journey where the knowledge on uncertainties is increased and moved as much towards 'Known Uncertainties' as possible. This will allow to minimize uncertainties and to increase the confidence in the obtained numbers.

4.2 Methods

4.2.1 Sampling

The top 2 cm of seabed sediments were collected from four stations (Figure 22) using a Haps corer and multiple grabs. Each station ended up with approx. 20 kg wet sediment stored in a plastic-free metal bucket, which was immediately transported to Aalborg University. The samples were then kept at 5°C until analysis.

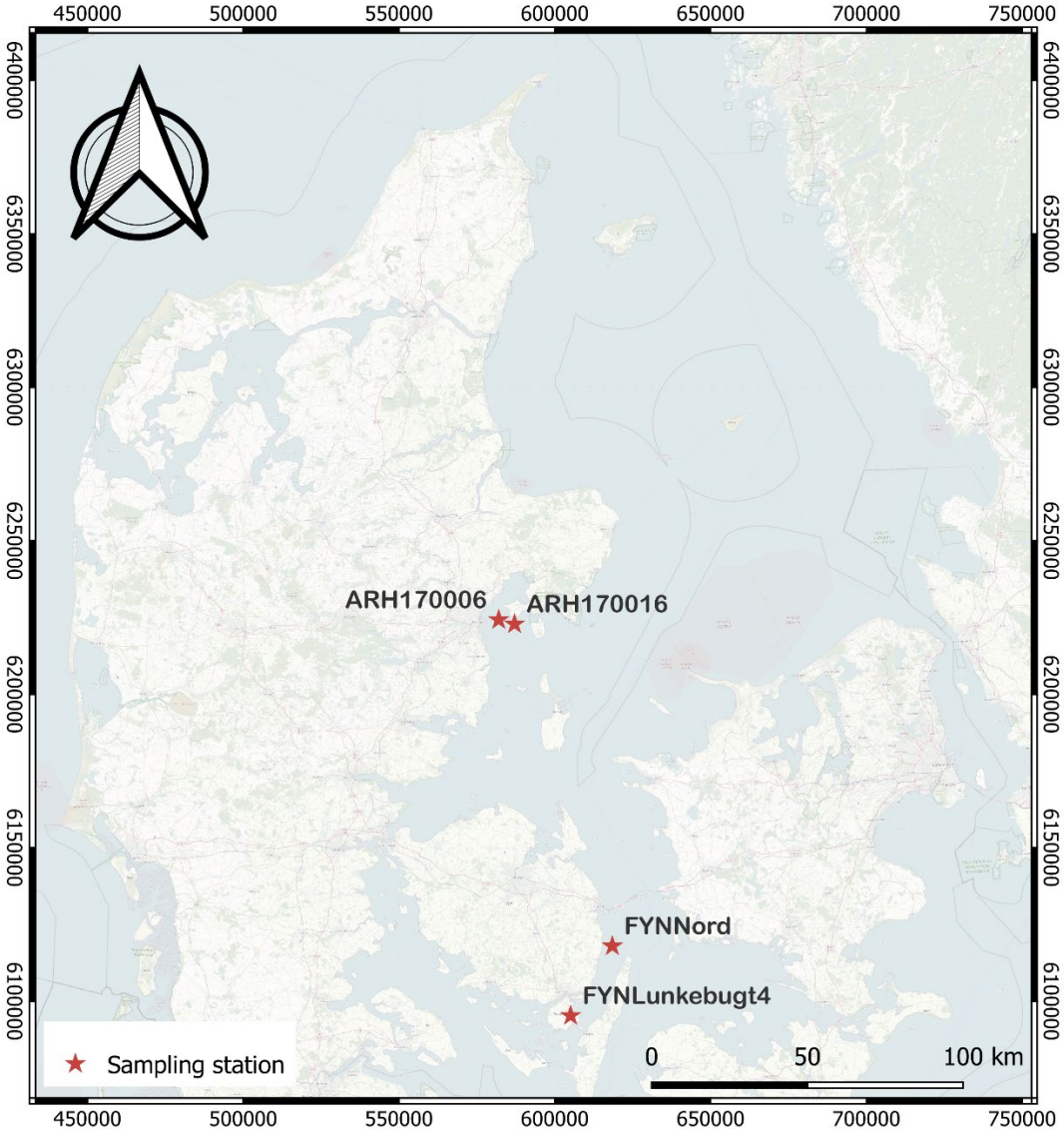


Figure 22. Sampling stations for the experiment

4.2.2 Subsampling in lab

The microplastic concentration in the 20 kg of sample in the metal bucket will have varied in all three dimensions. This variability was pragmatically assessed by reducing it to a two-dimensional variability, where the vertical heterogeneity was not assessed but overcome. Here for a stainless corer (length: 30 cm, Ø: 35 mm) was custom made for subsampling (Figure 23). It was inserted vertically into the bucket holding the sediment, collecting a full core from top to bottom, overcoming any vertical heterogeneity. To minimize the disturbance of the sediments while taking the core, the bottom of the corer was sharpened. The length of the corer was sufficient to accommodate the whole depth of the sediment in the bucket, with a few centimetres overhead space.

Figure 23. Sketch of the custom-made sediment corer

The horizontal heterogeneity was tackled by inserting multiple corers into the bucket at the same time, evenly covering the sediment surface. A total of 11 cores were taken as this was what could be fitted into the bucked. Of these the 6 were randomly taken into work and the rest stored for later use. This procedure was repeated trice, and a core from each of the three subsampling's were mixed into one. Hereby a typical subsampling strategy was mimicked where the three cores together yielded the amount of sediment which underwent sample preparation following the protocol shown in Figure 26. This hence led to 6 subsamples for each bucket which were then prepared in full (Figure 24). In addition, one blank sample containing 200 g washed and muffled (500°C) sand, was prepared for each bucket to assess the contamination generated during sample treatment.

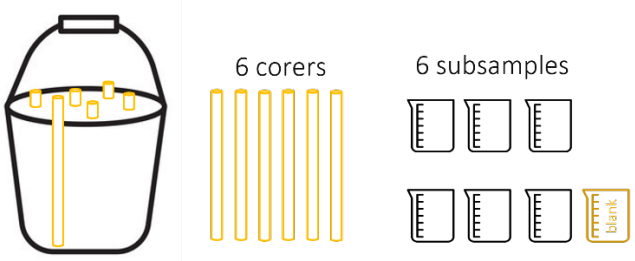


Figure 24. Diagram of subsampling design for sediments. One bucket sediment has 6 subsamples, plus one blank

Before the subsampling, the sediment in the bucket were well mixed with a pre-cleaned stainless-steel rod for at least 10 minutes. The corers were carefully inserted till they reached the bottom of the bucket. They were left for a few minutes to allow the captured sediments to settle, then the sediment was quickly transferred to pre-cleaned 5 L beakers. The total sediment amount extracted by the three consecutive subsampling's yielded approx. 0.5 kg wet weight, which was then taken into preparation (Figure 25).

Figure 25. Subsampling using custom-made stainless corers

4.2.3 Sample preparation

The sample preparation will cause some loss of microplastics, for example because particles stick to various surfaces, do not get separated completely during density separation, et cetera. To assess the efficiency of the microplastic extraction protocol (Figure 26), recovery experiments were conducted by adding well-known and easily recognizable plastic particles.

4.2.3.1 Sample preparation

Samples were prepared according to Liu et al. (2022). In short, the sample preparation and the extraction of microplastics followed the protocol outlined in Figure 26.

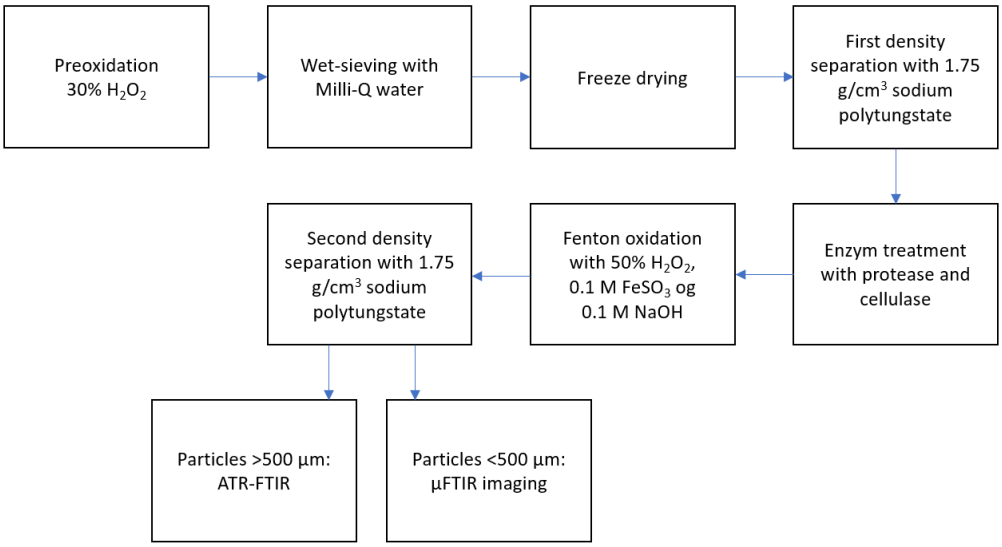


Figure 26. Protocol for sample preparation and microplastic extraction

4.2.4 Chemical analysis

Samples were prepared according to Liu et al. (2022). In short, the sample preparation and the extraction of microplastics followed the protocol outlined in Figure 27.

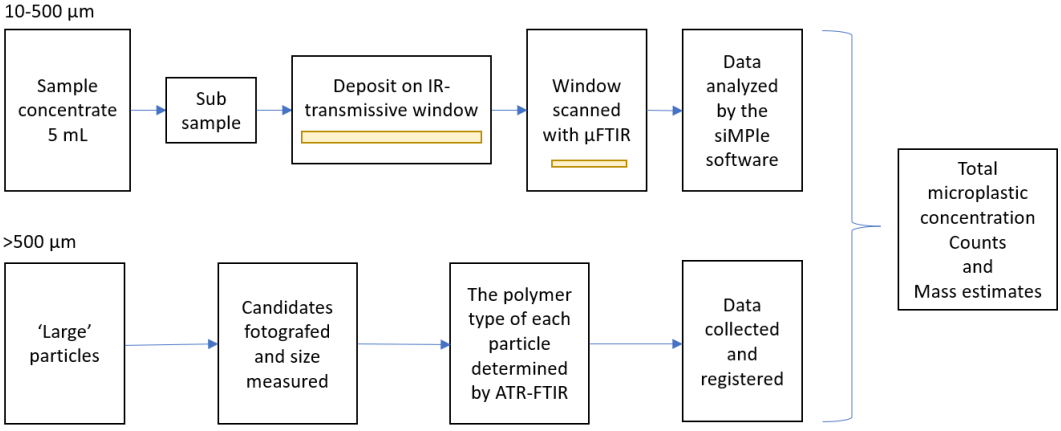


Figure 27. Protocol for chemical microplastic analysis

Only small aliquots of the 5 mL sample concentrate could be scanned in one go as marine sediment is a complex matrix which leaves interfering material in the concentrate even after excessive sample treatment. 3-5 aliquots out of the sample concentrates were scanned, yielding in the order of 10% of the extract analysed chemically.

4.2.5 Recovery

The efficiency of the extraction was assessed individually for each of the four times six subsamples. This was done by spiking a known number of readily identifiable microbeads (Figure 28) to the subsample before extraction and checking how many of them were covered after the full extraction process. As recovery test material, microbeads were selected with different polymer type and size. In total four types of microbeads were selected (Table 2): polypropylene (PE) of small size (45-63 µm) and large size (75-90 µm), and polystyrene (PS), also of two sizes (45-63 µm and 90-106 µm) ().

Figure 28. Microbeads selected as reference material to quantify the extraction recovery

Table 2. Microbeads for testing the extraction recovery

Polymer	**Density g/cm^3**	**Small size (µm)**	**Colour**
PE	0.98	45-63	light blue
PE	0.98	75-90	yellow
PS	1.01	45-63	dark blue
PS	1.01	90-106	red

The extraction recovery was assessed by counting a known amount of microbeads under a stereomicroscope (ZEISS, SteREO Discovery.V8) equipped with an Axiocam 105 color camera with a maximum magnification of 8 × (Figure 29), then adding that known number to the subsample (Figure 25). Each subsample was spiked all with four types of beads. After the extraction process, the recovery of beads was checked visually using the same stereomicroscope.

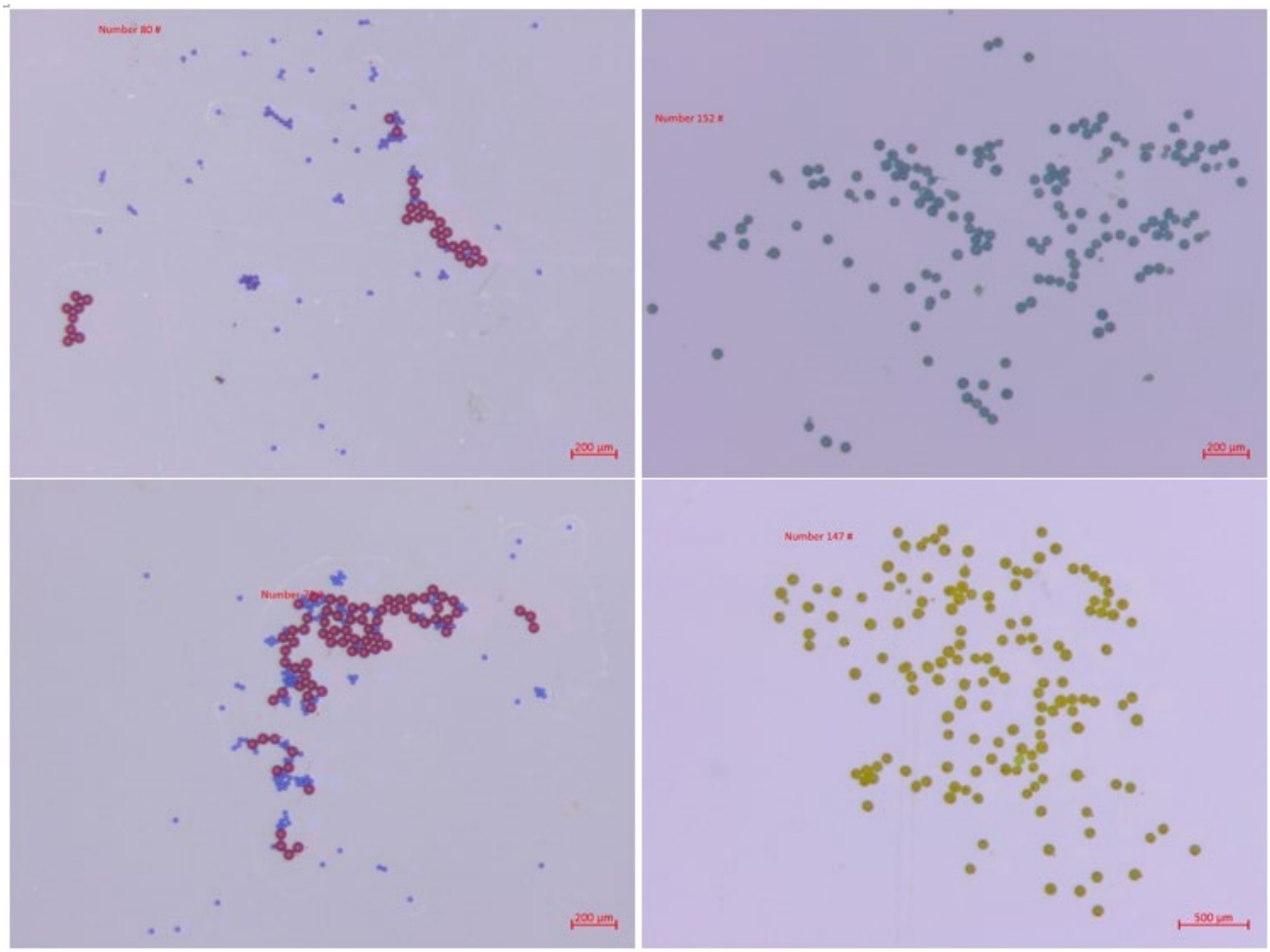


Figure 29. Counting microbeads under the microscope

4.2.6 Statistical analysis

All statistical analysis was done in R (4.2.2), using packages of ggplot2, RColorBrewer, ggbreak, scales, and readxl. Figures were generated by R (4.2.2) and QGIS (3.28.2).

4.3 Results

4.3.1 Concentration levels at the investigated stations

Without correcting the results for recovery within the individual subsamples, and without correcting them for blank values, the concentrations in the four samples ranged from 3204 to 10296 counts kg^{-1}, corresponding to 370 to 1866 µg kg^{-1} (Figure 30, Figure 31). In another study, Liu et al. (in prep) quantified microplastics in 19 sediment samples from different parts of the Danish marine environment and found 119 to 23340 counts kg^{-1}, corresponding to 5 to 6958 µg kg^{-1}. The samples collected for the present study were hence well within what was found for other Danish marine sediments.

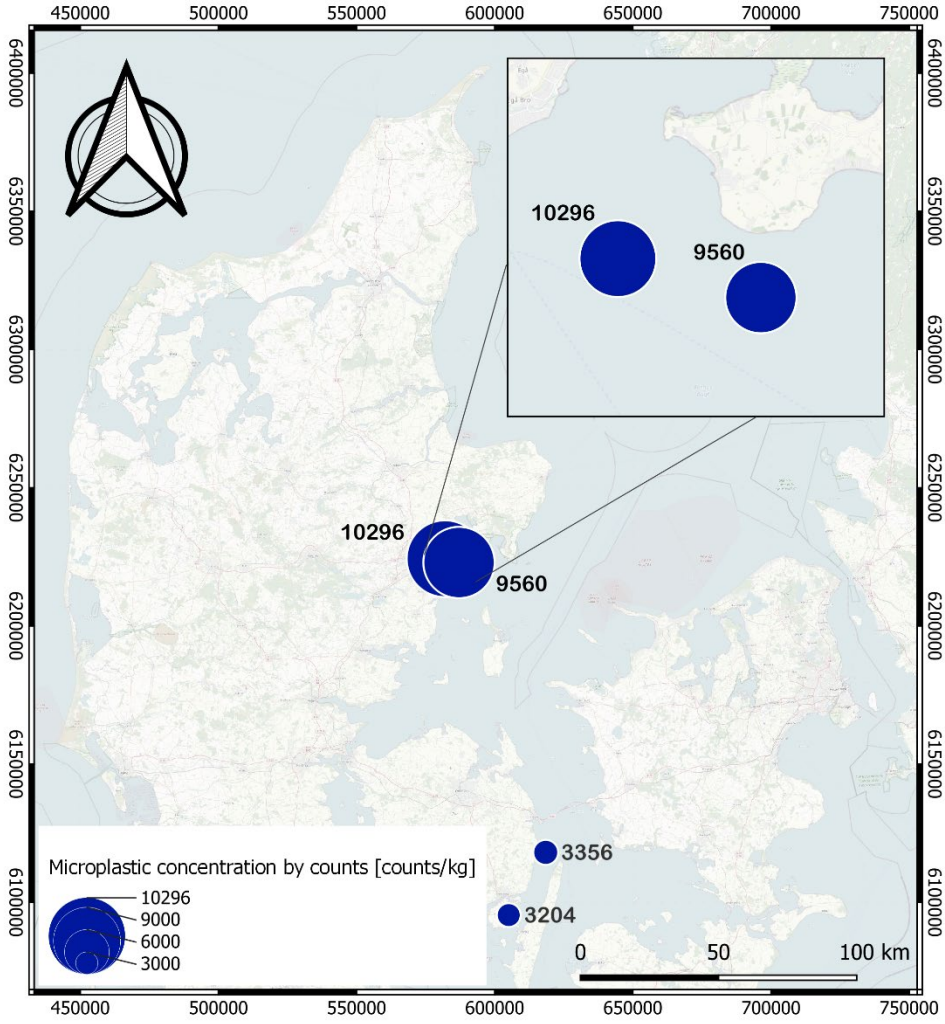


Figure 30. Microplastic concentrations as counts per kg of dry sediment in the four investigated stations

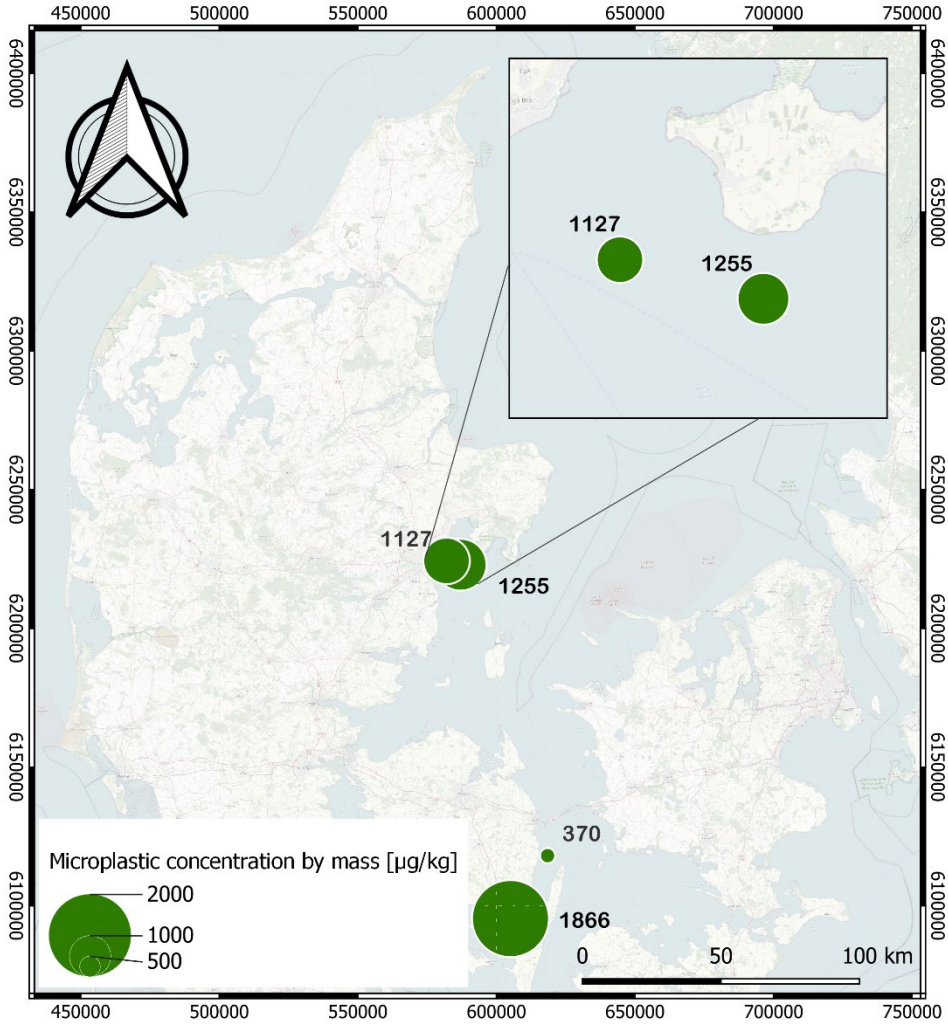


Figure 31. Microplastic concentrations as mass per kg of dry sediment in the four investigated stations

4.3.2 Variation between subsamples

4.3.2.1 Variation on microplastic concentration

Each of the 4 samples were analysed as 6 subsamples of approx. 0.5 kg wet sediments each, resulting in 24 analysed samples. In addition, one blank was analysed per sample. For each subsample, 3 or 5 aliquots were scanned, depending on how much extract could be deposited on the window (Appendix A).

