Project 18/UP6/02/Div

Micro and macro plastic litter at Belgian fisheries areas: sources, distribution and consequences

Micro-en macro-plastic afval in de Belgische visserijgebieden: bronnen, verspreiding en gevolgen

Final report

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Content

Nederlandse samenvatting

Tot 80% van alle afval in onze oceanen bestaat uit plastic (Gesamp, 2019). Naar schatting 4.8 tot 12.7 miljoen ton plastic komt jaarlijks in het marien milieu (Jambeck et al., 2015). Dit afval kan onderverdeeld worden volgens grootte. In een algemeen aanvaardde definitie is macro-afval groter dan 25 mm, gevolgd door meso-afval (5-25 mm), micro-afval (1µm – 5 mm) en nano-afval (<1 µm) (Gesamp, 2019). Marien afval kan nadelige effecten hebben op het mariene ecosysteem en de gezondheid van de mens: zeedieren kunnen afval inslikken of kunnen verstrikt geraken in afval wat kan leiden tot kwetsuren of sterfte. Afval kan de zeebodem bedekken of beschadigen, wat nadelig is voor benthische organismen, maar het kan eveneens diverse geochemische processen beïnvloeden. Daarnaast kunnen macro-, micro- en nanoplastics een vector zijn voor chemische contaminanten, invasieve soorten en potentieel pathogene micro-organismen (OSPAR, 2017b).

De afvalproblematiek is ook heel belangrijk voor de visserijsector. Visserij kan een bron zijn van afval in het mariene milieu, vb. door verlies van netten op de zeebodem of slijtage van spekkingmateriaal. Anderzijds ondervindt de visserijsector direct schade van afval in het mariene milieu: netten kunnen scheuren aan grotere stukken afval op de zeebodem; vissen of andere commercieel gevangen organismen kunnen kleinere microplastics opnemen.

Door de stijgende plasticproductie (Hammer et al.,2012), de persistentie van plastics (Derraik, 2002) en de onduidelijkheid over de effecten van macro- en micro-afval, is het noodzakelijk om de monitoring in het Belgisch Deel van de Noordzee uit te werken. Binnen de Europese Kaderrichtlijn Mariene Strategie (2008/56/EG; 2017/848/EU; KRMS) worden dan ook 2 primaire doelstellingen naar voren geschoven die stellen dat de samenstelling, hoeveelheid en ruimtelijke spreiding van afval op een niveau dient te zijn dat er geen schade is aan het mariene milieu voor zowel (1) afval aan de kust, de bovenlaag van de waterkolom en op de zeebodem (D10C1); en (2) micro-afval aan de kustlijn, in de bovenlaag van de waterkolom en in het zeebodemsediment (D10C2).
Binnen het marine plastics project, gefinancierd door EFMZV, ambieerden ILVO en OD Natuur-KBIN om (1) de bronnen en verspreiding van macro-afval in de Belgische visserijgebieden in kaart te brengen, (2) microplasticconcentraties in vis en visserijproducten van Belgische visserijgebieden op te meten, (3) monitoring van microplastics in Belgische mariene wateren en sediment voor te bereiden door uitwerking van een methodologisch protocol en verzameling van een referentiedataset (4) een efficiënt databeheer op te zetten voor data betreffende marien afval.

Macro-afval

Macro-afval op de zeebodem kan bestudeerd worden door de hoeveelheid afval in een sleepnet te registreren. Registratie gebeurt aan de hand van internationaal geharmoniseerde protocollen (OSPAR, 2017a; JRC, 2013). Twee unieke datasets werden gebruikt om de verspreiding van afval op de zeebodem van het Belgisch Deel van de Noordzee (BDNZ) en de Belgische visserijgebieden te bestuderen: afval geregistreerd tijdens milieumonitoringscampagnes van het ILVO (2013-2019) en afval geregistreerd tijdens de internationale boomkorvisserijcampagnes (beam trawl surveys, BTS; 2011-2019). In de milieumonitoringcampagnes wordt gebruikt gemaakt van een fijnmazig net (20 mm in de kuil), waarbij visslepen op korte afstand van elkaar liggen en waarbij zowel binnen als buiten de 12 nautische mijlszone wordt gesleept. Dit leidt tot een relatief hoog aantal afvalitems in het net, gemiddeld 12,7 ± 17 items per ha in de 12-mijlszone. Binnen de BTS-campagnes is het gemiddeld aantal afvalitems in het net lager, gemiddeld 2,2 ± 2,8 items per ha, omdat de maaswijdte van het gebruikte net groter is (40 mm in de kuil) en omdat er verder van de kust wordt gesleept. De BTS omvat evenwel een groot geografisch gebied omdat data beschikbaar is van verschillende landen. Hierdoor kan afval

op de zeebodem vergeleken worden voor de Noordzee, het Engels kanaal, de Keltische Zee en de Ierse Zee.

Macro-afval op de zeebodem bestaat voornamelijk uit plastic. 88% van alle afval dat teruggevonden werd in de visslepen van de milieumonitoringscampagnes bestond uit plastic. Voor de BTS was dit 77%. Er waren evenwel grote verschillen in de verspreiding van de specifieke afvalitems. Zwaardere plastics met een bron op het land, zoals plastic flessen en containers, worden voornamelijk binnen de 12 nautische mijlszone teruggevonden. Monofilament touwen van lage densiteit vertonen daarentegen een meer egale verspreiding over het BDNZ. Binnen het gebied dat bestreken wordt door de BTS, wordt de hoogste densiteit aan monofilament touwen teruggevonden voor de kust van Nederland.

Verschillende factoren beïnvloeden de verspreiding van macro-afval op de zeebodem. Niet alleen de lokalisatie van de afvalbron is belangrijk, maar ook hydrodynamische en geomorfologische factoren zullen een rol spelen. Bijgevolg is het niet steeds eenduidig om verbanden te leggen tussen menselijke activiteiten en de verspreiding van afval op de zeebodem. Binnen het Marine plastics project werd geen verband gevonden met zandextractie of windmolenparken. Ook was er geen duidelijk verband tussen visserij-activiteiten en de verspreiding van visserijgerelateerd afval. Toch bleek visserij een belangrijke bron van afval, aangezien tot 52% van het afval buiten de 12 nautische mijlszone op het BDNZ visserij-gerelateerd was.

Op het BDNZ was er een verhoogde concentratie aan afval op de baggerloswal BR&WZO, gelegen nabij de haven van Zeebrugge. In deze zone werden gemiddeld 61 ± 79 afvalitems per ha teruggevonden, ten opzichte van 15 ± 15 afvalitems per ha in zones nabij de loswal en 12 ± 14 afvalitems in verder afgelegen referentiezones. Ook hier kan het effect van het storten van baggerslib echter niet eenduidig onderscheiden worden van het effect van hydrodynamische processen zoals sedimentatie. Door de hoge hoeveelheid afval op deze plaats, raden we een meer diepgaand onderzoek aan naar de bronnen en processen die de accumulatie van afval op deze plaats kunnen verklaren.

Microplastics

Microplastics werden opgemeten in vis en visserijproducten van Belgische visserijgebieden alsook in water en sediment van het BDNZ.

Microplastics in vis en visserijproducten werden opgemeten met een gevalideerde methode, die toelaat om gekleurde plastics >50 µm en kleurloze, doorzichtige plastics >200 µm te analyseren. In alle vissen werden lage aantallen microplastics opgemeten, doorgaans lager dan de kwantificatielimiet, in zowel de visfilet als in het maag-darmstelsel van de vis. In grijze garnaal en noordzeekrab werden geen kwantificeerbare hoeveelheden aan microplastics opgemeten. Deze resultaten duiden aan dat er geen bioaccumulatie is van microplastics in vis, grijze garnaal en noordzeekrab. De blootstelling aan microplastics >50 µm door de consument is bijgevolg beperkt.

Voor de opvolging van microplastic in zeewater en sediment werd een accurate en betrouwbare methodologie uitgewerkt waarbij conform internationale richtlijnen een ondergrens van 100 µm gehanteerd werd. Standaardisatie is echter nog niet bereikt. Een grondig literatuuronderzoek (waarbij ook rekening werd gehouden met projectrapporten zoals van het JPI Oceans project BASEMAN), verschillende testen en informatie-uitwisseling via o.a. expertgroepen vormden de basis van een methodologisch protocol.

Zeewater- en sedimentstalen werden genomen op frequent bemonsterde monitoringsstations gelegen op een kust-offshore transect. Zeewater bemonsterd dicht bij de kust bevat een hoger aantal microplastics dan verder water van verder gelegen locaties. De haven vertoont het hoogste aantal met

1475 deeltjes per m^3, gevolgd door de stations ZVL en MOW1 (300 ± 177 microplastic partikels per m^3). De meer offshore gelegen stations (W05 & W08) hebben aanzienlijk lagere concentraties (30 ± 14 microplastic partikels per m^3). Deze resultaten zijn gebaseerd op stalen die genomen zijn via het continue zeewatercircuit aan boord van de RV Belgica, behalve voor de haven, waar de hoeveelheid materiaal in suspensie te groot is en enkel Niskinflessen gebruikt werden. Bemonstering via het zeewatercircuit laat accurate volumemetingen toe en is praktisch te combineren binnen bestaande multidisciplinaire monitoringscruises. De herhaalde metingen bij MOW1 resulteerden in aantallen variërend van 30 tot 360 microplastic deeltjes per m^3. Deze hoge variabiliteit is te wijten aan het mobiele karakter van microplastics, dat sterk beïnvloed worden door meteorologische en hydrodynamische omstandigheden.

Sediment werd bemonsterd met de Reineck box corer. Het aantal microplasticdeeltjes in MOW1, dicht bij Zeebrugge, (182,3 ± 128,7 microplastic partikels per kg drooggewicht) is ongeveer 9 keer hoger dan in het offshore station W08 (20,8 ± 4,2 microplastic partikels per kg drooggewicht). De meeste in het sediment geïdentificeerde deeltjes (61%) werden ingedeeld in de kleinste grootteklasse (100-350µm). Een deel van de variatie in de hoeveelheid microplastics die is waargenomen tussen de stalen genomen op MOW1 lijkt samen te hangen met verschillen in sedimentsamenstelling, nl. de hoeveelheid sediment met korrelgrootte kleiner dan 63 µm en organisch materiaal. Deze eerste resultaten zijn in overeenstemming met eerdere studies (Maes et al. 2017; Wang et al. 2018) die aantoonden dat meer plastic deeltjes aanwezig zijn wanneer het organische stofgehalte hoger is en de gemiddelde korrelgrootte kleiner. Aangezien de korrelgrootte van de zeebodem wordt beïnvloed door hydrodynamische krachten, benadrukten Wang et al. (2018) het belang van hydrodynamische effecten in de distributie en migratie van microplastics in intergetijdengebieden. De analyseresultaten van de drie substalen genomen uit eenzelfde sedimentstaal zijn gelijkaardig en zijn een indicatie voor een betrouwbare reproduceerbaarheid van de methodologie.

Hoewel de monitoring van beide matrices, oppervlaktewater en sediment, vereist is volgens de KRMS-specificaties, moet worden opgemerkt dat de concentraties in zeewater lager en meer variabel zijn, waardoor grote volumes zeewater moeten worden bemonsterd (Maes, 2017). Aangezien microplastics de neiging hebben te accumuleren in sediment (Kukulka et al., 2012; Woodall et al., 2014), is de opvolging van concentraties in de zeebodem aanbevolen voor monitoring op lange termijn.

De analyseresultaten werden gedocumenteerd met al de nodige meta-informatie. Hierbij werd rekening gehouden met reeds bestaande, internationale gegevensformaten. Aangezien deze formaten voor micro-afval nog niet optimaal (vnl. gebaseerd op macro-afval) en/of in testfase zijn, werd in het kader van dit project een template uitgewerkt. Het BMDC (Belgian Marine Data Centre) zal deze afvaldata beheren en zorgen voor de rapportage in regionale en EU-context.

1. WP1. Impact of fisheries on total plastic contamination

1.1. Introduction

Marine litter has been recognised as a global environmental concern. Vast quantities of plastic litter enter the ocean (Jambeck et al., 2015; Galgani et al., 2021). Marine litter may cause negative effects to the marine ecosystem as animals may swallow litter or get entangled, which can lead to injuries and even death of individual organisms. Litter may scour or smother the seafloor, which may impact fragile benthic habitats, reduce photosynthesis and prevent the movement of animals, gases and nutrients. Marine litter may also act as a vector for invasive species, transporting non-indigenous organisms into new areas where they can outcompete or prey upon native organisms. (OSPAR, 2017b). Within the Marine Strategy Framework Directive (MSFD), a primary aim is that composition, amount and spatial distribution of litter on the coastline, in the surface layer of the water column and on the seafloor are at levels that do not cause harm to the coastal and marine environment (2008/56/EG; 2017/848/EU). Monitoring of seabed litter is therefore essential.

Multiple techniques are available to assess seabed litter, each with specific advantages and disadvantages. Remotely operated vehicles equipped with cameras can be applied to record seabed litter, divers may collect or film litter along predefined transects or litter can be collected with fish trawls. Macrolitter monitoring by trawling has the advantage of covering a large area. It is typically included in fisheries survey cruises, which offers the advantage that monitoring is cost efficient. However, as cruises are not dedicated to litter collection, sampling locations are selected based on importance for fish stock assessments rather than for litter assessment. Moreover, trawling is prohibited at rocky areas and canyons. A trawl does not collect all litter items on the seafloor. The catchability is linked to the net size but also to the type of fishing net, limiting comparability between areas were different fishing techniques are applied (ICES, 2021).

At the Belgian fisheries areas, marine litter sampled by trawling is already recorded since 2011, following international OSPAR and MSFD guidelines (OSPAR, 2017a; JRC, 2013). The ILVO environmental monitoring surveys in the Belgian Part of the North Sea (BPNS) and the Belgian Fisheries surveys offer a vast dataset on seafloor macrolitter which can even be enlarged with data of the United Kingdom, Germany and the Netherlands to complete the view on litter distribution in the North Sea, English Channel, Irish Sea and Celtic Sea. Within the Marine Plastics project, the aim was to compile available seafloor litter data gathered within monitoring campaigns and to assess the distribution of litter items, considering not only total litter, but also specific litter categories such as plastic, metal or rubber, or even sub-categories. Links between spatial distribution of litter items and human activities such as fisheries, dredge disposal, sand extraction and offshore wind farms (OWF) were investigated.

1.2. Methods

1.2.1. Marine litter data source

For the assessment of seafloor litter in Belgian fisheries areas, two independent data sets were used: seafloor litter data collected (1) within the ILVO environmental monitoring campaigns and (2) within the international beam trawl survey (BTS). The ILVO environmental monitoring campaigns cover the BPNS. Seafloor litter is collected within the net, using an 8 m beam trawl with a cod end mesh size of 20 mm (stretched). The length of a fish track is about 1 nautical mile. The BTS covers the North Sea,

English Channel, Celtic Sea and Irish Sea. Beam trawl lengths can vary from 4 to 8m. Nets have a cod end mesh size of 40 mm (stretched).

Data is collected according to OSPAR (2017a) and MSFD (JRC, 2013) guidelines. Seafloor litter data from environmental monitoring campaigns was collected between March 2013 and March 2019. A total of 467 hauls were included in the original dataset (Figure 1.1). One haul lost its catch and was excluded. Another 5 hauls had partially missing data when it came to quantifying the number of litter items. These were excluded when analysing the number of litter items per area, leading to 461 observations including 29 tracks with no litter (6%).

BTS marine litter data was retrieved on the 22nd of January 2021 from the DATRAS Beam Trawl Survey (BTS) database publicly available on the website of the International Council for the Exploration of the Sea, ICES (https://datras.ices.dk/Data_products/Download/Download_Data_public.aspx). It is based on litter data submissions and haul information from Belgium, Germany, the Netherlands and Great Britain. Data between 2011 and 2019 was included (Figure 1.1).

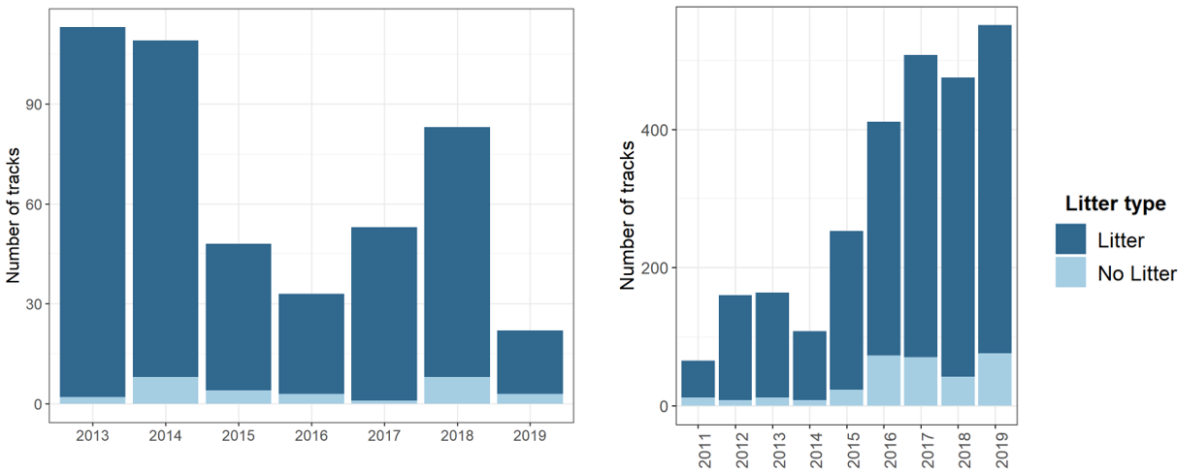


Figure 1.1: Total number of hauls with and without litter observations per year for (A) Environmental monitoring (N=461) and (B) BTS (N= 2695).

The number of items per haul were standardized by dividing by the swept surface area for each haul (length of the haul times the length of the beam). If no length was available, the distance between the shoot and haul coordinates of the track was calculated as follows:

Calculated Distance = Earth radius*acos(cos(as_radians(90- ShootLat)) *cos(as_radians(90-HaulLat)) + sin(as_radians(90- ShootLat)) * sin(as_radians(90-HaulLat)) * cos(as_radians(ShootLong-HaulLong))) *1000

1.2.2. BTS data cleaning

For the BTS survey data, on a total of 2800 hauls, 60 were labelled as invalid (HaulVal) in the original database. All invalid hauls were excluded. We understood that in the BTS database "LT-TOT" under the variable "PARAM" (LTREF = RECO-LT) denotes that no litter items were found in the haul (number of litter items ("LT_Items") equals 0). For 4 hauls, both "LT-TOT" and specific litter items were found in

the dataset. In this case, the record with “LT-TOT” was assumed to be a mistake and removed, while the haul was kept in the database.

In 41 hauls “LT-TOT” was mentioned while the number of litter items was missing (-9), making these hauls inconclusive. These hauls were all excluded in both analyses. As it is possible that mixing of the notation of zero hauls occurred (recording “-9” instead of “0”), this decision may have led to an underestimation of the amount of zero hauls. However, inclusion would lead to overestimation. One of the hauls labelled “-9” was labelled invalid and therefore already excluded from the analyses in the first step. As a result, another 40 hauls were excluded in this step.

Furthermore, 2 more hauls with at least one missing value (-9) for the number of items of any type of litter were excluded when considering the number of litter items, as these could not be used to calculate totals. We assumed that in this case no litter data were recorded, or data got lost.

When comparing lengths in the database with calculated distances using the formula above, 24 hauls had large deviations (at least 1.2 times larger or 0.8 times smaller). These tracks all belonged to the Netherlands’ data. In these few cases, it was decided to use the calculated distances instead of the lengths in the database.

Finally, 3 outliers with more than 100 items per haul were removed for the analysis of the litter items. This led to 2695 observations when considering the number of litter items, including 324 tracks with no litter (12%). To simplify matters the same observations were included in the presence/absence analysis.

Figure 1.2 shows the amount of tracks per country over the years. No tracks without litter were reported by Germany. This might indicate that hauls without litter were not reported or indicated as “-9” in data recording. The number of tracks without litter was lower in 2019 for the Netherlands compared to other years.

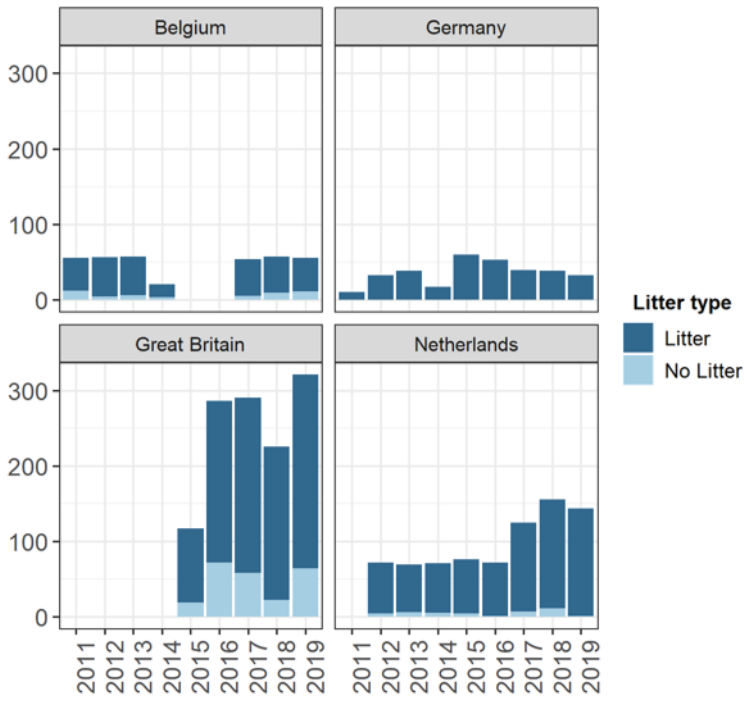


Figure 1.2 Number of BTS hauls (with and without litter observations) per country and per year (N = 2695).

1.2.3. Spatial visualization

The observations were pooled per spatial area, taking the midpoint of the track to allocate an observation to a grid cell. For environmental monitoring survey data of the BPNS, grid cells were 0.05 x 0.05 degrees, whereas grid cells of 0.5x0.5 degrees were applied for the BTS data. The number of hauls per grid cell are mapped in Figure 1.3. Figure 1.4 visualizes the haul locations of each data providing country.

The data were analyzed using two different approaches. For BTS and environmental monitoring data, all available detailed information was used to calculate the number of litter items per swept surface area and per litter type. For the BTS data, a second approach was also applied, calculating the risk of finding at least one item per haul using a dichotomous outcome on the presence or absence of litter in a haul.

Pooling the observations allocated to the same grid cell, the average number of litter items per 10,000m2 per grid cell was calculated and plotted on maps to be used as visual output of the analysis. For the presence/absence analysis, per grid cell, the number of hauls reporting litter was divided by the number of hauls observed, calculating the risk of finding litter in a haul. These risks were plotted on maps.

For environmental monitoring survey, 66 grid cells were covered. For the BTS survey, 256 grid cells were covered in the analysis of the number of litter items and in the presence/absence analysis. It was decided not to interpolate observations.

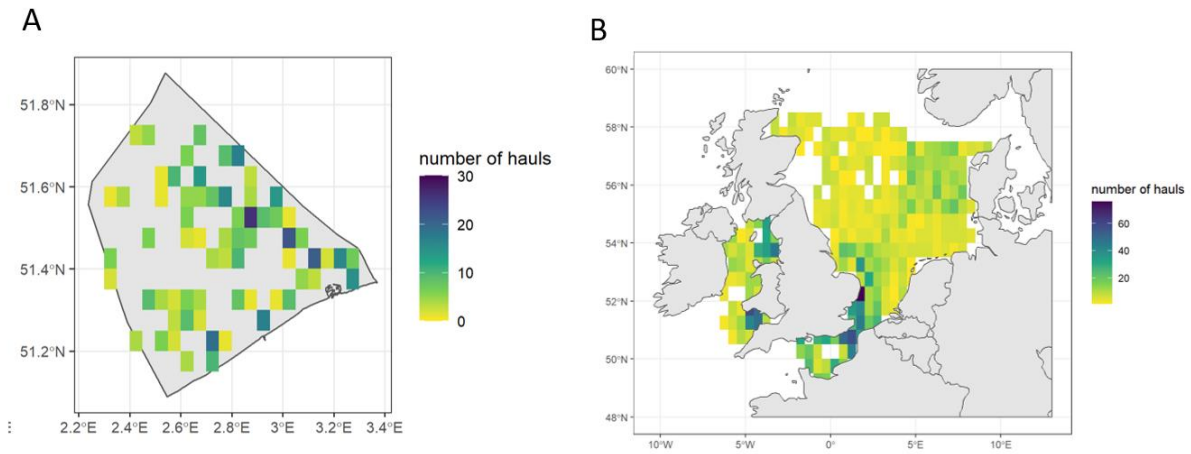


Figure 1.3: Spatial overview of the number of hauls per grid of all years combined for (A) Environmental monitoring (N=461) and (B) BTS (N=2695).

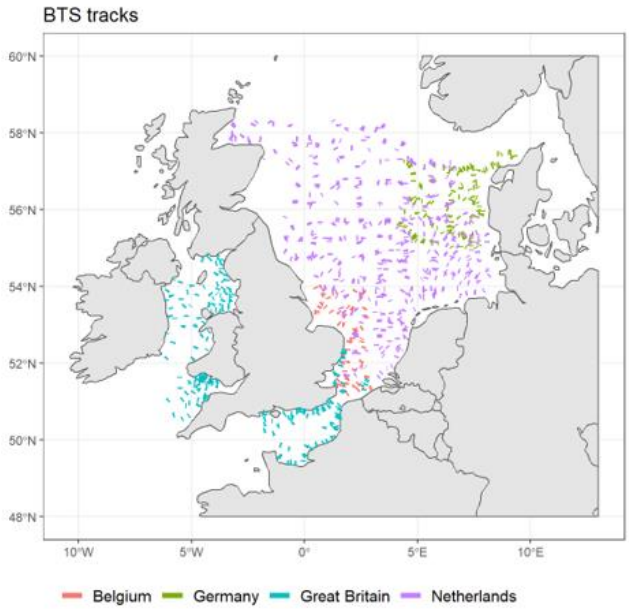


Figure 1.4: Spatial overview of the BTS hauls (observations) per country of all years combined (N=2695)

1.2.4. Litter categories and subcategories

Different litter categories and subcategories were explored, following the classification as described by OSPAR (2017a) and MSFD (JRC, 2013). Types include plastic (A), metals (B), rubber (C), glass and ceramics (D), natural Products (E) and miscellaneous objects (F). Within these different types of litter, 33 subtypes were identified within the environmental monitoring dataset and 40 subtypes were identified within the BTS dataset. A closer look was taken at the frequent occurrences. A complete list of subcategories can be found in annex 1.

A5, A6, A8, B3 and C3 were pooled to look at material coming from fishing activities. This is an indicative classification, as not all monofilaments (A5, A6) will results from fisheries activities. On the other hand, part of the synthetic ropes (A7) will also originate from fisheries activities.

1.2.5. Sediment type categorization

The midpoints of the fish tracks were linked to sediment information using the EMODnet-Geology substrate map on a scale of 1:250.000 (version October 2016) (Van Lancker, 2019). This data includes 5, 7 or 16 classes of the Folk classification. This classification makes distinctions based on the percentages of mud (< 63 µm), sand (63 µm – 2 mm) and gravel (fraction above 2mm).

Based on the Folk classification with 5 classes, 4 different types of substrate material were encountered (1. Mud to muddy Sand; 2. Sand; 3. Coarse substrate; 4. Mixed sediment). Since only 1 track location belonged to the fourth class, mixed sediment, this class was not included. The fifth class, bedrock and boulders, was not encountered. The North Sea in general is predominantly sandy and sand-rich areas are commonly close to a sediment source (e.g. a large river or a coastline marked by easily erodible sandstone) (Kaskela et al. 2019).

1.2.6. Fisheries activity data

Data on Bottom Fishing Intensity was retrieved from OSPAR special request to ICES (ICES 2018). Data for several years were pooled (2011-2017) and mapped (Figures 1.5-1.6), including all mobile bottom contacting gears (beam trawlers, dredge, demersal seine, otter trawl). ICES (2016) defines the swept area as the cumulative area contacted by a fishing gear within a grid cell over one year. The swept area ratio (SAR, also defined as fishing intensity) is the swept area divided by the surface area of the grid cell times 100. Two types of SAR are defined: surface (<2 cm penetration depth of the gear components) and subsurface (≥2 cm penetration depth of the gear components).

These fisheries data were allocated to a grid with cells of 0.05 x 0.05 degrees. As this is the size of the grid cells applied for mapping litter data of the environmental monitoring campaigns but smaller than used for the BTS survey litter data, the average number of litter items per 10,000m2 retrieved from the BTS surveys was also allocated to a 0.05 x 0.05 degrees grid cell. This made it possible to merge the fisheries and litter data and explore correlations between litter items and fishing hours, surface SAR and subsurface SAR. In total 962 grid cells were covered by both data sets (out of 1027 grid cells covered by BTS litter data).

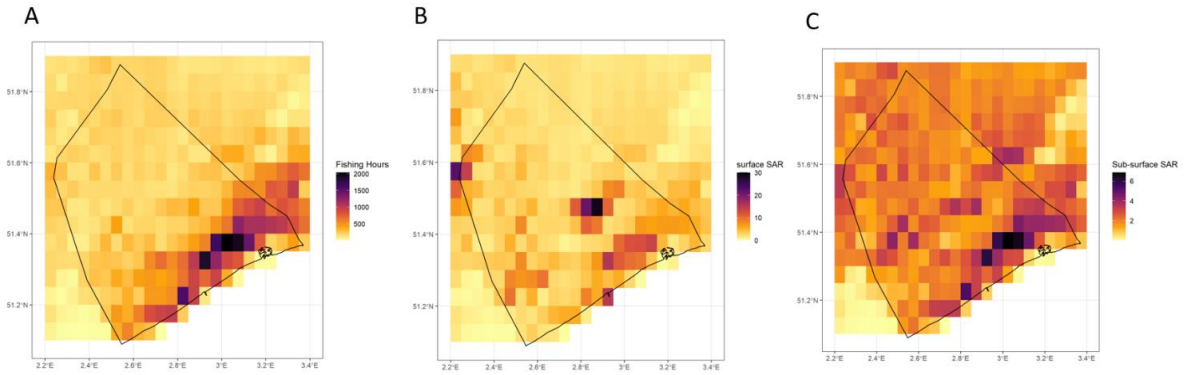


Figure 1.5: Spatial overview per grid cell of (a) the number of fishing hours, (b) the surface swept area ratio (SAR) and (c) subsurface SAR for the BPNS, data 2011-2017 retrieved from the OSPAR special request to ICES (ICES 2018).

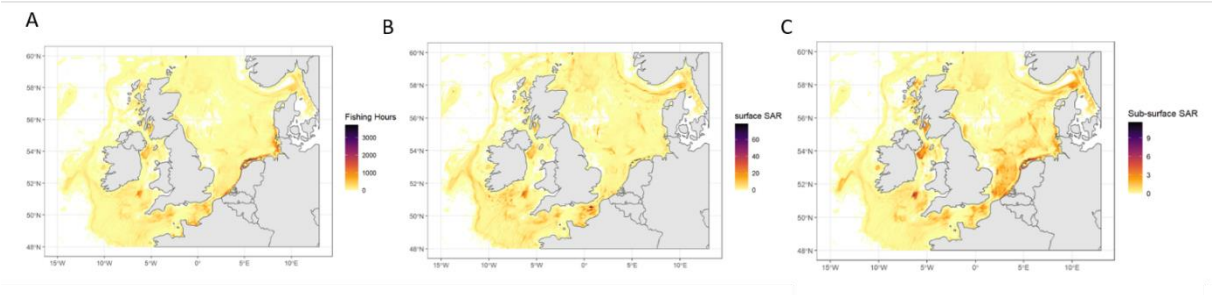


Figure 1.6: Spatial overview per grid cell of (a) the number of fishing hours, (b) the surface swept area ratio (SAR) and (c) subsurface SAR for the North-east Atlantic, data 2011-2017 retrieved from the OSPAR special request to ICES (ICES 2018).

1.2.7. Data from other human activities at sea

The fish tracks taken within the environmental monitoring campaigns are part of long-term monitoring on the effects of anthropogenic activities at sea such as dumping dredged material, OWF and sand extraction. As such, tracks made during these campaigns took place on reference sites ("reference"), near the occurrence of the activity ("nearby"/"low activity") and in the area where the activity took place (impact). The number of litter items per 10,000m2 was compared taking into account the exploitation locations. An overview of all tracks is provided in annex 2. For a more detailed geographical visualization, the midpoints of the tracks were plot (Figure 1.7). Geographical information on the marine spatial plan (MSP) for the Belgian part of the North Sea was retrieved in March and October 2020 from http://www.marineatlas.be/en/data. This data was used to visualize areas designated to the activities studied. Figure 1.8 gives an overview on the location of the 5 dredge disposal sites of the BPNS: LNP, BR&WOO, BR&WZE, BR&WS1 and BR&WS2.

Figure 1.7: Spatial overview of the midpoints of a haul according to the anthropogenic activities monitored between 2013 and 2019 (including where the activity itself occurs, near the occurrence of the activity and reference sites). (N=458) Note: some reference sites were used as a reference for several activities and may therefore be included more than once.

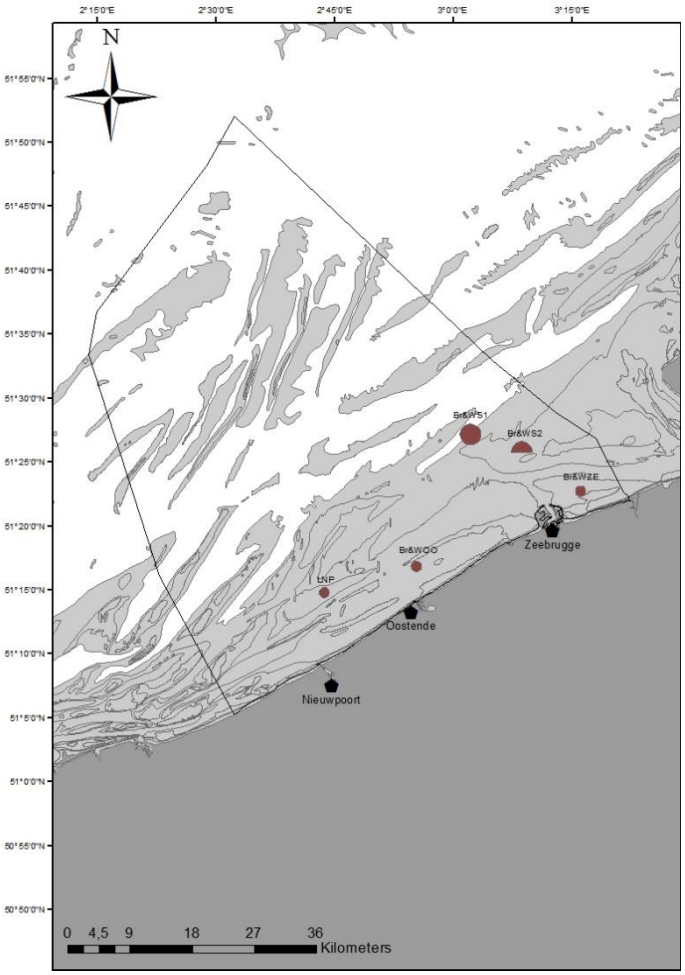


Figure 1.8: Spatial overview of the dredge disposal sites of the BPNS.

1.3. Results and Discussion

1.3.1. Marine litter in the BPNS

1.3.1.1. Temporal and spatial variation in total litter items

Within environmental monitoring campaigns in the BPNS, marine litter is monitored in a consistent way, applying a uniform way of litter categorization, since 2013. Figure 1.9 shows the litter distribution, combining all years litter data. Figure 1.10 shows the trend of litter items per ha, differentiating between the early march sampling campaign ("spring survey") and September-October sampling campaign ("fall survey").

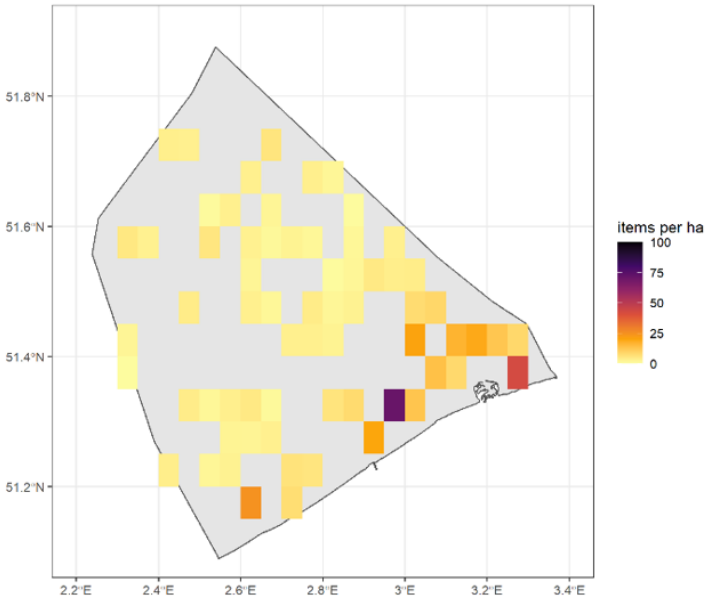


Figure 1.9: Average number of litter items per grid cell in the BPNS, pooled data of 2013 to 2019 (n=456).

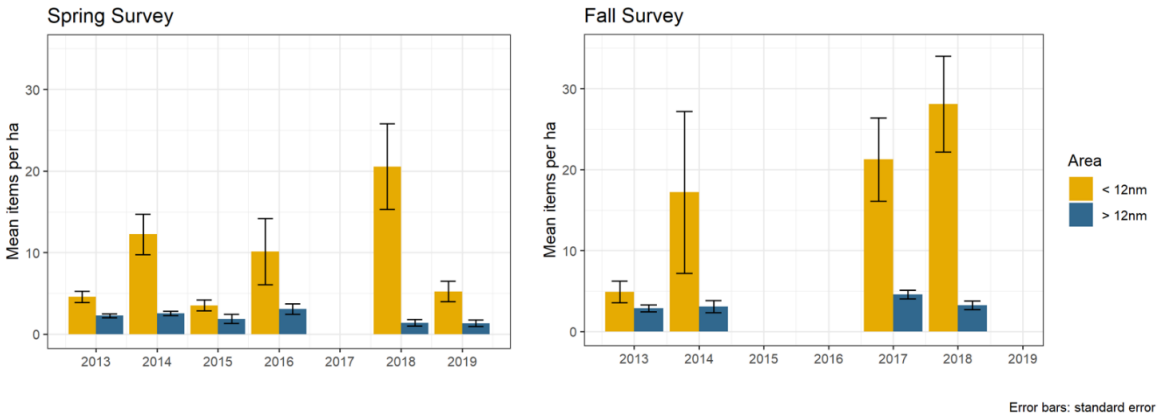


Figure 1.10: Average number of litter items within and beyond 12 nm of the Belgian coast, per year between 2013 and 2019 for spring survey and fall survey.

Largest number of litter items can be found at the coastal zone (Figures 1.9-1.10), with on average 12.7 ± 1.7 litter items per ha caught in the net of fish tracks taken within the 12 nautical miles (nm) zone compared to 2.8 ± 0.2 items per ha outside the 12 nm zone. This indicates an impact from land-based sources of marine litter or from marine activities within the 12 nm. However, current patterns and sedimentation may also play a role in the accumulation of litter. In the eastern part of the BPNS (Figure 1.9), the coastal area is a known sedimentation area (Fettweis et al., 2009). Sedimentation will increase when water velocities are low, resulting in fine sediments close to the coast. This will also affect litter settling at the coastal environment, with higher amounts of litter in mud to muddy sand regios (Figure 1.11).

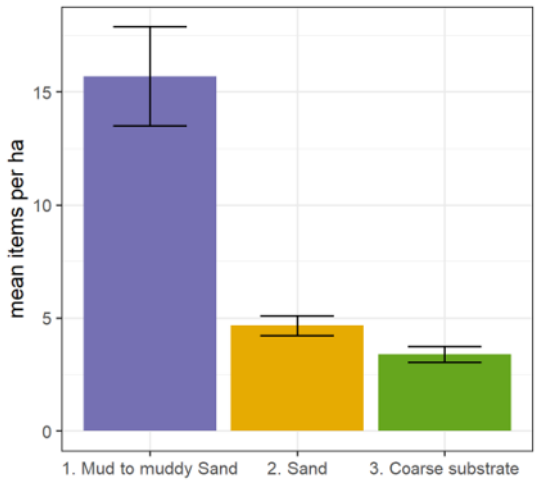


Figure 1.11: Average number of litter (± standard error) according to sediment type per substrate material based on the Folk pooled data, 2013-2019 (N=456). Error bars show the standard error of the average.

Analysing temporal trends in litter contamination is not straightforward (Figure 1.10), as average number of litter items strongly vary between years. In 2013, 2015 or 2019, yearly averages within the 12 nm zone were at maximum 5.3 ± 1.3 litter items/ha, while much higher yearly averages were noted for 2014, 2016, 2017 and 2018, with a maximum of 24.6 ± 4.0 litter items/ha within the 12 nm zone in 2018. Litter item counts in the net are highly variable (Maes et al., 2018; Kamman et al., 2018) and are affected not only by the amount of litter at a location, but also by other factors such as the type of net or the size of the fishery catch (Kamman et al., 2018). Moreover, numbers present annual averages over all fish tracks, but the selection of fish tracks will slightly vary between campaigns with a higher amount of tracks within fall campaigns (53 ± 5) compared to spring campaigns (41 ± 15), which impacts the effect of a single track to the average litter load.

1.3.1.2. Spatial variations in specific litter items

Of all litter items caught in the net within the environmental monitoring survey, 88% were plastic. The relative share of non-plastics is however different at coastal areas (<12nm) compared to offshore areas. In the BPNS, 13% of litter items in the net are non-plastics within the 12 nm zone compared to 8% outside this zone (Figure 1.12). The dominance of plastic in total marine litter contamination is coherent with other literature. Within the International Bottow Trawl Surveys (IBTS), recorded percentages of plastic items vary from 58% for the Celtic Sea, up to 68% for the Greater North Sea and 98% for the Eastern Bay of Biscay (Ospar, 2017b). On a larger scale, plastic is estimated to account for 80% of global marine litter items (UNEP, 2016).

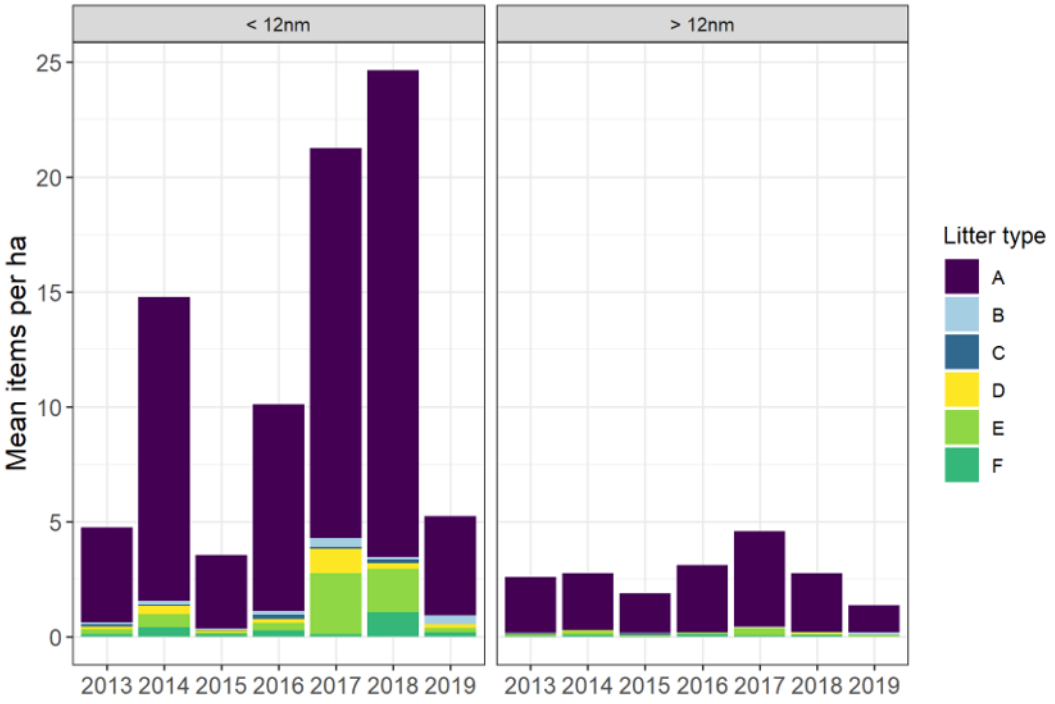


Figure 1.12: Average number of litter according to sediment type per substrate material based on the Folk pooled data, 2013-2019 (N=456). Error bars show the standard error of the average.

Within the litter category, sheets and filament fishing line (monofilament or entangled) are the dominating categories within as well as beyond the 12 nm zone (Table 1.1). This is in accordance with the work of Kamman et al. (2018) who found the same categories dominating within the IBTS of the North Sea. The relative contribution of these items is, however, not equally spread over the BPNS: the relative share of sheets, "crates and containers" and "caps and lids" is much higher in the coastal zone compared to the offshore area. In contrast, monofilaments have a high relative share at the offshore area. The source of the different items is a factor that impacts the spatial distribution as items from the categories sheets, "crates and containers" and "caps and lid" will mainly have a land-based origin whereas monofilaments are strongly linked to fisheries activities, e.g. by the use of dolly rope on nets. The physical characteristics of the litter item will also impact spatial distribution as shape, size and density will determine if an item rapidly sinks to the seafloor or is distributed over longer distances. As monofilaments from dolly rope are low density polyethylene plastics (Bekaert et al., 2015), they can be transported over longer distances along with the current.

Table 1.1: Overview of plastic litter items within and beyond the 12 nm zone. % is expressed as items of this category relative to the total amount of plastic litter items within this zone.

Litter category	Within 12nm zone		Beyond 12 nm zone	
	Number of items	% of plastic items	Number of items	% of plastic items
A1. Bottle	73	2.3	2	0.2
A2. Sheet	1525	47.9	267	28.7
A3. Bag	43	1.4	7	0.8
A4. Caps/lids	61	1.9	4	0.4
A5. Fishing line (monofilament)	718	22.5	450	48.3
A6. Fishing line (entangled)	405	12.7	70	7.5
A7. Synthetic rope	97	3.0	83	8.9

A8. Fishing net	16	0.5	7	0.8
A9. Cable ties	2	0.1	1	0.1
A10. Strapping band	8	0.3	3	0.3
A11. Crates and containers	69	2.2	3	0.3
A12. Diapers	0	0	0	0
A13. Sanitary towel/tampon	1	0	0	0
A14. Other	167	5.2	34	3.7
Total	3185	100	931	100

When non-plastic litter items are considered, natural products such as processed wood are recorded most, followed by items belonging to the groups miscellaneous and glass/ceramics. Categories metal and rubber each account for less than 10% of all non-plastic litter items (Table 1.2). No clear spatial difference can be found in relative abundance between the categories (Table 1.2).

Table 1.2: Overview of non-plastic litter items within and beyond the 12 nm zone. % is expressed as items of this category relative to the total amount of non-plastic litter items within this zone.

Litter category	Within 12nm zone		Beyond 12 nm zone	
	Number of items	% of plastic items	Number of items	% of plastic items
B. Metal	46	9.5	8	9.5
C. Rubber	27	5.6	8	9.5
D. Glass/ceramics	83	17.1	11	13.1
E. Natural products	238	49.0	39	46.4
F. Miscellaneous	92	18.9	18	21.4
Total	486	100	84	100

1.3.1.3. Litter contamination in relation to fisheries activities

The distribution of total litter items as well as fishing related litter items was studied for the BPNS. Fishing related litter items consisted of A5 and A6 (filaments), A8 (fishing net), B3 (fishery related metal) and C3 (rubber bobbins). Although the total amount of fisheries related items was higher within the coastal area (Figure 1.13), the relative share of these items was lower, being 31% in the coastal area compared to 52% beyond the 12 nm zone.

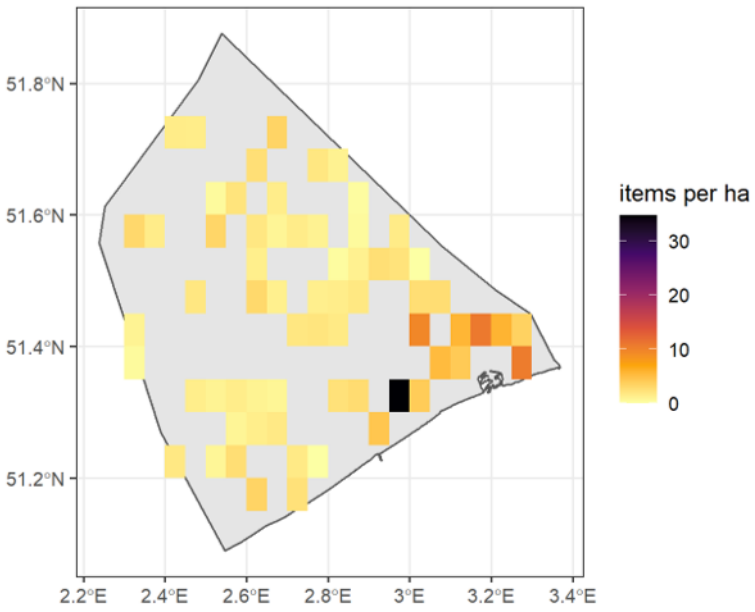


Figure 1.13: Average number of litter items from fishing material per ha per grid cell, pooled data 2013-2019.

Fishing activity, both expressed as number of fishing hours and intensity (swept area ratio, SAR), appeared correlated with both, the number of total litter items as well as the fishing material litter items (n=66). Results are not completely convincing as the correlation between the number of litter items and surface SAR was not statistically significant (correlation coefficient of 0.15 for total litter and 0.2 for fishing related litter). The correlation between number of fishing litter items and fishing hours (0.48) and number of fishing litter items and subsurface SAR (0.35) were statistically significant. However, the area with the highest fishing effort and intensity is also close to the coast (Figure 1.5) which makes it difficult to assess the net effect of fisheries activities in relation to other sources and effects, such as land-based sources or sedimentation effects.

1.3.1.4. Litter contamination in relation to other activities at sea

The effect of sand extraction, OWF and dredging on total litter contamination in the BPNS was assessed, comparing impacted locations with reference locations and nearby or lowly impacted locations (annex 2). Sand extraction and OWF did not affect the total litter contamination. At the sand extraction areas of the BPNS, an average of 2.4 ± 2.0 litter items/ha was found in the net compared to 2.7 ± 2.5 litter items/ha at the reference zone. Within OWF, 2.6 ± 2.5 litter items/ha were detected compared to 2.2 ± 2.0 litter items/ha at the reference area (Figure 1.14).

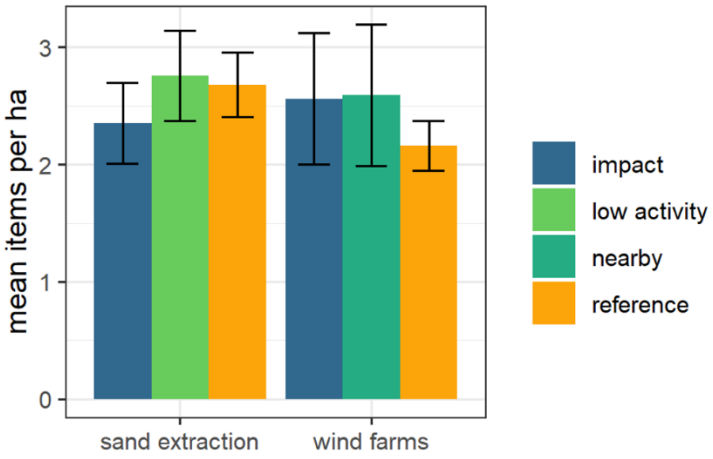


Figure 1.14: Average number of litter items per ha (± standard error) per year at sand extraction and OWF areas and corresponding reference areas (2013-2019).

The 5 dredge disposal sites in the BPNS are all located within the 12 nm zone (Figure 1.7). As a result, higher amount of average litter items are detected in the net (21.6 ± 38.6 items/ha) compared to sand extraction and OWF areas which are mainly located at the offshore area. Between the 5 dredge disposal sites, large differences can be seen with a highest average of 61.4 ± 79.2 litter items/ha in the impact zone of dredge disposal site Br&WZE and a lowest average value of 6.8 ± 3.3 litter items/ha in LNP (Table 1.3). Again, it is impossible to link differences univocally to 1 factor, as not only the dredge disposal intensity differs between the sites but also the sedimentation rate is different. In LNP, dredge disposal intensity was 0.08 ton dry matter (DM).m-2.year-1 from 2007 until 2017 while a much higher value of 1.84 ton DM.m-2.year-1 was recorded in Br&WZE (Lauwaert et al., 2019). However, this is not a prove that dredge disposal impacts the marine litter input as LNP is also located on a sandy area at the western part of the BPNS, while dredge disposal site Br&WZE is located at a sedimentation area at the eastern part of the BPNS, classified as "mud to muddy sand". At individual dredge disposal sites, no clear difference can be found between impact, nearby and reference areas (Table 1.3) on the disposal sites LNP, BR&WOO, Br&WS1 and Br&WS2. In Br&WZE, however, 61.4 ± 79.2 litter items/ha were caught in the net at the impact area, compared to 15.0 ± 14.6 and 11.5 ± 14.1 litter items/ha at nearby and reference zones, respectively. As the nearby zone is also a sedimentation zone, this data suggests a significant input of litter from the dredging activities in Br&WZE. Br&WZE is a dredge disposal site, mainly receiving dredge disposal from the harbour of Zeebrugge. It is also the site with highest dredge disposal intensity (Lauwaert et al., 2019). A more detailed source investigation at this local litter hotspot is therefore recommended.

Table 1.3: Average total litter contamination in different dredge disposal site of the BPNS, pooled data, 2013-2019 (N=191).

Location	Type	Number of items	Number of tracks	Average number of items/ha	Minimum number of items/ha	Maximum number of items/ha
LNP	Impact	90	9	6.8 ± 3.3	1.5	13.0
	Nearby	79	10	5.4 ± 3.1	2.0	10.2
	Reference	252	14	12.4 ± 15.1	0	59.9
Br&WOO	Impact	330	10	22.2 ± 24.6	5.2	76.1
	Nearby	177	7	16.5 ± 15.8	4.1	50.2
	Reference	250	17	10.0 ± 16.2	0.6	70.7
Br&WS1	Impact	253	17	9.8 ± 13.5	1.4	52.3
	Nearby	111	9	8.4 ± 4.3	2.7	15.7

	Reference	252	14	12.4 ± 15.1	0	59.9
Br&WS2	Impact	316	11	19.0 ± 21.2	1.3	68.2
	Nearby	270	12	15.2 ± 20.3	1.6	68.8
	Reference	379	23	11.5 ± 14.1	0.6	58.3
Br&WZE	Impact	625	9	61.4 ± 79.2	3.9	252.9
	Nearby	131	6	15.0 ± 14.6	4.2	43.6
	Reference	379	23	11.5 ± 14.1	0.6	58.3

1.3.1. Marine litter in the North Sea, English Channel Celtic Sea and Irish Sea

1.3.1.1. Temporal and spatial variation in total litter items

Amount of litter items caught in the net within the BTS (2011-2019) are shown within Figure 1.15. Figure 1.16 gives a view on the probability to find litter in the net. For 2695 BTS fish tracks, on average 2.2 ± 2.8 items per ha were caught with a median value of 1.4 items. This number is much lower than the amount of litter items caught in the net within the environmental monitoring campaigns of the BPNS, for which 12.7 ± 1.7 litter items per ha were found in the coastal zone and 2.8 ± 0.2 items per ha outside the 12 nm zone. This can be related to the fact that mainly tracks from outside the 12 nm are taken within the BTS, but also the mesh size of the net is different, which can lead to a lower catchability. Mesh size at the cod end is 40 mm for BTS campaigns compared to 20 mm for environmental monitoring.