Table 3 presents the raw data of the study, that is, data which is not corrected for recovery and blank values. It shows the amount of prepared subsample, the analysed fraction of the extract, count and mass of the identified microplastics.

For each subsample, the concentration was represented by averaging the concentration from the 3-5 scans of that subsample. A one-way ANOVA test was performed to assess if there is any significant difference between the subsamples. This was done for all for stations, both in terms of concentration by particle counts and mass. The test showed that when the concentration was measured by counts, only the most polluted station ARH170006 had no significant difference between subsamples (Appendix A, Table 11). But when it comes to the mass concentration, all stations showed no significant difference between the subsamples.

In terms of microplastic concentration (counts kg^{-1} and µg kg^{-1}, respectively), there was some variation between subsamples in terms of the concentration they yielded. Here we used relative standard deviation to the mean (RSD, %) as a measure of variation, as the absolute value based on the mean can be misleading when comparing across samples with different results. RSD value ranged from 17% to 44% when measuring concentrations as counts kg^{-1} from the four stations (Appendix A). In all cases, concentrations measured as mass kg^{-1} had much higher variation, with RSD ranging from 28% to 185%. ARH170006 always exhibited the lowest RSD between subsamples for counts as well as mass (17% and 28%, respectively). The highest RSD between subsamples for count concentrations was 44% at FYNLunkebugt4 and for mass concentrations it was 185%, also at FYNLunkebugt4. Appendix A presents all raw data down to the findings within the single scans.

In analytical chemistry, RSD is commonly used to assess the reproductivity and repeatability of an analytical method. However, the RSD value often depends on the concentration of analyte, where higher analyte concentration in general would yield a lower RSD value (Rivera and Rodríguez 2011). This is in line with the results from this study both in terms of particle counts and mass, as the lowest RSD always was found in the samples from the most polluted station ARH170006 (Figure 30), whilst the highest RSD was found in the samples from the least polluted station, FYNLunkebugt4. This suggests that one can expect higher variation between subsamples if the sample has a low content of microplastic, in other words, more subsample is needed to achieve lower RSD in less polluted samples.

Table 3. Subsamples analysed and microplastics identified

Station	Sub-sample	Amount of sediment [g DW]	Number of scans	Total fraction scanned [%]	Equivalent sample mass scanned [g DW]	Microplastic counts found in the scans [counts]	Microplastic mass found in the scans [µg]
FYNLunkebugt4	1	205.1	3	12	24.6	124	218.3
	2	212.6	5	20	42.5	188	32.3
	3	202.6	3	12	24.3	90	6.5
	4	232.1	3	12	27.9	54	1.8
	5	213.6	3	18	38.4	57	10.5
	6	176.7	3	12	21.2	56	20.3
Total		1242.7	20	86	178.9	569	289.8
Blank		218.2	1	4	8.7	4	0.04
FYNNord	1	134.8	2	12	16.2	83	14.4
	2	137.3	3	18	24.7	62	7.0
	3	134.4	3	12	16.1	31	3.2
	4	135.8	3	12	16.3	59	4.8
	5	148.1	3	12	17.8	71	6.4
	6	132.2	3	12	15.9	47	3.0
Total		822.6	17	72	98.9	353	38.8
Blank		203.2	1	6	12.2	1	0.04
ARH170006	1	113.4	5	10	11.3	112	6.8
	2	125.6	5	10	12.6	132	14.5
	3	117.7	5	10	11.8	85	15.8
	4	120.8	5	10	12.1	128	14.6
	5	118.6	5	10	11.9	148	17.7
	6	115.4	5	10	11.5	128	11.1
Total		711.5	30	10.0	71.2	733	80.6
Blank		218.3	1	4	8.7	2	0.03

ARH170016	1	96.2	3	12	11.5	41	7.3
	2	85.5	3	18	15.4	117	7.4
	3	100.0	3	12	12.0	106	14.7
	4	93.5	3	12	11.2	141	22.7
	5	99.5	3	12	11.9	136	23.6
	6	103.1	3	12	12.4	166	14.8
Total		577.8	18	12.9	74.5	707	90.5
Blank		218.4	1	6	13.1	6	0.25

The subsamples extracted as outlined in Section 4.2.2 were with a few exceptions rather homogeneous in terms of water content (Figure 32) and organic matter content (Figure 33). Only subsample 1 and 2 for FUNLunkebugt4 varied significantly in water content. FUNLunkebugt4 was also the station that held the least water content, which indicates that sediments with higher solids content would have higher variation between subsamples.

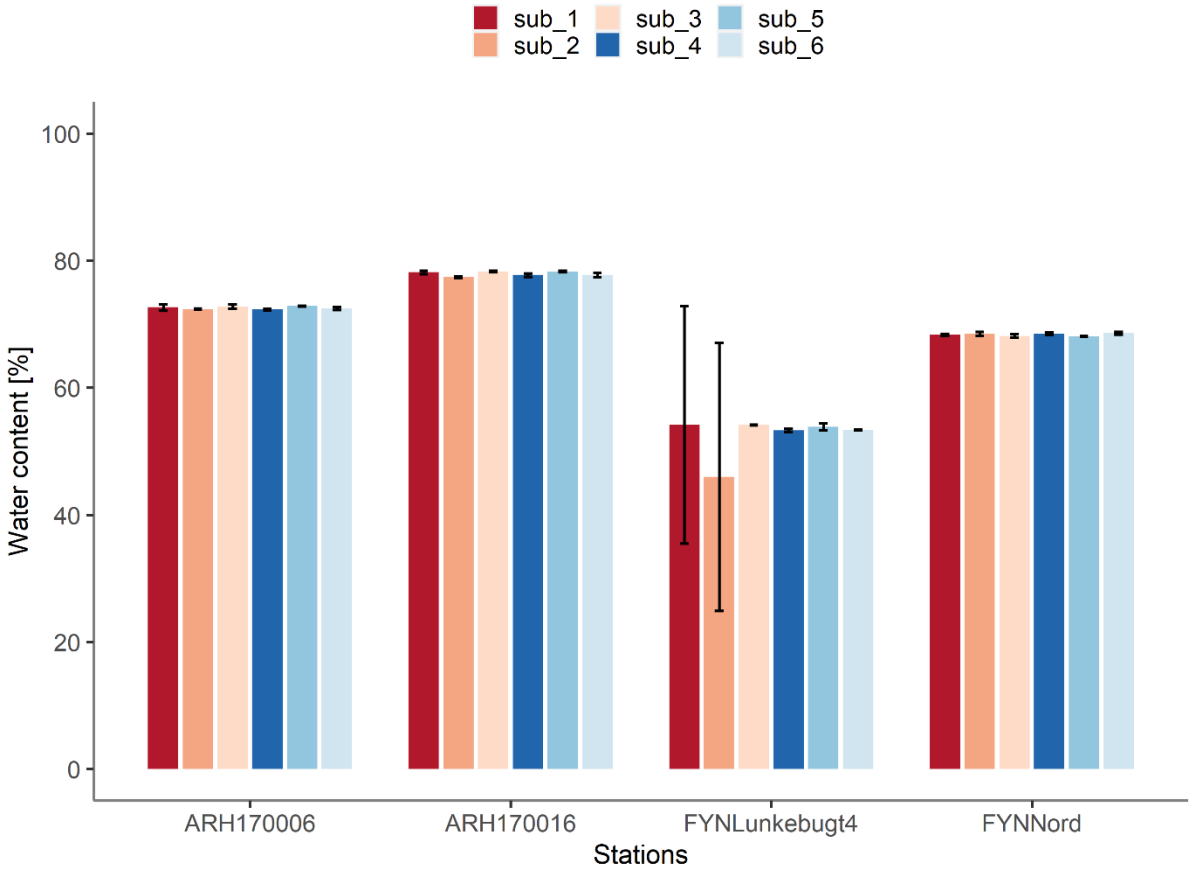


Figure 32. Water content measured for each subsample in triplicates

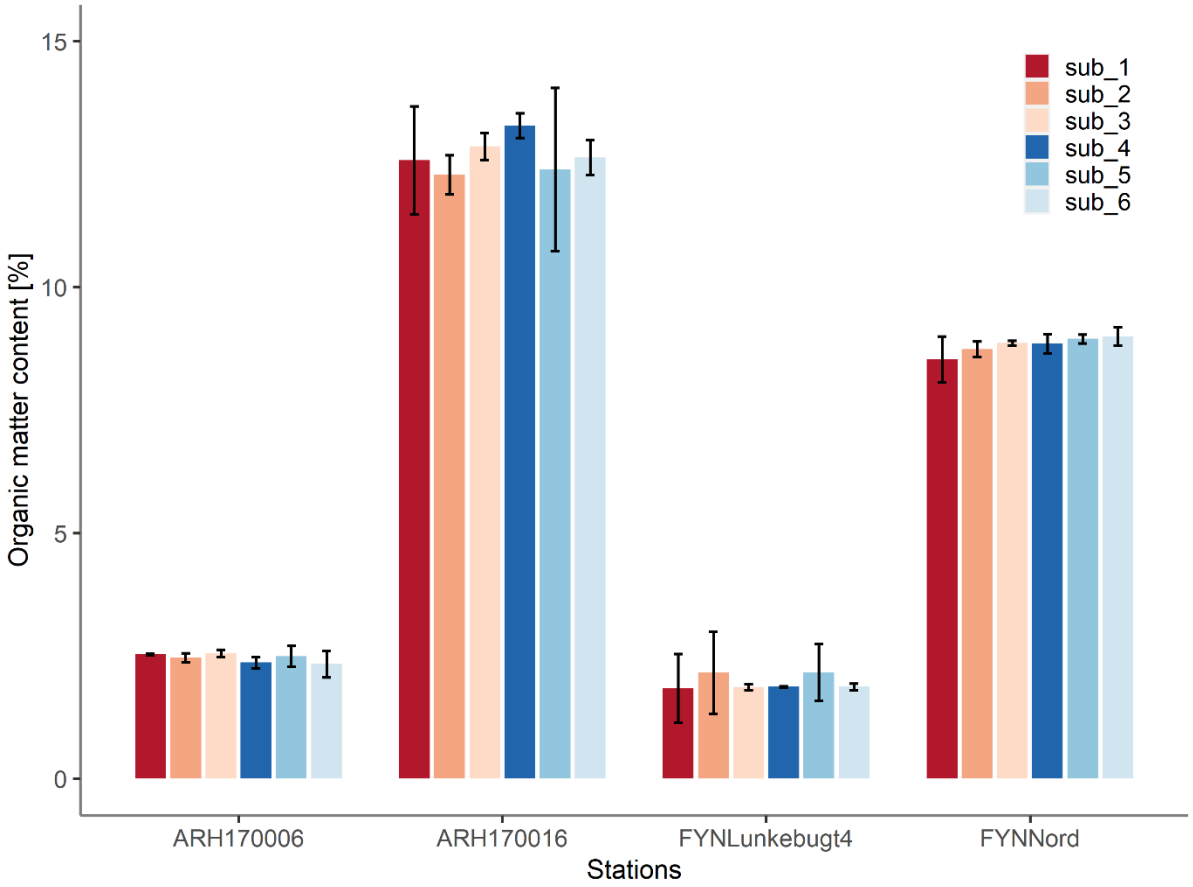


Figure 33. Organic matter content measured for each subsample in triplicate

4.3.2.2 Variation on polymers

In total 17 polymer types were found when lumping all the identified microplastics from the four stations. Lumping the values from all subsamples per station, it was found that the particle counts and the masses varied significantly between the stations. Polyester was the most abundant polymer at all stations (Figure 34), while some rare polymers were only detected in one or two of the stations, for instance Vinyl chloride copolymer and Poly(vinylpyrrolidone_co_vinyl acetate) were only found in ARH70016, each with one count (Appendix A,). Such low number means that there is a high uncertainty on such rare polymers, as one would need more particle counts to reliably say that the deducted concentration is the actual environmental concentration of that polymer. The variation between stations was even more expressed when measured by particle mass (Figure 35).

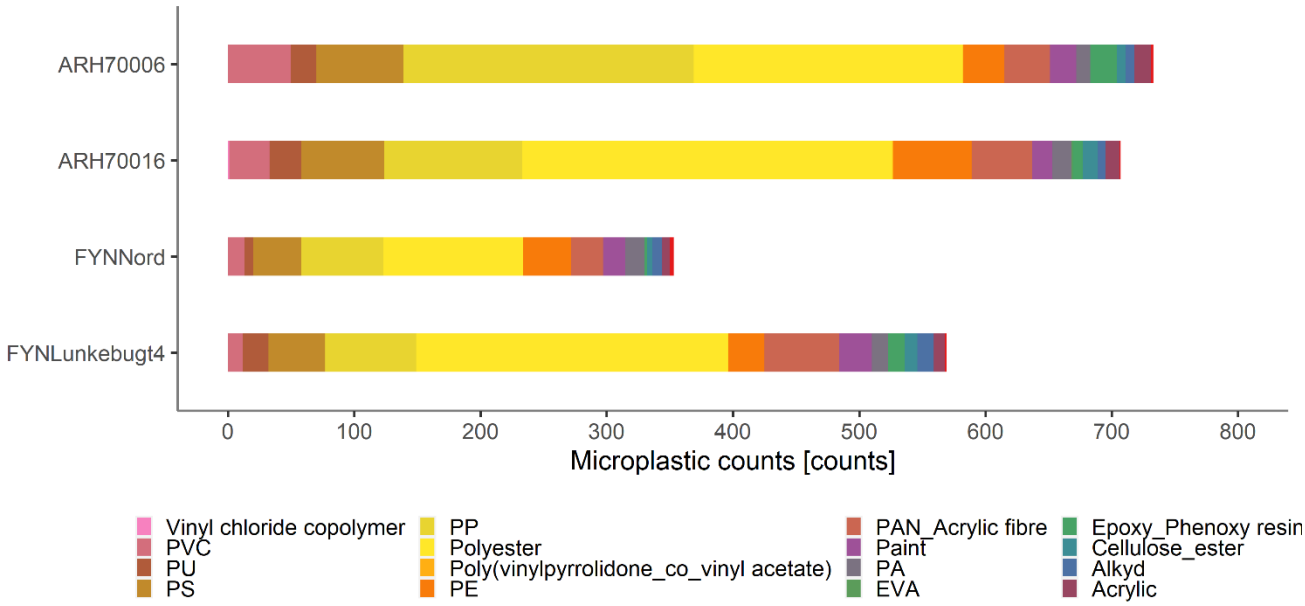


Figure 34. Polymer composition for each station, by particle counts

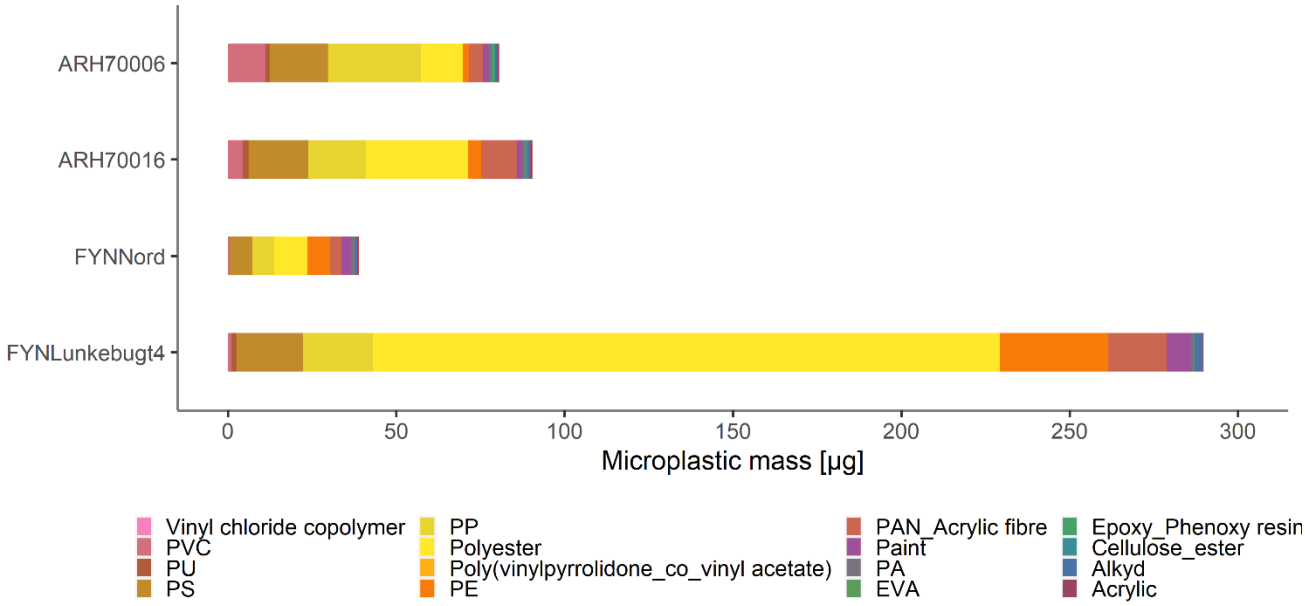


Figure 35. Polymer composition for each station, by particle mass

4.3.3 Variation between scans

3 or 5 aliquots of the extracts from each subsample were scanned (Table 3). Each such scan can be used to calculate a concentration in the original sample. For example, if the original sample taken into analysis was 200 g DW, of which 4% of the extract was deposited and analysed in a single scan, this would correspond to 200*0.04 = 8 equivalent grams of dry sediment scanned (column 'Equivalent sample mass scanned [g DW]' of Table 3). If for example 20 microplastics were identified in this scan, the resulting concentration would be 20/0.008 = 2500 counts kg^{-1}. Another scan would probably yield a different number and hence a different concentration. Combining all concentration calculated in this way, for all scans of all subsamples yields a distribution per collected sample (Figure 36, Figure 37).

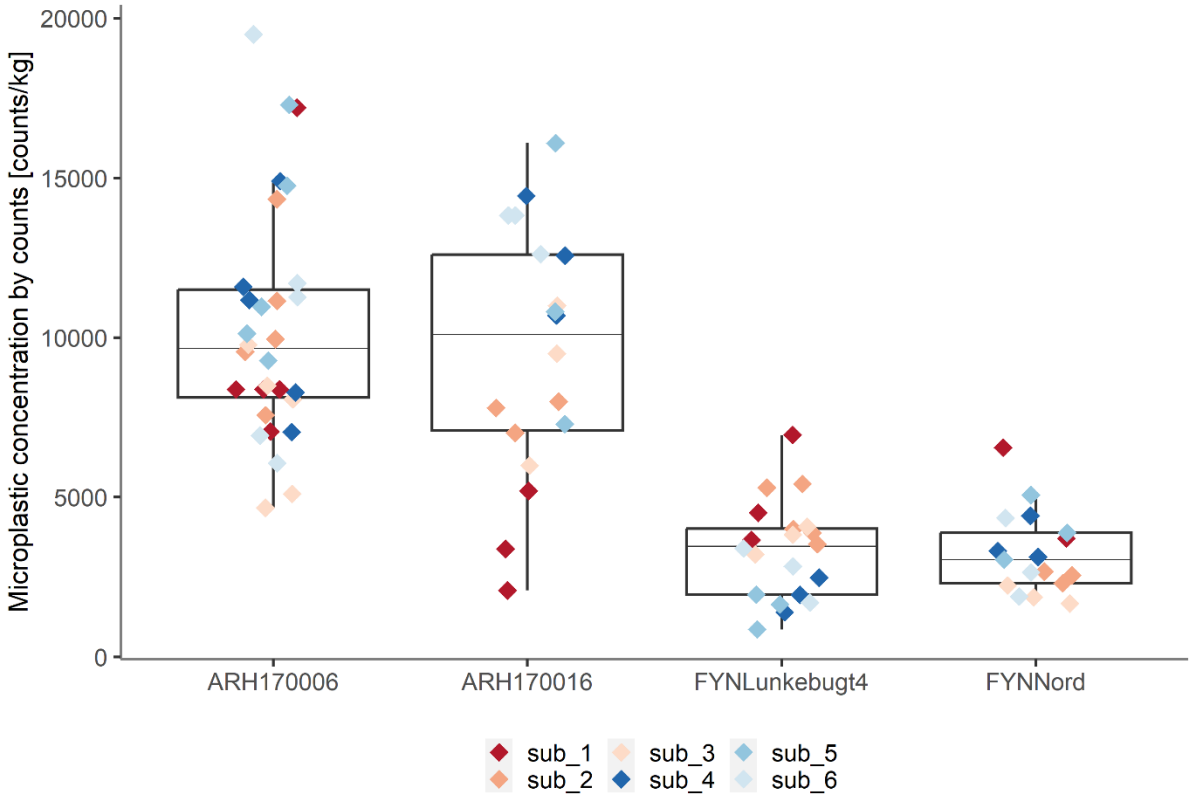


Figure 36. Boxplot of the microplastic counts from the four stations. The value of each scan is marked by a dot. The line in the centre of the box is the median, the bottom and top of the box are the 25 and 75 percentiles, respectively. The vertical lines below and above each box indicate the 5 and 95 percentiles, respectively.

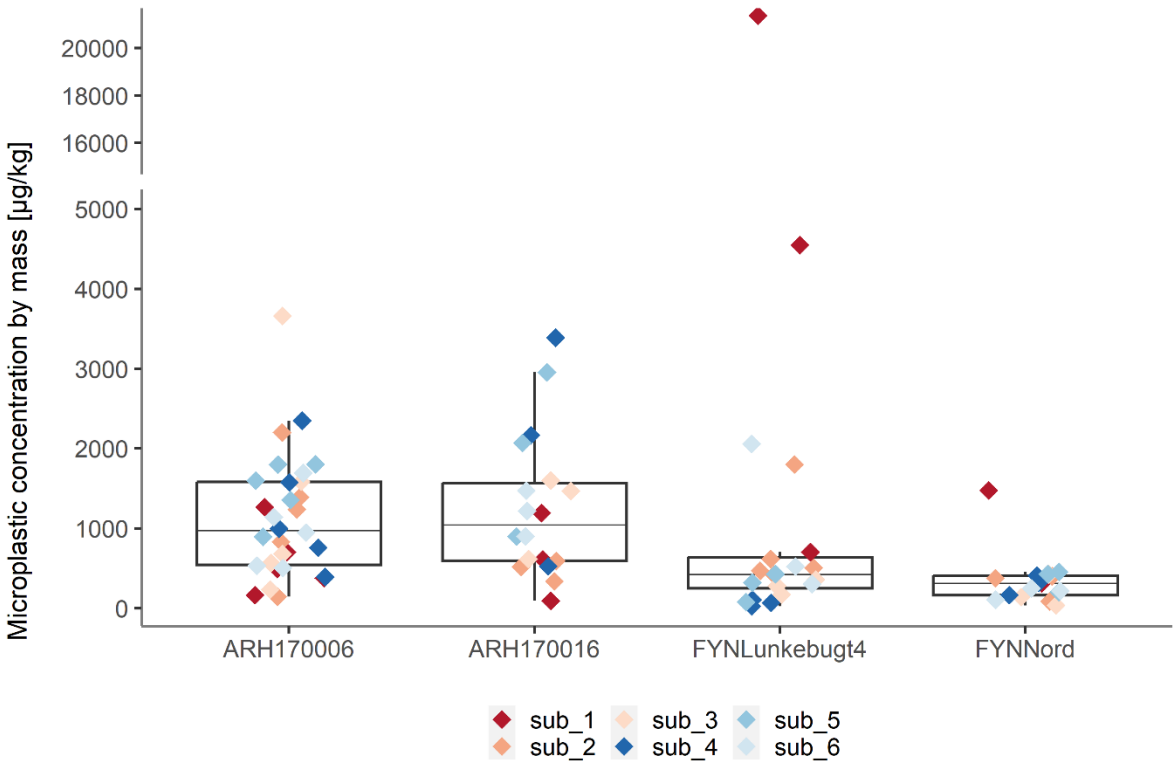


Figure 37. Boxplot of the microplastic mass per particle from the four stations. The concentration calculated by each scan is marked by a dot. The line in the centre of the box is the median, the bottom and top of the box are the 25 and 75 percentiles, respectively. The vertical lines below and above each box indicate the 5 and 95 percentiles, respectively.

The boxplots show that there is quite some variability between scans of a single sample and that relying on one subsample with one scan would introduce a quite high uncertainty. Exploding the figures into individual subsamples and the concentration they yielded for the 3-5 scans shows that variations between subsamples were quite significant especially for ARH170006 in terms of counts concentration (Figure 36, Appendix A), but the most significant variation in terms of mass concentration was in FYNLunkebugt4 (Figure 37, Appendix A) in mass concentrations.

For each station, the deviation between scans per subsample was compared with the variability between subsamples, in the measure of RSD (Figure 38, Figure 39). It shows that RSD between scans per subsample varied from 7% to 48% when concentration was measured by counts (Figure 38), and that it was much larger when measured by mass, with RSD ranging from 25% to 124% (Figure 39, Appendix A). After averaging RSD from scans, the highest value was found in ARH170006 when the concentration was measured by particle counts. In fact, ARH170006 always had higher RSD between scans than the RSD between subsamples, with one exception in mass concentration (Figure 38, Figure 39). Another station, ARH170016, had a similar level of microplastic concentration as ARH170006 both in terms of particle counts and mass, however, it had higher RSD between subsamples than that between scans. The only difference between the two stations was that ARH170016 had a higher deposited volume (4-6%) in scans, which was 2-3 times more than that for ARH170006 (2%). The picture held true when it comes to concentration in particle mass (Figure 39). This suggests that if a sample has high microplastic concentration, the overall variation is more likely attributed to the variation between scans. In other words, the volume of subsample matters less compared to the number of scans.

In any case, FYNLunkebug4 always had the highest variation between subsamples compared to the variation between scans, both by particle counts (Figure 38) and mass (Figure 39) despite the fact that the deposited volume for scans was similar to that of FYNNord and ARH170016. If comparing FYNLunkebug4 to FYNNord,

which had similar microplastic concentration in terms of particle counts (Figure 30), the high variation between subsamples could potentially be attributed to its low water content, which was 16% less than that of FYNNord (Figure 32). This may suggest that if the sediment is less watery, the degree of heterogeneity in subsamples increases as particles settle quickly after mixing. In other words, more subsample is needed to compensate for the high variability in subsampling for sediments with low water content.

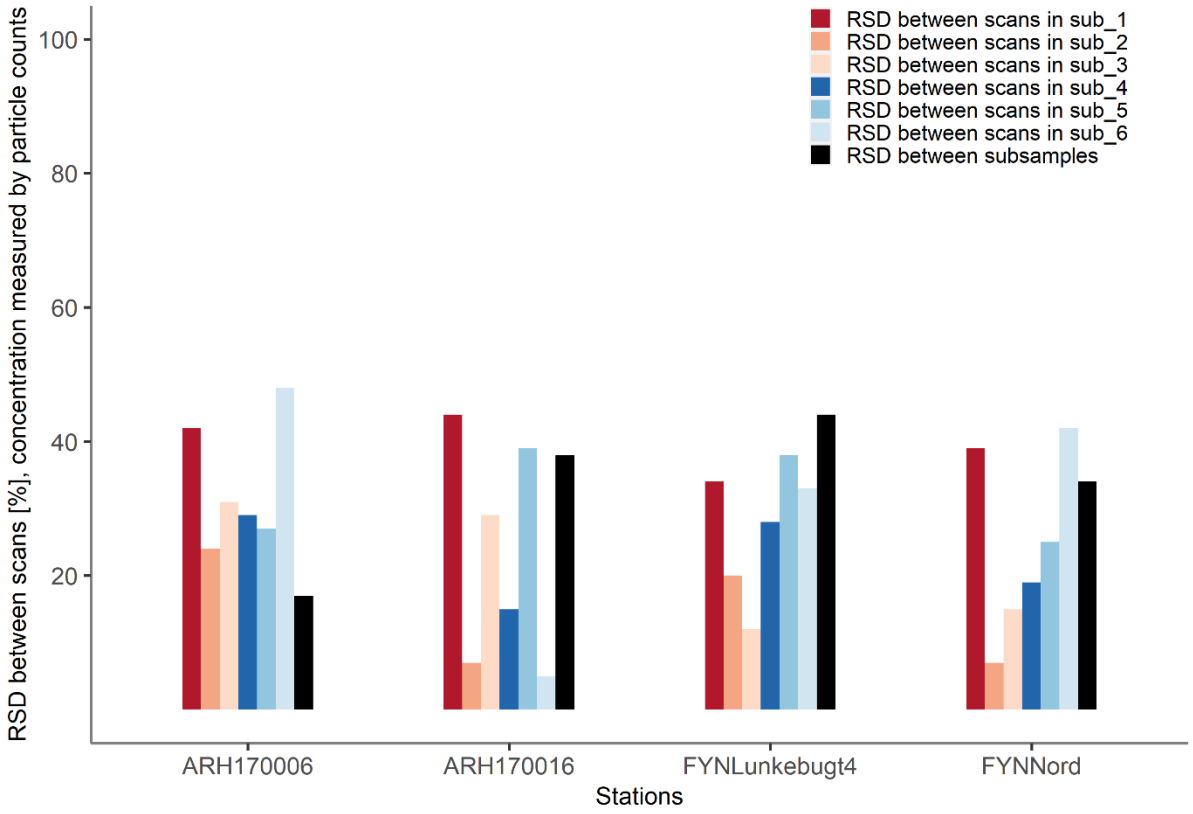


Figure 38. Relative standard deviation (RSD) on microplastic concentration between the scans, measured by particle counts

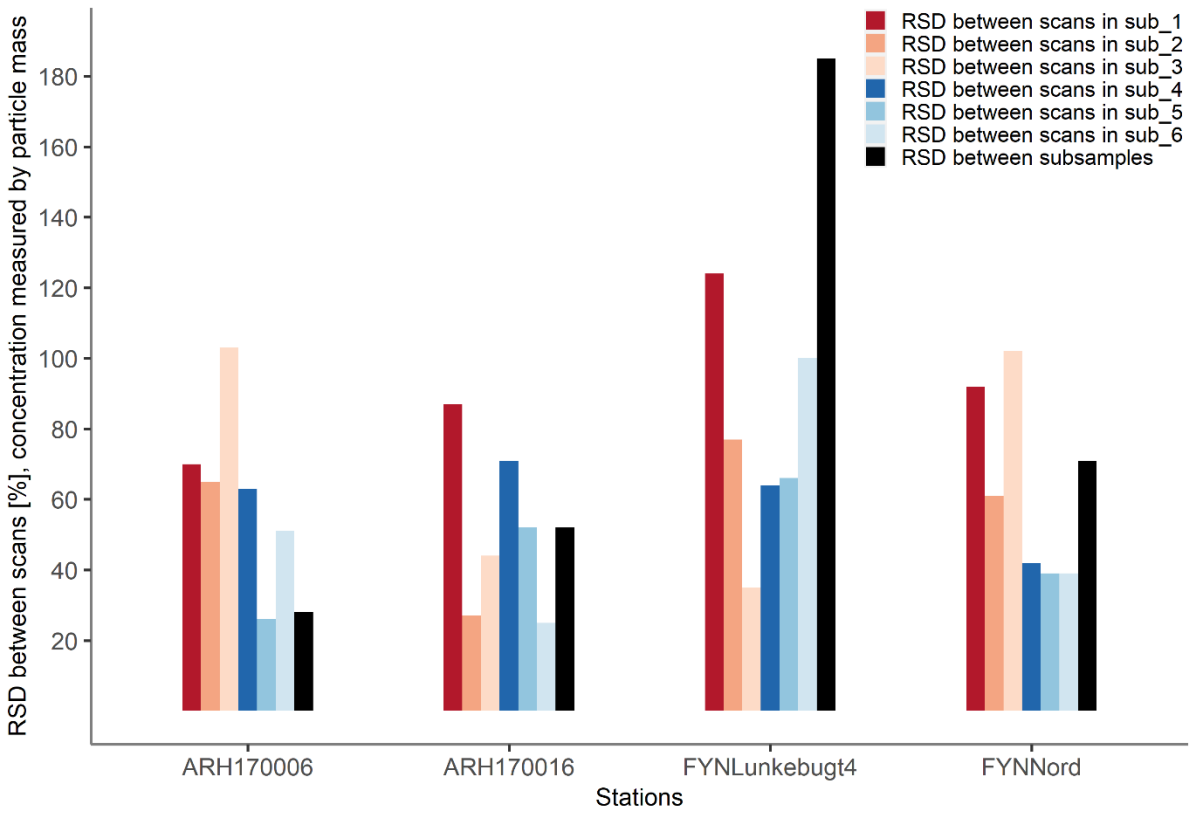


Figure 39. Relative standard deviation (RSD) on microplastic concentration between scans, measured by particle mass

The variation in microplastic concentration was most pronounced when measured as mass compared to counts. However, the average value per subsample varied between approx. a factor 1.7 and 3.8 for concentrations measured as counts (Figure 40), and was 2.5 and 139 when they were measured as mass (Figure 41). For the latter, FYNLunkebugt4 showed by far the largest variation between means.

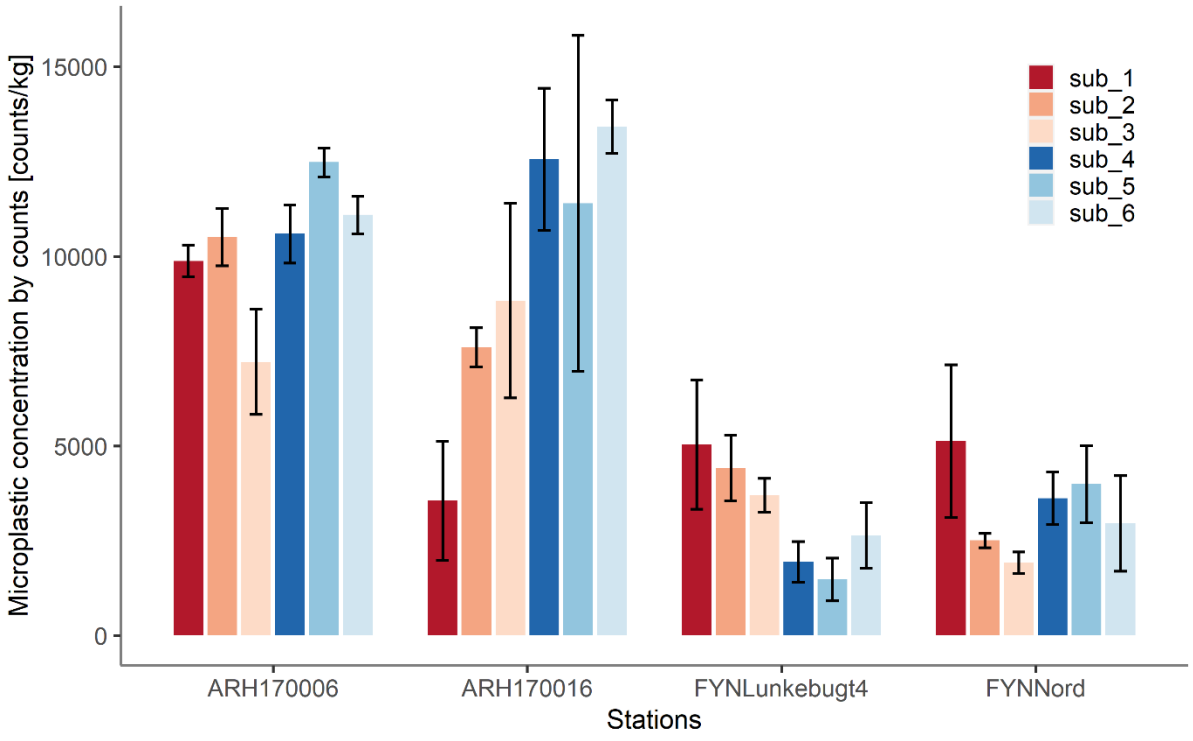


Figure 40. Variation on microplastic concentration by counts between the subsamples. The columns show the average value between scans. The error bars show the standard deviation between scans.

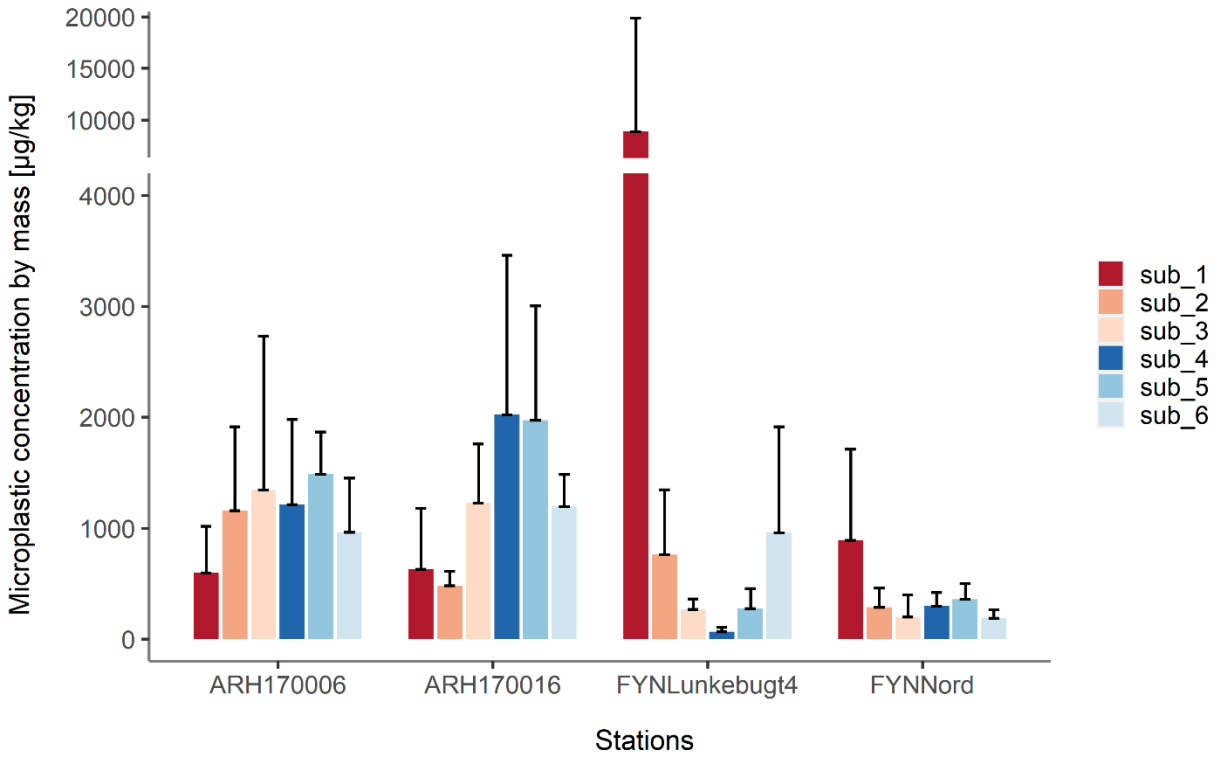


Figure 41. Variation on microplastic concentration by mass between the subsamples. The columns show the average value between scans. The error bars show the standard deviation between scans.

4.3.4 Relation between particle size and microplastic concentration

The large variability of mass concentrations compared to count concentrations is because particle mass comes in the third power of its dimension (assuming identical shapes). A few large particles will hence dominate the total mass in a sample. On the other hand, many small particles will dominate the counts, but not affect the mass much. Figure 42 and Figure 43 and show accumulated particle sizes distributions for all stations and how the distribution is even for the microplastic counts, while it is skewed towards the right for the microplastic mass. It is this skewedness which creates the higher variability for the mass concentrations compared to the count concentrations.