The map on litter distribution in the North sea, Celtic Sea, Irish Sea and English channel (Figure 1.15) does not reveal clear spatial trends, although somewhat higher litter amounts can be noted at the Dutch coast. These observations are in contrast to the results of the OSPAR intermediate assessment (OSPAR, 2017b), based on the IBTS data, were a north-south gradient was detected in the North Sea, which could be linked to difference in anthropogenic inputs, rivers, prevailing winds and/or currents (OSPAR, 2017b). The presence/absence analysis on BTS tracks, showing the probability of having litter items in the net (Figure 1.16) shows an opposite gradient as observed by the IBTS: based on BTS data, there is a higher probability to have litter in the net in the Northern Part of the North Sea compared to the Southern Part and the English channel. However, it should be noted that no zero litter catches were recorded from the German BTS tracks (Figure 1.16). This is possibly related to misreporting in the ICES database, biasing the presence/absence analysis. The ICES working group on marine litter states that it is difficult to perform temporal and spatial trend analyses based on litter items caught in the net during fisheries surveys due to the low number of items, the high variability and the fact that seafloor litter monitoring is mostly a secondary objective at fisheries surveys (ICES, 2021). Differences in registration and reporting between countries may occur, impacting the accuracy of the results (ICES, 2021).

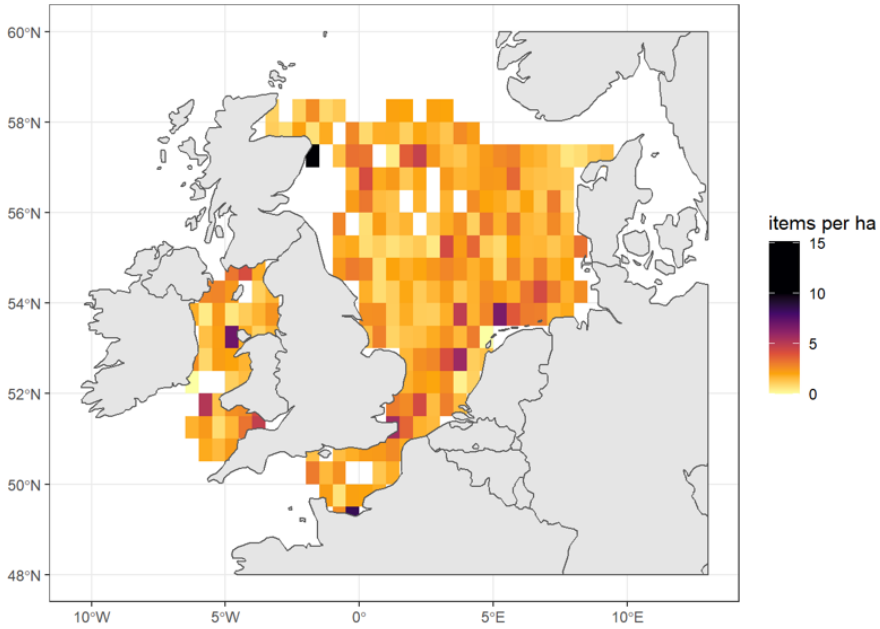


Figure 1.15: Average number of litter items per grid cell for the BTS, pooled data of 2011 to 2019 (n=2695)

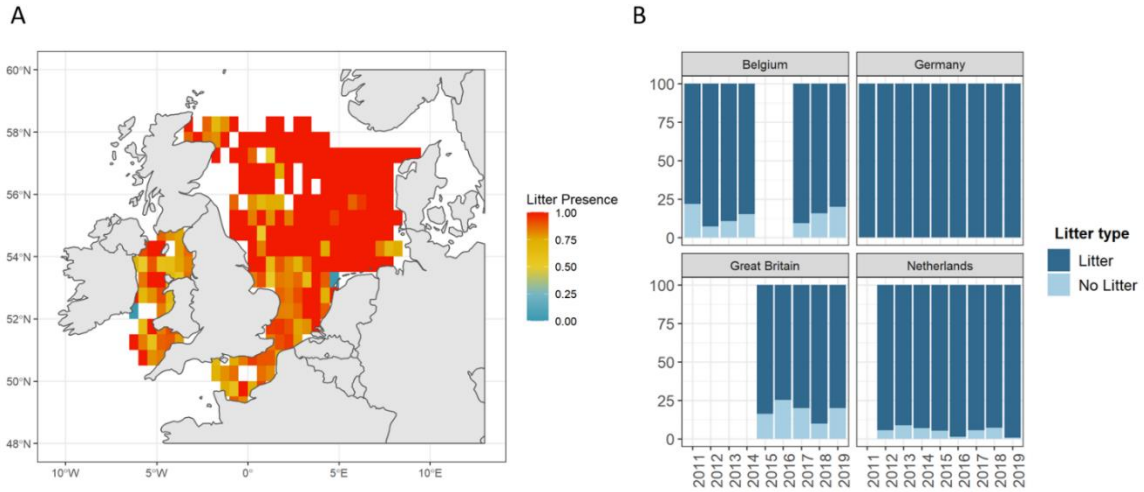


Figure 1.16: (A) Probability of finding litter in a haul per grid cell (based on presence or absence data) and (b) percentage of hauls with and without litter observations per country and per year (BTS, 2011-2019, n=2695)

1.3.1.1. Temporal and spatial variation in total litter items

Of all recorded litter items within the BTS (2011-2019), 77% of the items are plastic, followed by miscellaneous (6%), rubber (6%) and natural products (6%) (Figure 1.17). Within the litter category, dominant items are plastics sheets (A2, 25% of all plastic items), monofilament fishing line (A5, 22%), synthetic rope (A7, 11%), fishing line entangled (A6, 6%) and fishing net (A8, 2%). These results are in line with the results of the environmental monitoring survey in the BPNS and international studies on the North Sea based on IBTS data (OSPAR, 2017b; Kamman et al., 2018). The dominance of plastic is also reported at global scale (UNEP, 2016).

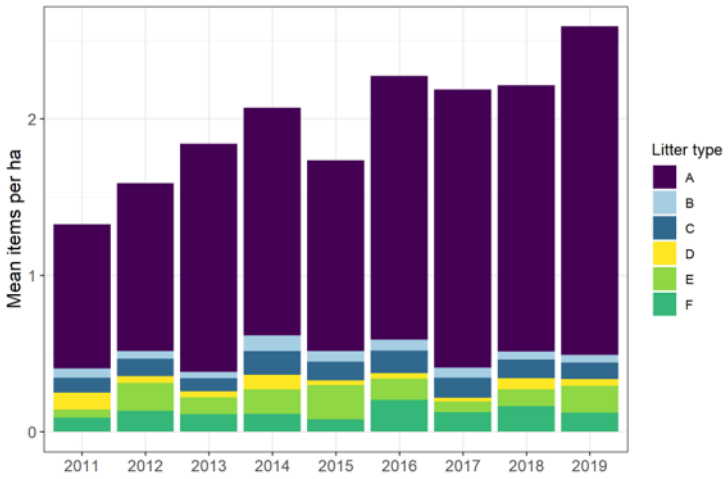


Figure 1.17: Average number of litter items per ha per year for the different litter material types, BTS, 2011-2019.

The distribution pattern of litter items is category dependent. In the North-West site of the BTS area, near Scotland, an increased occurrence of non-plastic items is recorded, with higher amounts of litter items per ha for metal, rubber, glass/ceramic, natural products and miscellaneous products (Figure 1.18). The category plastic reveals less pronounced spatial differences, although larger concentrations of plastic items can be found near the Netherlands. A more detailed look to the different plastic categories learns that especially ropes and filaments (A5, A6, A7) can be found in higher amounts near the Dutch coast (Figure 1.19) whereas bottles, crates and containers (A1, A11) and sheets (A2) show a more evenly distribution.

As fisheries activities are considered a major source of filaments, the link between fishing activity and total litter items and between fishing activity and fishing related litter items was investigated. The distribution of fishing related materials (Figure 1.20) closely follows the distribution of ropes (Figure 1.19c) as both grouped categories are dominated by monofilament fishing line and entangled fishing line. No clear relationship was found between the amount of litter items in the BTS area and fishing intensity. Correlation coefficients between total litter items or fishing related litter items on one hand and fishing hours, SAR and surface SAR on the other hand, were below 0.1. Although fisheries is recognized as an important source of marine litter (Garcia-Alegre et al., 2020), the lack of correlation between fishing effort and marine litter occurrence was also found by other authors (Buhl-Mortensen & Buhl-Mortensen (2018); Garcia-Alegre et al. (2020)). Different reasons may explain this lack of correlation. First issue is the lack of power. With an average value 2.2 ± 2.8 litter items per ha, numbers are low with a high variability, hampering the detection of correlations. Second, the spread of litter items is not only related to its sources, but also to hydrodynamic and geomorphological characteristics (Koutsodendris et al., 2008), which will lead to accumulation zones which can be further away from the source. E.g. deep sea canyons are known to have a higher density of marine litter, including derelict fishing gear (Cau et al., 2017; Pham et al., 2014). Third, fisheries activities themselves reduce litter density at the seafloor, due to delocalization during fishing operations (Lopez-Lopez et al., 2017).

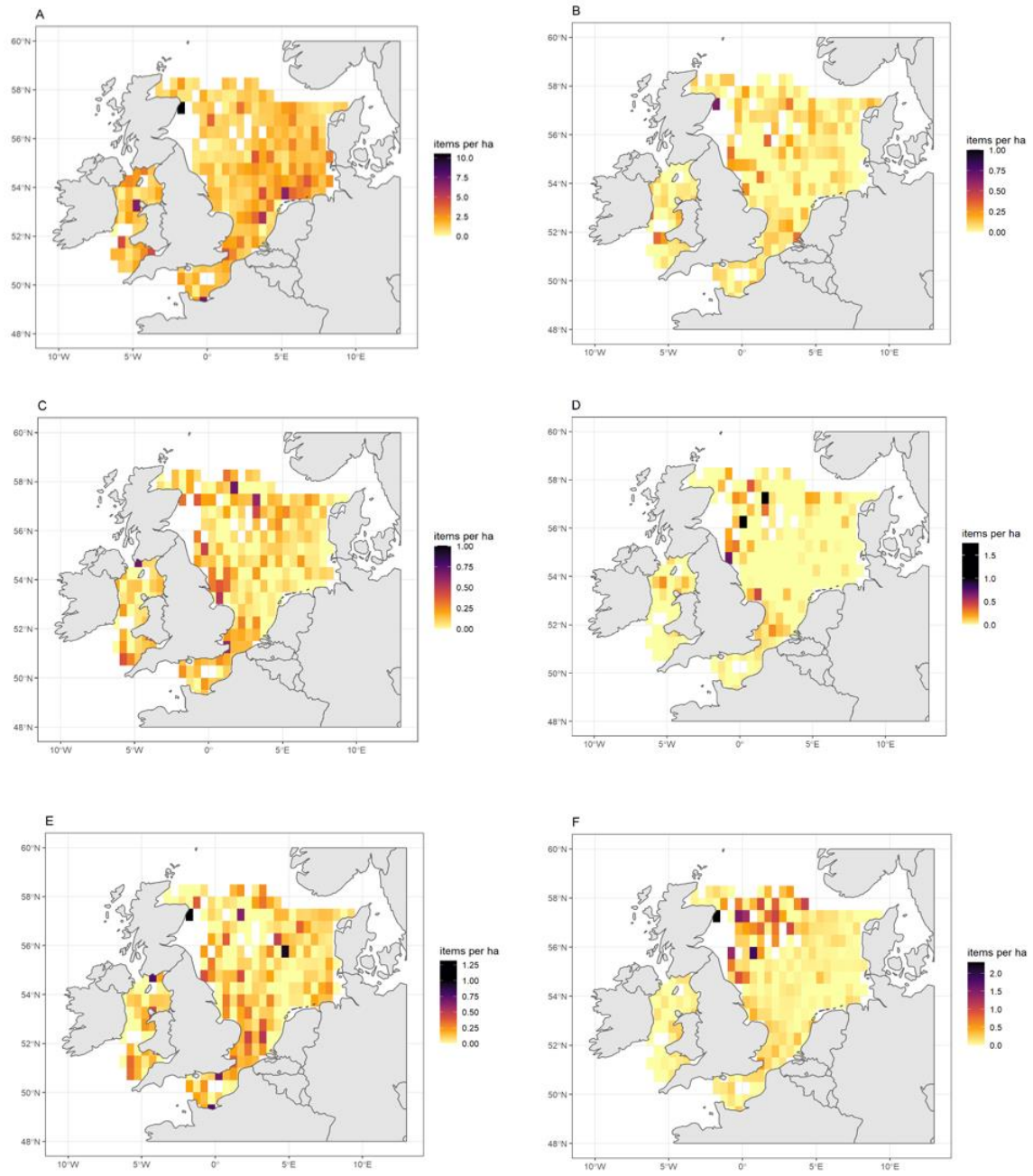


Figure 1.18: Average number of litter items per ha per grid cell for the different categories (BTS, pooled data, 2011-2019). (A) plastic (B) metal (C) rubber (D) glass/ceramic (E) natural products and (F) miscellaneous

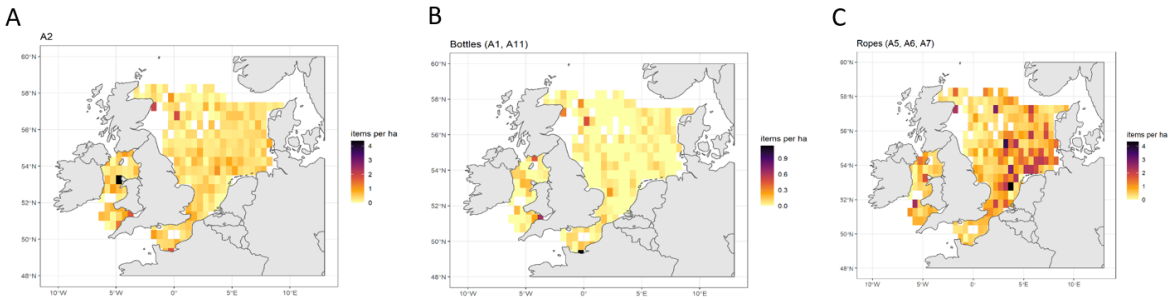


Figure 1.19: Average number of litter items per ha per grid cell for the different categories (BTS, pooled data, 2011-2019). (A) plastic sheets (B) bottles, crates and containers and (C) fishing line monofilaments, fishing line entangled and ropes.

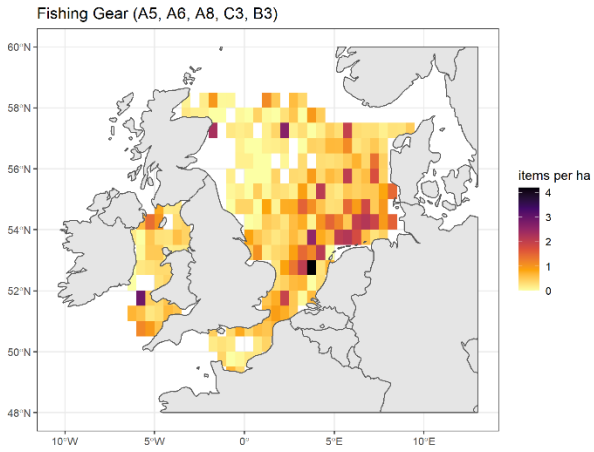


Figure 1.20: Average number of litter items from fishing material per ha per grid cell, pooled data 2011-2019.

1.4. Conclusions

Marine litter on the seafloor of the BPNS and Belgian fisheries areas is assessed, based on trawling data from environmental monitoring campaigns (2013-2019) and the international BTS (2011-2019). The environmental monitoring surveys offer an unique dataset as the regional density of fish tracks in the BPNS is high, with many tracks at a coastal environment sampled with a net with small mesh size. This leads to a relatively high number of litter items in the net, on average 12.7 ± 17 litter items per ha within the 12 nm zone. The amount of litter items caught within BTS fish tracks is much lower, on average on average 2.2 ± 2.8 items per ha, but the dataset offers the advantage of covering a large area, including North Sea, English channel, Celtic Sea and Irish Sea.

Litter on the seafloor of the BPNS and the BTS area mainly consists of plastic, but spatial differences occur in the distribution pattern of specific litter items. Heavier litter items with land-based sources such as bottles or crates and containers are especially found in the coastal area of the BPNS, whereas for example low density filament fishing line is more equally distributed over de BPNS. In the BTS area, filament fishing line has highest densities in front of the Dutch coast. Different factors may impact distribution patterns, as not only the location of the source plays a role, but also hydrodynamic and geomorphological characteristics will impact litter distribution. As a consequence, correlations between litter distribution and human activities are difficult to make. No increased amount of litter items was noted at sand extraction and OWF areas. Clear links between fisheries activities and fisheries related litter items could also not be made. Nevertheless, fisheries are an important source of marine litter with up to 52% of all litter items collected in the offshore area of the BPNS that can be linked to fisheries activities.

Dredge disposal site BR&WZE, located in the eastern coastal zone of the BPNS, was identified as a hotspot marine litter location. Also at this area, effects of dredge disposal cannot be unambiguously differentiated from the effect of hydrodynamic processes such as sedimentation. However, due to the high amount of litter items, we recommend a detailed investigation on the sources and the processes affecting litter accumulation in this area in order to tackle litter pollution of the marine environment and to efficiently remediate hotspot areas.

2. WP2. Microplastic contamination in the biotic environment

2.1. Introduction

Over the last decade, more than 400 papers have been published on the occurrence of microplastics in marine biota. The strong focus on microplastics in the marine environment can also be seen in food studies. Peer-reviewed A1-publications on microplastics occurrence in food are dominated by studies on seafood, followed by studies on drinking water and sea salt while little attention is paid to other, non-marine food matrices (EFSA, 2016, Robbens et al., 2021). Risks associated to microplastic contamination in food can be linked to direct toxicity from the uptake of small particles (Write & Kelly, 2017) or indirect toxicity, associated with the presence of chemical additives or pathogens on microplastics (OSPAR, 2017a). This information has reached the general public through press releases, reports, events and awareness campaigns (Otero et al., 2021). Question raises to what extent the seafood consumer is exposed to microplastics and its associated risks. Many studies report on microplastic contamination in fish, including the gastro-intestinal tract (GIT) (EFSA, 2016). As the GIT is often not consumed, this data is not always relevant from a consumer perspective. For marine biota as well as humans, microplastics are considered too big to transfer from the GIT to the blood or lymph system (Write & Kelly, 2017; Lusher et al., 2017), which limits consumer exposure. Moreover, microplastic occurrence data in seafood may be subject to overestimation when appropriate background measures were not taken during analysis (Hermsen et al., 2017). Debate is also ongoing on the risks of microplastic exposure, as exposure to chemical or pathogens is not only influenced by the concentration of the chemical or biological pollutant but also by its bioavailability (EFSA, 2016: Koelmans et al., 2013). In this study, the occurrence of microplastics in seafood from Belgian fisheries is studied. Distinction in microplastic concentration is made between the consumed part of a seafood product and the non-consumed part. The main aim was to assess the exposure of the consumer of Belgian fisheries products to microplastics.

2.2. Methods

2.2.1. Sampling

Based on relevance for Belgian fisheries, five fish species (sole (*Solea solea*), plaice (Pleuronectes platessa), brill (*Scophthalmus rhombus*), turbot (*Scophthalmus maximus*), cod (*Gadus morhua*)), brown shrimp (*Crangon crangon*) and edible crab (*Cancer pagurus*) were selected for the analysis of microplastics in fisheries products. Fish were sampled in five predefined Belgian fisheries areas: Southern North Sea (SNS), Central North Sea (CNS), English Channel (EC), Celtic Sea (CS) and Irish Sea (IS). The border between central North Sea and Southern North Sea was set at N52°36’ as samples were taken within the Belgian beam trawl survey (BTS) and a clear distinction could be made between sample locations below and above this line. We chose to sample undersize fish, which is bycatch during fisheries surveys such as the Belgian Demersal young fish survey (SNS, 2019), the Belgian BTS survey (CNS, SNS, 2019) and the English BTS survey (IS, 2019). Additional fish were sampled by ILVO seagoing observers in collaboration with commercial fishermen (EC, CS, 2019-2020). In some areas, not all fish species could be sampled (Table 2.1). For S. rhombus and S. maximus in SNS and CNS, insufficient fish were caught and additional GIT samples were taken on board during the campaign from undersized and sized fish (Table 2.1). Table 2.1 provides an overview of fish sampled per area, also indicating the

ICES areas (Figure 2.1) which correspond to each predefined area. *C. crangon* was sampled in the Southern North Sea. *C. Pangurus* in Central and Southern North Sea.

Table 2.1: Fish sampled for microplastic analysis. Sampling areas are based on ICES areas (Figure 2.1).

Area	ICES area	Species	Total number of fish caught	Weight (g)	Length (cm)	GIT weight (g)
SNS	IVc	*S. solea*	29	53-114	19-23	1.1-5.4
		P. platessa	19	88-167	21-25	1.7-5.5
		S. rhombus	4	[1]	[1]	14.2-35.7
		S. maximus	5	[1]	[1]	7.4-43.1
		G. Morhua	6	176-533	32-36	12.4-25.6
CNS	IVb-IVc	*S. solea*	28	57-128	18-24	0.9-4.6
		P. platessa	18	98-169	21-25	2.4-7.4
		S. rhombus	10	[1]	[1]	2.4-19.0
		S. maximus	5	[1]	[1]	27.3-74.1
		G. Morhua	20	27-53	14-16	1.3-5.7
EC	VIId/VIIe	*S. solea*	28	71-145	21-25	1.4-7.0
		P. platessa	26	113-207	22-27	1.4-5.8
CS	VIIf/VIIg/VIIh	*S. solea*	35	73-121	21-25	1.6-3.4
		P. platessa	21	98-157	22-27	3.0-10.2
IS	VIIa	*S. solea*	22	91-132	21-24	1.2-5.3
		P. platessa	11	94-196	22-26	3.7-14.5
		S. rhombus	10	192-397	24-29	4.4-15.6
		G. Morhua	1	390	32	25.7

[1] Stomach dissection on board from fish >27 cm

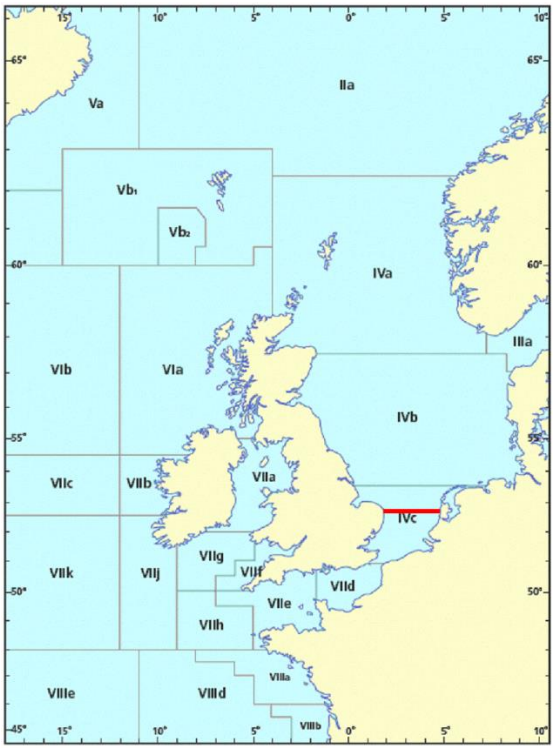


Figure 2.1. Separation of the Northwest Atlantic according to the ICES divisions (image © ICES) with in red, the line dividing CNS and SNS at N52°.36.

2.2.2. Analysis protocols

For all species, microplastic analysis was done on an edible part and a part that is not commonly consumed by European consumers. For fish, the filet was selected as edible part and the GIT as non-consumed part. For shrimps, analysis was done on the peeled tail muscle and on total shrimps. Crab meat was divided in the white edible meat within the claws and the brownish non-consumed inner organs of the crab.

Entire fish, shrimp and crab were stored on board at -20°C. Fish and crab were stored in a bag, shrimp were stored in a metal container. For samples of *S. rhombus* and *S. maximus* in ICES area IVb and IVc, no individuals were caught, and stomach samples were dissected on board and immediately wrapped in aluminium foil before storage at -20°C.

Fish and edible crab were dissected in a fume hood at the laboratory. Brown shrimp samples were also peeled in a fume hood. Fish samples did not include fish specimens with plastic or other materials in the mouth, nor everted or empty stomachs. All samples were stored at -20°C in metal containers after dissection/peeling. Samples were pooled to have approx. 20 - 30 g of sample (see Tables 2.2-2.5). For fish filet and the white meat of crab claws, a validated standard method was applied, able to analyse colored microplastics of 50 µm and larger and colorless microplastics of 200 µm and larger. A brief summary of main validation data is provided within annex 1. For fish GIT, edible crab GIT and shrimp, an additional density separation step was added to the standard protocol to separate dense particles from microplastics.

The analysis was initiated by a digestion with 100 ml 10% KOH / 10g sample for 24 h at 60°C. This digestion was done on a stirrer at a speed of 150 rpm. For the fish filet and the crab claws, the liquid sample was filtered directly after digestion through a paper filter (VWR 415) with particle retention of 12 - 15 µm and a pore size of 31 - 50 µm. For the fish GIT, crab GIT and shrimps, the particles in the samples were allowed to settle down for 24h at room temperature. The upper liquid layer was poured over a filter (VWR 310) with particle retention of 13 µm and a pore size of 25-60 µm. The remaining sample was brought into suspension again by 75 mL of a saturated sodium tungstate solution, prepared by dissolving 700 g of Na2WO4.2H2O in 1 L of demineralized water, followed by a settling phase of 24 h and decanting the liquid over the same filter (the density separation step was performed twice). After filtration, the filters were transferred to a petri dish and analyzed under a stereomicroscope (Leica M205), applying magnification factor 40 x. Particles were tentatively identified to be plastic by the hot needle test, i.e. by pointing a heated needle to the particle and evaluating if the particles melts or curls (Marine & Environmental Research Institute, 2015). Although indicative, validation showed that this technique allows differentiation between natural, semi-synthetic and synthetic fibres when performed by experienced lab technicians (annex 1).

All results were expressed in particles per 100 g of sample. For final results, the average blank signal, obtained from the procedure blanks, was subtracted. A limit of quantification (LOQ) was determined below which no quantification of particles in a sample could be done. The LOQ was calculated as three times the standard deviation of 18 procedure blank runs, analyzed in 2019 and 2020, and set at 1.8 particles per analysis.

2.2.3. Quality control

Stringent quality control measures were taken throughout the entire procedure. As background control measures, all materials were maximally covered. The equipment and devices used in the fume hood were daily cleaned with wet white cotton towels, and all glassware was rinsed 2 times with filtered water before use. Filtered water and solvents were prepared by filtration through a paper folding filter (VWR 310, particle retention 13 µm). Laboratory technicians wore white cotton clothing, a cotton white lab coat, and washed hands between each procedure step. No other lab members were allowed in the dedicated analysis area. Procedural blanks were checked daily, verifying the quality of the analysis and the contamination control measures taken. Air blanks were taken by placing petri dishes with wet paper filter open to the air during dissection. As a positive control, recovery tests with spiked particles were also included weekly.

2.3. Results and Discussion

The results of the microplastic analysis in fish GIT (Table 2.2), fish filet (Table 2.3), *C. crangon* (Table 2.4) and *C. pagurus* (Table 2.5) are presented below. For each analysis, limit of quantification is equal to 1.8 particles per filter. Depending on the sample weight, this leads to a LOQ of 3.9 to 9.6 particles per 100g of sample.

Table 2.2: Microplastics observed in fish GIT of five different fish species, sampled in the southern North Sea (SNS), central North Sea (CNS), English channel (EC), Celtic Sea (CS) and Irish Sea (IS).