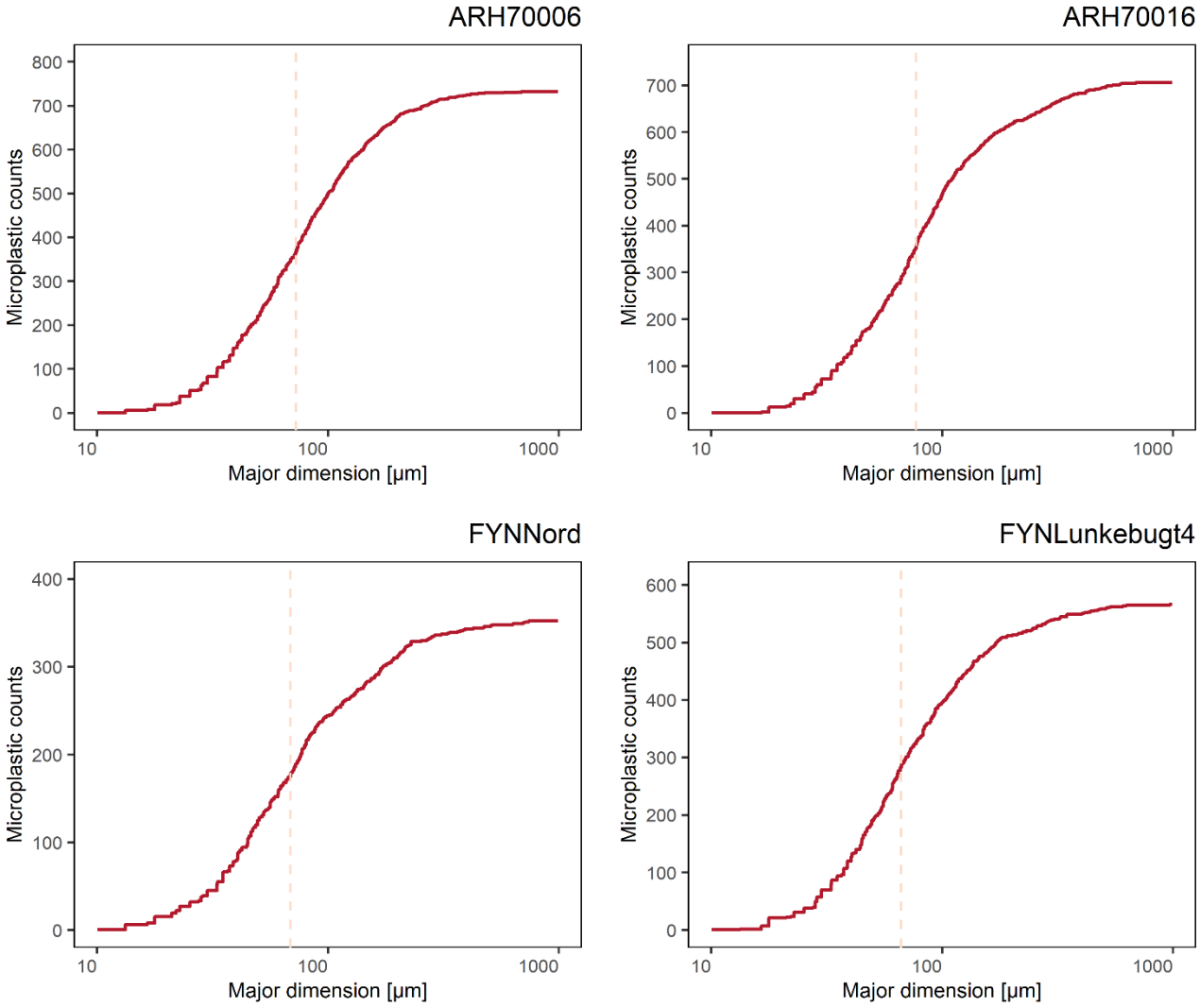


Figure 42. Size distribution of identified microplastics measured as counts. The dashed grey line represents the median value of particle's major dimension. X-axis is log10 scaled

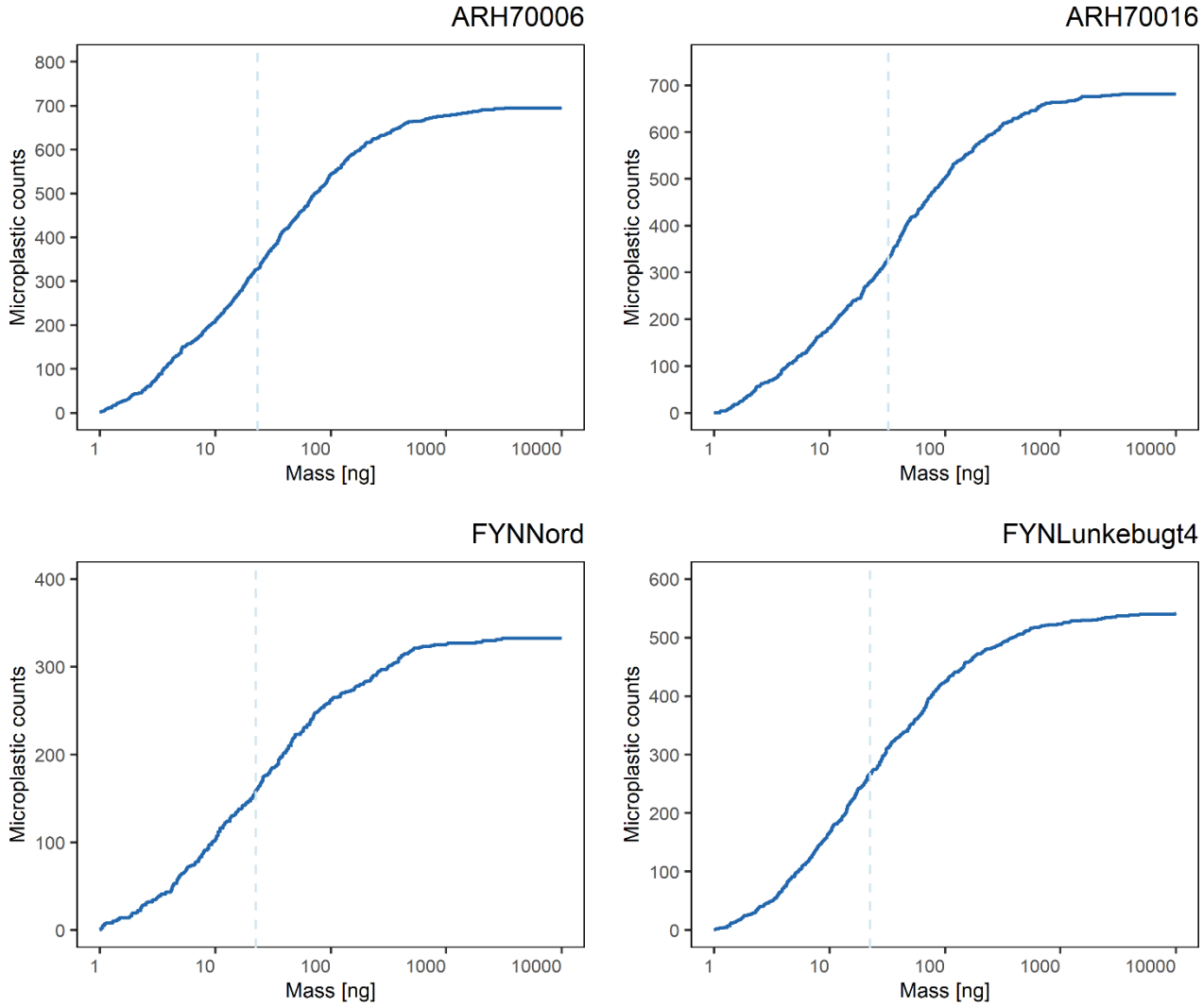


Figure 43. Mass distribution of identified microplastics measured as counts. The light bule dashed line represents the median value of particle mass. X-axis is log10 scaled

In order to minimize variability caused by the skewedness in size, we sorted particle's major dimension into five size classes. The size classes were determined in line with HELCOM, aiming to generate more comparable results across studies. In specific, the five classes are: < 20 µm, 20-50 µm, 50-100 µm, 100-300 µm, and 300-1000 µm. The microplastic concentration was further interpreted within each size class.

Most of the identified microplastics sat in the range of 50-100 µm by counts, and this applied to all four stations (Figure 44). Among them, ARH70006 had the highest number (279 counts), while FYNNord had the lowest (119 counts). This corresponded to a microplastic concentration by counts of 10296 counts kg^{-1}, and 3359 counts kg^{-1}, respectively (Figure 45). In all cases, the smallest size class, 10-20 µm, had the least particle counts, whilst the largest size range, 300-1000 µm had slightly higher counts. The lower particle number in the small size range was also reported by (Primpke et al. 2020), however that was only observed for the smallest possible particles, namely those covering one pixel in their µFTIR imagincountsg (11 µm). The number of particles constituting two pixels or more (>22 µm) continued to increase with decreasing size. We believe that the main reason here for was that Primpke et al. used much lower decision thresholds for the chemical identification of particle material than in our case, and also a different identification algorithm. The low number of small particles in our case is probably attributed to the much stricter decision threshold in comparing the sample spectra with the database, which was chosen to minimize false positives and increase confidence in polymer

identification. I.e., we prefer to err on the conservative side. The issue of struggling when identifying small particles is mainly due to the analytical technique we apply, namely µFTIR imaging in transmission or transflection mode, where the IR spectra get noisier when particles get small and thin. This is because thin particles absorb less IR light than thicker ones, hence yielding less signal in the IR spectrum. The low count in large size is in line with many other studies, for instance Haave et al. (2019), who reported that the number of microplastics decreased significantly above 100 µm in size in sediments from Byfjorden in Norway. Nevertheless, particles > 100 µm found in this study had higher concentration by counts than that in Byfjorden by one-two orders of magnitude.

The problem with identifying smaller particles can in principle be addressed in the identification algorithm used to interpret the µFTIR imaging scans. One can chose to set the global decision thresholds lower, or one could selectively set the decision threshold lower for small particles compared to larger ones. Both approaches will yield a higher number of identified small MPs. While this will reduce the false negative identification below roughly 50 µm (i.e., amend the issue of 'finding' less very small particles), it comes at a cost of a higher false positive identification rate, i.e., declaring more non-plastic particles to be of plastic. What to choose hence becomes a question of whether the analysist prefers to err on the side of caution. I.e., to err towards an increase in false negative identification of the small particles or to err towards an increase in false positive identification of the small particles.

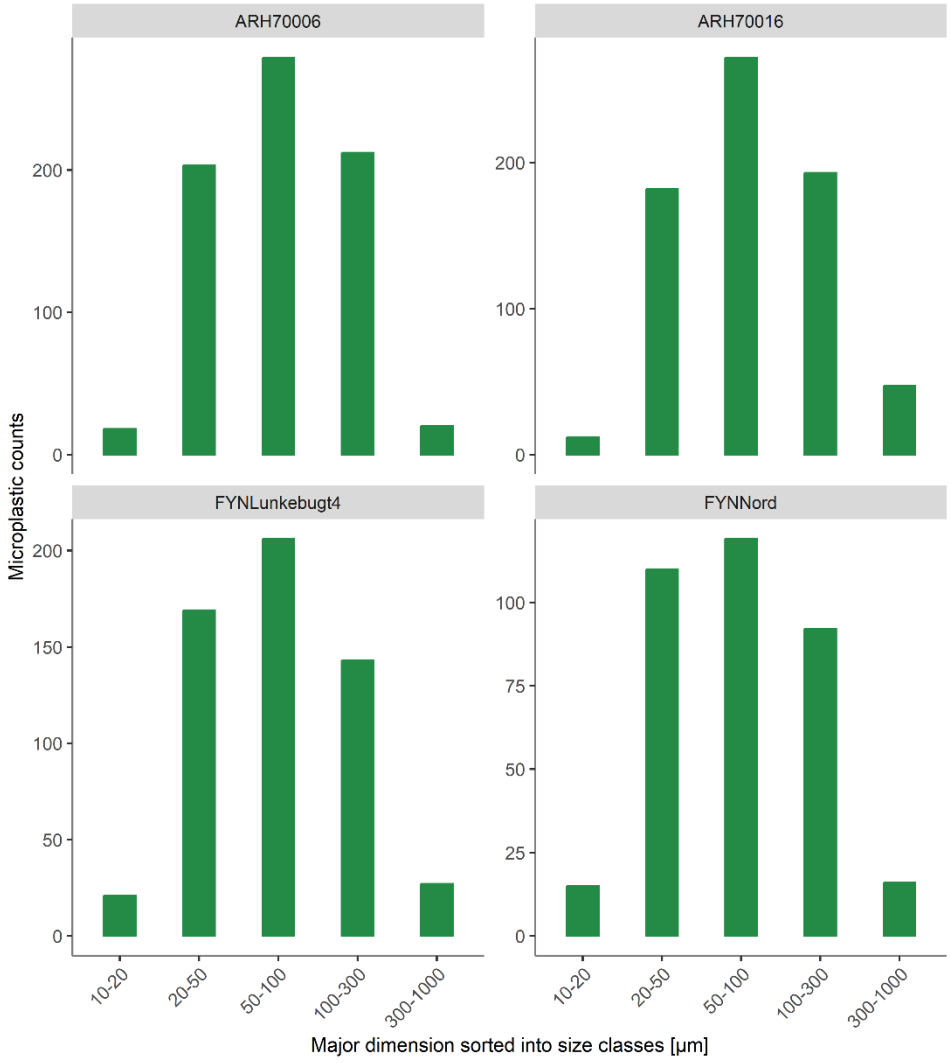


Figure 44. Microplastic counts in four stations, with the particle's major dimension sorted into five size classes

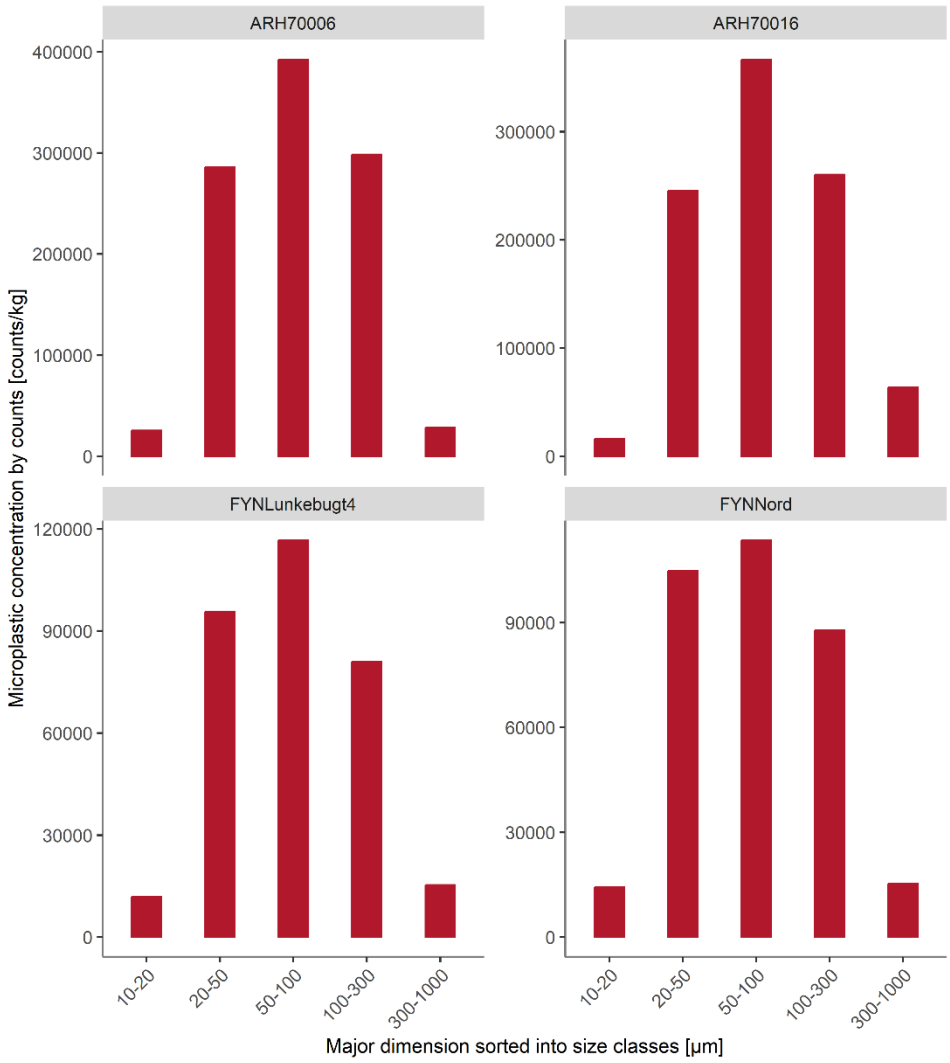


Figure 45. Microplastic concentration by counts, with the particle's major dimension sorted into five size classes

The microplastic concentration showed a different picture when measured by mass. In specific, particles between 100-300 µm constituted most of the mass concentration (Figure 46), followed by the size range of 300-1000 µm. The concentration decreased with the decrease in size, where the concentration for size 10-20 µm seemed negligible. The concentration difference in particle counts and particle mass provided clear evidence that interpreting results by lumping particles into one size class can easily hinder revealing important details in the sample, as large particles generate huge variability in particle mass. Hence it is suggested to sort the particles into multiple size classes before discussing the results.

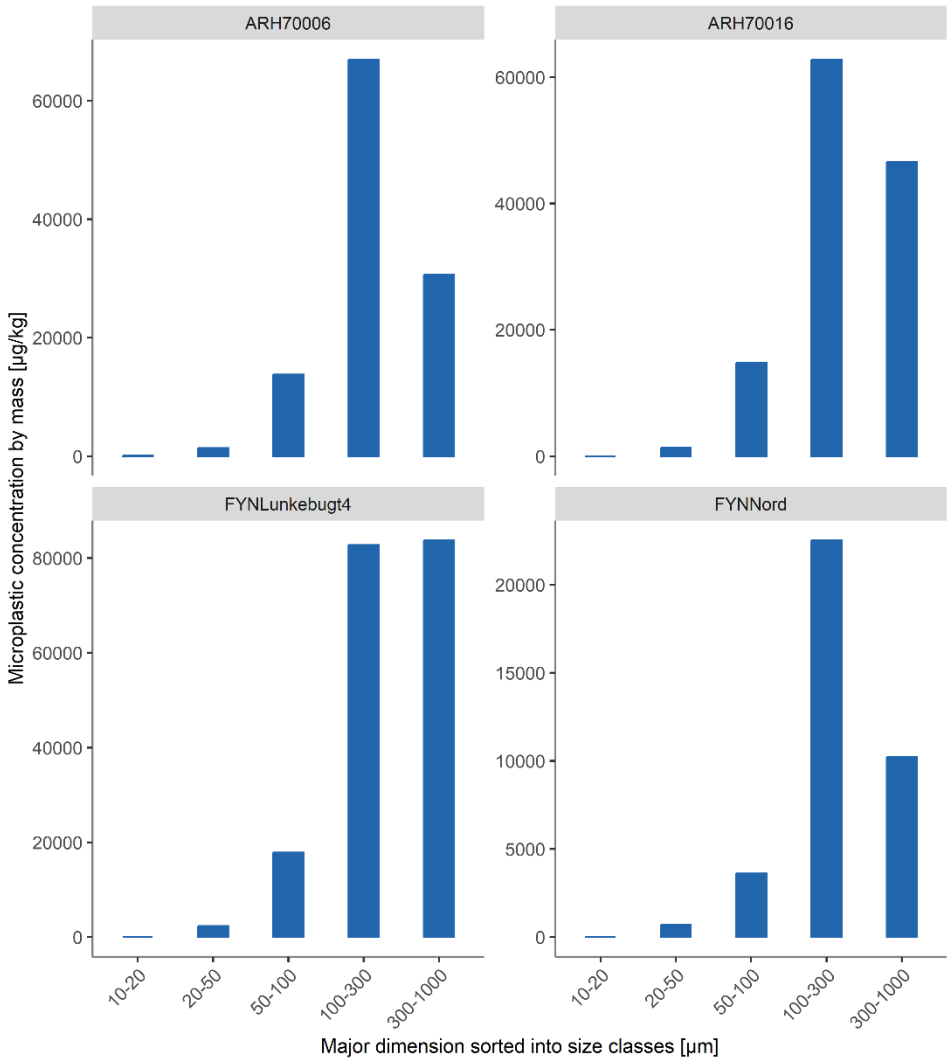


Figure 46. Microplastic concentration by mass, with the particle's major dimension sorted into five size classes

Some final issues must be mentioned related to identifying the smallest and largest particles. For the smallest particles, it seems likely that recovery of the very small particles is poorer than the recovery of the larger ones, a conclusion which was also drawn in Section 4.3.5. This might well be even more pronounced for naturally occurring MPs compared to the virgin beads added to test recovery in the present study. For the very large particles, the issue is mainly that there are very few of them, and it becomes rather random if one is found in a sample or not.

The take home message from the discussion of particle sizes versus determined concentrations can be condensed as follows when using µFTIR imaging as analytical tool:

- One can rightfully expect that microplastic numbers must increase with decreasing particle size. The rate of increase is probably by some sort of exponential function. However, the analytics show that this only holds true down to roughly 50 µm particles. There are strong explanations why the analytical method struggles to identify the smallest microplastics. One can 'amend' this by using a more nonconservative approach to chemical identification of the smallest particles, or one can 'be conservative' and only accept those where there is high certainty on the chemical identification. Whatever approach is chosen, the uncertainty on the identification increases drastically below roughly 50 µm particles. The only way around this issue would be to apply an analytical method which is more sensitive to the small particles, e.g., µRaman imaging which does not have the same issues with

chemical quantification of small particles. However, the method has other drawbacks, here among that it would be very problematic to at the same time analyse larger particles, and that the method requires more effort (i.e., cost).

- In line with the above discussion, large microplastics occur much more seldom than small ones. While a few such particles do not affect the microplastic counts much, they heavily affect the microplastic mass, as mass comes in the third power of size. The only way to amend this issue is by finding enough large microplastics, so that the identified particles are representative for the sampled site. These particles, however, are rare, which means that large sample volumes must be analysed, which in many cases is not realistic in terms of costs. The mass of particles below roughly 50 µm contribute insignificantly to the overall mass, while the particles >100 µm dominate the mass. To achieve a better mass quantification, one could hence choose to focus on larger sample volumes and only analyse down to roughly 100 µm in size.
- There hence is a schism between analysing microplastics with the goal of count versus mass quantification, the targeted size range, and keeping costs down. At the end of the day, how exactly to approach microplastic analysis of marine sediments remains a choice based on the specific requirements.

4.3.5 Extraction recovery

The chemical analysis of a subsample entails other uncertainties than the number of scans of the extract. A main uncertainty relates to the efficiency of the sample treatment protocol. To assess the size of this, the recovery was determined as presented in section 4.2.5. The recovery varied between stations as well as between subsamples from the same station (Figure 46). The highest recovery was found in station ARH170006, with an average of 93% (Appendix A).

The recovery study indicated that it was more likely to have a stable recovery when the water content was high, and the organic matter content was low (Figure 48). This statement shall though be taken with some reservation as it is based on only one station. No correlation between detected microplastic concentrations and recovery could be identified (Figure 49).

Recovery is often used to correct values obtained by the analysis. For example, FynLunkebugt4, subsample 1, had a recovery of 60%. One could hence choose to correct the concentrations by a factor 1/0.6. However, it is debatable if this is the best approach as microplastics is a diverse group of particles and materials, and a recovery obtained by ‘simple’ particles such as virgin beads is not necessarily identical to the recovery of naturally present microplastics. As the ground truth of the latter is problematic to determine, specialist opinions are divided on this point.