Sample number	Species	Number of GIT /sample	Sampling area	Sample weight (g)	Number of particles per 100g
TUR 1	*S. maximus*	2	CNS	19.8	< LOQ
TUR 2	*S. maximus*	2	CNS	27.7	< LOQ
TUR 3	*S. maximus*	1	CNS	37.4	< LOQ
TUR 4	*S. maximus*	2	SNS	18.8	< LOQ
TUR 5	*S. maximus*	1	SNS	19.6	< LOQ
TUR 6	*S. maximus*	2	SNS	29.7	9.2
PLE 1	*P. platessa*	4	IS	28.4	< LOQ
PLE 2	*P. platessa*	3	IS	23.3	< LOQ
PLE 3	*P. platessa*	4	IS	31.1	< LOQ
PLE 4	*P. platessa*	6	CNS	21.0	< LOQ
PLE 5	*P. platessa*	6	CNS	27.6	< LOQ
PLE 6	*P. platessa*	6	CNS	22.3	< LOQ
PLE 7	*P. platessa*	7	SNS	24.5	< LOQ
PLE 8	*P. platessa*	6	SNS	24.2	< LOQ
PLE 9	*P. platessa*	6	SNS	24.9	15.1
PLE 10	*P. platessa*	8	EC	21.5	< LOQ
PLE 11	*P. platessa*	7	EC	22.3	< LOQ
PLE 12	*P. platessa*	8	EC	21.1	< LOQ
PLE 13	*P. platessa*	4	CS	24.5	< LOQ
PLE 14	*P. platessa*	5	CS	25.1	< LOQ

PLE 15	*P. platessa*	5	CS	25.7	< LOQ
SOL 1	*S. solea*	9	CNS	18.7	< LOQ
SOL 2	*S. solea*	10	CNS	23.4	< LOQ
SOL 3	*S. solea*	9	CNS	19.5	< LOQ
SOL 4	*S. solea*	10	SNS	26.6	< LOQ
SOL 5	*S. solea*	10	SNS	25.1	< LOQ
SOL 6	*S. solea*	9	SNS	28.5	9.6
SOL 7	*S. solea*	8	IS	26.2	< LOQ
SOL 8	*S. solea*	7	IS	21.6	< LOQ
SOL 9	*S. solea*	7	IS	27.8	< LOQ
SOL 10	*S. solea*	7	EC	21.7	< LOQ
SOL 11	*S. solea*	8	EC	22.8	< LOQ
SOL 12	*S. solea*	10	EC	21.6	< LOQ
SOL 13	*S. solea*	9	CS	21.4	< LOQ
SOL 14	*S. solea*	11	CS	23.8	< LOQ
SOL 15	*S. solea*	9	CS	22.0	< LOQ
BLL 1	*S. rhombus*	4	IS	23.2	< LOQ
BLL 2	*S. rhombus*	3	IS	27.6	< LOQ
BLL 3	*S. rhombus*	3	IS	35.4	< LOQ
BLL 4	*S. rhombus*	4	CNS	31.2	< LOQ
BLL 5	*S. rhombus*	4	CNS	25.7	< LOQ
BLL 6	*S. rhombus*	2	CNS	25.8	< LOQ
BLL 7	*S. rhombus*	1	SNS	34.7	< LOQ
BLL 8	*S. rhombus*	1	SNS	26.0	< LOQ
BLL 9	*S. rhombus*	2	SNS	30.5	< LOQ
COD 1	*G. morhua*	1	IS	25.7	< LOQ
COD 2	*G. morhua*	11	CNS	28.8	< LOQ
COD 3	*G. morhua*	10	CNS	24.8	< LOQ
COD 4	*G. morhua*	2	SNS	35.5	< LOQ
COD 5	*G. morhua*	2	SNS	37.7	< LOQ
COD 6	*G. morhua*	2	SNS	36.6	< LOQ

Table 2.3: Microplastics observed in fish filet of five different fish species, sampled in the southern North Sea (SNS), central North Sea (CNS), English channel (EC), Celtic Sea (CS) and Irish Sea (IS).

Sample number	Species	Number of filet /sample	Sampling area	Sample weight (g)	Number of particles per 100g
PLE 1	*P. platessa*	1	IS	28.6	< LOQ
PLE 2	*P. platessa*	1	IS	23.7	< LOQ
PLE 3	*P. platessa*	1	IS	25.0	12.1
PLE 4	*P. platessa*	1	CNS	33.6	6.0
PLE 5	*P. platessa*	1	CNS	28.8	< LOQ
PLE 6	*P. platessa*	1	CNS	29.6	< LOQ
PLE 7	*P. platessa*	1	SNS	32.3	6.3
PLE 8	*P. platessa*	1	SNS	32.4	< LOQ
PLE 9	*P. platessa*	1	SNS	33.0	< LOQ
PLE 10	*P. platessa*	1	EC	32.3	< LOQ
PLE 11	*P. platessa*	1	EC	25.5	< LOQ
PLE 12	*P. platessa*	1	EC	25.5	< LOQ
PLE 13	*P. platessa*	1	CS	23.9	< LOQ

PLE 14	*P. platessa*	2	CS	24.4	< LOQ
PLE 15	*P. platessa*	1	CS	26.0	< LOQ
SOL 1	*S. solea*	2	CNS	27.7	< LOQ
SOL 2	*S. solea*	2	CNS	23.5	< LOQ
SOL 3	*S. solea*	2	CNS	26.9	< LOQ
SOL 4	*S. solea*	1	SNS	24.7	< LOQ
SOL 5	*S. solea*	1	SNS	30.5	< LOQ
SOL 6	*S. solea*	1	SNS	32.7	< LOQ
SOL 7	*S. solea*	2	IS	23.9	< LOQ
SOL 8	*S. solea*	2	IS	26.2	< LOQ
SOL 9	*S. solea*	1	IS	25.4	< LOQ
SOL 10	*S. solea*	1	EC	27.7	< LOQ
SOL 11	*S. solea*	1	EC	27.0	< LOQ
SOL 12	*S. solea*	1	EC	28.0	< LOQ
SOL 13	*S. solea*	1	CS	23.9	< LOQ
SOL 14	*S. solea*	1	CS	22.1	< LOQ
SOL 15	*S. solea*	1	CS	22.7	< LOQ
BLL 1	*S. rhombus*	1	IS	27.3	7.4
BLL 2	*S. rhombus*	1	IS	26.7	7.6
BLL 3	*S. rhombus*	1	IS	24.9	< LOQ
BLL 4	*S. rhombus*	1	CNS	33.0	< LOQ
BLL 5	*S. rhombus*	2	CNS	26.2	< LOQ
BLL 6	*S. rhombus*	2	CNS	32.9	< LOQ
COD 1	*G. morhua*	1	IS	29.0	< LOQ
COD 2	*G. morhua*	2	CNS	25.3	< LOQ
COD 3	*G. morhua*	2	CNS	24.0	< LOQ
COD 4	*G. morhua*	1	SNS	29.4	< LOQ
COD 5	*G. morhua*	1	SNS	25.1	< LOQ
COD 6	*G. morhua*	1	SNS	28.1	< LOQ

Table 2.4: Microplastics observed in brown shrimp, sampled in the Southern North Sea

Sample number	Species	Sample	Sample weight (g)	Number of particles per 100g
Shrimp P1	*C. crangon*	Peeled tail muscle	26.7	< LOQ
Shrimp P2	*C. crangon*	Peeled tail muscle	25.1	< LOQ
Shrimp P3	*C. crangon*	Peeled tail muscle	26.2	< LOQ
Shrimp U1	*C. crangon*	Unpeeled shrimps	20.0	< LOQ
Shrimp U2	*C. crangon*	Unpeeled shrimps	21.1	< LOQ
Shrimp U3	*C. crangon*	Unpeeled shrimps	22.4	< LOQ

Table 2.5: Microplastics observed in edible crab, sampled in the southern (SNS) and central North Sea (CNS).

Sample number	Species	Number of crabs /sample	Sample	Sampling area	Sample weight (g)	Number of particles per 100g
CRE C1	*C. pagurus*	1	Claw meat	CNS	46.5	< LOQ
CRE C2	*C. pagurus*	2	Claw meat	CNS	23.6	< LOQ
CRE C3	*C. pagurus*	1	Claw meat	CNS	26.2	< LOQ

CRE C4	*C. pagurus*	2	Claw meat	SNS	28.1	< LOQ
CRE C5	*C. pagurus*	1	Claw meat	SNS	28.8	< LOQ
CRE C6	*C. pagurus*	1	Claw meat	SNS	25.6	< LOQ
CRE G1	*C. pagurus*	1	GIT	CNS	32.4	< LOQ
CRE G2	*C. pagurus*	1	GIT	CNS	22.8	< LOQ
CRE G3	*C. pagurus*	1	GIT	CNS	25.8	< LOQ
CRE G4	*C. pagurus*	1	GIT	SNS	33.1	< LOQ
CRE G5	*C. pagurus*	1	GIT	SNS	24.6	< LOQ
CRE G6	*C. pagurus*	1	GIT	SNS	36.2	< LOQ

The number of particles detected in fish, brown shrimp and edible crab was low, as the number count did not exceed the LOQ for most samples. For fish GIT samples, only 3 out of 51 samples had a value higher than LOQ with a maximum of 15.1 particles/100 g in a sample of P. platessa of the southern North Sea. As this sample was a pooled sample of 6 fish GIT, average amount of particles per individual fish GIT in this sample was 0.6. For fish filet, only 5 out of 42 samples had a value above LOQ with a maximum of 12.1 particle per 100g. For brown shrimp and edible crab, no value higher than LOQ was recorded.

Since the number of microplastics larger than 50 µm is low in fish filet, crab meat and shrimp tail muscle, the consumption of Belgian fisheries products will not lead to a large exposure to microplastics of this size range. This is as expected, as microplastics of this size cannot cross epithelium membranes such as in the gastro-intestinal tract (Gouin, 2020; Lusher et al., 2017). Transfer of plastics through epithelium membranes is especially described for nanoplastics (1-100 nm) and small microplastics (<5-10 µm) (Abbasi et al., 2018; Avio et al., 2015; Collard et al., 2017; EFSA, 2016; Write & Kelly, 2017), but analytical methods to determine non-spiked nanoplastics in complex matrices such as fish GIT are currently still lacking.

Remarkably, the number of particles are also low in the GIT of fish. Many studies have reported on the presence of microplastic in fish, with a large variety in data, from <5% of fish (e.g. Foekema et al., 2013; Liboiron et al., 2016) to >90% of the fish with microplastics in the GIT (e.g. Jabeen et al., 2017). Differences between fish may result from feeding behavior, size, tropic level and ecological niche, but also geopgraphical differences may impact the amount of microplastics in fish GIT (Claessens et al., 2011; Rummel et al., 2016; Ory et al., 2017; Beer et al., 2018; Kühn et al., 2020). Next, also the quality of the analysis can play a big role. Many studies reporting high incidence lack appropriate quality control (Hermsen et al., 2017). The results of our study are in line with other studies on North Sea fish reporting low incidence of microplastics in the GIT such as Foekema et al., 2013 (2% of samples), Kühn et al., 2020 (1.8% of samples) and Hermsen et al., 2017 (0.25% of samples). Our results indicate that microplastics > 50 µm do no bioaccumulate in fish GIT. Due to the low number of microplastics found, no relevant comparison between species or areas could be done in this study.

In contrast to fish, only little data is available on microplastic occurrence in *C. crangon* and *C. pagurus*. Devriese et al. (2015) found on average 68 fibers per 100 g of brown shrimps from the English Channel and North Sea. The results of this study are, however, much lower. Korez et al. (2020) report strong depuration of particles from *C. crangon* within 48h. Similar to fish GIT, low microplastics numbers in *C. crangon* and *C. pagurus* suggest that microplastics do no bioaccumulate in this species.

2.4. Conclusions

The microplastic occurrence in seafood from Belgian fisheries was assessed, applying a validated method for microplastic analysis. Colored microplastics >50 µm and colorless microplastics >200 µm could be determined by the applied method. In most of the fish samples, for GIT as well as filet, values did not exceed the limit of quantification. Also in samples of *C. crangon* and *C. pangurus*, limit of quantification was not exceeded. These results indicate that microplastics do no bioaccumulate in fish, brown shrimp and edible crab. The exposure to microplastic by consuming seafood from Belgian fisheries products is limited.

3. WP3. Microplastic contamination in the abiotic environment

3.1. Introduction

Microplastics are defined as "synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 µm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water" (Frias and Nash, 2019). They are persistent and due to the enormous production and frequent use, widespread in the environment. A dedicated follow up in different compartments and at different locations in the Belgian part of the North Sea is needed. The evaluation should allow to determine whether concentrations are below levels that cause harm to the marine environment as is stated in the Marine Strategy Framework Directive (MSFD, 2008/56/EC; 2017/848/EU; Descriptor 10, criterion 2). The harmful effects of microplastics are however still unclear and need further research. Microplastics are ingested by marine organisms and, depending on the size and shape, may translocate from the gastrointestinal tract to other tissues. Some studies have shown negative effects on specimen exposed to very high levels of microplastics (Van Colen et al., 2020; EPHEMARE, 2019; PLASTOX, 2019).

Although the level causing harm is still hard to define, data and knowledge need to be collected to monitor the evolution of the pollution level. Objectives and measures to reduce litter and microlitter are being defined at different levels: at the European Union level with a.o. the MSFD and the Plastic Strategy, the UN Environment Programme and the Sustainable Development Goals and several actions at the federal[1] and Flemish[2] level. The effectiveness of these measures must be evaluated. The MSFD revised specifications state that microplastics should be monitored in the surface layer of the water column and in the seabed sediment and may additionally be monitored along the coastline.

The aim of this work package is to elaborate a monitoring approach for microplastics in sediment and in the water column with as first step the development of a methodology. The suitable methods strongly depend on the size range of particles that will be considered. While the smallest microplastics are those representing the highest risk for the marine biota, the methodologies to identify microplastics up to the micron-level are extremely challenging, costly, and laborious. For monitoring purposes, a practical and feasible approach must be elaborated and consequently a lower size limit of 100 µm is being put forward in international guidelines and harmonization efforts (Frias et al., 2018, Gago et al., 2018, MSFD Technical Subgroup on Marine Litter, 2013). Guidelines are in development at EU and OSPAR level, and the status of these are closely followed and contributed to by participation to the dedicated groups. However, since no consensus exist yet for several steps involved in the

[1] Actieplan marien zwerfvuil (belgium.be)
[2] Vlaams Integraal Actieplan Marien Zwerfvuil (ovam.be)

determination of microplastics, a thorough literature study has been done to identify and compare the possible options.

3.2. Literature overview of methodological approaches

3.2.1. Microplastic water and sediment sampling methods

For sampling of seafloor sediment, the van Veen grab and the box corer, are both recommended in the Baseman protocol (Frias et al., 2018), and commonly used. The box corer sampler seemed to be a better option thanks to its minimal impact in surface deformation, maintaining the sediment integrity and allowing an easier calculation of the volume of sediment collected (Frias et al., 2018). The risk of losing microplastics during sampling is also lower. Therefore, it was chosen to use the Reineck box corer sampler.

For the seawater matrix, different devices for microplastic sampling are being used. The most commonly used are the Manta trawl and the Neuston net. The main advantage of these net-based sampling devices is that they can sample large volumes of water quickly. Besides that, many disadvantages have been highlighted. First, the tow speed limit is approximately 3 knots and sampling must occur during calm sea conditions. Then, there is the limiting factor of the mesh size that affects the size of the collected particles (Gago et al., 2018; Hidalgo-Ruz et al., 2012). The determination of the exact volume sampled can be difficult and there is also the problem of diminishing mesh size during sampling due to the clogging of the pores. According to Green et al. (2018), compared to bulk samples, sampling with net-based methods leads to an underestimation up to 3 times lower microplastic concentrations. The last major disadvantage is the non-negligible risks of sample contamination (Lenz et al. 2018).

Niskin bottles attached to a CTD-Rosette sampler are also commonly used to sample water. The main advantages of this method are that unlike nets, there are no clogging problems; the exact volume of collected water is known and it is easier to control and minimise sample contamination. This method is also better to collect small plastic particles and fibres than the net-based method. The only major disadvantage of Niskin bottles method is the low volume of water collected. (Di Mauro et al. 2017; Gago et al., 2018.; Green et al. 2018).

Another method, less frequently used but also recommended in the Baseman protocol (Gago et al. 2018), is the one used by Desforges et al. (2014) and Lusher et al. (2014). This method uses the vessel's continuous seawater inlet pump to collect subsurface seawater. Compared to Niskin bottles, this method allows to collect higher volumes of water while keeping the advantages mentioned for the Niskin method (exact volume known, no clogging, minimisation of air contamination…). Other advantages to choose this method within the framework of a monitoring project are its low costs and easy implementation in multidisciplinary cruises because it does not interfere with other vessel operations. (Desforges et al. 2014; Gago et al., 2018.; Lusher et al. 2014; 2015)

Based on this comparison of the different methods, we choose to take samples from the seawater inlet and in parallel some samples with Niskin-bottles.

3.2.2. Microplastic separation and pre-treatment methods

For the sediment matrix, the most common method to extract the microplastics from the sediments is to do what is called a density separation step. The principle is to exploit the fact that plastic particles have a lower density than sediment grains by mixing the sediment sample with a specific solution. Therefore, the sediments sink to the bottom when on the contrary low-density plastic particles float and can be retrieved. For that step, different methods and density separation solutions are used.

The most frequently used density separation method is based on an adaptation of the "Thompson et al. 2004" method which consists of putting a certain amount of sediments in a beaker, adding a density solution, stirring the mixture, letting the suspension settle and then filtrating the supernatant which contains the researched microplastics (Frias et al., 2018; Hidalgo-Ruz et al., 2012; Maes et al., 2017). Based on the same principle of density separation, the Munich Plastic Sediment Separator (MPSS) is a device recommended for larger sediment volumes (>1L) (Frias et al., 2018; Hidalgo-Ruz et al., 2012; Imhof et al., 2012). Because of this interesting possibility to analyze a large volume of sediment, the MPSS was chosen to be tested for this monitoring project.

The most common density solution used for the density separation is a solution of saturated NaCl ($1.2 g/cm^3$). Other solutions with different densities can also be used but for monitoring purposes sodium chloride and sodium tungstate dihydrate are recommended by the baseman protocol as they are economical and safe methods (Frias et al., 2018, Hidalgo-Ruz et al., 2012). The main disadvantage of NaCl solution is its lower density which leads to a poor recovery of denser plastics. Sodium chloride solution was used for the first set of MPSS tests (see 3.4.2.1). For subsequent tests, it was decided to use a denser solution. Sodium Bromide (NaBr) was chosen because of its high density (d= 1.52 g/ cm^3) that allows to recover denser plastics (Table 3.1), its low toxicity and its ease of use (less viscous) compared to ZnCl (oral communication G. Gerdts).

Table 3.1: Densities of common plastics. Polymers with lower densities are retained by the solutions: Sodium chloride: 1.2 g cm^{-3}; Sodium tungstate dihydrate: 1.4 g cm^{-3} and Sodium bromide: 1.5 g cm^{-3}. (Frias et al., 2018)

Polymer	Abbreviation	Density (g cm^{-3})
Polystyrene	PS	0.01 – 1.06
Polypropylene	PP	0.85 – 0.92
Low-density polyethylene	LDPE	0.89 – 0.93
Ethylene Vinyl Acetate	EVA	0.94 – 0.95
High-density polyethylene	HDPE	0.94 – 0.98
Polyamide	PA	1.12 – 1.15
Nylon 6,6	PA 6,6	1.13 – 1.15
Poly methyl methacrylate	PMMA	1.16 – 1.20
Polycarbonate	PC	1.20 – 1.22
Polyurethane	PU	1.20 – 1.26
Polyethylene terephthalate	PET	1.38 – 1.41
Polyvinyl chloride	PVC	1.38 – 1.41
Polytetrafluoroethylene	PTFE	2.10 – 2.30

The separation of microplastics from water is easier than from sediments. The Baseman protocol recommends to first do an alkaline digestion with 10% potassium hydroxide if organic content is too high for direct filtration. If the sample still contains undigested organic matter, a second digestion is recommended with 15% hydrogen peroxide. The digested sample is then filtered on a glass fiber filter (Gago et al., 2018).

For this project we decided to implement the Baseman protocol recommendation, but as research is still evolving, other digestion processes will be considered in the future. A recent article from Pfeiffer and Fischer (2020), investigated different digestion protocols (agents, temperature, times) for microplastics sample processing. The results of this study show that the best result for organic matter digestion and synthetic polymer resistance is achieved by using NaClO oxidizing agent.

3.3. Sampling sediment and seawater in the Belgian part of the North Sea

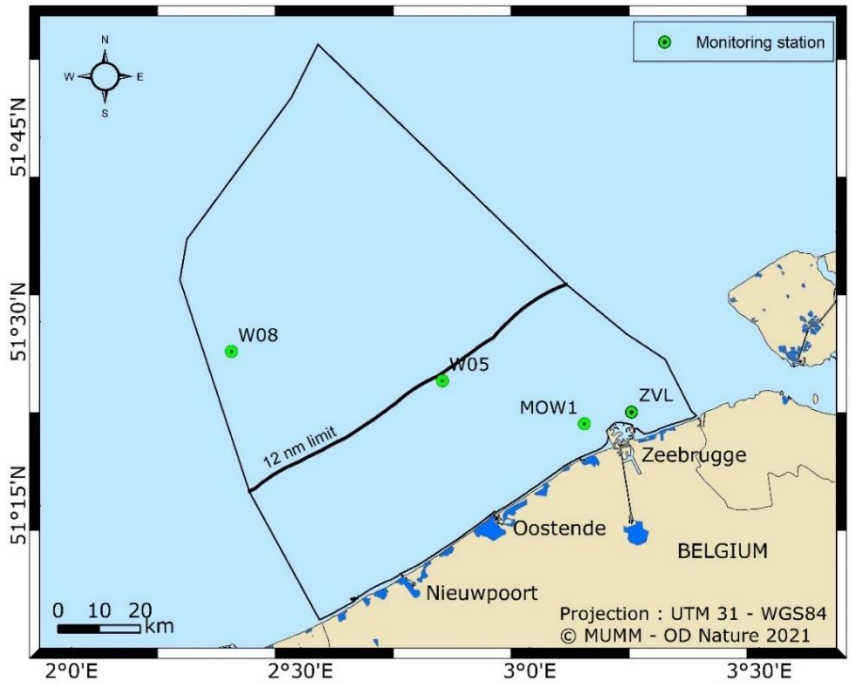


Figure 3.1: Sampling stations in the Belgian Part of the North Sea (BPNS)

The first sampling campaign took place the third week of February 2020. Due to the bad weather conditions only stations close to the coast could be visited. A second campaign was conducted the 2nd week of July 2020. Due to ship technical problems, the station MOW1 could not be sampled as planned during that cruise. An overview of the sampling events is shown in Table 3.2, and the sampling locations are shown in Figure 3.1.

Table 3.2: Sampling specificities

Station	Date	Latitude	Longitude	Sediment		Water	
				Reineck box corer	Sampling depth (m)	Seawater inlet pump	Niskin
Port of Zeebrugge	19/02/2020	51°N 21.397’	3°E 12.229’	1 sample	21,1	-	40L
ZVL	19/02/2020	51°N 22.670’	3°E 13.347’	1 sample	14,9	100L	100L

MOW1	18-19/02/2020	51°N 21.345’	3°E 07.582’	3 samples	13,5-8,8	2x100L,2x 200L	100L
WO5	07/07/2020	51°N 24.610	2°E 49.471	3 samples	23,5	2 x 100L	-
W08	08/07/2020	51°N 27.368’	2°E 21.042	3 samples	24	2 x 100L	-

The choice of sampling in these stations was based on the coastal-offshore transect characterized by a high salinity gradient. The three stations MOW1, W05 and W08 are regularly visited during monitoring cruises and as such provide a wide range of simultaneously collected data.

Measures were taken to minimize and record possible contamination. Glass and metal tools were used to collect and store the samples. All material was rinsed three times with milliQ water. Clothes of natural origin (cotton, wool) were worn during sampling. During each sampling event, a control filter was placed in an open petri dish to monitor airborne contamination.

3.3.1. Water

On the seawater inlet in the vessel’s laboratory, pipes equipped with a flowmeter were installed to enable precise recording of the volume sampled. The “seawater inlet pump” continuously collects water from an inlet located in the front of the vessel at a depth of 3 m. Water samples with the seawater pump (Figure) were taken in 4 of the 5 stations. Due to the very high turbidity the seawater pump cannot be used in the harbour. The volume of water collected varied between 100 and 200 liters. The sampled volume could be adapted depending on the amount of suspended matter, to allow sufficient microplastic particles collection but without having too much material on the sieve.

Water samples with 5l Niskin bottles were taken in the stations close to the coast, since the highest number of microplastics are expected there, near the surface (-0.5m).

The water was sieved on board with two stacked stainless-steel sieves having a pore diameter of 63µm and 100µm. The material on the filter was transferred in labelled glass jars and stored at -20°C.

To test the recovery rate of particles collected on the sieve, a positive control has been carried out. Red microbeads of around 500µm were put on the sieve and followed the seawater pump sample processing procedure.

Figure 3.2: Water sampling. Left: Seawater inlet pump equipped with a flowmeter. Middle: water filtration from the inlet through metal sieves (100µm, 63µm), covered with aluminium foil to prevent air contamination. Right: Rosette with the 5l Niskin bottles.

3.3.2. Sediments

Three replicates of sediment samples were taken in every station with the Reineck box corer (Figure 3.3). The upper layer (5-10 cm) of the sediment samples was directly put in labelled glass jars and stored at -20°C until further analysis.

Figure 3.3: Sampling with Reineck box corer

3.4. Sample pretreatment and microplastic separation

3.4.1. Water

For the water matrix, the sample pre-treatment to digest the organic matter (OM) is done following the method recommended in the Baseman protocol (Gago et al., 2018). It consisted of an alkaline digestion with potassium hydroxide (10% KOH) at 40°C for 72h. The digested sample was directly filtered on a glass fiber filter (retention 1.2µm) and the filter transferred into a glass petri dish. The recovered particles were then visualized with a stereo microscope. For quality control of contamination, each batch of sample series was accompanied by a procedure blank also called negative control, similar to the sample preparation but without sample.

3.4.2. Sediments

3.4.2.1. Density separation tests

Two types of separation methods were tested and evaluated by assessing the recovery rate of spikes, microplastic particles added to sediment samples. First tests were performed using the Munich Plastic Sediment Separator. As the tests with the MPSS did not lead to a satisfactory recovery level , another, more classic and commonly used separation method was also tested.

Munich Plastic Sediment Separator (MPSS)

General protocol

The Munich Plastic Sediment Separator was installed in the laboratory (see Figure 3.4). The first step was the cleaning of the device by filling it with 30 liters of the density solution (without sample), let it run and settle to allow the removal of possible solution contaminations. In parallel, the sediment sample was defrosted, homogenised, weighted and spikes were added. When the sediment sample contained a lot of organic matter, a digestion with hydrogen peroxide (10% H_20_2) was done at room-temperature during 18h. After this digestion the sample was rinsed thoroughly, on a 63µm stainless steel sieve, to remove any hydrogen peroxide residue. After the introduction of the sample in the MPSS, the device was filled, closed and the rotor on the bottom was switched on for a determined time (Table 3.3). Then, the rotor was switched off and the device with the sample was left to settle for another determined time. The valve from the sample chamber was then closed, disconnected from the rest of the device and taken to our workspace for filtration allowing to recover the top floating particles on a filter. The filter was then transferred into a glass petri dish for visual identification and recovery assessment of the particles. After each MPSS run, an important and time-consuming step was to empty the device, to filter the solution (20µm sieve) to allow its later reuse, and to clean all the parts. This protocol was elaborated based on different sources (HYDRO-BIOS 2015-2018; Imhof et al., 2012; Lorenz et al., 2019; Gago et al.,2018; Bergmann et al., 2017; Have et al., 2019; information exchange with experts). The detailed protocol is available in annex.

Results and discussion

For testing the efficacity of the device (Figure 3.4, a-b), two types of spikes were used: blue fragments (1 to 2mm) made of HDPE and red microbeads (500 to 600µm) made of polyethylene. Ten recovery tests were done with different conditions. Two solutions of different densities: NaCl (d=1.2 g/ml) and NaBr (d=1.52 g/ml) were used. Tests with and without sediments, with different mixing and settling times have been run. The results, together with the test parameters are presented in Table 3.3.

Table 3.3: MPSS recovery test results and test conditions

	Density solution	**Sediments**	**Mixing time**	**Settling time**	**Spikes recovery Blue fragment** (HDPE)	**Red microbead** (PE)
Test 1	NaCl	Yes	50’	1h	60%	NA
Test 2	NaCl	Yes	50’	24h	0% *fail	
Test 3	NaCl	Yes	50’	2h	30%	0%
Test 4	NaCl	Yes	3h	Overnight (~18h)	30%	0%
Test 5	NaBr	No	1h	1h	60%	30%
Test 6	NaBr	No	1h	1h	30%	30%
Test 7	NaBr	Yes (beach sand)	3h	3h	100%	60%
Test 8	NaBr	Yes (W08)	1. 1h 2. 1h	1. 4h 2. 42h	1. 20% 2. 30%	1. 0% 2. 10%
Test 9	NaBr	Yes (beach sand)	3h	Overnight (~18h)	60%	60%
Test 10	NaBr	Yes (W05)	4h	Overnight (~18h)	1. 40% 2. 80%	1. 20% 2. 70%

First tests did not allow to recover sufficient plastic reference particles, and some problems were encountered. The test n°2 shows a 0 % recovery because a leak during the settling time caused the emptying of the sample chamber. Another small leakage was observed from the sample chamber (Figure 3.4, c-d), this problem was not large enough to cause a significant loss of liquid, however if the MPSS was filled with a more toxic solution (e.g.$ZnCl_2$), it would pose a risk to the operator.

The recovery rate of the test n°7 was the highest. This test was done using undigested beach sand which, due to the presence of organic matter, led to a large amount of foam in the sample chamber. Our hypothesis is that the foam helped the spikes to rise by trapping them. But this foam led to difficulties during the filtration step, considerably reduced the readability of the filter and made the solution very dirty, leading to a heavy cleaning step. For the test n°8, after the poor recovery of the supernatant filtration, a second run was done with high settling time. But even after this second extraction the total recovery rate was not satisfying.

The most important problem encountered during all these tests was the adhesion of the spikes to the walls of the device. These adhesions were mainly observed on the irregular parts of the MPSS: at the spots of large diameter reduction and on the rubber rings (Figure 3.4, f-h). The supplier’s advice was to knock on the device walls in order to enhance the rise of the air bubbles stuck to the inner walls and at the same time bring up the plastic particles. While some bubbles reached the surface, it had no impact on the rise, and recovery, of the spikes. Advice from experienced users of the MPSS was put in practice: use of NaBr as density solution; pre-digestion with H_2O_2; 20 minutes of pre-mixing with NaBr before the introduction in the MPSS; increase of the mixing time; increase of the settling time. This advice helped to increase a little the recovery rate, especially the switch from NaCl (average recovery:

20%) to NaBr (average recovery: 43%), but still did not lead to satisfactory levels. The same problem of particle adhesion was notified by other users who also performed many experiments.

Since static electricity forces might be involved in the adherence of plastics to the metal walls, a metal cable was placed to connect the device to a grounding point (tests n° 8, 9, 10) in order to discharge the device from potential electrostatic charges. Unfortunately, this technique also did not show any effect.

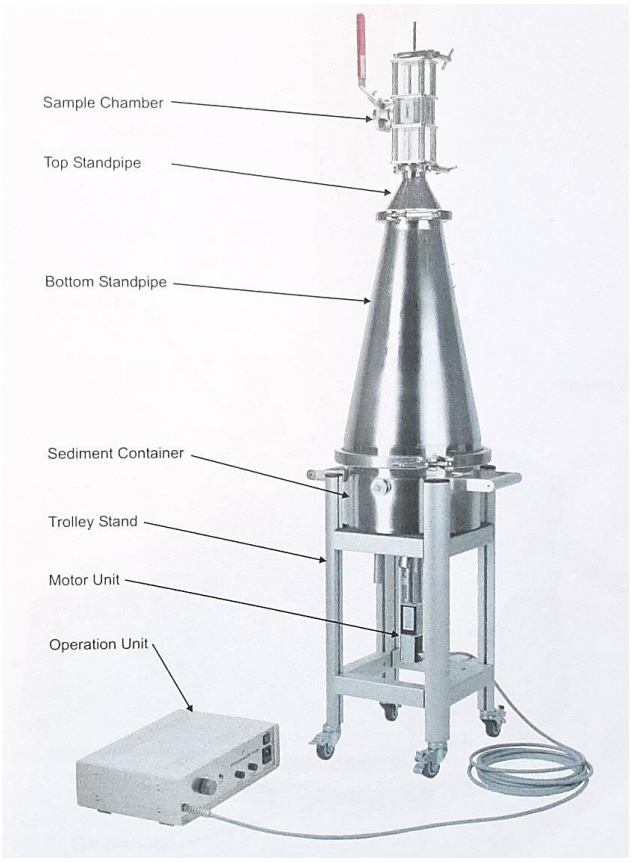
For the test n°10, as the adhesion of the particles on the upper part walls was the major problem, a second step was done after the sample chamber filtration. This second steps consisted of the rinsing of the "top standpipe" to recover the spikes stuck on the walls. The recovery rate doubled, approaching the 80%, which is currently being considered as requirement in regional recommendations. Drawback of this step is the increase in risk of air contamination which reduces for an important part the advantage of the closed device.

A first attempt to add surfactant to reduce surface tension with the hope to reduce particles adhesion was unsuccessful because the reagent (SDS) reacted with NaBr by creating white precipitates (Figure 3.4, j). The search for other surfactants could be considered in future.

Other disadvantages were encountered with the use of the MPSS. Although this device is interesting because of its ability to extract plastics from a large volume of sediments at once, it implies that a large amount of solution has to be made (>30 liters). The maintenance of this large volume is labour intensive because it must be carefully filtered and stored to avoid air contamination. The large size of this device also implies that the different pieces are heavy and difficult to handle during the cleaning process. In addition, this device does not allow for replicates or procedural blank in parallel of the sediment analysis.

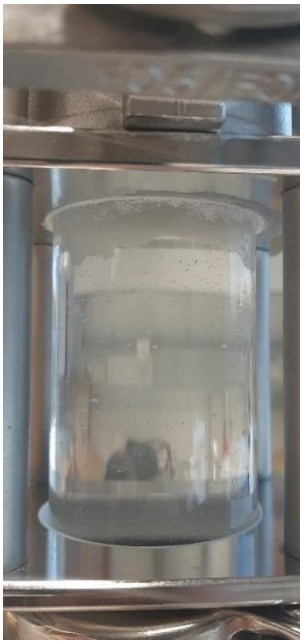
Our recovery results are considerably lower than the 100% and 95.5% reported by Imhoff et al. (2012) for respectively large (1-5mm) and small microplastic particles (<1 mm) from clean riverine sediments. Similarly, Zobkov et al. (2017), tested the MPSS and reported low recovery rates (13-39%) for microplastics extraction from marine bottom sediments.

a


Sample Chamber
Top Standpipe
Bottom Standpipe
Sediment Container
Trolley Stand
Motor Unit
Operation Unit

b

c



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e

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h

Figure 3.4: a-b: Components of the MPSS; c-d: Slight leakage from the ball valve; e: Foam in the sample chamber; f-h: Spikes (blue and red) stuck on different MPSS parts; i: Cleaning of the density solution (20µm sieve) before storage in glass jars; j: Reaction of NaBr + SDS (1%) =white precipitate

Classic density separation method - modified method of Thompson et al. (2004)

General protocol

The sediment sample was defrosted and homogenised. When the sample contained a lot of organic matter, a digestion with hydrogen peroxide (10% H_20_2) was done at room-temperature during 18h. After this digestion, the sample was rinsed thoroughly, on a 63µm stainless steel sieve, to remove any hydrogen peroxide residue. The sediment subsample (25g; 100g) was transferred in a glass beaker were the saturated NaBr solution was added in a ratio of at least three times the volume of the sample. The suspension was mixed for 2 minutes using a metal spoon. After mixing, the sample was let to settle for 1 hour, allowing the dense sediment particle to sink while the lighter particles float at the surface. The supernatant was then poured into a filtration set and filtered through a glass fiber filter (1.2 µm retention). The density separation steps were repeated two more times in order to ensure a total extraction of the particles from the sediment. The filter was then transferred in a glass petri dish for visual identification and recovery assessment of the particles. For every sediment analysis, 3 subsamples were analysed in parallel and one procedural blank accompanied every batch of subsamples. This protocol was elaborated based on different sources recommendations (GESAMP

2019, Gago et al., 2018; Maes et al., 2017; Marusa et al., 2015; Zhang et al., 2020; Hidalgo-Ruz et al., 2012). The detailed protocol is available in annex.

Results and discussion

Five tests of spikes recovery (see Table 3.4) were done based on density separation in a beaker. Five types of spikes with different shapes, size and polymer densities were used: HDPE fragments (1 - 2mm); PE microbeads (500 to 600µm & approximately 100µm); PP fragments (1 - 2mm) and PET film (1 - 2mm). The recovery tests were done with different sediment weight (25g and 100g) and with saturated NaBr (d=1.52 g/ml) solution. This solution has the advantage to allow the flotation of dense plastics like PET with a density around 1.4 g/ml.

Table 3.4: Recovery test results and test conditions using the modified method of Thompson et al. (2004). [1]Extracted from sediments, [2] Retrieved on filter. ww= wet weight; dw=dry weight

	Density solution	**Sediment weight**	**Mixing time**	**Settling time**	**Spikes recovery**				
					Blue fragment (HDPE)	Red microbead (500-600µm) (PE)	Red microbead (100µm) (PE)	Brown fragment (PP)	Blue film (PET)
Test 1	NaBr	25g dw	2'	1h	100%[1]	100%[1]	/	/	/
Test 2 "classic"	NaBr	25g dw	2'	1h	100%[1]	100%[1]	/	/	/
Test 2 "overflow"	NaBr	25g dw	2'	1h	100%[1]	100%[1]	/	/	/
Test 3 "classic"	NaBr	25g dw	2'	1h	100%[1]	100%[1]	100%[1]	100%[1]	/
Test 3 "Erlenmeyer overflow"	NaBr	25g dw	2'	1h	100%[1]	100%[1]	20%[1] (not visually clear)	100%[1]	/
Test 4	NaBr	100g dw	2'	1h	100%[1] 80%[2]	100%[1] 86%[2]	100%[1] 66%[2]	100%[1] 86%[2]	100%[1] 93%[2]
Test 5	NaBr	100g ww	2'	1h	100%[1] 73%[2]	100%[1] 93%[2]	100%[1] 66%[2]	100%[1] 86%[2]	100%[1] 100%[2]
Control + (MOW1)	NaBr	100g ww	2'	1h	100%[1] 40%[2]	100%[1] 80%[2]	100%[1] 100%[2]	100%[1] 80%[2]	100%[1] 100%[2]
Control + (W08)	NaBr	100g ww	2'	1h	100%[1] 100%[2]	100%[1] 80%[2]	100%[1] 100%[2]	100%[1] 80%[2]	100%[1] 100%[2]

During our first tests (1 to 3), only the first steps of the above protocol were done to test the efficiency of the separation of the spikes from the sediments without performing the filtration step. The recoveries in table 3.4 marked with '1' represent the recovery of spikes from the sediments by analysis of the supernatant. As such these results correspond to the spikes retrieved from the sediments after three separations steps. These first excellent results indicated that the extraction method using the beaker seemed to be an effective approach.

For tests 2 and 3, another technique to recover the supernatant was tested in parallel of the classic supernatant pouring, the overflow approach (Mani et al. 2019). The method consists of adding an excess of density solution to induce the overflow of the supernatant, thereby drawing the floating particles out of the container. This technique also appears to be effective, but the pouring of the solution over the edges of the container means that this technique is more vulnerable to contamination.

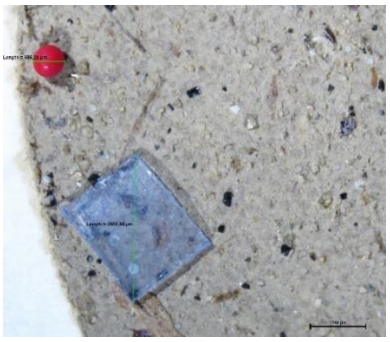
The recoveries for tests 4 and 5 were done with three beakers in parallel therefore, the total recoveries in the tables are based on the mean of the three replicates recoveries. The other tests and positive controls showed very good results with 100% spikes extracted from the sediment after three repetitions of density separation. Good final recoveries on the filter were reached for the different spikes, between 66 and 100%.

As all the particles are always extracted from the sediment and the container, the lower final recovery percentages are due to particles adhering to the edges of the filter set during the last filtration step. With the visible spikes it is possible to examine the edges of the filtration set and so to recover the totality of the particles. This last rinsing step is thus very important to ensure a maximal recovery of plastic particles and improvements will be considered in future to reduce this adherence problem (e.g. rinsing step with acetone or surfactant). Further validation could be done by performing additional recovery tests using transparent spikes. Besides that, a further reduction of inorganic particles should be aimed for to facilitate the microplastics identification. For that, elements such as a decrease in the quantity of subsample (to 50g) and an increase in the mixing/settling time can also be evaluated to achieve the best results (Figeras & gago 2020).

a

b

c



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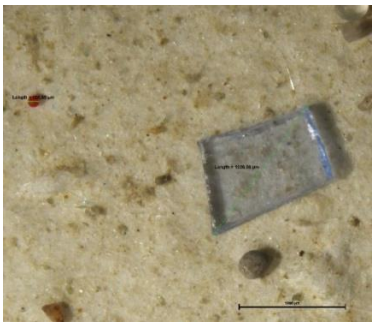
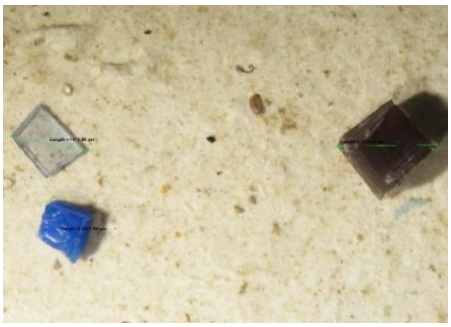


e



f

g

Figure 3.5: a: digestion of the sample by addition of H_2O_2, the reaction is exothermic & create bubbles; b-e: spikes from positive controls (b-c:MOW1, d-e:W08); f-g: subsamples of 100g in a beaker with addition of density separation solution (NaBr), plus one blank sample.

3.5. Analysis – Identification

The particles collected on the filter and placed in close petri dish, were visually examined under a stereomicroscope equipped with digital camera. The suspected particles were photographed and their characteristics (type, size, colour) were noted. The existing criteria used to suspect a particle were:

- clear and homogeneous color
- no natural structures visible (e.g. cells)

- unnatural bending
- fibers must have equal thickness throughout their length
- fibers must have no fraying at their ends

The suspected particles were then submitted to the hot needle test (HNT). This test consists of the heating of a needle until it is red hot and then bring it close to the particle. Plastic particles will bend, curl or melt at the approach of the hot needle, while non plastic particles like wool or cotton will show no reaction or will burn (Hidalgo-Ruz et al., 2012; Lusher et al. 2018, 2020; Battaglia et al., 2020; Whitaker et al., 2019). Therefore, only the particles which were positive to the hot needle test were recorded and classified by type (fiber, film, fragment…), size and colour.

As the visual characterization by itself is not fully reliable, it is advised to use a chemical analysis technique in complement. For validation, a small subset of particles was analyzed by VLIZ using micro-Fourier transform infrared spectroscopy (µFTIR, Spotlight 200i FT-IR microscope) for identification of the polymer type. Polytetrafluorethylene (PTFE) filters have been used. Spectra were captured in transmittance mode between 4000-450 cm^{-1}, with a resolution of $4cm^{-1}$.

Quantification of microplastics by manual counting is highly time-consuming, especially for samples with high number of microplastics and other remaining particles. Automated techniques are needed for better and faster analyses.

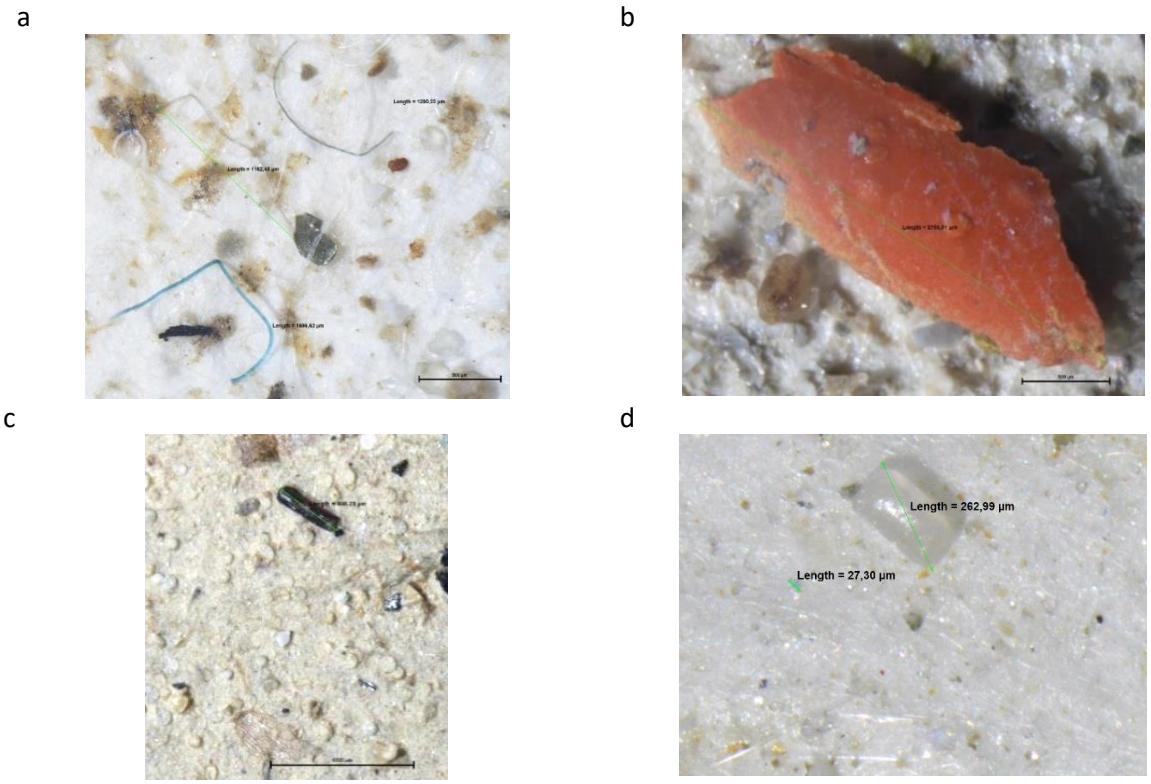


Figure 3.6: Microplastics detected in water (a-b) & in sediments (c-d).

3.6. Quality control and validation

3.6.1. Validation by µFTIR

Of the 40 particles analyzed with µFTIR by VLIZ, 2 were lost; 4 black fragments could not be identified because black particles absorb most of IR light; 8 had no clear identification, 5 were identified as non-plastic and 21 were confirmed as plastic particles. Out of the 5 particles identified as non-plastic, 3 of them were negative at the HNT (thus confirmed by FTIR) and 2 showed confusing reaction to the HNT. Thus, the analysis of these particles was important to confirm their non-plastic nature. For the 8 uncertain identifications, the highest chemical correspondence, the HNT result and the resemblance to other identified particles was examined to determine if the particle had to be taken into account as plastic or no. For the 4 unidentified black fragments, we considered them as non-natural because they were positive to the HNT and because black rubbery fragments can be present in microplastic samples and contain carbon black known to completely absorb the IR beam (Brate et al., 2020).

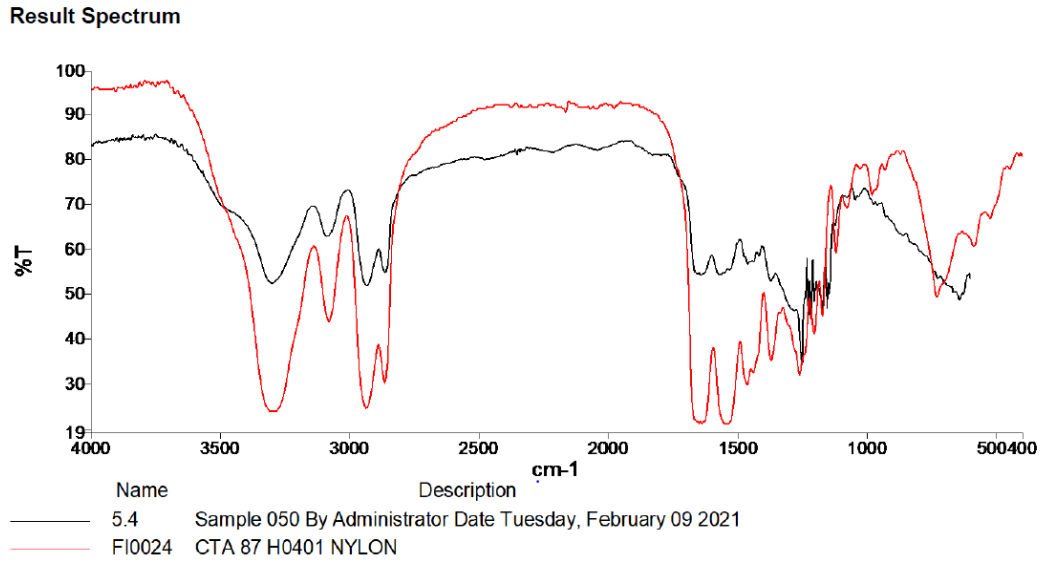


Figure 3.7: Example of a µFTIR spectrum for nylon (source: VLIZ)

3.6.2. Quality control

For quality control and contamination prevention, some measures have been taken (Frias et al., 2018; Gago et al., 2018 (Baseman protocols)):

- Use of glass and metal material
- Material decontamination: cleaning and rinsing of all material with MilliQ water
- Mitigation of cross-contamination:
    - Limiting air exposure by covering samples and equipment
    - Avoid wearing synthetic clothing
- Air contamination control: filters were placed at the side of the sampling places on the vessel to monitor airborne particles
- Contamination control: each batch of sample was accompanied by a procedure blank also called negative control, which followed the same steps as the sample but without sample

Based on the procedural blank samples, in case of a contamination, a particle average is calculated per type and colour and is subtracted from the number of microplastics observed in the corresponding samples.

3.7. Methodological protocols for microplastics in sediment in seawater

The Figure 3.8 represent an overview of the resulting general protocol applied from the sampling to the analyse. The detailed protocols are provided in annex.

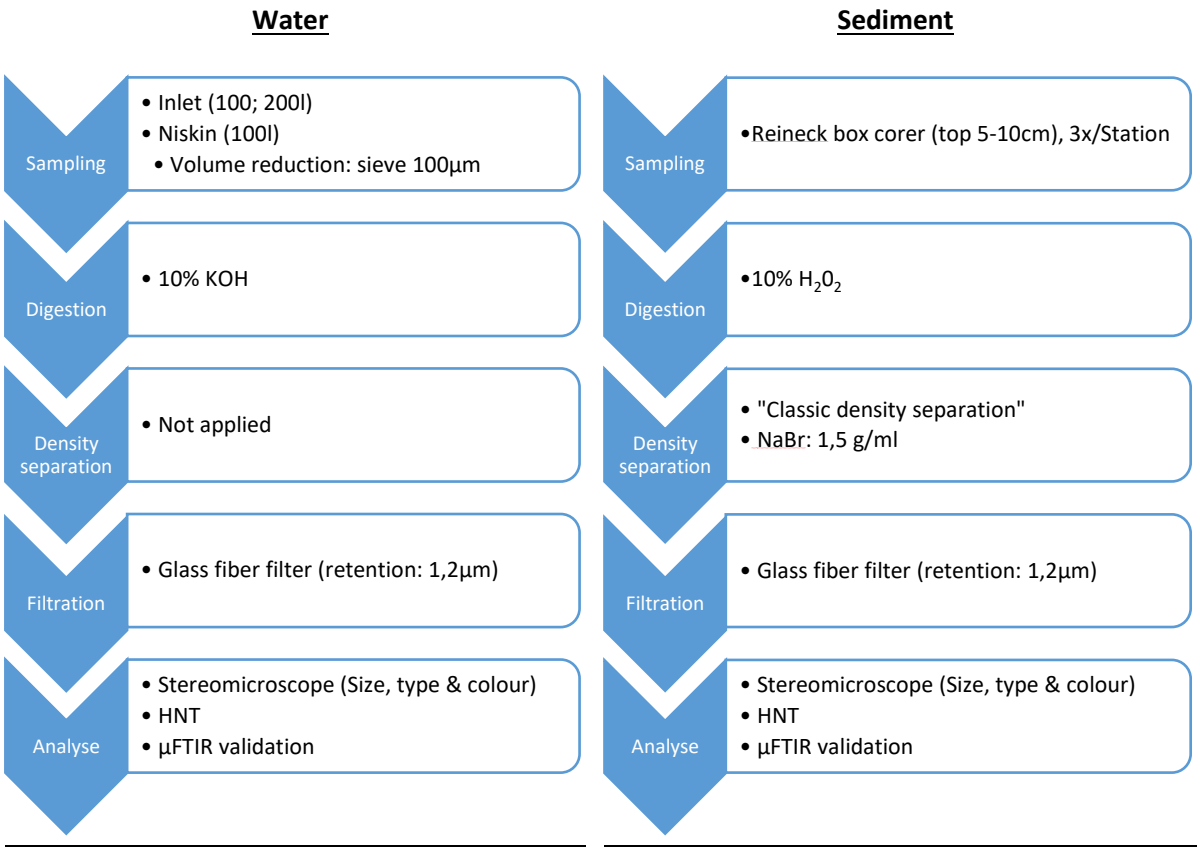


Figure 3.8: Schematic flowchart of sample processing

3.8. Results

In the following part, in accordance with the current recommendations for monitoring, the analysis of the results only considers the microplastics with a size between 100µm and 5mm. Any particle smaller than 100µm or bigger than 5mm has been excluded.

3.8.1. Microplastics in water

3.8.1.1. Microplastic particle abundance

An overview of the microplastic particle abundance is given in Figure 3.9. Table 3.5 and Figure 3.10 show the results for all seawater samples collected at five locations with 2 different sampling methods. All microplastics are reported by type category (fiber, fragment, film, microbead, foam), size (measured at largest cross-section) and colour for each sample.

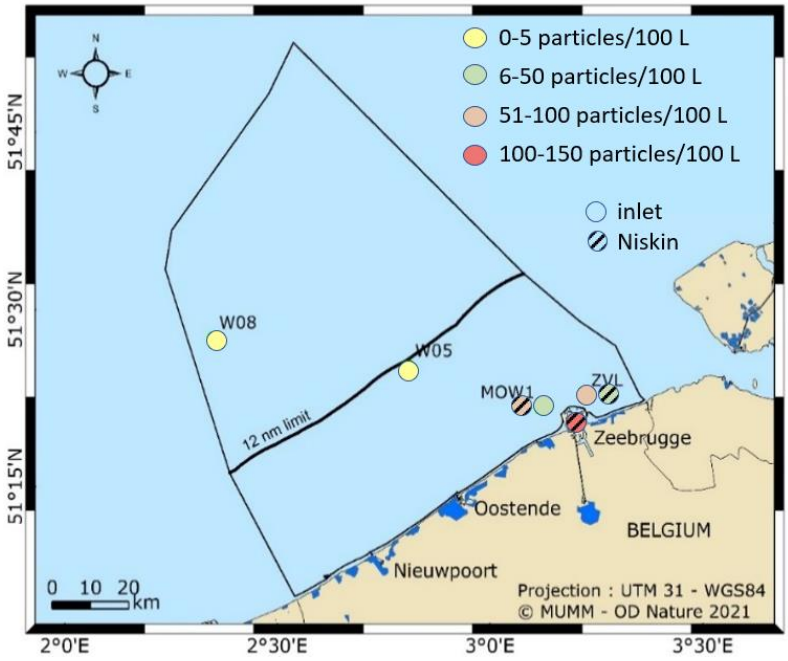


Figure 3.9: Microplastics particle number in seawater in the Belgian Part of the North Sea (BPNS)

Table 3.5: Microplastic particle number per 100 L: average, standard deviation, minimum and maximum in water via continuous vessel inlet and Niskin samples from five locations in the Belgian part of the North Sea (BPNS).

	Number of samples	Average (particles $100L^{-1}$)	Standard deviation	Minimum (particles $100L^{-1}$)	Maximum (particles $100L^{-1}$)
MOW1					
inlet	4	24,8	15,3	3	36
Niskin	1	99	0		
W05	2	3,5	2,1	2	5
W08	2	2,5	0,7	2	3
ZVL					
inlet	1	51	0		
Niskin	1	47	0		
Zeebrugge Port (Niskin)	1	147,5	0		

Twelve samples were collected by two different sampling methods on five different stations and on two cruises. Microplastics particles were found in all samples. A total of 455,5 microplastic particles were identified ranging between 2 to 147,5 particles per 100 litres.