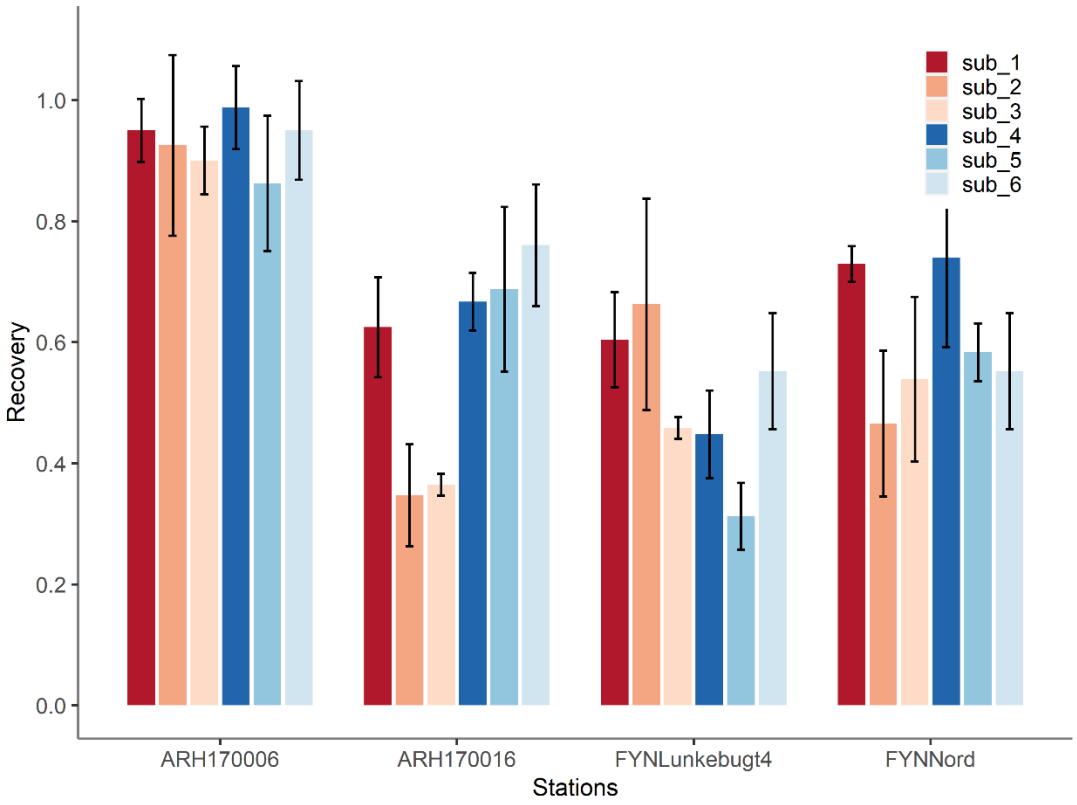


Figure 47. Extraction recovery for the four stations, quantified for each subsample

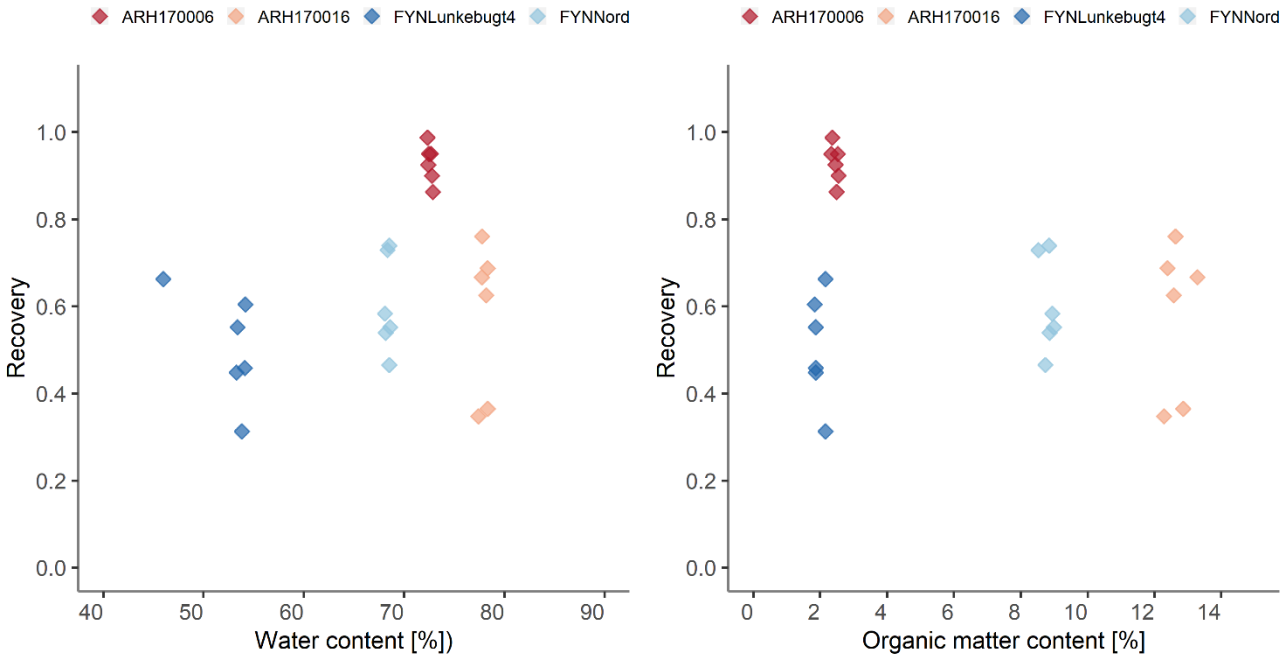


Figure 48. Correlation between the extraction recovery and water content, as well we organic matter content

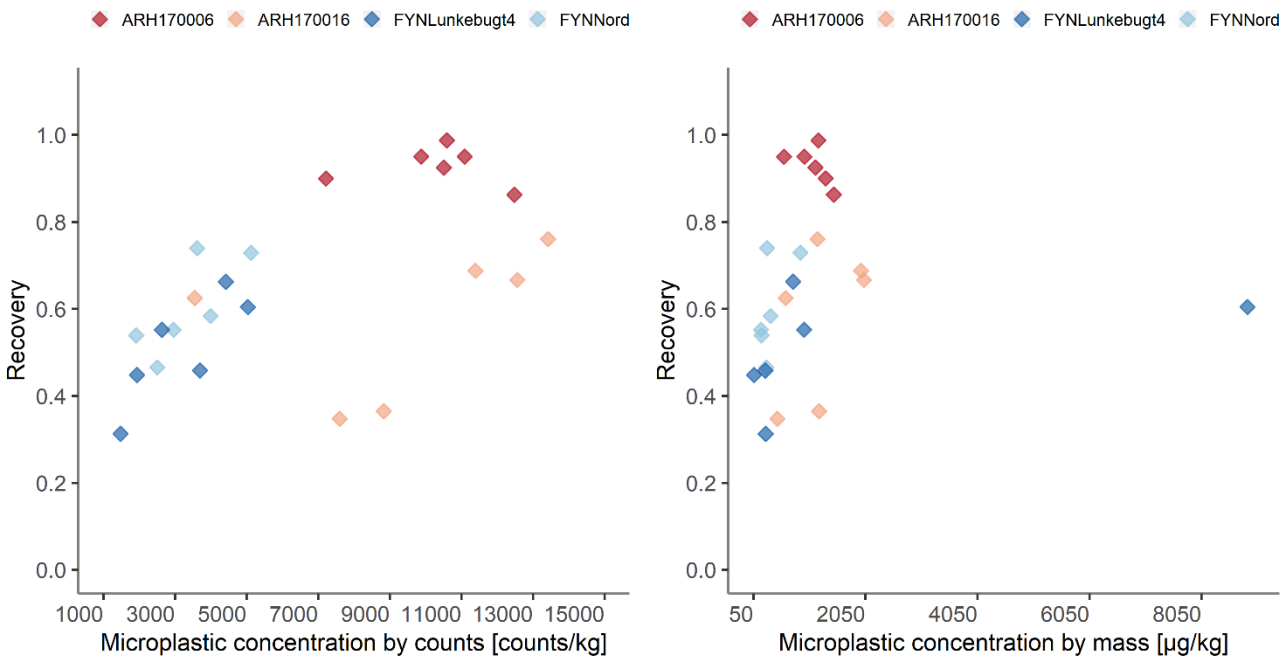


Figure 49. Correlation between the extraction recovery and microplastic concentration by counts, as well as by mass

The recovery varied between the polymer type and size of the beads (Figure 50). For the same polymer type, larger beads had relatively higher recovery than the smaller beads, except large PE beads in FYNLunkebugt. When the size range was the same, heavy beads (PS) tended to yield higher recovery than the light beads (PE). Overall, large PS beads had the highest recovery in all stations. This suggest that using a single type or size of beads as the reference material for recovery test in microplastic extraction may not be representative, if possible, a larger variety of reference materials should be used.

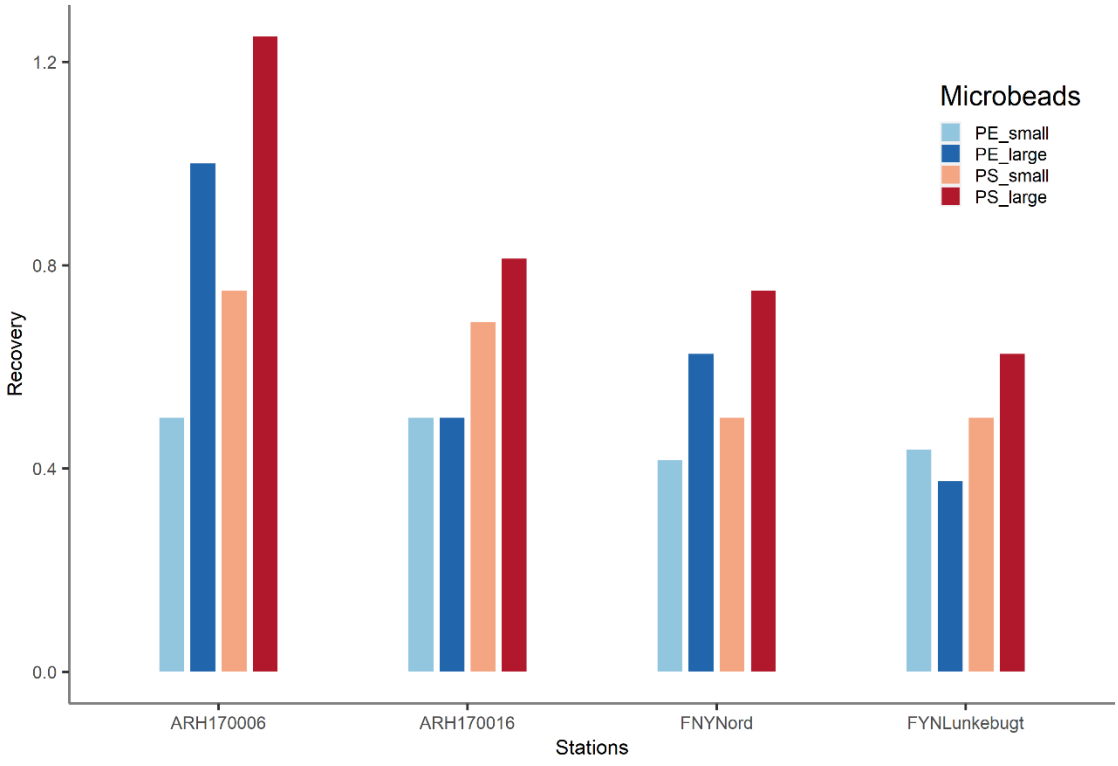


Figure 50. Recovery calculated for each type of beads

4.3.6 Blanks

Contamination in the laboratory is a further source for uncertainty in the analysis, especially when working on samples with very low levels of microplastics. In the present study, the contamination as measured by the blanks was quite small compared to the levels found in the samples (Table 3). In cases where very low microplastic levels are attempted quantified, this uncertainty can though dominate the analysis.

4.4 Discussions

The relative variability between subsamples and individual scans depends on the level of microplastic concentration in the sediment, as well as the basic characteristics (e.g., water content) of the sediment. In specific, if the sediment has a high microplastic content, the variability comes more from the scans but less so from the subsamples. In other words, sediments with high microplastic concentration can benefit from analysing more scans to obtain a more reliable results, but not necessary from more subsamples. In the case of less polluted sediment, especially coupled with low water content, it becomes necessary to take more subsample to decrease the overall variability, as it is the main source of uncertainty.

However, when it comes to reality, the actual level of microplastic concentration is difficult to know beforehand, which leaves the sampler nowhere to give a good "guess" on the necessary subsamples. Theoretically speaking, the number of subsamples analysed matters, partly to obtain a more accurate average value, and partly to obtain a more precise value. At the same time, a significant part of the extract should be scanned to improve the precision of the subsample analysis. Unfortunately, this leads to an approach where the effort to analyse a single sample becomes quite substantial. Current practice is to analyse one subsample applying up to three scans. Increasing this to analysing for example six subsamples would six-double the analysis costs. Increasing the number of scans is less costly, albeit there are limits to this too. It would in principle be ideal to scan all the extract from a subsample (Figure 27). However, a complete clean-up of marine sediment samples is not doable for a complex environmental matrix like marine sediments, and interfering material (particles) will be present even after extensive sample preparation. This means that only small aliquots of extract can be deposited per scan, which then means that many scans would be needed to chemically analyse the whole extract. There are in practice limits to how many scans can be done, as each scan requires quite some machine-time. In the present study, one scan took approx. 5 hours, and 2-3 scans per day could be done when working in shifts. Hence, doing 3 scans tied down a machine for at least a day. Increasing the number of scans for example to 20 would allow between half and all extract from a subsample to be analysed. It would however tie down a machine for approx. 2 weeks, which would of course increase costs per sample analysed. How large a fraction to scan, or better, how many aliquots to scan, is hence a matter of cost.

Nevertheless, a rough estimation on how much sample to take can still be obtained by several ways with the aim to reduce variability, for instance referring to the data from stations which are geographically close to the desired sampling location. Taking the Danish marine environment as an example, though stations close to land have microplastic concentration varying by an order of one magnitude (Liu et al, 2022, in prep), the ones far from land are at a similar level of concentration, with differences of only a few hundred counts kg^{-1}. So, if the targeted station is geographically close to any of these already investigated stations, in other words, whether the targeted station is similarly close to land, or similarly far from land, one could give a rough estimation on the concentration level. Another approach is to have the water content analysed first, then decide if more subsample is necessary. This can effectively lower the effort for sample processing while still obtaining a reliable result.

The uncertainty on the analysis of the large composite samples of the present study was however substantially smaller than the uncertainty on sampling within an area as reported by Liu et al. (2022; in prep), who studied variations in two areas covering approx. 1 km^2 each. One was in Kattegat east of Strandby, while the other was

in Odense Fjord close to its mouth. They report that for microplastics measured as counts, the concentrations in the sampled areas varied within a factor 10.3 and 4.6, respectively. With respect to microplastics measured as mass, the variation in the Strandby area was more than three orders of magnitude, while it varied two orders of magnitude for the Odense area. Seen in this light, it would not make sense to increase the analytical accuracy and precision without also increasing the representativeness of the sampling, cf. section 4.1.

Assuming the relative uncertainties of this study and the studies of Liu et al. (2022; in prep) hold, the first place in the ranking of the uncertainties becomes sampling in the field, which seems to be by far the largest potential source of uncertainty. Following that, the uncertainty in subsampling the collected samples in the laboratory, as well as the uncertainty in extracting and chemically analysing the extracts depends on microplastic concentration level and the basic characteristics of the sediment. In short, if the sample is high in microplastic content, the analytics cause more uncertainty than subsampling. If the sample is low in microplastic content and low in water content, then subsampling causes more uncertainty. But overall, it is way less significant compared with the sampling in the field.

This leads to a discussion on how best to minimize the total uncertainty on microplastic quantification for the purpose of monitoring in marine sediments. Obviously, emphasis must be put on sampling, but also what happens in the laboratory once the sample has been collected plays a major role. At the same time, it must be affordable to analyse marine sediments for microplastics. Hence, simply measuring many samples by many subsamples by many scans is not a viable approach.

Albeit ship time is expensive, at is probably worthwhile to invest a bit more time in sampling a composite sample covering a reasonable area. It then is worthwhile to optimize the subsampling in the lab. In principle it would be preferred to analyse many individual subsamples, however, this is costly. Hence it might be more appropriate to take a pragmatic approach, for example taking many small cores from a larger sample, mixing them, and then let that sample go into analysis. In terms of the chemical analysis, it would probably be worthwhile to increase the number of scans, at least to a minimum of three per sample, preferable more, even though this will increase the cost a bit.

It also seems necessary to sort the particles into multiple size classes before interpreting the results, because large particles can cause increased uncertainty on the determination of mass concentration, and because small particles are inherently uncertain to quantify, and hence can cause increased uncertainty on the determination of count concentration. In addition, depending on the type of technique applied, the analytical method generates uncertainty which can be different between size classes. Hence it is important to sort the size into classes, and the results ought to be presented within each class with its own uncertainty. When comes to which size classes to use, recommendation can be referring to, for instance, HELCOM or OSPAR, so results can be more comparable across studies. The study also proved that the choice of reference material in recovery test affects the results significantly. Generally speaking, larger and denser polymers achieve higher recovery than the smaller and lighter ones. But in environmental samples, both types of polymers are expected to be present, hence using a single type of reference material to represent recovery for all types of polymers is insufficient, which could further introduce uncertainty to the results.

5 WP2

5.1 Screening analysis based on Nile Red Staining for Large microplastic particles

Different analytical techniques are currently considered for microplastic identification and quantification for different particles size fractions. In general, the larger size fractions (> 300 µm) are often visually identified by means of optical microscopy, which is a laborious and rather subjective method with relatively high risk for false positives and negatives (Kotar et al., 2022). It has been proposed that faster screening methods using staining techniques and fluorescence microscopy can improve the visual identification of plastic particles, that afterwards can be supplemented with chemical identification for polymer characterization (e.g., FTIR analysis). This screening strategy has been proposed as a potential monitoring method by OSPAR and HELCOM sea convention (HELCOM 2022a,b; OSPAR, 2022; Bakir et al., 2023).

Nile Red (NR) was developed as a low cost and fast approach for the detection and quantification of microplastics in environmental samples with its feasibility successfully demonstrated. NR is a dye that is adsorbed onto the polymer surface and can fluoresce under certain light conditions, creating a fluorescent tag to the target plastic particles (Maes et al., 2017). Its uses has been demonstrated in experimental studies and also applied in a large-scale environmental sample pool, as observed in the spatial and temporal assessment of microplastics in United Kingdom (England and Wales) seafloor sediment, which is under the OSPAR framework (Kukkola et al., 2022).

NR staining methods still requires an element of visual identification under a microscope, but with improved perception of plastic particles due to the characteristics of the dye to colour the polymer. In contrast, the dye also stains organic material that actively act an interfering particle as a common false positive. Although the fluorescent signal for stained organic material being slightly different from the plastic, there is still a risk for that be overlooked as polymer. This means that further method development for this promising staining technique is needed, such as the development of an automated protocol for sorting plastic from organic material using the fluorescent signal; as well as the use of computer-driven solutions for polymer sorting and their count and size distribution. Nevertheless, a more cost-effective approach could be applied by using a photography camera to capture the fluorescent image to increase the throughput analysis by selecting the polymers using image processing. In summary, these topics were investigated in this WP. The proposed approach combining both digital camera and imaging processing for a screening analysis of microplastic using Nile Red staining offer substantial benefits for more automated methods, speeding up analysis and improving quality in visual polymer assignment.

A protocol evaluating different staining conditions of microplastic particles was developed and a photobox prototype which obtained particle images in a more accessible way was produced. It is believed this photobox can greatly benefit monitoring activities on large-scale sample pools. The obtained images were used to start the development of an automatic method for microplastic identification and their differentiation of interfering particles, such as organic material.

5.1.1 Method

Fluorescence staining methods provide a simple and sensitive approach to improve visual identification of plastic particles and differentiate them from interfering inorganic and organic particles. Nile Red is a fluorescence dye with demonstrated efficiency in terms of adsorption and signal intensity for this purpose (Maes et al., 2017). In this study, 0.01 mg mL^{-1} of NR in ethanol was prepared and applied to a steel filter system containing microplastic particles > 1 mm (PE, PP, PET and PA) with a mixture of spiked organic materials (marine plants, wood, proteins, etc.) that acted as interfering particles. The filter system (Figure 51) was placed in a petri dish and the NR solution incubated for 15 min. Subsequently, 100 mL of MilliQ water were flushed into the system to remove residual dye. Throughout the method development and for all steps of the filtration, the

filter system was covered with aluminum foil and kept in dark. After incubation, the filter was removed from the system and oven-dried (40°C) before image measurements.

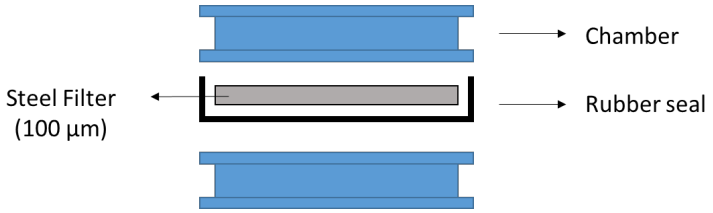


Figure 51. Steel filter system used for Nile Red staining. This filter is placed in the middle of two metal chambers with a rubber O-ring sealing

An initial test was conducted to determine the condition in which optimal fluorescence signal was obtained at high background contrast, e.g., where the polymer was highlighted as much as possible in a dark frame background. To achieve that, a wooden photobox prototype was created for image acquisition (Figure 52). Blue and UV light (LED Party Panel RGB + UV, Eurolite) were evaluated for excitation and both orange and red camera lens filters (Heliopan) used to count the NR emission signal. Digital images were taken using a Canon M50 Mark II camera and EF-M 28 mm f/3.5 macro lens (Canon). Different exposure times that the camera collected light from the sample (shutter speed) were also evaluated for image acquisition applying ISO-100.

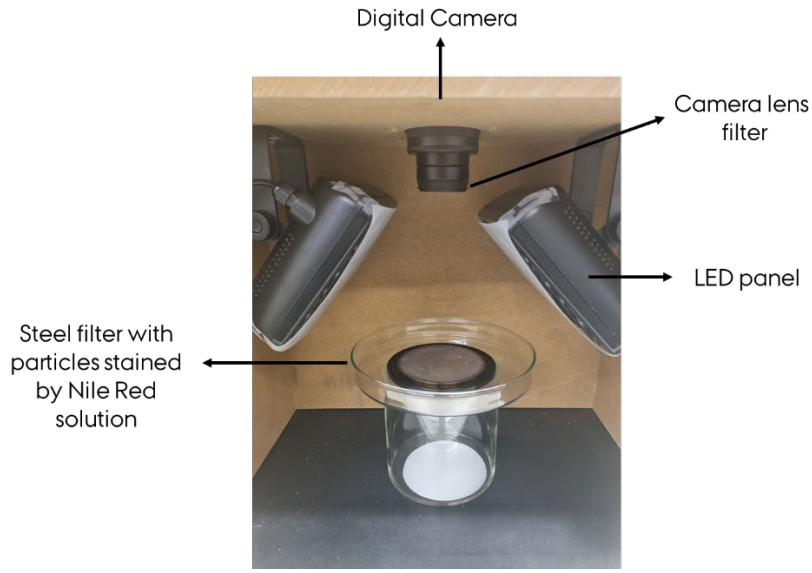


Figure 52. Photobox prototype (inside) created for acquisition of Nile Red staining digital images

Following this, an automatic method applying digital images for screening analysis of microplastic was evaluated. This strategy combined image pre-processing followed by image segmentation and object measurement. For image pre-processing, colour models were evaluated to discriminate the plastics from interfering particles. Colour models are mathematical descriptions of how colours can be represented as tuples of numbers, typically as three values. The Red-Green-Blue (RGB) colour model is the most common and it was compared to Hue-Saturation-Value (HSV) colour model (Yan et al., 2021). The latter provides a more intuitive and flexible way to represent colour by providing a better separation of colour information, as well as a better representation of colour range when compared to simplistic RGB colour model. For that reason, HSV is often preferred for image processing and computer vision applications. Image segmentation was carried out by selecting appropriate colour channels within a colour model that highlighted properly the plastic material. This strategy was validated by applying the method on a marine sediment sample collected east of Skagen, Denmark.