The results show that the stations closer to the coast have higher average microplastics numbers than the stations further away (Table 3.5, Figure 3.10). The harbour, where only Niskin sampling was possible, shows the highest results with 147,5 particles per 100L followed by the ZVL dredge disposal site with 51 particles per 100L (n=1) and MOW1 station with 24,8 ± 15,3 particles per 100L (n=4) based on the vessel inlet samples. The more offshore stations (W05 & W08) show considerably lower particle values than those near the coast, respectively 3,5 ± 2,1 and 2,5 ± 0,7 particles per 100L (n=2).

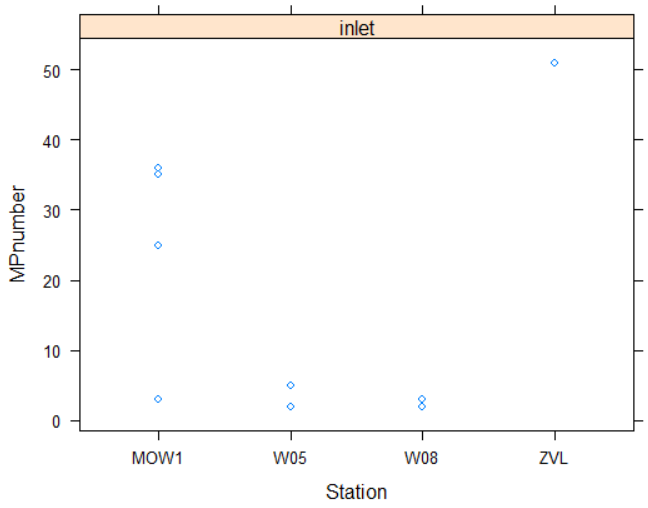


Figure 3.10: Microplastic particle number per 100 L in water samples from five locations, with the continuous vessel inlet sampling technique.

When the samples within a same station are compared (Table 3.5, Figure 3.10), a high variation can be observed at MOW1. Four inlet samples have been taken at different times in a time range of approximately 6 hours resulting in numbers ranging from 30 microplastic particles per m^3 up to 360.

A very high variability of microplastics in water of the North-East Atlantic has already been reported (Maes et al., 2017) with numbers ranging from a few ten to a few thousand in different transects. Several meteorological factors like wind and currents influence the distribution of these particles. In addition, tidal change leads to a shift of currents and a higher input of water from the Scheldt can be observed in the Belgian Part of the North Sea (http://odnature.naturalsciences.be/marine-forecasting-centre/fr/maps/surface_sea_water_velocity/bcz). Since hydrodynamic conditions are known to have major influences on floating plastics, and as the major input of microplastics to the sea is via land and rivers, it is important to take into account the currents, state of the sea and tidal state during sampling, especially for stations close to the coast as they are more impacted by terrestrial influence (Wang et al. 2018; Cole et al. 2011).

Based on these first results at MOW1, the lowest number of microplastic particles was observed during ebb. For future data analysis, these parameters should be annotated as additional meta-information in the data set.

3.8.1.2. Sampling gear comparison: continuous vessel inlet vs Niskin

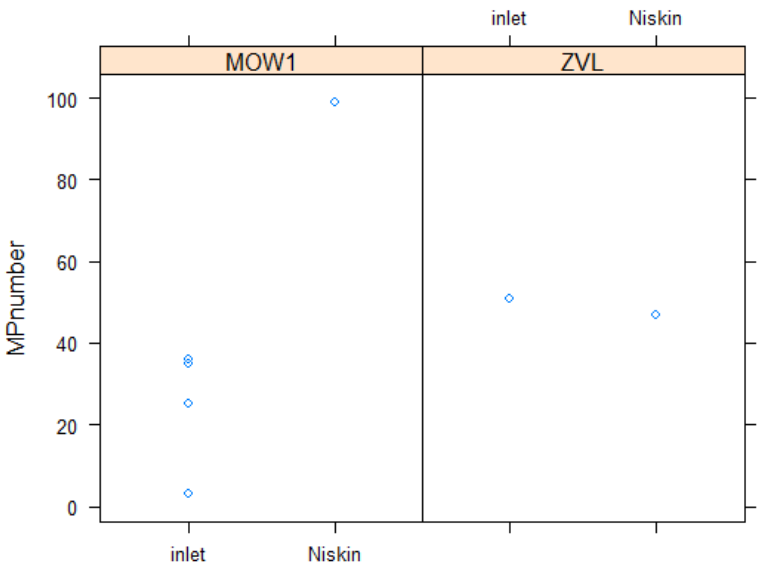


Figure 3.11: Microplastic particle number per 100 L in water samples, comparison of continuous vessel inlet and Niskin at two stations (MOW1 & ZVL).

For ZVL station, a good correspondence in microplastics number seems visible between the two sampling techniques: Niskin or inlet (Figure 3.11).

For MOW1 station, a clear difference is visible between the number of particles obtained through the inlet sampling (average of 24,8 ± 15,3 particles per 100L) and the numbers derived via Niskin sampling (99 particles per 100L) (Figure 3.11, Table 3.5). Due to the lack of replicas based on Niskin samples, no conclusion can be made with regards to the significance of this difference.

The difference seen between the two gears at MOW1 may be due to different factors such as meteorological and hydrodynamical conditions and sampling depth. There is a slight difference between the two techniques: -0.5m for the Niskin and -3m for the inlet. However, it is known that the BPNS is characterized by a thorough vertical mixing due to strong currents. In addition, more contamination is possible with the Niskin as the handling is more complicated and the sample possibly more subject to air contamination than with the inlet.

The analysis of the particle types indeed shows that many fibres are present in the Niskin sample but, in general, the distribution of particle types via inlet or Niskin appears to be comparable (Figure 3.12). The particle size distribution of Niskin and inlet do not differ (Figure 3.13) (Wilcoxon rank=16124, p-value=0.0002).

No difference in microplastics numbers seems observed between the two sampling techniques for ZVL station. The large differences at MOW1 can probably be explained by the known large variability in the concentration of microplastics at the water surface (Maes et al. 2017). To gain more insight in the temporal variation in the Belgian Part of the North Sea and the comparability of both sampling approaches, more data is required.

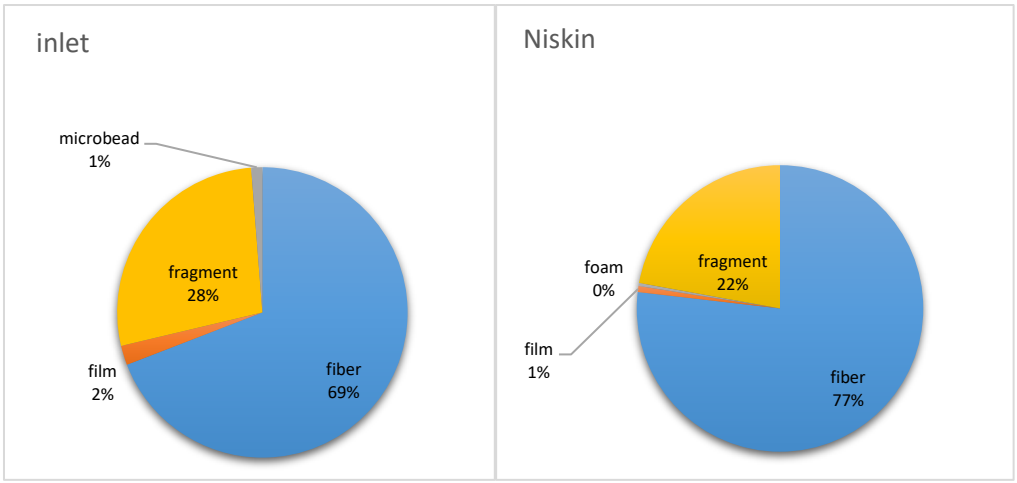


Figure 3.12: Microplastic particle type distribution (percentage) in function of the sampling gear: continuous vessel inlet (left) or Niskin.(right)

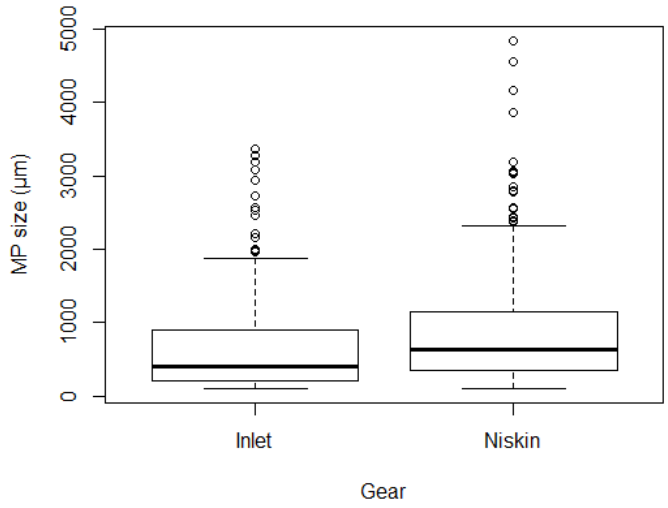


Figure3.13: Size of the microplastic particles in µm in function of the sampling gear: continuous vessel inlet or Niskin.

3.8.1.3. Particle characteristics (types; size; colour)

Five different types of particles (fibers, fragments, film, microbead and foam) were encountered with the vast majority being fibers (74,1%) or fragments (24%) (Figure 3.14).

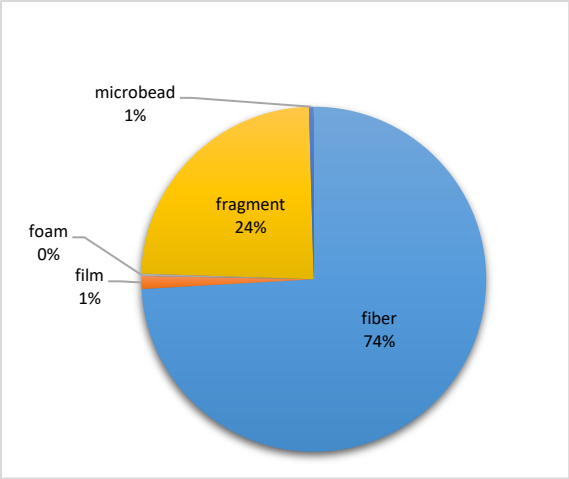


Figure 3.14: Microplastic particle type distribution (percentage) in water samples.

The particles were, more or less evenly, classified into different size classes: 100-350µm (26%), 350-1000 (43%) and 1-5mm (31%) (see annex 0).

Of all the colours observed, blue (25,7%) followed by black (22,8%), transparent (16,3%) and red (15,3%) were most abundant (see annex 0).

The particle characteristics size and type are more important to consider than the colour. Colour is subject to operator interpretation. The type and size are more important for assessment of potential impact on the fauna. Indeed, the thin size of the fibers make them accessible to a higher number of organisms (Wang et al. 2018; Frias et al. 2010).

3.8.1.4. Conclusion & comparison with other studies

Based on the current dataset of the continuous vessel inlet pump samples, microplastic particle abundance in subsurface water of the BPNS ranges between 20 and 510 particles per m^3. Microplastics were encountered in every sample, even at a distance of around 40 km from the coast. While considering the coastal/offshore difference (inlet samples), the coastal stations (MOW1 & ZVL) show a higher average, 300 ± 177 particles/m^3, than the offshore stations (W05 & W08) 30 ± 14 particles/m^3. The repeated measurements at MOW1 show a large variation which is typical for microplastics in water. They are highly mobile and hence influenced by meteorological and hydrodynamical conditions.

At first sight, these results seem higher than the amounts detected in previous studies (Table 3.6). But in most of these studies, a different sampling technique - manta trawl or plankton net – was used. These sampling techniques collect particles closer to and on the water surface and more importantly use different mesh sizes. As Lindeque et al. 2020 demonstrated, the microplastic abundance in the marine environment is underestimated when the sampling is done with nets of larger mesh-size: a net of 100µm finds 2.5 more microplastics than a net of 333µm. The numbers derived via the net with mesh size of 100 µm by Lorenz (2019) are closer to the values obtained in this study. Their results for locations close to the BPNS (at a distance of 8 to 8 km from the coast) range between 0-10 slightly lower than the results obtained for W05 and W08.

No standardized approach exists yet at the EU or international level. As a consequence, every study has its own specificities and comparison between them is difficult.

Table 3.6: Comparison of the current results with results from research in the same region or with the same technique (continuous vessel inlet pump).

Microplastic particles/m³ (min-max or average ± sd)	Location	Specificities	Sources
20 - 510 180 ± 190 Coastal: 30-510 ; 300 ± 177 Offshore: 20-50 ; 30 ± 14	Belgian Part of the North Sea (BPNS)	Continuous vessel inlet pump (at -3m), 100µm	Current study
0 - 3,5	North Sea	Manta trawl, 300µm	Mintenig et al. 2014
0 -1,5	North-East Atlantic	Manta trawl, 300µm	Maes et al. 2017
0,1 245,4	Southern North Sea	Neuston net, 100µm	Lorenz et al. 2019
0.24 – 8.9	Close to & in BPNS		Lorenz et al. 2019 (Suppl.)
0 - 22,5 2,46 ± 2,43	Offshore Ireland	Continuous vessel inlet pump (at -3m), 200µm	Lusher et al. 2014
0 - 11,5 2,68 ± 2,95	Arctic waters, south & south-ouest of Svalbard	Continuous vessel inlet pump (at -6m), 200µm	Lusher et al. 2015
279 ± 178	Offshore NE Pacific	Continuous vessel inlet pump (at -4,5m), 62,5µm	Desforges et al. 2014
1710 ± 1110	Coastal Vancouver Island	Continuous vessel inlet pump (at -4,5m), 62,5µm	Desforges et al. 2014

3.8.2. Microplastic in sediments

3.8.2.1. Microplastic particle abundance

An overview of the microplastic particle abundance is given in Figure 3.15. Table 3.7 shows the results for all sediment samples collected at two locations.

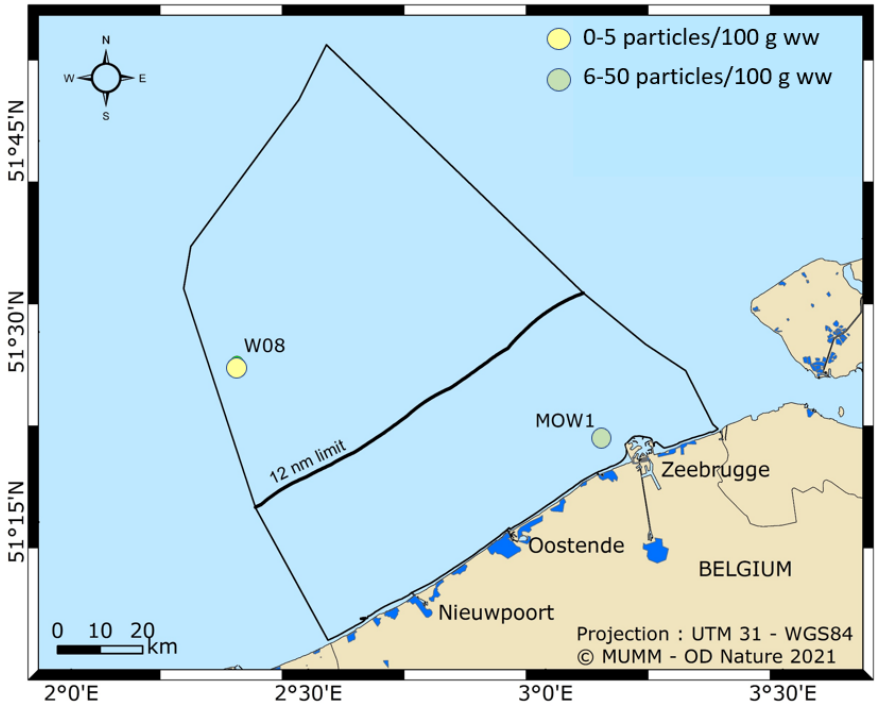


Figure 3.15: Microplastics particle number in sediment in the Belgian Part of the North Sea (BPNS).

Due to time constraints a selection of samples has been analysed based on the coastal-offshore gradient with station MOW1 representing the monitoring station close to Zeebrugge and the Scheldt Estuary and W08 an offshore monitoring station.

Table 3.7: Microplastic particle number per 100g ww (wet weight), average, standard deviation (sd), minimum/maximum. Microplastic particle concentration per 1kg dw (dry weight = % of ww). Total organic carbon (TOC%) and silt percentage in sediment samples from two locations in the Belgian part of the North Sea (BPNS).

	Average (particles/100g ww)	Standard deviation (sd)	Minimum (particles/ 100gww)	Maximum (particles/ 100gww)	Average (particles/kg dw) ± sd	TOC %	Silt %
MOW1 sed1	9	3	6	12	114,5 ± 38,2	0,182	1,43
MOW1 sed2	8	1	7	9	101,7 ± 12,7	0,092	0
MOW1 sed3	26	6,9	22	34	330,7 ± 88,1	0,185	5,13
Total MOW1	**14,33**	**10,12**			**182,2 ± 128,7**		
W08 sed1	1,3	1,5	0	3	16,6 ± 19,1	0,045	0
W08 sed2	1,7	1,2	1	3	20,8 ± 14,4	0,031	0
W08 sed3	2	2	0	4	25 ± 25	0,031	0
Total W08	**1,67**	**0,33**			**20,8 ± 4,2**		

Six samples (3 core replicates per station: 'sed1', 'sed2','sed3') were collected on two different stations during two cruises. For each core sample, 3 subsamples of 100 g were analysed after homogenisation. Microplastics particles were found in 89% of the subsamples (16 out of 18). A total of 144 microplastic particles were identified ranging between 0 to 34 particles per 100g ww (Figure 3.17).

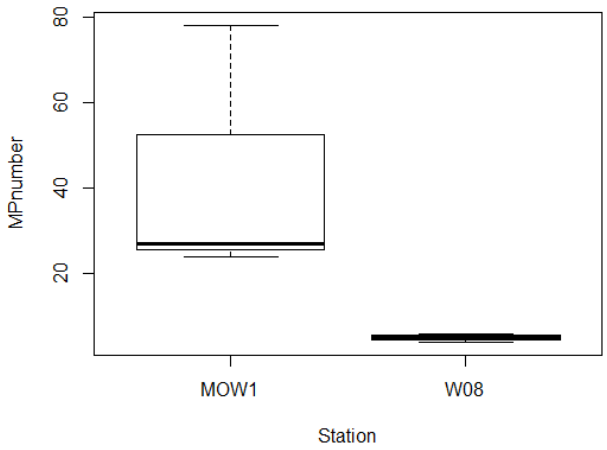


Figure 3.16: Microplastic particle number per 300g wet weight in sediment samples (n=3) from two locations.

Figure 3.16 shows the number of microplastics for the total amount of sediment analysed (300 g ww of the 3 subsamples) at the two stations. The results from sediment samples show that MOW1 station, close to the coast, has higher microplastics values (14,33 ± 10,12 particles/100g ww) than the W08 offshore station (1,67 ± 0,33 particles/100g ww) (Table 3.7). Despite the small number of samples to compare (3 core replicates), this coastal/offshore difference is confirmed with a confidence of 90% (Wilcoxon rank=9, p-value=0.1 = minimum value possible for 3 replicates). This coastal/offshore difference was expected due to the proximity of human influence for MOW1 station.

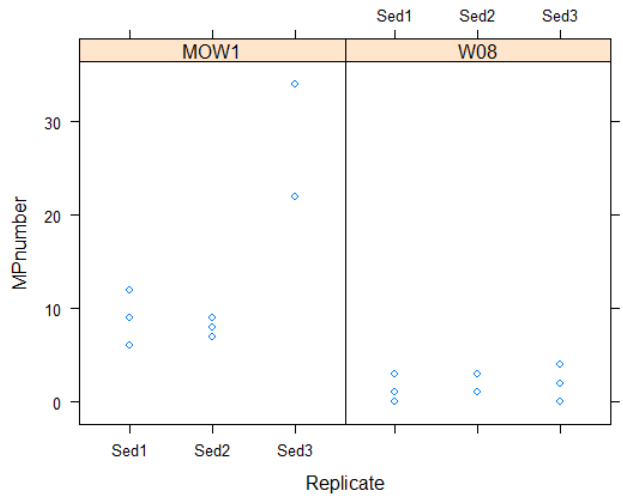


Figure 3.17: Microplastic particle number per 100g ww in sediment samples from two locations.

When the three core replicates (sed1, sed2 & sed3) within a same station are compared, variation is seen between replicates. With an average of 14,3 particles per 100g ww and a standard deviation of ± 10,12, the replicates from MOW1 shows an important variation (Figure 3.17, Table 3.7). Because of the small microplastic numbers found, it is difficult to draw conclusions for W08 samples. While concentrations of microplastic abundance in sediment are expected to vary to some extent, part of this variation could be due to differences in sediment composition between the samples at a given location. When considering the sedimentological characteristics (Figure 3.18), it can be noted that e.g. the amount of sediments smaller than 63 µm differs between the different cores taken at MOW1.

For their part, the results for the three subsamples of 100g taken after homogenisation within a core show a good correspondance. This correspondence is a good indication for repeatibility of the method used.

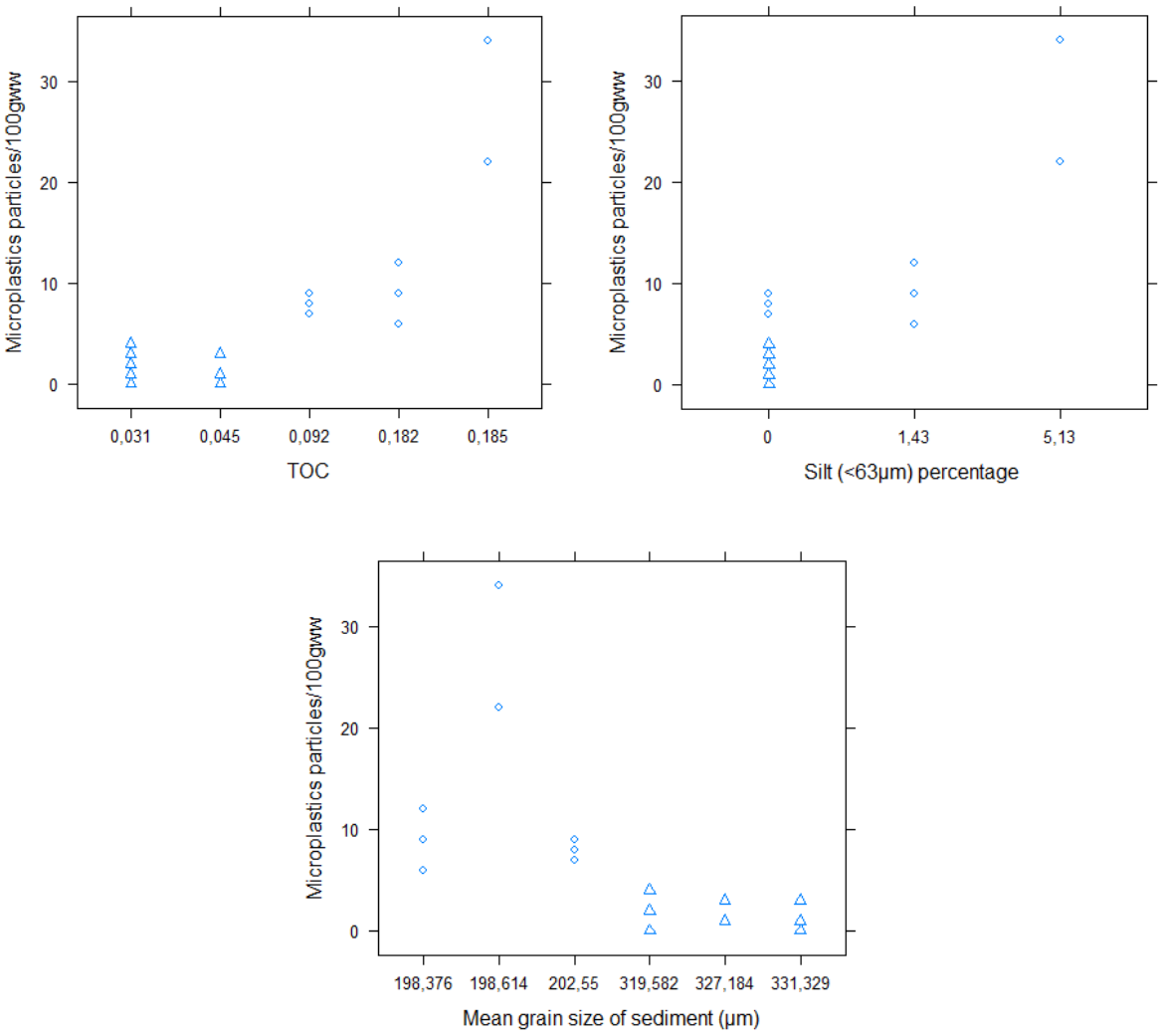


Figure 3.18: Microplastic abundance in sediments in function of sediment characteristics: Total Organic Carbon (TOC%); silt percentage (<63µm) & mean grain size (µm); at two locations: MOW1=circles; W08=triangles.

Based on large amount of data, Maes et al. 2017, found that the microplastic abundance is positively correlated with the total organic carbon and the silt percentage of the sediment, and negatively correlated with the mean grain size of the sediment. And indeed, these first results (Figure 3.18) are in

line with the former studies (Maes et al. 2017; Wang et al. 2018) that showed that more plastic particles were found when the TOC was higher and at locations with smaller average grain size. Grainsize of the seabed being influenced by hydrodynamic forces, Wang et al. 2018 highlighted the importance of the hydrodynamic effect in the distribution and migration of microplastics in intertidal zones. For our actual results, the variation of organic matter content and mud percentage in MOW1 samples appear to explain the high variation in microplastic abundance between the different cores replicates.

3.8.2.2. Particles characteristics (types; size; colour)

Three different types of particles (fibers, fragments and microbead) were encountered with the vast majority being fragments (60%) followed by fibers (39%). Only one microbead was found and no film or foam was found in the sediment samples. The distribution of the microplastic shapes in sediment appears to be different than in the water matrix where the particle types are dominated by fibers (Figure 3.19). This difference between the two matrixes is also consistent with results found in other studies (Leslie et al., 2017; Ryckesbusch 2017-2018; Lorenz et al., 2019). On the opposite, Maes et al. 2017 reports mainly fibers in the sediments and mainly fragments at the sea surface.

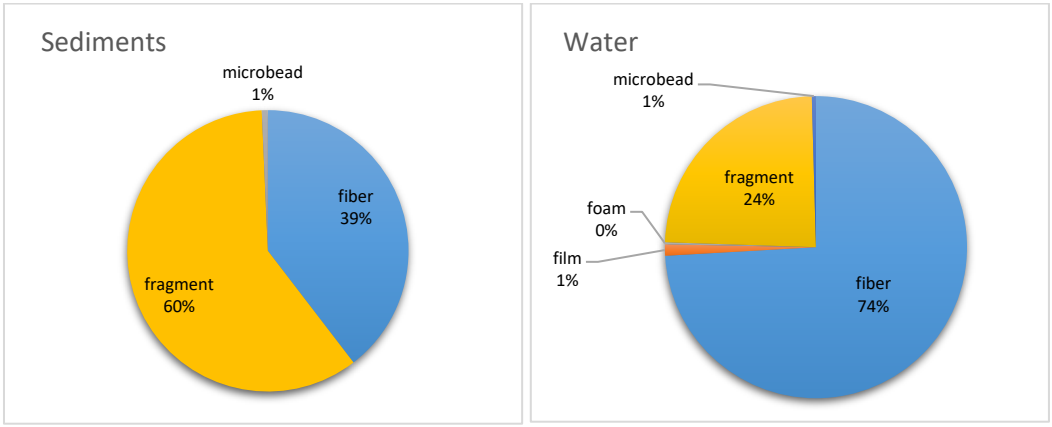


Figure 3.19: Microplastic types in percentage in sediment samples (left) and in water samples (right).

The particles were classified into different size classes: 100-350µm (61%), 350-1000 (19%) and 1-5mm (19%) (Figure 3.20: Microplastics size classes repartition (left) and size in function of the type (right) in sediment samples.Figure 3.20). Microplastics in sediments show a higher abundance of small size microplastics. This result is expected as fragments show smaller general size than fibers that are generally very thin but measured by their longest dimension. This difference in size is also consistent

with results found in another study where they report larger microplastic in water samples than in sediment samples (Wang et al. 2018).