5.1.2 Results and Discussion

An initial test was conducted to determine the conditions in which the images should be taken for this analytical method. Several criteria were evaluated, and the three main factors identified to obtain optimal fluorescence signal were light source, filter, and shutter speed. In a first assessment, blue light demonstrated the best performance on emphasising the polymers particles, whereas the orange filter could better differentiate them from spiked organic material. Furthermore, shutter speed at 0.5 seconds gave appropriate contrast between the particles and the background. Therefore, these setting were applied in this study. Figure 53 shows the visual image and stained particles under the different factors initially evaluated, and image selected (in Red) for the following imaging procedure using colour models.

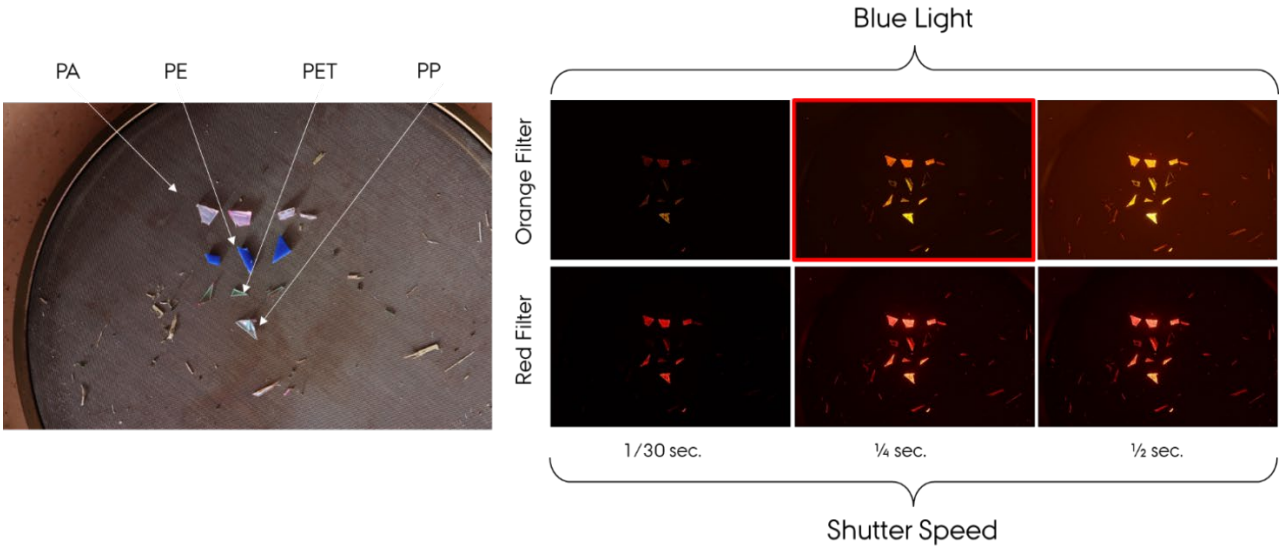


Figure 53. Visual and Nile Red-stained images of PA, PE, PET, and PP particles. Fluorescent images obtained with blue light using red and orange filters and applying different shutter speeds. The image highlighted in red was selected for further image processing

Two different colour models were evaluated in this work: RGB and HSV. The latter is an alternative colour representation of the commonly RGB model and it was designed to better represent the eye-tracking system. Figure 54 shows the representation of each colour channel present by both RGB and HSV colour models.

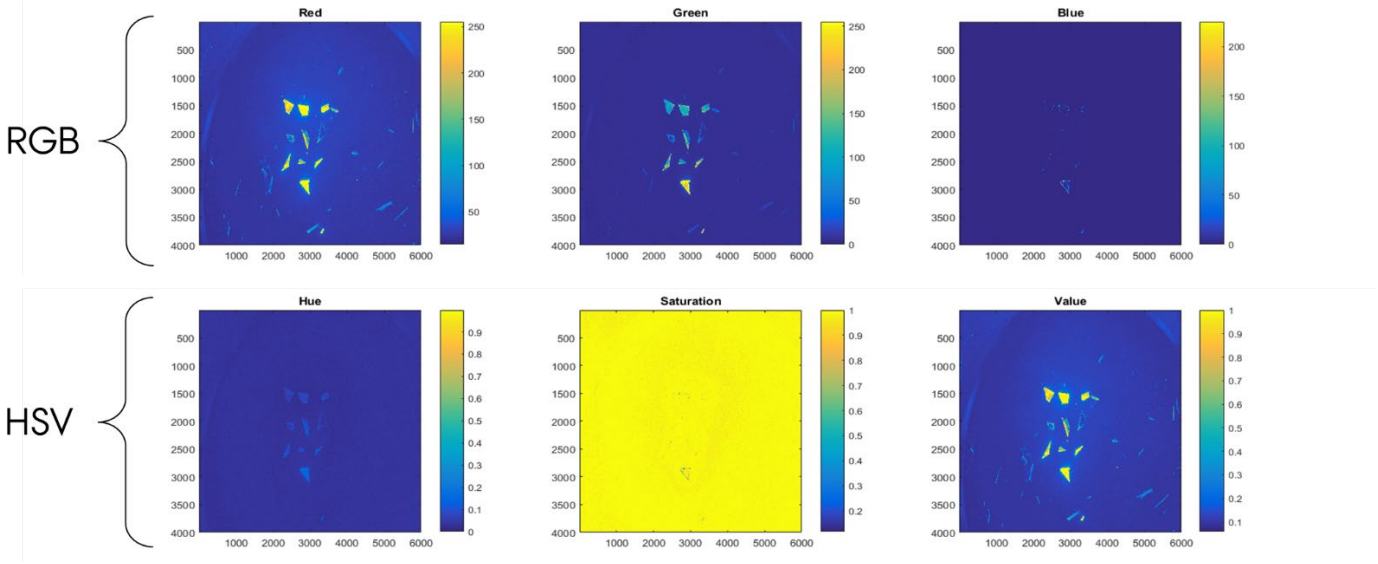


Figure 54. Nile Red-stained image decomposed in three colour channels for RGB and HSV colour models

Figure 54 shows that each colour channel feature different information from the visual image, where the colour bar displayed for each image refers to colour intensity. Some colour channel, for instance Red and Value are similar with organic material clearly visible. On the other hand, Hue demonstrated that the polymer information is predominant, opposed by Blue and Saturation. By having a close look at the Hue channel for outlier pixels, the polymer particles can even be highlighted by simply removing them. This can be followed by selecting a pixel value threshold for image segmentation, as shown in Figure 5549. This highlights the importance of examining outliers when dealing with digital images, which is related to the camera sensor and colour model transformation.

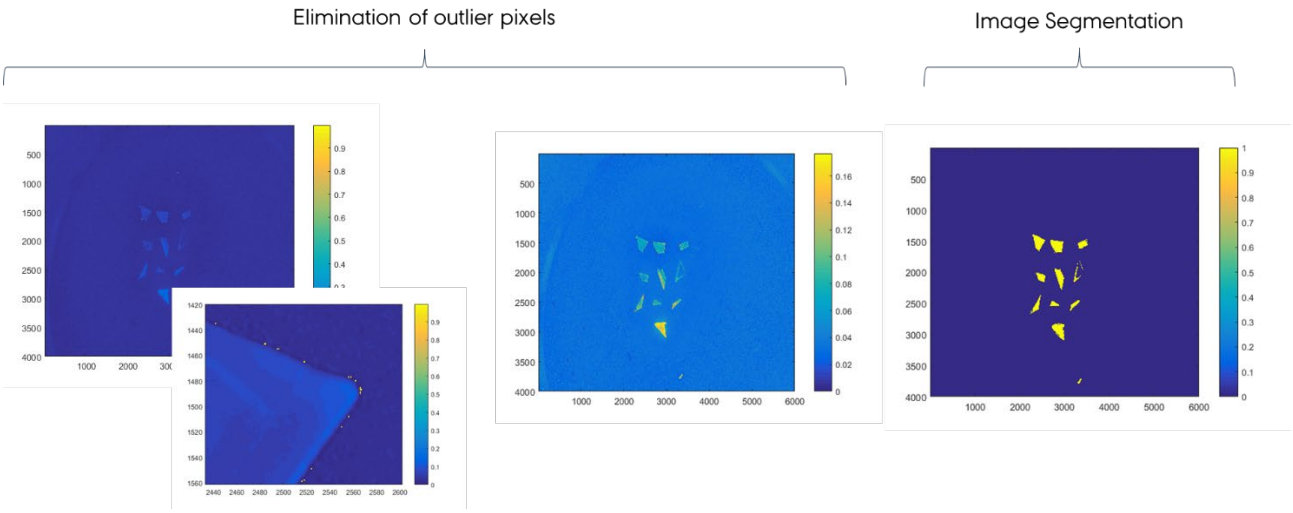


Figure 55. Visualization of outlier pixels and image segmentation on the Hue colour channel of a Nile Red-stained image

Doing a few image processing steps allows image segmentation, separating target particles from the background and interfering elements. This can greatly improve the visual analysis of microplastics by directing the target particles that should be further validated chemically. This automatically driven strategy was applied to a sediment sample for performance evaluation, as it can be seen on Figure 56. The selected particles were handpicked and validated chemically using infrared spectroscopy. The particles were positively identified as plastic, and they were PE, PVC, and paint flake. This demonstrates the strategy feasibility to correctly sort the plastic particles from interfering materials.

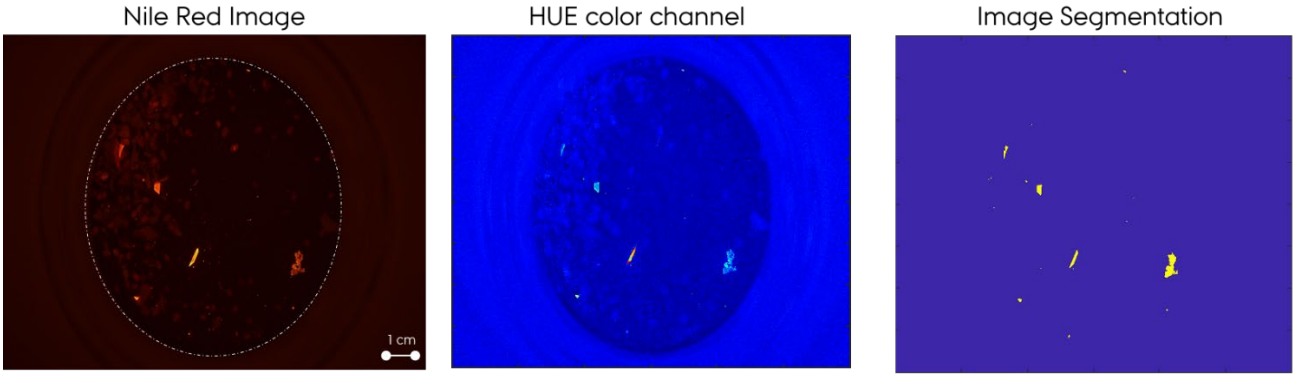


Figure 56. Automated image processing using Nile Red applied to a sediment sample (Skagen, DK)

This automated image process approach improves the visual analysis of large microplastics (> 1 mm), which is still a common procedure for particle identification. This screening strategy reduces the bias and false positive rates on the selection of particles by discriminating the interfering particles. In addition, it improves the

throughput analysis by directing the particles to be handpicked and further analysed, despite of general information about the plastic particles being already the method output. The proposed strategy can be effectively utilized for microplastic analysis in various environmental compartments, including sediment, water (via mantra trawling), and biota, provided that appropriate sample processing is carried out. Nile Red, as suggested by both OPAM and HELCOM, offers a promising method for monitoring activities due to its simplicity and cost-effectiveness, as evidenced by the results of this study. While the study's focus was on particles larger than 1 mm, this approach can be extended to smaller particles, as long as they can be selected manually for further analysis without requiring higher magnification equipment. Moreover, there is potential to use this technique for characterization of polymers, however further investigations are needed.

5.2 Machine Learning Strategy for Microplastic Characterization and Quantification for small microplastic particles

The common pipeline for microplastic identification comprises several steps ranging from sampling to sample processing to separating and purifying MPs from the media prior the polymer characterization (Mattsson et al., 2021). After the sample processing, the microplastics are usually membrane filtered for further characterization, where the polymeric particles are chemically identified, listed, and reported, for instance, according to the categories established on AMAP and OSPAR monitoring guidelines. Several analytical techniques have been applied for microplastic characterization and infrared microscopy (µ-FTIR hyperspectral imaging) is the most common and currently the state-of-the-art for identification of small microplastic (< 300 µm) (Elkhatib and Oyanedel-Craver, 2020; Mattsson et al., 2021). This technique has the advantage of collecting chemical and spatial information of several particles at the same time by automated mapping of a sample, allowing the analysis of small microplastics without manual sorting and the estimation of particle features such as their area and diameters (Löder et al., 2015).

µ-FTIR hyperspectral imaging produces complex and large amounts of information (millions of spectra), which suggests the use of automatic analyses to transform huge dataset into information. There are available different approaches on how to deal with such dataset, ranging from library search approach (correlation to a reference library) to more advanced machine learning strategies (Löder et al., 2015; da Silva et al., 2020). The latter often applies several data pre-processing strategies, exploratory analysis, and multiclass models that embrace the reality of plastics that are more common in the environment. Therefore, it is proposed that with the correct data pre-treatment and appropriate selection of hyperspectral data analysis approaches, we can fuel the development of methods to automate the quantification and identification of microplastics from an environmental sample, including the validation steps as a central aspect of the method to performance evaluation. The latter also brings benefits for how different methods can be compared in monitoring activities by referring to the validation attributes.

This WP investigates and develops an analytical method for characterization of MPs using µ-FTIR imaging and machine learning. Different multivariate techniques (PCA, SIMCA and PLS-DA) were orderly evaluated to retrieve the microplastic information of small microplastic (< 300 µm) of µ-FTIR imaging measurements. It is proposed that with the correct data pre-treatment and appropriate selection of hyperspectral data analysis approaches, we can fuel the development of a method to automate the quantification and identification of MPs from environmental samples. The results of these studies are likely to lead to improved data processing protocols and the development of more robust analytical identification methods, also with a focus on data quality assurance and quality control, including validation processes to reduce bias and sources of error.

5.2.1 Method

Microplastics of the most common commercial plastics (PE, PET, PMMA, PVC, PC, PUR, PA, PS, ABS and PBT) was produced and kept as a reference to aid with the identification of plastic taken from the environment.

These polymers were grounded into small pieces using a metal grinder. The material was sieved and particles from 10 to 300 µm were placed on a Silicon (Si) filter, which is a quite newly developed membrane that allows the IR fingerprint region to be collected in transmission measurements. One membrane for each polymer and a mixture of all microplastics spiked with natural matter were produced and analysed using a µ-FTIR hyperspectral imaging system (Cary 620 FTIR microscope coupled with a Cary 670 FTIR spectrometer from Agilent Technologies).

A machine learning approach applying hierarchical analysis (HA) was evaluated to retrieve the microplastic information of hyperspectral images. HA combines different multivariate models/methods to sort the different spectral information in subsequent levels to obtain the target particles in a fully automated method. This helps to provide insights of multivariate models appropriated for different aspects of the analytical pipeline based on spectroscopy and machine learning. The Figure 57shows the workflow proposed and developed for microplastic characterization.

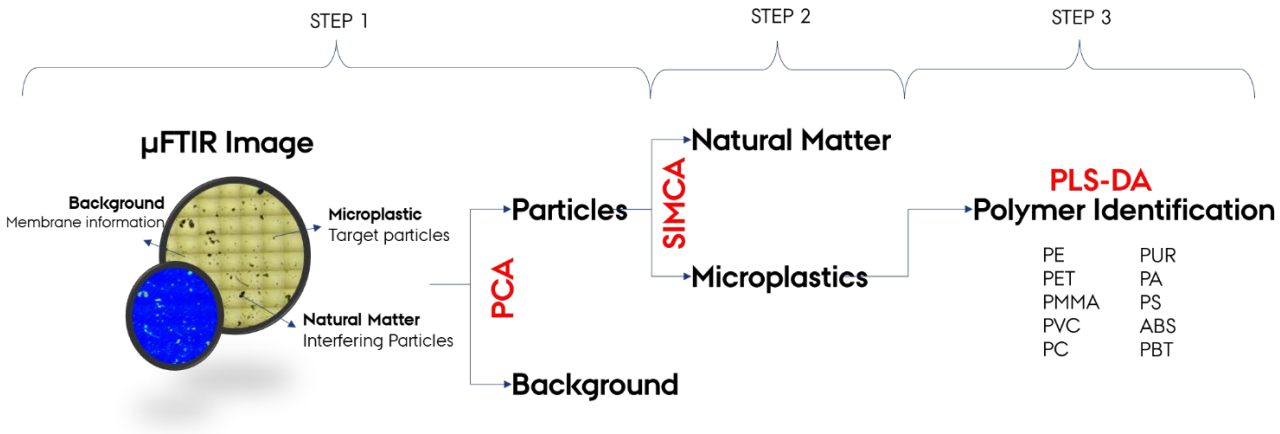


Figure 57. Workflow of the FTIR data processing and the multivariate technique applied in each step on the hierarchical analysis

Principal Component Analysis (PCA), which provide the source of data variability, was firstly applied for selection of the region of interest, i.e., particle information. Soft Independent Modelling Class Analysis (SIMCA) was subsequently applied to sort out the natural matter and microplastic information (STEP 2, Figure 57), where the latter was further used for polymer discrimination applying Partial Least Squares-Discriminate Analysis (PLS-DA - STEP 3, Figure 57). STEP 2 and 3 are classification models and were validated based on true positive rate (sensitivity, Sn), true negative rate (Specificity, Sp) and misclassification applied to an image with a mixture of polymer and interfering particles (Fielding and Bell 1997) with a workflow described in Figure 58.

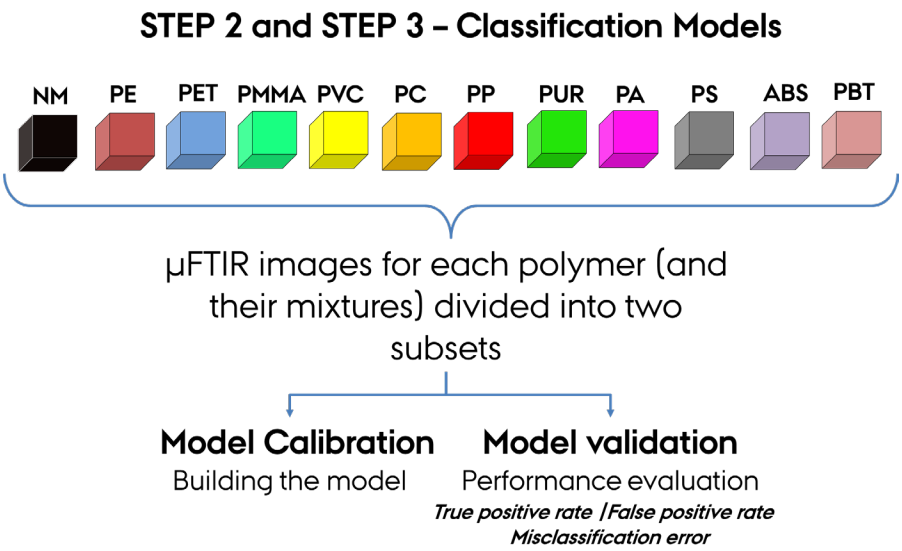


Figure 58. Workflow applied in this work for the development of classification models (STEP 2 and STEP 3) where the sample set was subdivided into calibration and validation. The later was used for performance evaluation of the models

Detailed information about the particles was obtained by using the spatial information of the image for particle counting and size distribution. The latter were further investigated to assess their variability in relation to the two common ways of reporting the particle size: (1) Length (Maximum diameter) and (2) Filter Cut-off (Minimum Diameter). Feret diameter was calculated for an imaging and the number of particles was reported based on both maximum and minimum diameter for comparison and discussion on how these numbers can affect the reported number of particles.

5.2.2 Results and discussion

Hierarchical analysis was a feasible approach to deal with three major information contained in the dataset: background (membrane), MPs (target information) and Natural Matter (interfering particles, which often occur despite sample processing). Different spectra pre-processing was evaluated, and the best results were obtained with 1st derivative (Savitzky-Golay, 2nd order polynomial and 15 window width) and normalization (Inf-Norm). Moreover, the sample spectra were cut off in the range of 2600–2000 cm^{-1} to eliminate the CO_2 signal that is not chemically related to the sample.

PCA successfully selected the region of interest by removing any pixels not related to the particles in the image, which reduces processing time and the risk for false positive identification. This spatial pre-processing was made using the score frequency histogram obtained from PCA realized on the hyperspectral images. Both SIMCA (STEP 2) and PLS-DA (STEP 3) models showed great average sensitivity (Sn ≅ 1) and specificity (Sp ≅ 1) for sorting natural matter and discriminating the polymer types. As for misclassification error, an average of 3% and 0.2% were obtained on STEP 2 and STEP 3, respectively. These evaluation parameters are used to estimate the probability of pixels belonging or not belonging to the correct target category and are good examples of method robustness evaluation that can be applied when reporting and comparing microplastic data in different analytical techniques/methods, i.e., validation and performance data. Figure 59 shows the results of one sample imaging containing a mixture of plastics and natural matter in each step of the hierarchical analysis.

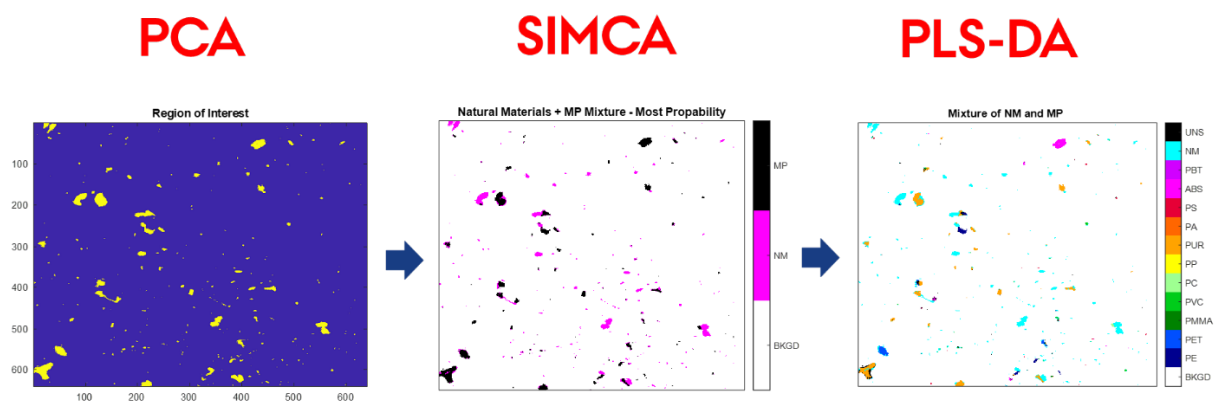


Figure 59. Results of one image sample containing a mixture of plastic and natural matter in each step of the hierarchical analysis

The results clearly show the discrimination of the major content information in Si filter. This methodology can be applied for different sample matrixes, as long as the sample is previously processed for microplastic separation and purification. The proposed method can speed up data analyses, improve quality and reproducibility in polymer assignments, as well as it demonstrates an approach to fully harness the potential of µ-FTIR hyperspectral imaging for quantifying and qualifying microplastics. Finally, morphological information about the samples can be obtained using the predicted final images, for instance, particles size and their frequency, as seen in Figure 60. Here, the size distribution was based on the maximum Feret diameter and all spectra from the predicted particles can be examined for QA/QC.

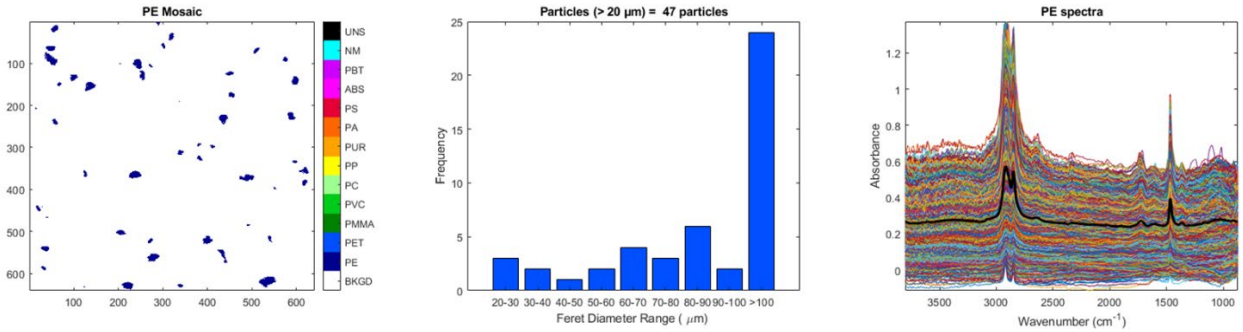


Figure 60. PE predicted image with the particle counts and size distribution. Infrared spectra of all PE particles displayed

Particle counts and size distributions are usually defined by a size range, e.g., 50–100 µm and/or 1–5 mm. However, these numbers vary greatly depending whether the reporting data is based on the individual particle's diameter (longest dimension – length) or mesh size of the filters used in sample processing (smallest dimension – filter cut-off). This is especially important for reliable comparison of produced datasets in both research and monitoring activities for assessment of microplastic pollution. Feret diameter is often used for this purpose where the maximum and minimum diameter are obtained referring to particle's length and filter cut-off, respectively. A comparison of particle counts obtained using is both Max. and Min. Feret (microplastic > 50 µm) were carried out to a predicted image containing only PE particles, Figure 61. The result shows that the number of particles was halved when the filter cut-off was used for calculating particle frequency. This suggests the necessity for standard definitions on how the particles size and frequency should be calculated and/or reported in research and monitoring activities to be able to compare results, since it can vary significantly on the strategy applied. There are no standard procedure guidelines available regarding particle counts. However, it sheds light on the importance of at least reporting the chosen calculation rather than stating only the number of particles identified.

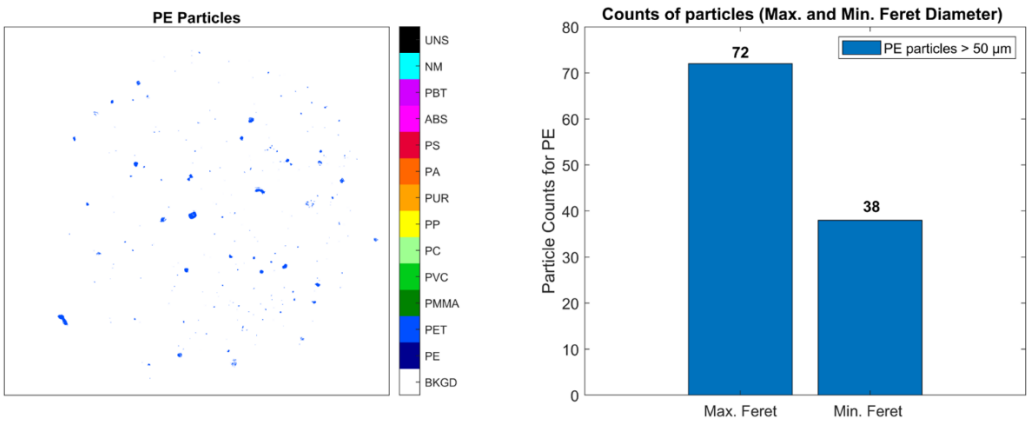


Figure 61. Predicted image for PE and their particle counts (> 50 µm). Particle amount calculation based on maximum and minimum Feret diameter

The approach presented in this study offers a wealth of information that can aid in the characterization and tracing of microplastics from different sources in environmental samples. However, it is essential to properly process and extract microplastics from the target matrix to obtain accurate results. This strategy can be effectively implemented for monitoring activities using the parameters presented here. In case data from another instrument or source is used, a calibration transfer must be established before conducting any analysis. Nonetheless, the method can be replicated using all the provided information in any other µFTIR instrument. Furthermore, the approach can be updated to include other polymers or classes, depending on the research needs.

6 WP3

Evaluation of sampling methods for microplastics in surface water and water column.

The most appropriate sampling technique for microplastics is defined by the targeted compartment (e.g., beaches, sublittoral sediments, sea surface, water column) as well as the subsequent processing and analysis capabilities. The latter sets the bar for what can be achieved in terms of analytical output, such as how small microplastics can be reliably identified, if polymer types can be identified, if particle size and shape can be identified, et cetera. What shall be achieved in terms of microplastic analysis must hence be considered when planning a monitoring strategy.

6.1 Sampling with different techniques

Taking bulk-samples of sea surface water (e.g., Ng et al., 2006; Norén, 2007) or the water column, e.g., with Niskin bottles attached to a rosette sampler (Bagaev et al., 2017; Courtene-Jones et al., 2017; Kanhai et al., 2018), is the exception. Typically, the sea surface is sampled by trawling a net alongside a vessel for a certain amount of time. The two most common net versions are manta trawls and neuston nets with a mesh size of around 300 µm (e.g., Collignon et al., 2012; de Lucia et al., 2018; Tamminga et al., 2018; Vianello et al., 2018; Frias et al., 2020; Ferrero et al., 2022), which are also recommended by the Marine Strategy Framework Directive (MSFD) guidelines (Hanke et al., 2013; Gago et al., 2018). Examples of other devices which have been used are the AVANI trawl (Eriksen et al., 2018), plankton (WP2) nets (Gorokhova, 2015; de Lucia et al., 2018), and a neuston catamaran (Löder et al., 2015; Kirstein et al., 2016; Lorenz et al., 2019). For a volume-reduced sampling of the water column, either sub-surface trawls with bongo nets (Doyle et al., 2011; Beer et al., 2018; Rist et al., 2020) or special 'multi-level-trawls' have been performed (Reisser et al., 2015; Kooi et al., 2016). Alternatively, a device termed the Continuous Plankton Recorder (Thompson et al., 2004) or various pumping systems coupled to filtering units with varying mesh size have been used (e.g., Lusher et al., 2014; Enders et al., 2015; Kanhai et al., 2017; Zobkov et al., 2019; Rist et al., 2020; Tekman et al., 2020; Liu et al., 2023; Kuddithamby et al., 2023). The advantage over bulk-sampling methods is that a larger water volume can be sampled. Another aspect to consider when using volume-reduced sampling with nets and pumps is that the size of the smallest microplastics to quantify is already influenced during sampling by the mesh or filter size used.

Historically, most studies target microplastics floating at the sea surface since these are more easily accessible. Figure 6256 provides a selection of studies (n=26) reporting microplastic concentrations in surface and sub-surface waters (n=44). Apart from the sampling location, the sampling method, e.g., Manta Trawl, Neuston net, plankton net, pump-filtration system with the respective mesh size is noted. The average sampled volume is indicated, if provided by the authors, as well as the analysed size range. Utilized filter or mesh sizes divided on ranges were 10–50 µm (n=7), 51–150 µm (n=11), 151–250 µm (n=3), 251–350 µm (n=20), and 451–550 µm (n=3). Approximately half of the studies (n=26) provided the average sampled volume, which ranged from 5 L (Di Mauro et al., 2017) to 614 m^3 (de Lucia et al., 2014). Most of the selected studies (n=18) applied spectroscopic methods, i.e., FTIR or Raman, to verify at least a subset of visually pre-selected microplastics. A few of these studies (n=6), utilized FPA-based FTIR imaging or automated single-particle exploration coupled to µRaman to analyse small microplastics (11–500 µm), which makes the analysis independent of bias from visual pre-selection (Cabernard et al., 2018; Lorenz et al., 2019; Rist et al., 2020; Tekman et al., 2020; Kuddithamby et al., 2023; Liu et al., 2023).

Figure 62shows that, while not conclusively so, there is a tendency that studies using a smaller size limit for sampling and identification also detect more microplastic particles. This is most notable when comparing studies conducted in the same oceanographic region. For the central western Mediterranean, for example, Suaria et al. (2016), sampling with a 200 µm net and analysing microplastics down to 200 µm, found average

concentrations one order of magnitude higher than de Lucia et al. (2014), sampling with a 500 µm net and analysing microplastics down to 500 µm. A similar observation can be made from studies focusing on the English Channel and the North Sea. While Maes et al. (2017) sampled with a 333 µm net and reported an average microplastic concentration of 0.14 counts m^{-3}, not specifying the analysed size range, Lindeque et al. (2020), sampling with a net with the same mesh size, reported a concentration of 4 counts m^{-3} (11–5000 µm). In turn, when sampling with a 100 µm net, Lindeque et al. (2020) reported an average microplastic concentration of 10 counts m^{-3} (11–5000 µm), which is the same order of magnitude as reported Lorenz et al. (2019) (27 counts m^{-3}, 11–5000 µm). In general, very few studies on marine surface waters have included microplastics down to 11 µm in their analysis. Cabernard et al. (2018) analysed a subset of the samples analysed by Lorenz et al. (2019) with single-particle exploration µRaman and found, in the size range of 10–5000 µm, microplastic concentrations of 38–2621 counts m^{-3}. These were an order of magnitude higher than the ones reported by Lorenz et al. (2019) (5–245 counts m^{-3}). Cabernard et al. (2018) ascribed this difference to the analysis technique and identification approach, which might indicate that single-particle exploration µRaman had an advantage in automatic identification, especially of small-sized microplastics.

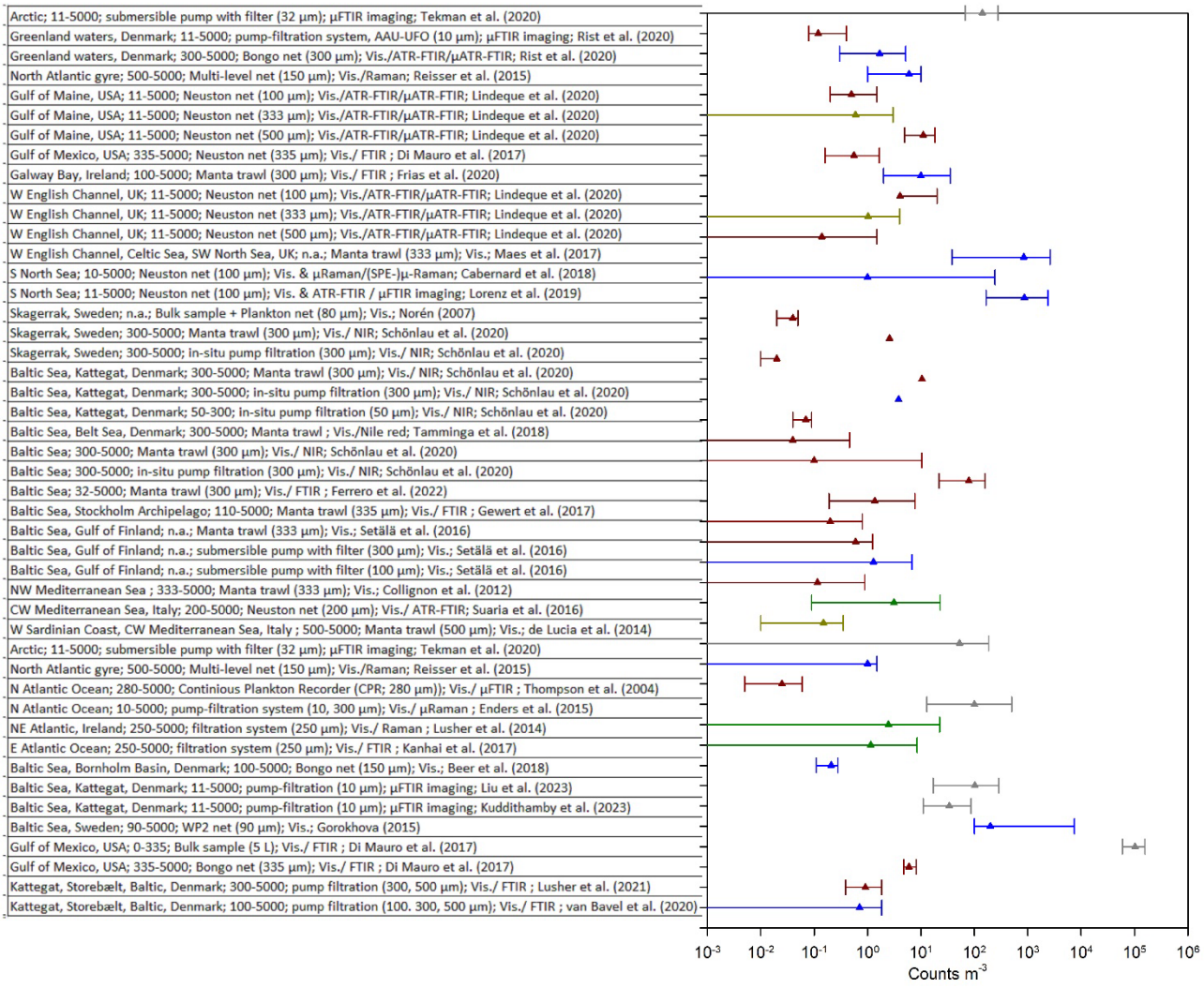


Figure 62. A selection of studies having analysed the concentration of microplastics in marine waters. The bars show the recorded range of microplastic concentrations, and the dots mark the mean concentrations in particles per m^3 (counts m^{-3}). The studies are grouped according to oceanographic region. The greyscale of the bars refers to the mesh size used from 10–50 µm (gray), over 50–150 µm (blue), 150–250 µm (green), 250–350 µm (red) to 450–550 µm (yellow). Note that the studies used quite different analytical methods, of which some are better than others in discerning small microplastics. Partly after Lorenz (2021).

Another study analysing microplastics in the size range 10–5000 µm and using Raman spectroscopy and presented in Figure 62, was done by Enders et al. (2015). They found microplastic concentrations of 13–501 counts m^{-3} with the highest concentration recorded in the English Channel and the second highest in the North Sea close to the English Channel (approx. 400 counts m^{-3}). This is in the same order of magnitude as the highest concentration (245 counts m^{-3}) recorded in the study of Lorenz et al. (2019), detected at a station near the English Channel as well. The slightly higher concentration might be explained by the sampling setup since Enders et al. (2015) used a pumped system with 10 µm filters and Lorenz et al. (2019) used a 100 µm mesh Neuston net and analysed down to 11 µm in size, hence microplastic concentrations in the size range 11–100 µm (on average 26.4 ± 53 counts m^{-3}) should be considered as semi-quantitative and are likely higher than reported.

Another study (Tekman et al., 2020) used a pumped system as well with 32 µm filters and recorded microplastics in size range of 11–5000 µm in Arctic surface waters, showing that the lowest microplastic concentration (113–262 counts m^{-3}) in these waters was in the same order of magnitude as the highest in the southern North Sea (245 counts m^{-3}, Lorenz et al., 2019) and that the highest (1287 counts m^{-3}) was even one order of magnitude higher. The discrepancy might for one be explained again with the smaller mesh size of the sampling equipment or, as indicated by Tekman et al. (2020), by the Arctic Ocean being an accumulation zone for microplastic pollution.

Another issue for inter-study comparison is the reporting unit. Some studies provide concentrations in microplastic counts per m^3, other provide abundances in microplastic counts per km^2 and some do both. This highlights the importance of acquiring high-resolution data, e.g., the sampled water volume and surface area that allow for the conversion of microplastic counts per m^3 to microplastic counts per km^2 and vice versa, to facilitate comparison between different studies. A further issue relates to measuring plastic items as counts, as one 'big' microplastic particle will count as much as one 'small' particle. While there are good reasons to quantify microplastics by how many items there is in an environment, this unit is less suited for other purposes, for example when comparing to sources of microplastics. If it for example is known that a certain source contributes X tons of plastic per year into the marine environment, measuring counts in the environment cannot readily be linked back to that source contribution as there is no simple conversion factor between microplastics counts and microplastic mass.

6.2 Experimental comparison of sampling techniques

Comparing data from sampling with nets which followed the latest international recommendations (AMAP, 2021; HELCOM BLUES, 2022; JRC, 2022) showed significantly less variability compared to the variability depicted in Figure 62(Section 6.2.1).

Comparing sampling applying nets versus sampling by pumped filtration has only been done by very few studies. In sections 4.3.2.1 and 4.3.2.2, two examples of datasets are given which illustrate the difference when sampling the same water bodies in parallel.

6.2.1 Case study: Coastal waters around Sjælland

A study was carried out in 2022 for establishing a monitoring strategy to assess the abundance of floating microlitter in the surface layer of Danish coastal marine waters. The study applied the latest international recommendations (AMAP, 2021; HELCOM BLUES, 2022; JRC, 2022) for routine sampling, analysis, and data reporting of MPs (Simon et al., 2023).

Microlitter > 300 µm was collected at seven coastal locations around Sjælland, Denmark, using a manta trawl. The collected microlitter particles were sieved into size fractions > 5 mm, 1–5 mm and 0.3–1 mm and first assessed visually to hand-pick microplastic-like particles and fibres. The number and concentrations of identified microlitter at each sampling station are listed in . The median microplastic concentration was 0.057 particles m^{-3}, and a maximum concentration of 0.213 particles m^{-3}, indicating low contamination levels of the investigated marine surface waters. This level is also comparable with findings in other published studies from the Baltic Sea (Tamminga et al., 2018; Scönlau et al., 2020) and by Lorenz et al. (in preparation) as shown in .

Spectroscopic characterisation of a subset of selected MP-like particles and fibres by FTIR-ATR technique followed the visual assessment for validation. The plastic origin of 90% of the particles subjected to FTIR-ATR was confirmed. The rest of the MP-like particles and fibres were identified as cellulose, protein, or their material could not be determined based on their collected IR spectra. Most MPs were of PE (65%), including PE-acrylic acid co-polymer, while the second most abundant polymer type was PP (24%).

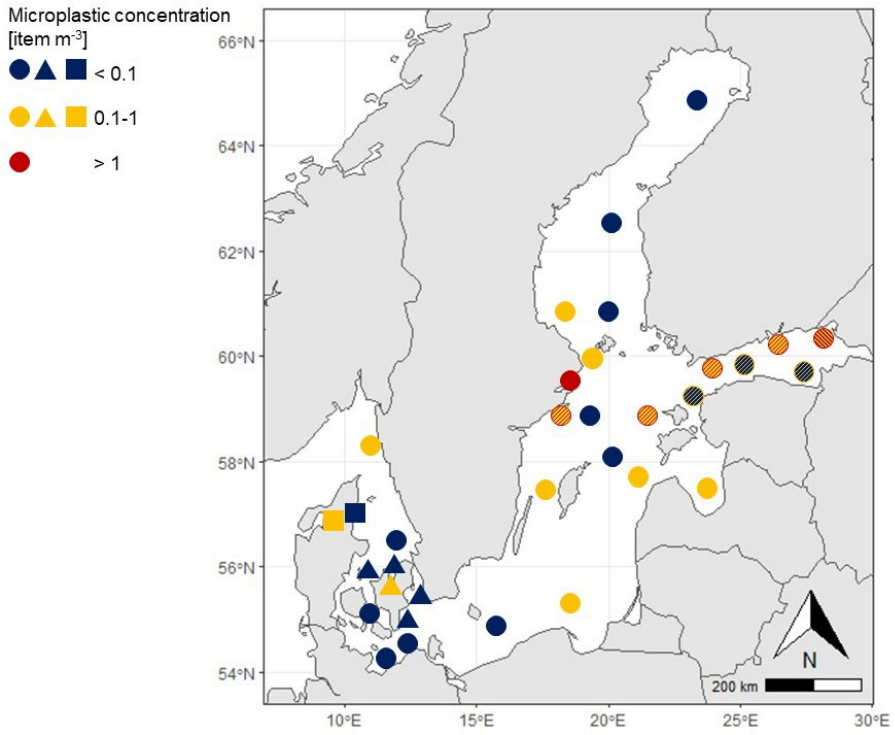


Figure 63 . Microplastic concentrations in the Baltic Sea by manta trawl surveys in different scientific studies in 2015-2022 (reviewed) marked with circles and compared to recent data from Danish coastal waters marked with triangles and squares (Simon et al., 2023; Lorenz et al., in preparation). The red-yellow and blue-yellow striped points indicate that concentrations both below and above 1 and below and above 0.1 item m^{-3} were measured, respectively.