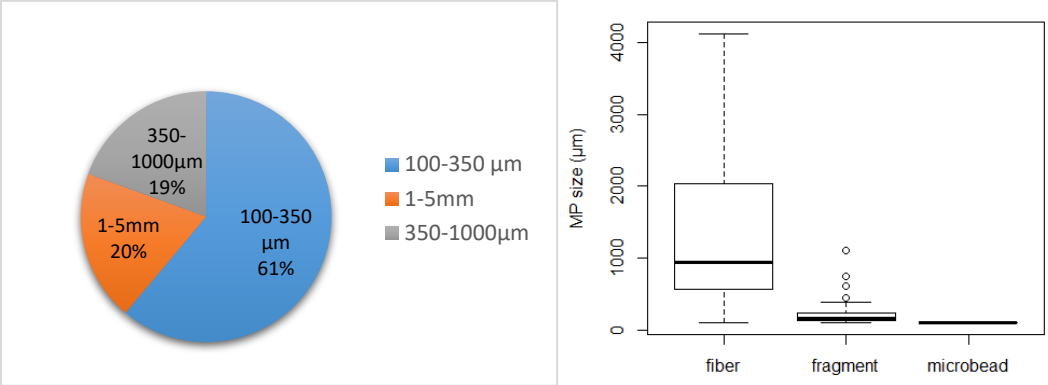


Figure 3.20: Microplastics size classes repartition (left) and size in function of the type (right) in sediment samples.

Of all the colours observed, black (51%) followed by transparent (30%), blue (6%), and red (7%) were the most present (see annex 0). The colour found in sediments are the same as in the water matrix but in different proportions.

3.8.2.3. Conclusion & comparison with other studies

To conclude, microplastic concentration in sediments from the BPNS is within a range of 20,8 ± 4,2 offshore to 182,2 ± 128,7 particles/kg dry weight near the coast. Concentrations are higher in the coastal station MOW1 and these first results, show similarly to other studies, a higher number in samples where the sediment fine fraction and organic matter is higher.

Table 3.8 provides an overview of the results of other studies in the Belgian and Dutch part of the North Sea. As can be noticed from the table, every study uses another methodology with differences in e.g. density separation solution and mesh size of sieve used, both influencing the number of particles retrieved and hampering a proper comparison between locations. Nevertheless, our results are in the same range with those of previous studies in the BPNS.

Table 3.8: Comparison of the current results with results from research in the same region

Microplastics in particles/kg dw (range or average ± sd)	Location	Specificities	Sources
16,6 - 330,7 Coastal: 182,2 ± 128,7 Offshore: 20,8 ± 4,2	Belgian Part of the North Sea (BPNS)	NaBr; mesh size of sieve:63µm; reported particles: 100µm-5mm; upper 5-10 cm	Current study
97,2 ± 18,6	Belgian Part of the North Sea (BPNS)	NaCl; 38µm-1mm,	Claessens et al. 2011
54 – 330	Belgian Part of the North Sea (BPNS)	NaCl; upper 5cm	Maes et al. 2017
8,3 - 99,5 39,4 ± 16,7	Belgian Part of the North Sea (BPNS)	NaI, upper 4-10cm; Mesh size of sieve: 32 µm	Ryckebusch, 2017-2018
100 - 3600	Dutch North Sea Coast	NaCl; 10µm-5mm	Leslie et al., 2017

234,5 ± 254,3 2,8 1188,8 22,2 – 106,5	Southern North Sea Close to & in BPNS	$ZnCl_2$; Mesh size of sieve: 20 µm; reported particles:11 µm- 5mm ; upper 5cm; MPSS	Lorenz et al. 2019 Lorenz et al. 2019 (suppl.)

3.9. Conclusion

This study allowed to elaborate a methodological approach that can be applied for future long-term monitoring in line with European requirements.

Microplastic sampling of seawater via the continuous vessel inlet pump proved to be practical and easy to combine within existing multidisciplinary monitoring cruises. It allows to record sampled volume accurately and did not pose constraints caused by the state of the sea. Lusher (2014) did not observe significant effects of vessel speed and sea state on the number of particles collected. A first comparison with Niskin sampling showed similarities in particle types and sizes while for total abundance of microplastics this was the case for one of the two stations compared.

The retrieval of microplastics out of bulk test sediment samples using the MPSS was not satisfactory. Different tests have been performed with several adaptions aimed to increase the recovery. Tests according to the classic density separation method yielded better results and consequently this method was applied to the actual field samples. Since this method does not allow to process large quantities of sediment simultaneously, three times an amount of 100 g of wet sediment originating from one homogenised sediment core sample was analysed. The first results of the triplicate analyses are closely related and confirm the feasibility and repeatability of the chosen approach.

The resulting dataset of microplastics abundance in seawater and sediment provides useful baseline information.

In both matrixes, coastal locations showed higher concentrations of microplastic particles than offshore locations. In seawater, this concentration was around 10x higher for the average of coastal inlet samples (300 ± 177 particles/m^3), compared to offshore (30 ± 14 particles/m^3). The highest number was found near the sludge disposal site, east of Zeebrugge. In sediment, the number of particles at MOW1 was about 9 times more than at W08. These results were expected as the levels of microplastics are known to be correlated with the anthropogenic activities (Wang et al. 2018, Cole et al.2011).

Different distributions of particles shapes are seen in both compartments. In sediments, more fragments were found, whereas fibers were dominant in water.

Finally, a high variability of microplastic numbers is notifiable between replicates at a same station in both compartments. This variability seems to be correlated to environmental characteristics (hydrodynamic, organic matter, …). For the water matrix, the highlight is on the known variability of microplastics related to water mass sampling. This variability could be due to temporal aspects linked to natural factors like tide, wind and currents. More data would be needed to evaluate the possible effects of these factors and how these could lead to recommendation for sampling conditions and timing. For the sediment matrix, the spatial heterogeneity of sediment composition can explain part of the variability and confirms the need of sampling replicates to correctly characterise a location. Sediments with high organic carbon content are possible hotspots and more focus should be put on

these areas (Maes et al.2017). For further data interpretation it is important to log the related meteorological, hydrodynamical and sedimentological parameters as meta-information.

While the monitoring of both matrices, surface water and sediment, is required according to the MSFD specifications, it must be noted that concentrations in seawater are lower and more mobile requiring high volumes of seawater to be sampled (Maes, 2017). Since the microplastics tend to accumulate in sediment (Kukulka et al., 2012; Woodall et al., 2014), the seabed sediment appears to be the priority matrix for long-term monitoring.

3.10. Next Steps and recommendations

More information on the spatial distribution of microplastics in sediment of the Belgian Part of the North Sea needs to be gathered. On short term the analysis of samples taken at W05 and at ZVL is foreseen. In addition, more locations need to be considered in order to take into account point sources (e.g. Nieuwpoort, east of Zeebrugge). A proper selection of regular monitoring sites should be made taking into account that floating and settled microplastic particles are more abundant in coastal areas and estuaries. These locations need to be sampled on an annual basis at a minimum to allow for trend analysis. Since microplastics in general tend to accumulate in sediment and less variation of the concentration is observed compared to water, the follow-up of concentration in sediment is highly recommended for long term monitoring. Hereby the focus should be put on muddy areas since hotspots are most likely encountered over there. Besides the determination of microplastic abundance, the analysis of sedimentological characteristics is important.

The applied methodology will be further validated and developed. Based on the current results, it is expected that higher volumes of seawater could be sampled and analysed, especially offshore, to be more representative. Additional tests including the use of different types of spikes, are recommended to have a more accurate assessment of the extraction of microplastics out of sediment. In addition, a more automated way for the microplastics identification is recommended, ideally including the polymer identification. When microplastic sizes and numbers need to be registered, µFTIR and µRAMAN are approaches that can be considered, with LOD of approximately 10-25 µm down to 0.5 µm respectively (Bessa et al., 2019; Primpke, 2020). Automated approaches can be based on particle detection algorithms or chemical imaging of the whole filter. Other techniques, like pyrolysis-GC-MS, are able to provide information on polymer composition (incl. additives) and masses but are generally performed and/or recommended to be performed in a second step as these methods are destructive and do not provide information on numbers and sizes (Frias et al. 2018; Primpke, 2020).

Methodological improvements as well as harmonisation and standardization work need to be closely followed. The EU Horizon 2020 project EUROqCHARM is expected to provide valuable information as well as material to evaluate the quality of the analyses (a.o. interlaboratory exercises, reference material). Indicator development is ongoing at the regional level for microplastics in sediment.

Transport behaviour and hotspot identification of microplastics within the BPNS could be achieved by coupling field data in sediments and in the water column (over seasons and tide) with models able to simulate the transport and distribution of microplastic particles.

4. WP4. Data management

The gathered data need to be compiled and archived in a consistent way to enable future usage and exchange in national and international context. Marine contaminant data over several decades is stored in the central database of the Belgian Marine Data Centre (OD Nature-RBINS) and has been reported yearly to ICES for import in the DOME, the Marine Environment Database and use in a regional context. The data concerning litter in the marine environment, can be integrated in the same structure, after some modifications for specific data characteristics. Figure 4.1 shows the simplified scheme of the core structure with several levels common for different datatypes. The same approach is taken by ICES (Figure 4.1). Samples are described using detailed information on location, sampling device, time, amount of material sampled and sampling depth.

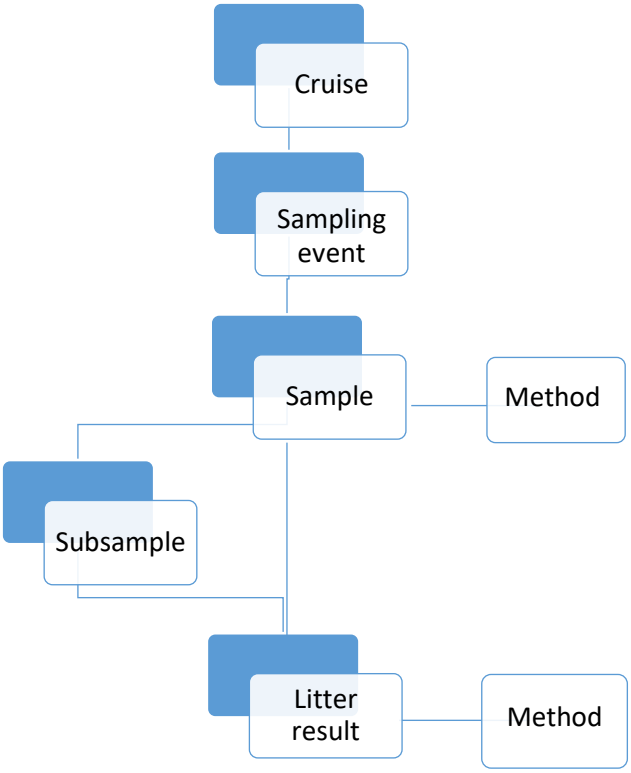


Figure 4.1: General simplified structure for litter data

The required meta information depends on the environmental matrix sampled. For biota and sediment, the notion of a subsample is introduced to describe respectively the size and/or weight of individual(s) or sediment slice being analysed. The importance of methodological information cannot be underestimated. In the case of microplastics factors like the mesh size or the density separation solution used determine which particles are retrieved and analysed, and as such the final microplastic densities reported. Also, the equipment used for separation, the digestion method (solution, mesh size of sieve used for rinsing) and the order of the sample pretreatment steps need to be registered (first digestion followed by density separation or the other way)

Besides the regional context, data in IDOD are also made available in European data infrastructures like EMODnet Chemistry. Direct exchange between ICES and EMODnet Chemistry is already established for some datasets. While many data initiatives exist, for microplastics the formats are not yet well established. Few data so far are ingested in these structures and some amendments are to be expected. For example, the controlled reference lists related to the methodology will need to be expanded. Both, the ICES- and the EMODnet Chemistry[3] proposed data formats have been consulted. Some items for further considerations have been shared with the OSPAR expert group on microplastics with the aim to specify common requirements for data management regarding microplastics in sediment. Consequently, the update of the IDOD database at RBINS has not yet taken place. Instead, the data collected have been stored in excel following a detailed data template (see 4.2).

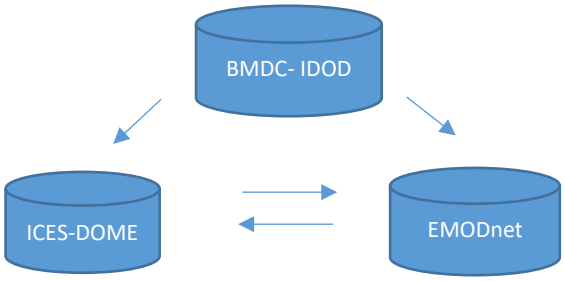


Figure 4.2: data exchange between national, regional and EU data repositories

4.1. Existing data formats

4.1.1. ICES DOME database

ICES has 2 databases containing litter data. Data collected during fishery trawl surveys are stored in DATRAS, while data collected during environmental monitoring cruises are stored in DOME, the Marine Environment Database. The amount of microlitter data is currently very limited in DOME, but more datasets are expected. Micro-, meso- and macrolitter can be submitted in a hierarchical (Environment Reporting Format version 3.2. multi-record (ICES, 2019) or a simplified format (excel-based tables). The format is used for litter on the seafloor, in the water column and on the water surface or on a beach (excluding the OSPAR beach monitoring data).

[3] https://www.emodnet-chemistry.eu/marinelitter

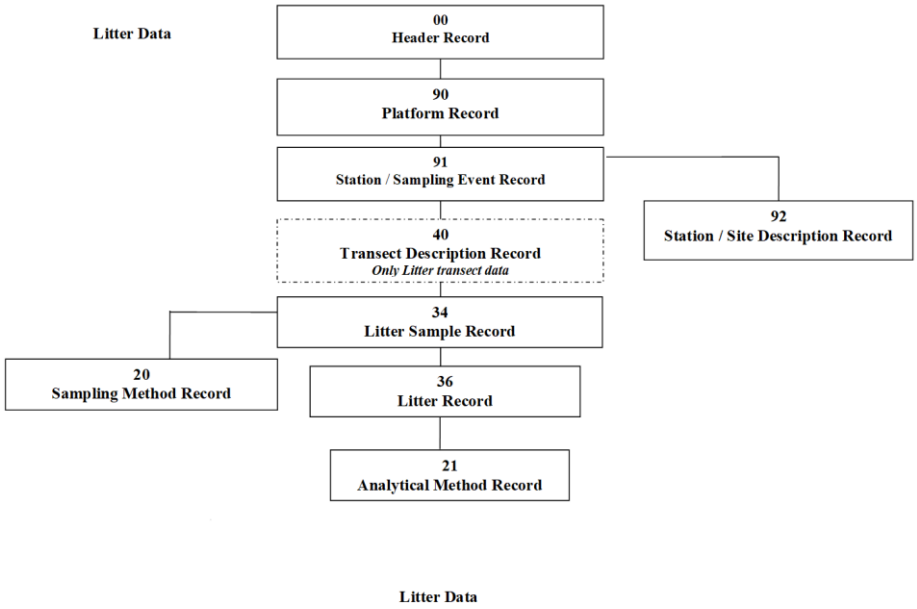


Figure 4.3: Hierarchical structure for litter data in the ICES DOME database

An online data screening utility provides automated data quality control (formats, vocabularies, logical errors, and specific requirements). Additional quality assurance is provided by the ICES Working Group on Marine Litter (WGML). The ICES vocabulary server provides controlled references. Vocabularies exist for general common fields like laboratories, ships, stations and monitoring programmes.

The sample record ('RECID34') describes the litter sample (and biological community sample). It contains a.o. fields for:

- the number of subsamples combined if the sample is an aggregation of several hauls, sediment cores or grabs.
- the volume of the sample.

Litter record 'RECID36' describes the litter data, with fields dedicated to the portion of the sample analysed:

- Subsample identification to link to individual specimen or pool of same species or sediment grab portion or sediment slice,
- Matrix describes a.o. the portion of sediment analysed, eg 'sediment untreated total' or the tissue analysed e.g. stomach,
- Minimum and maximum depth of sample.

And litter related fields*:*

- Parameters are derived from reference list for litter parameters with a.o. UNEP lists for macro litter and OSPAR beach litter categories. For micro litter entries exist in:
    - TG Litter Reference containing some codes for microplastics (e.g. disks pellets < 5mm, cylindrical pellets < 5mm, filament < 5mm, …)
    - RECO-LT list, an open-ended ICES litter list starting with 'LT', for microlitter starting from code 'LT239 <5 mm fragment'. Codes exist for some shapes and combination of shapes: fragment, fiber and particle;

- Value to register the number of litter items ('0' to be entered when no litter found in a sample),
- Type of polymer, an open-ended list,
- Litter size category as optional field with entries for different size categories e.g. every 100 µm up to 500µm,
- Litter properties, an open-ended list containing a list of codes for colours and shapes (e.g. fiber, fragment, pellet, film, sphere). These codes can be attached, so it can be reported for example with or without colour.

The simplified format for litter (excel-based) contains the dedicated litter-related fields. But in case, other analyses, like contaminant concentrations, are performed on the same subsample, the litter data must be entered together with the other parameters in the "Simplified Format for Contaminants & Microlitter" where the litter-related fields are not available. In this case parameters codes can be requested to ICES that may include any combination of the litter field options. This will lead to a growing list with entries for any combination of shape, size category and polymer type. Especially when reporting in the extended format, where the same information would need to be recorded in different fields, which may lead to inconsistencies during data encoding.

4.1.2. EMODnet data structure

EMODnet is a long-term EU initiative of DG MARE. EMODNET Chemistry has developed a pan European Marine Litter Database. The database contains data of beach and sea floor litter from a variety of sources, including existing International and Regional Sea Conventions, and data submitted by EU Member States, EMODnet partners and external research or monitoring projects. For beach litter and seafloor litter the format is based on OSPAR and ICES (DATRAS), for microlitter the format is based on the SeaDataNet/EMODnet data formats. A first proposal of guidelines and formats has only been created in spring 2021 (M. Vinci, 2021).

Litter fields include:

- Minimum and maximum sampling depth,
- Sampling effort to register the amount of sampled sediment. It can be the survey area in m^2, the wet weight (ww) of the sample in kg, the dry weight (dw) of sediment in kg or the volume in litres,
- Microlitter type with entries for generic (microplastic items of non-plastic man-made microparticles) or specific types (microplastic pellets, filaments, films, styrofoam, non-plastic filaments (natural fibres, rubber), …),
- Microlitter count: the number of items collected (dimensionless),
- Microlitter weight as additional/optional field,
- Width of sample collector for seawater,
- Size of sample collector (mesh size) for seawater,
- Optional fields include shape (e.g. rounded, subrounded, angular, flat, cylindrical, discoid, …), colour (eg black/grey, blue/green, brown/tan, white/cream, yellow, orange/pink/red, multicolour, colourless, baseman others), transparency ('transparent/translucent' and 'opaque') and polymer type,
- % sand, clay, silt, total organic carbon and water content are foreseen as additional/optional fields,
- Additional fields to allow for comparability:
    - % of water is necessary for the reporting in wet/dry weight of sediment

- in case of sampled volume, dry weight of the sampled volume should be provided
- in case of sampled area, the sampled volume and weight might be provided to allow comparability.

EMODnet makes use of controlled reference vocabularies available at https://vocab.nerc.ac.uk/collection / or https://vocab.seadatanet.org/search. Additional terms have been included to describe micro-litter characteristics (the so-called H vocabularies).

4.1.3. Conclusions

The existing formats take into account the general sampling characteristics. While the amount of material sampled is foreseen in either volume or weight, not necessarily the total amount is analysed for microplastics. Often several smaller subsamples are taken after homogenisation for the analysis (e.g. 3 x 25g; 3 x100g up to a total of 1 kg). A field to report the total weight of sediment that has been analysed is recommended and even mandatory if the individual particles, and not the number per kg, are being reported.

Litter size provides important information related to lower size targeted during the analysis and should be mandatory information in addition to information on mesh size of sieve if used in the methodological records.

Code lists, especially related to methodology are not yet complete for microplastics and differ between the two formats e.g. shape of the microplastic particle.

An additional consideration involves the annotation of potential polymer identifications based on FTIR or RAMAN.

With regards to meta information, storage of grain size and amount of organic matter for microplastics in sediment, as well as sea state, and preferably also tide and currents are recommended.

4.2. Project data template & datasets

Based on the existing IDOD-structure and templates, and the ICES format for litter, a data template for usage within the project has been established. For every particle identified as microplastic, colour, size and shape has been recorded. In addition, the photo ID has been noted for internal usage. Code lists of ICES have been used as basis and new categories have been defined where these do not correspond to the commonly used categories that are being put forward in the draft guidelines (e.g. size categories).

	Sample ID	Sub-sample ID	Replicate nr	upper-depth (cm)	lower-depth (cm)	Parameter code	Value	Unit	Type	Colour	Analysis date (dd/mon/yyyy)	Project Acronym	Photo ID	µFTIR filte
2	2020-04 MOW1 Sed1	1		0	10	LitterDiameter-SPM	2366	µm	fiber	transparent	07/12/2020;	EFMZV-MarinePlastics	Captured4	µFTIR 6.1
3	2020-04 MOW1 Sed1	1		0	10	LitterDiameter-SPM	201	µm	fragment	white	07/12/2020;	EFMZV-MarinePlastics	Captured4 bis	µFTIR 6.2
4	2020-04 MOW1 Sed1	1		0	10	LitterDiameter-SPM	2605	µm	fiber	red	07/12/2020;	EFMZV-MarinePlastics	Captured14	
5	2020-04 MOW1 Sed1	2		0	10	LitterDiameter-SPM	747	µm	fiber	blue	08/12/2020;	EFMZV-MarinePlastics	Captured4	
6	2020-04 MOW1 Sed1	2		0	10	LitterDiameter-SPM	1109	µm	fiber	other (brown)	08/12/2020;	EFMZV-MarinePlastics	Captured28	
7	2020-04 MOW1 Sed1	3		0	10	LitterDiameter-SPM	218	µm	fragment	other (pink)	09/12/2020;	EFMZV-MarinePlastics	Captured3	
8	2020-04 MOW1 Sed1	3		0	10	LitterDiameter-SPM	620	µm	fiber	black	09/12/2020;	EFMZV-MarinePlastics	Captured4	
9	2020-04 MOW1 Sed1	3		0	10	LitterDiameter-SPM	603	µm	fragment	other (brown)	09/12/2020;	EFMZV-MarinePlastics	Captured5	
10	2020-04 MOW1 Sed2	1		0	10	LitterDiameter-SPM	913	µm	fiber	black	14/12/2020;	EFMZV-MarinePlastics	Captured19	
11	2020-04 MOW1 Sed2	1		0	10	LitterDiameter-SPM	384	µm	fiber	blue	14/12/2020;	EFMZV-MarinePlastics	Captured21	
12	2020-04 MOW1 Sed2	1		0	10	LitterDiameter-SPM	1071	µm	fiber	red	14/12/2020;	EFMZV-MarinePlastics	Captured33	
13	2020-04 MOW1 Sed2	2		0	10	LitterDiameter-SPM	153	µm	fragment	black	15/12/2020;	EFMZV-MarinePlastics	Captured7	µFTIR 7.1
14	2020-04 MOW1 Sed2	2		0	10	LitterDiameter-SPM	354	µm	fiber	black	15/12/2020;	EFMZV-MarinePlastics	Captured7	µFTIR 7.2
15	2020-04 MOW1 Sed2	2		0	10	LitterDiameter-SPM	107	µm	fragment	transparent	15/12/2020;	EFMZV-MarinePlastics	Captured11	
16	2020-04 MOW1 Sed2	2		0	10	LitterDiameter-SPM	489	µm	fiber	blue	15/12/2020;	EFMZV-MarinePlastics	Captured14	
17	2020-04 MOW1 Sed2	2		0	10	LitterDiameter-SPM	2036	µm	fiber	red	15/12/2020;	EFMZV-MarinePlastics	Captured15	
18	2020-04 MOW1 Sed2	2		0	10	LitterDiameter-SPM	311	µm	fiber	black	15/12/2020;	EFMZV-MarinePlastics	Captured26	

Sheet1

Figure 4.4: example of data registered in the template

Without considering the density separation test samples and the control samples, a total of 30 samples have been analysed. The resulting microplastic data set is described in annex.

The BMDC will serve as repository for the described litter datasets and will report the data to ICES and EMODnet Chemistry.

5. Recommendations

Based on the project results, following recommendations are put forward by the project partners.

Recommendation 1: Alternative fishing line monofilaments

Although spatial correlations between fisheries activities and fishery-related litter items are difficult to make, the MarinePlastics project showed that fisheries largely contribute (>50 %) to the total amount of litter items on the seafloor in the Belgian part of the North Sea (BPNS) and the wider Belgian fishery areas. The largest contribution came from fishing line monofilaments (up to 30 items per ha), which are commonly used as dolly rope to protect the fishing nets. Non-plastic and degradable alternatives for dolly rope do exist or should be developed. It is highly recommended to force the implementation of such alternatives for dolly rope, but also for monofilament fishing lines.

Recommendation 2: Detailed source investigation on macro and microlitter hot spots in the Belgian Part of the North Sea.

The project results indicate the presence of at least one marine litter hot spot in the BPNS. However, the marine litter source is not yet unambiguously identified, and the impact of processes altering litter distribution, such as water currents, sedimentation processes or litter delocalization by fisheries, are not yet fully understood. A detailed study on marine litter hotspots, investigating the impact of different sources and modelling litter transport processes is advised. Additionally, both macrolitter and

microplastics concentrations were much higher in the nearby coastal zone. The correlation between the distribution of both macrolitter and (degraded) microplastics should be investigated as well.

Recommendation 3: Validate quality-assured, cost-efficient and harmonised monitoring methodologies for microplastics analysis

For routine long-term monitoring, a validated methodology for the determination of microplastics is of primary importance. The methodology elaborated in the frame of the MarinePlastics project needs further validation and improvement, taking into account evolving analytical possibilities, new knowledge, cost-efficiency and harmonisation efforts at the regional and EU level. Laboratory intercomparison exercises need to be developed and implemented to assess the quality of the analysis method.

Recommendation 4: To establish a long-term monitoring programme for microplastics in the Belgian Part of the North Sea

A monitoring network needs to be defined with annual sampling, at least in the nearby coast, allowing us to follow-up the evolution of microplastics contamination in the BPNS. As the type of microparticles differs in seawater and sediment (fibers vs. fragments), both matrices should be monitored, although it is recommended to focus especially on the seabed, since microplastics tend to accumulate in the sediment. A detailed study on the behaviour and distribution of microplastics in the marine environment, including a modelling approach, is essential to localise potential microplastic hotspots (e.g. muddy areas) in the Belgian marine waters and the elaboration of risk-based monitoring approach in the frame of the MSFD.

This monitoring programme needs to be accompanied by a secure, long-term and well-documented data management to allow the evaluation of differences between locations and evolution over time.

Recommendation 5: To communicate that Belgian fisheries products are a safe food source with regard to microplastics

The project results clearly revealed that microplastics >50 µm are almost not found in the edible parts of fish and crustaceans from the Belgian fishery areas. As the concentrations of microplastics were also low in the non-edible parts, we conclude that microplastics do not accumulate in seafood from the Belgian fishery. Therefore, it is important to restore or increase the public awareness that concentrations of microplastics in seafood from the Belgian fishery areas are very low, thereby ensuring a qualitative product. Additionally, further research on the occurrence and risks of smaller particles (<10 µm) and nanoplastics, which can potentially pass the gastro-intestinal wall into the blood and filets, is recommended.