Similar to other internationally published studies, the present study finds that inter-sample variability can be high. Thus, sampling and analysing at least 2-3 replicate samples from each site should be implemented more widely in monitoring frameworks. Additionally, more than one sampling event per year per site should be considered. It was also concluded that a minimum sampling volume of 100 m^3 of surface water is required to collect a representative sample in line with recommendations in the international monitoring guidelines for manta trawl surveys.

Table 4. The concentration of visually identified microplastic fibres and particles and the calculated sampled volume of each analysed sample.

Sample	Sample volume [m^3]	Concentration [item m^{-3}]		
		Fibres	Particles	Total
Køge Bugt, Brøndby st T1	193	0.047	0.010	0.057
Køge Bugt, Brøndby st T2	173	0	0.006	0.006
Sejerøbugten, Gudmindrup	222	0.036	0	0.036
Køge Bugt, Kofoeds enge T1	183	0.005	0.005	0.011
Køge Bugt, Kofoeds enge T2	185	0.005	0.07	0.076
Østfalster, Pomlenakke	172	0.035	0.017	0.052
Roskilde Bredning, Risø	184	0.011	0.005	0.016
Roskilde Vig East I T1	184	0.027	0.016	0.043
Roskilde Vig East I T2	126	0.095	0.095	0.191
Roskilde Vig West I T3	162	0.056	0.037	0.093
Roskilde Vig West I T1	135	0.081	0.030	0.111
Roskilde Vig East II T1	149	0.047	0.134	0.181
Roskilde Vig East II T2	85	0.047	0.166	0.213
Roskilde Vig West II T1	140	0.057	0.021	0.078
Roskilde Vig West II T2	130	0.031	0	0.031

6.2.2 Case study: Greenland

Sampling was done with horizontal tows of a bongo net just below the surface (300 µm) and the AAU-UFO pump-filtration system (10 µm stainless steel mesh) at 5 m water depth in the Nuup Kangerlua fjord in Greenland (Rist et al., 2020). The results are presented in , showing approx. a factor 1000 difference in mean concentrations. There seemed to be no clear correlation between concentrations obtained by the two techniques (Table 5). If any, there might be a weak positive correlation between counts obtained by the two techniques. However, using one to estimate the other seems problematic.

Table 5. Results from a case study conducted in the Nuup fjord in Greenland (Rist et al., 2020). Sampling was conducted with the AAU UFO pump filtration system (10-300 µm) and in parallel with a bongo net (mesh size 300 µm)

Transect	AAU-UFO pump (Microplastics 11-500 µm) Microplastic [counts m^{-3}]	Bongo-net (Microplastics 300-5000 µm) Microplastics [counts m^{-3}]
ST.1	107	0
ST.2	91	0.12
ST.3	68	0.11
ST.4	278	0.08
ST.5	241	0.4
ST.6	178	0.23
Mean	160.5	0.157

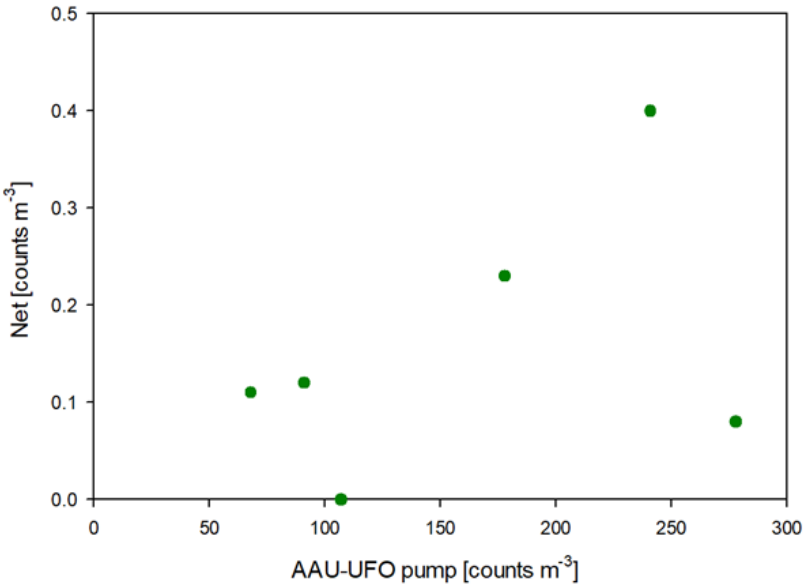


Figure 64. Comparison of concentrations found by samples collected by AAU-UFO pump system and Bongo-net, Greenland

6.2.3 Case study: Limfjord

A second study applying a Manta net for surface water sampling and the same pumped filtration system as in the Greenland study (Rist et al., 2020) showed similar large differences in concentrations (Table 6). The difference in mean concentration between the two sampling techniques was more than three orders of magnitude. There seemed to be no clear correlation between concentrations obtained by the two techniques (Figure 65). If any, there might be a weak negative correlation between counts obtained by the two techniques. However, using one to estimate the other seems problematic.

Table 6. Preliminary results from a case study in the Limfjord in August 2020 (Lorenz et al., in prep). Sampling was conducted with the AAU UFO pump filtration system (10-300 µm) and in parallel with a Manta net (mesh size 300 µm).

	AAU-UFO pump (Microplastics 11-500 µm)		Bongo-net (Microplastics 300-5000 µm)	
Transect	Microplastics [counts m^{-3}]	Sampled volume [m^3]	Microplastics [counts m^{-3}]	Sampled volume [m^3]
1	143	0.77	n.d.	134.86
2	201	1.20	0.21	237.00
3	68	0.63	0.14	140.00
4	54	1.00	0.16	243.00
5	107	0.71	0.32	96.00
6	1720	1.00	n.d.	n.d.
7	1188	1.01	0.06	94.00
8	600	0.90	0.04	336.00
Mean	510	0.90	0.155	182.98

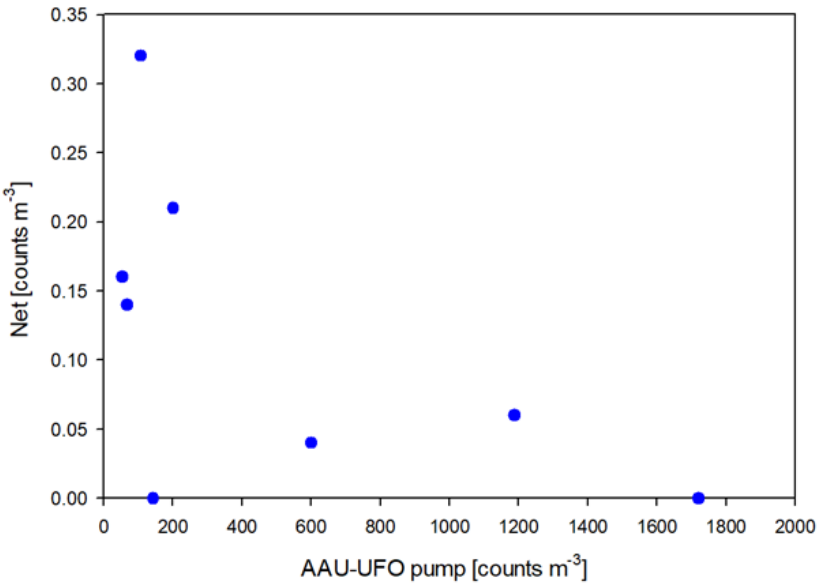


Figure 65. Comparison of concentrations found by samples collected by AAU-UFO pump system and Manta net, Limfjord

6.2.4 Other studies comparing pump and net sampling

A few other studies have looked into the comparison of pump water samples and net samples. Tamminga et al. (2019) investigated the representativeness of pump water samples versus manta sampling in freshwater environments and found that for sample volumes of 3 m^3 and considering only microplastics >300 µm, pump samples showed generally higher microplastics concentrations than net samples. Similar results were found by Schönlau et al. (2020) for Baltic Sea surface waters, comparing Manta net and pumped filtration for microplastics >300 µm. A study by Setälä et al. (2016), comparing samples from a manta net (333 µm) and a submerged pump (300 µm), found higher microplastic concentrations for pumped samples but the difference was not significant.

6.3 Discussion

The comparison of different sampling techniques shows that the variability in results is large, some of it being due to the sampling and some of it being due to the subsequent analysis. Most, if not all, of the studies were done for other purposes than marine monitoring, and opportunistically using them as input to marine monitoring must hence be done with care.

Following the latest recommended standards reduces the variability significantly. It can in this case not be excluded that the observed variability is due to differences in actual concentrations, and only to a lesser degree due uncertainties caused by sampling and analysis. There is though no solid proof hereof.

Comparing sampling with nets to sampling using pumped filtration show that these methods yield quite different results which are not readily comparable. Which technique to use should hence be governed by the target of the study. Which approach is the better, or even the least costly, in terms of monitoring spatial and temporal variations in microplastics content in the marine environment cannot be evaluated based on the information available.

7 References

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8 Appendix A

Table 7. Detailed microplastic concentration and recovery for ARH170016. RSD stands for relative standard deviation (%)

ARH170016	Wet weight (g)	Water content (%)	Organic matter content (%)	Concentrated volume (mL)	Aliquots for analysis	Aliquot volume (mL)	Microplastic counts found in the scans [counts]	Microplastic mass found in the scans [µg]	Microplastic number concentration [counts/kg]	Microplastic mass concentration [µg/kg]	Recovery
Sub_1	96.15	78.19	12.58	5	1	0.2	8	2.35	2080	611	0.53
					2	0.2	13	0.35	3380	91	0.66
					3	0.2	20	4.58	5200	1190	0.69
					average	0.2	14	2.43	3553	631	0.63
					RSD between scans [%]				44	87	
Sub_2	85.52	77.41	12.29	5	1	0.3	36	3.02	7016	589	0.44
					2	0.3	41	2.66	7990	519	0.27
					3	0.3	40	1.72	7795	335	0.33
					average	0.3	39	2.47	7600	481	0.35
					RSD between scans [%]				7	27	
Sub_3	100.03	78.34	12.86	5	1	0.2	44	5.86	10997	1465	0.38
					2	0.2	24	2.45	5998	613	0.34
					3	0.2	38	6.40	9497	1598	0.38
					average	0.2	35	4.90	8831	1225	0.37
					RSD between scans [%]				29	44	
Sub_4	93.53	77.75	13.29	5	1	0.2	47	8.10	12563	2164	0.66
					2	0.2	54	12.67	14434	3387	0.72
					3	0.2	40	1.96	10692	524	0.63
					average	0.2	47	7.58	12563	2025	0.67
					RSD between scans [%]				15	71	

Sub_5	99.46	78.34	12.40	5	1	0.2	43	3.57	10808	897	0.78
					2	0.2	64	8.23	16087	2069	0.53
					3	0.2	29	11.75	7289	2953	0.75
					average	0.2	45	7.85	11395	1973	0.69
					RSD between scans [%]				39	52	
Sub_6	103.08	77.77	12.64	5	1	0.2	57	5.01	13824	1216	0.69
					2	0.2	57	6.08	13824	1474	0.72
					3	0.2	52	3.70	12612	896	0.88
					average	0.2	55	4.93	13420	1195	0.76
					RSD between scans [%]				5	25	
					Average RSD between scans [%]				23	51	
					RSD between subsamples [%]				38	52	

Table 8. Detailed microplastic concentration and recovery for FYNNord. RSD stands for relative standard deviation (%)

FYNNord	Wet weight (g)	Water content (%)	Organic matter content (%)	Concentrated volume (mL)	Aliquots for analysis	Aliquot volume (mL)	Microplastic counts found in the scans [counts]	Microplastic mass found in the scans [µg]	Microplastic number concentration [counts/kg]	Microplastic mass concentration [µg/kg]	Recovery
Sub_1	134.84	68.35	8.53	5	1	0.3	-	-	-	-	0.83
					2	0.3	30	2.51	3708	311	0.75
					3	0.3	53	11.91	6551	1473	0.71
					average	0.3	42	7.21	5130	892	0.76

					RSD between scans [%]				39	92	
Sub_2	137.31	68.53	8.74	5	1	0.3	19	3.28	2306	398	0.40
					2	0.3	22	0.69	2670	84	0.60
					3	0.3	21	3.06	2549	371	0.40
					average	0.3	21	2.34	2508	284	0.47
					RSD between scans [%]				7	61	
Sub_3	134.42	68.16	8.87	5	1	0.2	10	0.18	1860	34	0.56
					2	0.2	9	2.27	1674	422	0.34
					3	0.2	12	0.74	2232	137	0.66
					average	0.2	10	1.06	1922	198	0.52
					RSD between scans [%]				15	102	
Sub_4	135.82	68.54	8.85	5	1	0.2	17	0.89	3129	164	0.59
					2	0.2	18	1.71	3313	314	0.91
					3	0.2	24	2.22	4418	409	0.63
					average	0.2	20	1.61	3620	296	0.71
					RSD between scans [%]				19	42	
Sub_5	148.09	68.09	8.95	5	1	0.2	18	2.69	3039	454	0.69
					2	0.2	23	1.18	3883	199	0.63
					3	0.2	30	2.52	5064	426	0.53
					average	0.2	24	2.13	3995	360	0.62
					RSD between scans [%]				25	39	
Sub_6	132.19	68.61	9.00	5	1	0.2	10	0.55	1891	105	0.59
					2	0.2	23	1.13	4350	214	0.53
					3	0.2	14	1.30	2648	246	0.66
					average	0.2	16	0.99	2963	188	0.59
					RSD between scans [%]				42	39	

					Average RSD between scans [%]				25	63	
					RSD between subsamples [%]				34	71	

Table 9. *Detailed microplastic concentration and recovery for FYNLunkebugt4. RSD stands for relative standard deviation (%)*

FYNLunke-bugt4	Wet weight (g)	Water content (%)	Organic matter content (%)	Concentrated volume (mL)	Aliquots for analysis	Aliquot volume (mL)	Microplastic counts found in the scans [counts]	Microplastic mass found in the scans [µg]	Microplastic number concentration [counts/kg]	Microplastic mass concentration [µg/kg]	Recovery
Sub_1	205.10	54.18	1.84	5	1	0.2	37	37.31	4510	4548	0.59
					2	0.2	30	175.27	3657	21364	0.69
					3	0.2	57	5.76	6948	702	0.53
					average	0.2	41	72.78	5038	8871	0.60
					RSD between scans [%]				34	124	
Sub_2	212.55	45.99	2.16	5	1	0.2	45	3.56	5293	418	0.88
					2	0.2	33	4.29	3881	505	0.50
					3	0.2	34	5.23	3999	615	0.50
					4	0.2	46	3.97	5410	467	0.63
					5	0.2	30	15.31	3529	1801	0.81
					average	0.2	38	6.5	4422.4	761.2	0.7
					RSD between scans [%]				20	77	
Sub_3	202.62	54.13	1.87	5	1	0.2	33	1.37	4072	170	0.47
					2	0.2	26	2.89	3208	357	0.47
					3	0.2	31	2.24	3825	277	0.44
					average	0.2	30	2.17	3702	268	0.46

					RSD between scans [%]				12	35	
Sub_4	232.11	53.29	1.87	5	1	0.2	18	0.96	1939	103	0.41
					2	0.2	23	0.63	2477	68	0.53
					3	0.2	13	0.20	1400	21	0.41
					average	0.2	18	0.60	1939	64	0.45
					RSD between scans [%]				28	64	
Sub_5	213.55	53.84	2.16	5	1	0.3	25	5.47	1951	427	0.29
					2	0.3	11	0.95	859	74	0.38
					3	0.3	21	4.08	1639	318	0.27
					average	0.3	19	3.50	1483	273	0.31
					RSD between scans [%]				38	66	
Sub_6	176.66	53.39	1.87	5	1	0.2	20	2.13	2830	301	0.53
					2	0.2	12	3.67	1698	519	0.47
					3	0.2	24	14.53	3396	2056	0.66
					average	0.2	19	6.78	2641	959	0.55
					RSD between scans [%]				33	100	
					Average RSD between scans [%]				27	78	
					RSD between subsamples [%]				44	185	

Table 10. *Detailed microplastic concentration and recovery for ARH170006. RSD stands for relative standard deviation (%)*

ARH170006	Wet weight (g)	Water content (%)	Organic matter content (%)	Concentrated volume (mL)	Aliquot for analysis	Aliquot volume (mL)	Microplastic counts found in the scans [counts]	Microplastic mass found in the scans [µg]	Microplastic number concentration [item/kg]	Microplastic mass concentration [µg/kg]	Recovery
Sub_1	113.39	72.68	2.53	5	1	0.1	19	1.11	8378	487	1.00
					2	0.1	19	0.37	8378	163	1.00
					3	0.1	19	2.87	8378	1266	0.94
					4	0.1	39	0.85	17197	375	0.94
					5	0.1	16	1.59	7055	699	0.88
					average	0.1	22	1.4	9877	598	1.0
					RSD between scans [%]				42	70	
Sub_2	125.58	72.41	2.46	5	1	0.1	25	3.49	9954	1388	1.00
					2	0.1	28	2.08	11148	828	0.88
					3	0.1	24	3.10	9556	1235	0.69
					4	0.1	19	0.36	7565	141	1.00
					5	0.1	36	5.52	14333	2200	1.06
					average	0.1	26	2.9	10511	1158	0.9
					RSD between scans [%]				24	65	
Sub_3	117.74	72.79	2.55	5	1	0.1	19	8.62	8069	3660	0.88
					2	0.1	23	1.60	9767	678	0.94
					3	0.1	20	3.73	8493	1583	0.94
					4	0.1	11	0.55	4671	233	0.81
					5	0.1	12	1.33	5096	565	0.94
					average	0.1	17	3.2	7219	1344	0.9
					RSD between scans [%]				31	103	
Sub_4	120.8	72.33	2.36	5	1	0.1	20	3.80	8278	1574	1.00
					2	0.1	17	2.40	7036	994	1.06
					3	0.1	27	0.94	11175	390	1.00
					4	0.1	36	5.67	14901	2347	1.00
					5	0.1	28	1.83	11589	756	0.88
					average	0.1	26	2.9	10596	1212	1.0

					RSD between scans [%]				29	63	
Sub_5	118.58	72.88	2.50	5	1	0.1	35	4.26	14758	1798	1.00
					2	0.1	26	4.27	10963	1802	0.88
					3	0.1	22	3.21	9276	1353	0.75
					4	0.1	41	2.12	17288	895	0.75
					5	0.1	24	3.78	10120	1595	0.94
					average	0.1	30	3.5	12481	1489	0.9
					RSD between scans [%]				27	26	
Sub_6	115.4	72.48	2.33	5	1	0.1	45	3.91	19497	1693	0.88
					2	0.1	16	1.23	6932	531	1.00
					3	0.1	26	2.18	11265	944	1.06
					4	0.1	14	1.16	6066	502	0.88
					5	0.1	27	2.63	11698	1141	0.94
					average	0.1	26	2.2	11092	962	1.0
					RSD between scans [%]				48	51	
					Average RSD between scans [%]				33	63	
					RSD between subsamples [%]				17	28	

Table 11. *Microplastic counts and mass found in each station, grouped by polymer type*

	ARH170006	**ARH170016**	**FYNNord**	**FYNLunkebugt4**

Polymers	Particle counts found in the scans [counts]	Particle mass found in the scans [µg]	Particle counts found in the scans [counts]	Particle mass found in the scans [µg]	Particle counts found in the scans [counts]	Particle mass found in the scans [µg]	Particle counts found in the scans [counts]	Particle mass found in the scans [µg]
ABS	2	0.13	1	0.03	3	0.07	1	0.07
Acrylic	13	0.44	11	0.64	6	0.35	9	0.31
Alkyd	7	0.73	6	0.60	8	0.61	13	2.26
Cellulose_ester	7	0.08	12	0.66	4	0.05	10	0.09
Epoxy_Phenoxy resin	21	0.93	8	0.30	2	0.06	13	0.26
EVA	0	0.00	1	0.01	0	0.00	0	0.00
PA	11	0.78	15	0.71	15	1.16	13	0.69
Paint	21	1.82	16	1.80	18	2.91	26	7.37
PAN_Acrylic fibre	36	4.12	48	10.59	25	3.28	59	17.25
PE	33	1.84	62	3.70	38	6.63	29	32.37
Poly(vinylpyrrolidone_co_vinyl acetate)	0	0	1	0	0	0	0	0
Polyester	213	12.40	293	30.35	111	9.93	247	186.04
PP	230	27.60	109	17.17	65	6.49	72	20.91
PS	69	17.34	66	17.78	38	6.67	45	19.72
PU	20	1.09	25	1.62	7	0.17	20	1.50
PVC	50	11.26	32	4.39	13	0.47	12	0.99
Vinyl chloride copolymer	0	0	1	0.10	0	0	0	0
SUM	733	80.55	707	90.45	353	38.84	569	289.82

Table 12. One way ANOVA test on microplastic concentration between subsamples, both by counts and mass. If $p < 0.05$, there is significant difference between the subsamples

	P value of the test on microplastic number concentration [item/kg]	P value of the test on microplastic mass concentration [µg/kg]
ARH170006	0.352	0.554
ARH170016	< 0.05	0.154
FYNLunkebugt4	< 0.05	0.131
FYNNord	< 0.05	0.184