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8. Annexes

Annex 1: Litter categories and subcategories

Type	Description_Type	PARAM	Description_PARAM
A	Plastic	A1	Plastic bottle
A	Plastic	A2	Plastic sheet
A	Plastic	A3	Plastic bag
A	Plastic	A4	Plastic caps/lids
A	Plastic	A5	Plastic fishing line (monofilament)
A	Plastic	A6	Plastic fishing line (entangled)
A	Plastic	A7	Synthetic rope
A	Plastic	A8	Plastic fishing net
A	Plastic	A9	Plastic cable ties
A	Plastic	A10	Plastic strapping band
A	Plastic	A11	Plastic crates and containers
A	Plastic	A12	Plastic diapers
A	Plastic	A13	Sanitary towel/tampon
A	Plastic	A14	Other plastics
B	Metals	B1	Cans (food)
B	Metals	B2	Cans (beverage)
B	Metals	B3	Fishing related metal
B	Metals	B4	Metal drums
B	Metals	B5	Metal appliances
B	Metals	B6	Metal car parts
B	Metals	B7	Metal cables
B	Metals	B8	Other metal
C	Rubber	C1	Boots
C	Rubber	C2	Balloons
C	Rubber	C3	Rubber bobbins (fishing)
C	Rubber	C4	Tyre
C	Rubber	C5	Glove
C	Rubber	C6	Other rubber
D	Glass/Ceramics	D1	Jar
D	Glass/Ceramics	D2	Glass bottle
D	Glass/Ceramics	D3	Glass/ceramic piece
D	Glass/Ceramics	D4	Other glass or ceramic
E	Natural products	E1	Wood (processed)
E	Natural products	E2	Rope
E	Natural products	E3	Paper/cardboard
E	Natural products	E4	Pallets
E	Natural products	E5	Other natural products
F	Miscellaneous	F1	Clothing/rags
F	Miscellaneous	F2	Shoes
F	Miscellaneous	F3	Other

Annex 2: Categorisation of environmental monitoring campaign fish tracks

Overview of fish track locations within environmental monitoring, indicating the zone (within or beyond the 12 nautical mile zone) and location category. The latter is linked to anthropogenic activity (dredge disposal, sand extraction, offshore wind farms). Ref. = reference, Low act. = low activity.

Location	Lat. 1 (North)	Long. 1 (East)	Lat. 2 (North)	Long. 2 (East)	Zone	Activity 1	Sample category	Activity 2	Sample category
115s	51°09.263'	2°36.896'	51°09.747'	2°38.291'	<12nm	Dredge dis.	Ref.	NA	NA
120s	51°10.939'	2°41.568'	51°11.128'	2°43.135'	<12nm	Dredge dis.	Ref.	NA	NA
140biss	51°20.752'	3°02.689'	51°20.287'	3°01.271'	<12nm	Dredge dis.	Ref.	NA	NA
140triss	51°20.128'	3°00.643'	51°19.667'	2°59.193'	<12nm	NA	NA	NA	NA
215s	51°16.123'	2°37.265'	51°16.854'	2°38.356'	<12nm	Sand ext.	Low act.	NA	NA
230s	51°18.126'	2°50.351'	51°18.783'	2°51.556'	<12nm	Dredge dis.	Ref.	NA	NA
315s	51°17.990'	2°27.707'	51°18.803'	2°28.637'	>12nm	Sand ext.	Ref	NA	NA
330s	51°25.599'	2°47.639'	51°26.040'	2°49.079'	>12nm	OWF	Ref	NA	NA
340s	51°29.617'	2°59.425'	51°30.201'	3°00.731'	<12nm	NA	NA	NA	NA
415s	51°23.611'	2°19.676'	51°24.420'	2°20.631'	>12nm	NA	NA	NA	NA
421s	51°29.050'	2°28.208'	51°28.131'	2°27.574'	>12nm	NA	NA	NA	NA
820s	51°34.636'	2°21.181'	51°35.530'	2°21.904'	>12nm	NA	NA	NA	NA
830s	51°42.259'	2°26.496'	51°43.067'	2°27.445'	>12nm	NA	NA	NA	NA
840s	51°44.097'	2°38.672'	51°44.257'	2°40.266'	>12nm	NA	NA	NA	NA
1401s	51°16.666'	2°54.798'	51°17.121'	2°56.222'	<12 nm	Dredge dis.	Impact	NA	NA
1402s	51°16.009'	2°56.001'	51°16.477'	2°57.414'	<12nm	Dredge dis.	Nearby	NA	NA
2251s	51°14.567'	2°43.160'	51°15.101'	2°44.510'	<12 nm	Dredge dis.	Impact	NA	NA
2252s	51°14.494'	2°44.071'	51°15.082'	2°45.362'	<12nm	Dredge dis.	Nearby	NA	NA
7001s	51°22.801'	3°14.957'	51°22.940'	3°16.544'	<12 nm	Dredge dis.	Impact	NA	NA
7002s	51°23.598'	3°14.920'	51°23.757'	3°16.503'	<12nm	Dredge dis.	Nearby	NA	NA
7003s	51°22.672'	3°15.004'	51°22.825'	3°16.587'	<12nm	Dredge dis.	Impact	NA	NA
7101s	51°25.788'	3°07.576'	51°26.413'	3°08.826'	<12nm	Dredge dis.	Impact	NA	NA
7102s	51°26.382'	3°06.591'	51°26.961'	3°07.897'	<12nm	Dredge dis.	Nearby	NA	NA
7103s	51°26.043'	3°7.530'	51°26.043'	3°9.134'	<12nm	Dredge dis.	Impact	NA	NA
7104s	51°26.288'	3°7.526'	51°26.288'	3°9.130'	<12nm	Dredge dis.	Impact	NA	NA
7105s	51°26.979'	3°9.130'	51°26.268'	3°10.258'	<12nm	Dredge dis.	Nearby	NA	NA
7802s	51°27.906'	3°03.130'	51°28.620'	3°04.255'	<12nm	Dredge dis.	Nearby	NA	NA
7803s	51°27.248'	3°00.890'	51°27.950'	3°02.032'	<12nm	Dredge dis.	Impact	NA	NA
7804s	51°26.765'	3°01.568'	51°27.475'	3°02.699'	<12nm	Dredge dis.	Impact	NA	NA
7805s	51°28.218'	3°01.173'	51°27.520'	3°00.023'	<12nm	Dredge dis.	Nearby	NA	NA
B03s	51°25.394'	3°12.255'	51°25.488'	3°13.851'	<12nm	Dredge dis.	Ref.	NA	NA
B04s	51°26.602'	3°13.357'	51°26.834'	3°14.917'	<12nm	Dredge dis.	Ref.	NA	NA
B07s	51°25.897'	3°18.560'	51°25.746'	3°16.975'	<12nm	Dredge dis.	Ref.	NA	NA
BRN01s	51°18.182'	2°35.702'	51°18.902'	2°36.811'	<12nm	Sand ext.	Low act.	NA	NA
BRN02s	51°18.322'	2°34.920'	51°17.621'	2°33.779'	<12nm	NA	NA	NA	NA
BRZRs	51°14.143'	2°31.766'	51°14.906'	2°32.795'	<12nm	NA	NA	NA	NA
GB01s	51°25.382'	2°44.578'	51°24.963'	2°43.122'	>12nm	Sand ext.	Ref.	OWF	Ref.
GB02s	51°26.512'	2°48.254'	51°26.05'	2°46.831'	>12nm	Sand ext.	Ref.	OWF	Ref.
HB6s	51°37.912'	2°35.282'	51°36.922'	2°35.048'	>12nm	Sand ext.	Ref.	NA	NA
HB8s	51°37.992'	2°34.526'	51°36.996'	2°34.394'	>12nm	Sand ext.	Ref.	NA	NA

HB9s	51°40.582'	2°36.227'	51°41.579'	2°36.361'	>12nm	Sand ext.	Ref.	NA	NA
HB10s	51°33.682'	2°38.948'	51°32.787'	2°38.229'	>12nm	Sand ext.	Impact	NA	NA
HB11s	51°31.053'	2°37.481'	51°32.028'	2°37.834'	>12nm	Sand ext.	Impact	NA	NA
KB1s	51°18.569'	2°40.600'	51°17.678'	2°39.862'	<12nm	Sand ext.	Impact	NA	NA
KB2s	51°18.614'	2°41.458'	51°19.605'	2°41.242'	<12nm	Sand ext.	Impact	NA	NA
KBR01s	51°13.322'	2°34.262'	51°14.087'	2°35.290'	<12nm	Sand ext.	Ref.	NA	NA
KBZ01s	51°16.173'	2°39.373'	51°17.036'	2°40.181'	<12nm	Sand ext.	Low act.	NA	NA
LWO11s	51°22.461'	3°07.703'	51°23.313'	3°06.865'	<12nm	Dredge dis.	Ref.	NA	NA
LWO12s	51°22.900'	3°08.900'	51°22.900'	3°08.900'	<12nm	Dredge dis.	Ref.	NA	NA
MIC3s	51°28.816'	2°40.109'	51°30.056'	2°42.527'	>12nm	NA	NA	NA	NA
MIC4s	51°36.558'	2°32.043'	51°38.109'	2°33.703'	>12nm	NA	NA	NA	NA
ODC01s	51°18.721'	2°29.819'	51°19.632'	2°30.481'	>12nm	Sand ext.	Impact	NA	NA
ODR01s	51°14.169'	2°25.561'	51°15.008'	2°26.430'	<12nm	Sand ext.	Ref.	NA	NA
STP01s	51°22.702'	3°03.446'	51°23.321'	3°04.709'	<12nm	Dredge dis.	Ref.	NA	NA
TB1s	51°29.645'	2°47.399'	51°30.048'	2°48.869'	>12nm	Sand ext.	Impact	NA	NA
TB2s	51°29.340'	2°45.360'	51°30.000'	2°46.740'	>12nm	Sand ext.	Impact	NA	NA
Track2	51°32.859'	2°54.316'	51°32.358'	2°55.267'	>12nm	NA	NA	NA	NA
Track3	51°32.878'	2°55.259'	51°32.408'	2°56.268'	>12nm	NA	NA	NA	NA
Track5	51°33.759'	2°58.943'	51°34.388'	2°59.978'	>12nm	NA	NA	NA	NA
Track6	51°33.722'	2°57.948'	51°34.349'	2°58.975'	>12nm	NA	NA	NA	NA
WBB01s	51°34.139'	2°46.197'	51°34.852'	2°47.326'	>12nm	OWF	Ref.	NA	NA
WBB02s	51°34.177'	2°44.642'	51°35.057'	2°45.406'	>12nm	Sand ext.	Ref.	OWF	Ref.
WBB02bs	51°33.937'	2°44.752'	51°33.021'	2°44.105'	>12nm	Sand ext.	Ref.	OWF	Ref.
WBB03s	51°34.743'	2°43.902'	51°35.621'	2°44.674'	>12nm	OWF	Ref.	NA	NA
WBB04s	51°38.380'	2°50.989'	51°39.263'	2°51.746'	>12nm	OWF	Nearby	NA	NA
WBB05s	51°38.646'	2°49.248'	51°39.628'	2°49.554'	>12nm	OWF	Impact	NA	NA
WBB05bs	51°40.020'	2°48.986'	51°40.954'	2°49.559'	>12nm	NA	NA	NA	NA
WBB06as	51°38.887'	2°47.955'	51°39.825'	2°48.515'	>12nm	OWF	Impact	NA	NA
WBB06bs	51°41.133'	2°48.498'	51°40.195'	2°47.939'	>12nm	OWF	Impact	NA	NA
WBB07s	51°39.730'	2°46.824'	51°40.673'	2°47.360'	>12nm	OWF	Impact	NA	NA
WBB08s	51°39.598'	2°45.518'	51°40.512'	2°46.172'	>12nm	OWF	Nearby	NA	NA
WG2s	51°27.170'	2°50.670'	51°27.625'	2°52.099'	>12nm	NA	NA	NA	NA
WOH01s	51°35.076'	2°41.772'	51°36.052'	2°42.121'	>12nm	Sand ext.	Ref.	OWF	Ref.
WOH02s	51°37.225'	2°41.192'	51°36.240'	2°40.916'	>12nm	Sand ext.	Ref.	OWF	Ref.
WOH03s	51°37.922'	2°40.282'	51°36.935'	2°40.028'	>12nm	Sand ext.	Ref.	OWF	Ref.
WT1biss	51°30.754'	2°54.598'	51°30.348'	2°53.130'	>12nm	Sand ext.	Low act.	OWF	Ref.
WT2biss	51°31.815'	2°53.863'	51°31.402'	2°52.396'	>12nm	NA	NA	NA	NA
WT2triss	51°31.883'	2°53.411'	51°31.457'	2°51.957'	>12nm	Sand ext.	Ref.	OWF	Ref.
WT3s	51°31.582'	2°50.098'	51°31.953'	2°51.590'	>12nm	Sand ext.	Ref.	OWF	Ref.
WT3biss	51°32.365'	2°52.616'	51°31.831'	2°51.257'	>12nm	Sand ext.	Ref.	OWF	Ref.
WT7	51°32.462'	2°59.280'	51°33.117'	3°00.497'	>12nm	OWF	Nearby	NA	NA
WT9	51°33.824'	2°56.816'	51°34.479'	2°58.032'	>12nm	OWF	Nearby	NA	NA
WT10	51°33.564'	2°54.241'	51°33.134'	2°52.965'	>12nm	OWF	Nearby	NA	NA
WT11	51°31.864'	2°58.167'	51°31.290'	2°56.909'	>12nm	OWF	Nearby	NA	NA

Annex 3: Summary of method validation data

For method validation, water and mussel tissue was spiked with red polystyrene beads (size 106-125 µm and 500-600 µm) and colorless polyethylene beads (size 106-125 µm and 500-600 µm). For each type and size, spiking was done with 10 and 40 particles per sample. Validation was done by 2 lab technicians on 6 days with 2 independent analyses per sample per day.

Accuracy (%) was determined as: $100 + ((X_{av} - C_{ref})/C_{ref} * 100)$ with X_{av} the average measured value and C_{ref} the spiked amount of reference particles. Precision was determined as CV_R (%) = $S_r/X_{av}*100$ with Sr the standard deviation of the measured value.

As can be seen in the tables below, predefined quality criteria were met for colored particles as well as colorless particles of 600 µm. As criteria were not met for colorless particles of 100 µm, accuracy and precision analyses was also done on colorless particles of 200 µm (2 days, 2 independent measurements/day). For 200 µm colorless beads, criteria were met with an accuracy of 87.5% and a precision of 9.5%.

Table A1: Accuracy of the method, applying spiked water and mussel samples.

Beads		Matrix	X_{av} (#)	C_{ref} (#)	Ac. (%)	b (%)	± 20%
600 µm	Colorless	Water	9.8	10	97.5	-2.5	OK
		Mussel	9.3	10	92.5	-7.5	OK
	Red	Water	10.0	10	100.0	0.0	OK
		Mussel	9.8	10	98.3	-1.7	OK
	Colorless	Water	38.2	40	95.4	-4.6	OK
		Mussel	36.7	40	91.7	-8.3	OK
	Red	Water	39.8	40	99.4	-0.6	OK
		Mussel	39.9	40	99.8	-0.2	OK
Beads		Matrix	X_{av} (#)	C_{ref} (#)	Ac. (%)	b (%)	± 30%
100 µm	Colorless	Water	3.3	10	33.3	-66.7	NOK
		Mussel	3.7	10	36.7	-63.3	NOK
	Red	Water	8.5	10	85.0	-15.0	OK
		Mussel	8.6	10	85.8	-14.2	OK
	Colorless	Water	19.0	40	47.5	-52.5	NOK
		Mussel	18.8	40	46.9	-53.1	NOK
	Red	Water	36.4	40	91.0	-9.0	OK
		Mussel	32.6	40	81.5	-18.5	OK

Table A2: Precision of the method, applying spiked water and mussel samples.

Beads		Matrix	C_{ref} (#)	X_{av} (#)	S_R (#)	CV_R (%)		± 20%
600 µm	Colorless	Water	10	9.8	0.5	4.6	6.7	OK
			40	38.2	3.4	8.9		
		Mussel	10	9.3	1.0	10.4	10.0	OK
			40	36.7	3.5	9.5		
	Red	Water	10	10.0	0.0	0.0	0.8	OK
			40	39.8	0.6	1.6		
		Mussel	10	9.8	0.4	4.0	2.3	OK
			40	39.9	0.3	0.7		

100 µm	Colorless	Water	10	3.3	2.4	71.6	53.8	NOK
			40	19.0	6.8	36.0		
		Mussel	10	3.7	1.8	49.8	43.5	NOK
			40	18.8	7.0	37.2		
	Red	Water	10	8.5	1.3	15.5	13.6	OK
			40	36.4	4.3	11.7		
		Mussel	10	8.6	1.3	15.3	17.6	OK
			40	32.6	6.5	19.8		

Next to accuracy and precision, robustness and specificity of the method was evaluated within validation and a limit of quantification was determined, based on the analyses of procedure blanks.

Within specificity tests, hot needle test proved reliable in making distinction between synthetic polyester and polyacryl fibres compared to semi-synthetic rayon and natural cotton, jute, linen and wool.

Annex 4: Microplastic sampling protocol

SAMPLING PROTOCOL FOR MICROPLSTICS COLLECTION IN WATER COLUMN & SEDIMENTS

1. Introduction

This sampling protocol describe how microplastics are sampled in the water column and in sediments on board of RV Belgica. For water collection, the method uses the vessel's seawater inlet pump to collect subsurface seawater on sieves, to filter large (100-200L) amount of water and reduce the volume of the final sample. For sediment collection, the sampling device used is the Reineck box corer sampler.

2. Procedures to mitigate background contamination

- Use glass, metal or aluminium material for sampling and sample storage.
- Clean the working area with fiber-free tissue or paper.
- Wear polymer-free clothing or cotton coveralls and take notes of the type and colour of clothes each person is wearing.
- Rinse all material (sieves, funnel, glass jars,…) thoroughly with MilliQ water. After rinsing, dry up-side-down or cover all material to avoid air contamination.
- Cover the sample as much as possible.
- A control filter, in open petri dish, is placed around the sampling working area to take into account airborne contamination on each sampling event.
- Note the colour and take a sample of the boat deck paint to compare if suspicion of contamination.

3. Water sampling collection (with water ship inlet)

Preliminary actions

- Prepare the sampling gear: pipes equipped with a flowmeter are connected to the water ship inlet to enable water collection and precise recording of the volume sampled.
- Check the proper drainage of the sink drain.
- Do a positive control sample: place spikes on the sieves and follow the sampling procedure.
- Just before every water sampling event, let the water flow through the pipes to ensure that the that the water collected is not water that has remained stagnant in the pipes.
- For every sampling event, complete the sampling sheet (see sampling sheet section).

Sampling

- Place the Sieves (63 and 100µm) in cascade below the inlet equipped with a flowmeter (Figure 1).
- Cover the top sieve (100µm) with aluminium foil to minimise airborne contamination.

- Put the air control filter in a petri dish, open the petri when sampling is at open air (when not covered by aluminium foil)
- Note the initial time and the flowmeter start number in the sampling sheet.
- Open the tap inlet and let it flow through the sieve during 10/20min to filter 100 or 200 L (depending on the turbidity/suspended matter).
- Close the inlet tap, write the final time and the flowmeter end number in the sampling sheet.
- Use MilliQ water to concentrate the material in one part of the sieve.
- With the help of a funnel, and with a small amount of MilliQ, rinse the sieve into a glass bottle.
- Close the bottle (if the bottle have a plastic lid: put an aluminium foil between the sample and the lid)
- Label the bottle with the unique sample ID.
- Store the bottle in the freezer (-20°C).

➔ Repeat the sampling procedure 3/4 X per station (at different tidal states)

Figure 1: Water sampling. Left: Seawater inlet equipped with a flowmeter. Right: water filtration from the inlet through metal sieves (100µm, 63µm), covered with aluminium foil to minimise air contamination.

4. Sediment sampling collection

Preliminary actions

- Prepare the sampling gear: Reineck box corer, glass jar.
- For every sampling event, complete the sampling sheet (see sampling sheet section).

Sampling

- Put the air control filter in a petri dish, open the petri when sampling is at open air (from the emergence of the Reineck until the closure of the jar).
- Note the sampling time in the data sheet.
- When Reineck is on board: detach it and place it on the table.
- If there is water at the top of the sediments: drain it carefully with a tube and store it into a glass jar.
- With a metal spoon, take the top 5cm of the sediments and place it in the glass jar.
- If large items: rinse them with milliQ, discard organic matter and keep if it is plastic.
- Close the jar (if it have a plastic lid: put an aluminium foil between the sample and the lid)
- Label the jar with the unique sample ID.
- Store the jar in the freezer (-20°C).

➔ Repeat the sampling procedure 3X per station.

5. Sampling sheet

Sample ID	Campaign Code	**Station Code**	**Sampling device**	Start date/time (UTC)	End date/time (UTC)	**Start date/time (CET)**	**End date/time (CET)**	Number of samples	Volume (l)	Remarks	Clothes color	Tidal state

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Annex 5: Microplastic analysis protocol

ANALYSIS OF MICROPLASTICS IN WATER & SEDIMENTS

1. Introduction

This protocol describes how microplastics down to 100 µm can be determined from water and sediments. The method is based on digestion of organic matter by potassium hydroxide (KOH) or hydrogen peroxide (H_2O_2) depending on the matrix. A density separation step is done to separate microplastics from sediments. The density separation step can be done with different techniques, here two different methods will be presented. Determination of microplastics is done visually by stereomicroscopy and confirmation of a subsample by µ-FTIR.

2. Procedures to mitigate background contamination

- Working in a clean room and if possible, under a fume hood.
- Use glass, metal or aluminium material.
- Clean the working area with fiber-free tissue or paper.
- Wear polymer-free clothing or cotton coveralls and take notes of the type and colour of clothes each person is wearing.
- Filter (retention of 20 µm or lower) all solvents and solutions before use.
- Rinse all material thoroughly with MilliQ water. After rinsing, dry up-side-down or cover all material to avoid air contamination.
- Cover the sample as much as possible.
- Include positive and negative control samples (see quality control section).

3. Preliminary actions

Defrost the samples. Prepare filtered 1O% H_2O_2 or KOH solution for the digestion step. For the density separation step, prepare the sodium bromide (NaBr) solution, to achieve a density of ~1.5 g.cm-3 . For filtration, use a glass fiber filter with a particle retention of 1.2 µm.

4. Water sample digestion and filtration

Potassium hydroxide (KOH) is corrosive chemical, hand it with care, under fume hood with gloves and lab coat.

- Add 10% potassium hydroxide with a ratio of 1:3 volume sample: solution.
- Positive and negative control samples analysed in parallel (see quality control section).
- Place the mixture in temperature-controlled oven at 40°C until all visible organic material is digested (from 24h to max 72h).
- If there is too much inorganic suspended matter, do a density separation step (see section 5.B).
- Pour the sample over the filter for filtration, with MilliQ, rinse 3 times the sampling bottle and the filtration kit walls.

- Transfer the filter into a closed and labelled petri dish for microplastic determination.
- Allow the filter to dry at room temperature or at 40° for 48h.

5. Sediment sample digestion, density separation and filtration

Hydrogen peroxide (H_2O_2) is corrosive chemical, hand it with care, under fume hood with gloves and lab coat.

A) **MPSS** (Figure 2)

Cleaning of the MPSS:

- Assemble the MPSS (see Hydrobios operational manual) and fill it with only the sodium bromide solution inside, no sediments.
- Start the rotor for 1h, followed by a settling phase of 5h.
- Close the ball valve, discharge of the supernatant from the sample chamber and rinse with milliQ.

Sediments handling before introduction in the MPSS:

- Homogenise the sample thoroughly, with a metal spoon.
- Take subsamples for TOC and grain size analyse.
- Transfer the homogenised sample in a large beaker, weight the sample. For the recovery tests, add the spikes to the sediments.
- Add 100 ml of 10% hydrogen peroxide.
- Stir during 1 min. The digestion creates a lot of bubbles and foam at the beginning, if the foam goes too high in the beaker stir again.
- Let the digestion proceed for 18h at room temperature, under fume hood.
- Rinse the sediments thoroughly with MilliQ and through a metal sieve of 63 µm.
- Put the rinsed sediments in a beaker and add sodium bromide solution, mix and let it soak for 20 min.

Density separation in the MPSS:

- Turn the rotor on, carefully put the sample in the MPSS from the top of the “bottom standpipe” (Figure 2), close the rest of the MPSS and fill it to the top (see Hydrobios operational manual).
- Let it mix for 4h, from time to time, knock on the MPSS walls to help the release of particles and bubbles sticking to the inside surface.
- Turn the rotor off and let it settle overnight (>12h).
- Close the ball valve and filtrate the sample from the sample chamber (see Hydrobios operational manual), rinse the sample chamber walls with MilliQ.
- Transfer the filter into a closed and labelled petri dish for microplastic determination.
- Allow the filter to dry at room temperature or at 40° for 48h.
- Empty the MPSS, filter the density solution on 20 µm metal sieve, disassemble and clean the MPSS.

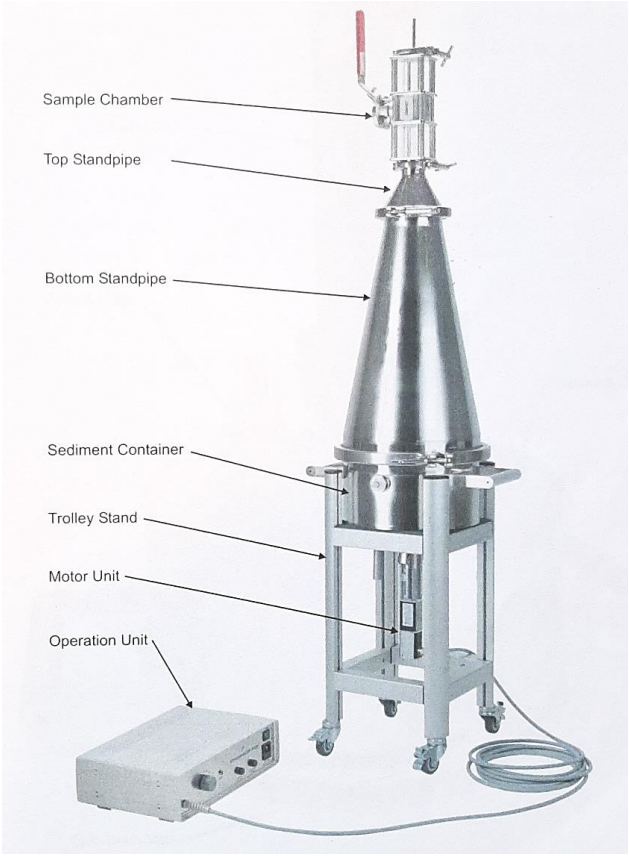

Figure 2: Components of the MPSS

B) Modified method of Thompson et al. 2004 - "Classic" density separation method in a beaker

- Homogenise the sample thoroughly, with a metal spoon.
- Take subsamples for TOC and grain size analyse.
- Transfer ~500g ww of the homogenised sample in a large beaker and add 100 ml of 10% hydrogen peroxide.
- Stir during 1 min. The digestion creates a lot of bubbles and foam at the beginning, if the foam goes too high in the beaker stir again.
- Let the digestion proceed for 18h at room temperature, under fume hood.
- Rinse the sediments thoroughly with MilliQ and through a metal sieve of 63 µm.
- Take one subsample for wet weight/dry weight calculation.
- Put 100 g ww of sediments in a beaker. In parallel, run 2 other subsamples, the positive and the negative control samples (see quality control section).
- Add the sodium bromide solution, 3 times the volume of the sediment.
- Stir for 2 min and let it settle for 1h.
- Pour the supernatant layer over a filter in a filtration set.
- Repeat the last 3 steps 2 more times, with the decantation of the supernatant on the same filter.
- Rinse 3 times the filtration kit walls with MilliQ.
- Transfer the filter into a closed and labelled petri dish for microplastic determination.
- Allow the filter to dry at room temperature or at 40° for 48h.

➔ This protocol is still in evolution (digestion process, amount of g ww/volume of density solution, time of stirring/settling).

6. Microplastic determination

The particles collected on the filter are visually examined under a stereomicroscope equipped with digital camera. The suspected particles are photographed and their characteristics (type, size, colour) are noted. The existing criteria used to suspect a particle are:

- clear and homogeneous color
- no natural structures visible (e.g. cells)
- unnatural bending
- fibers must have equal thickness throughout their length
- fibers must have no fraying at their ends

Then the suspected particles are submitted to a hot needle test, where the point of a hot needle is brought close to the particle. Plastic particles will bend, curl or melt at the approach of the hot needle, while non plastic particles will show no reaction or will burn (wool, cotton)(Hidalgo-Ruz et al., 2012; Lusher et al., 2020).

As the visual characterization by itself is not fully reliable, it is advised to use an analytical technique in complement. Therefore, for validation and polymer identification, a small subset of particles is analyzed by VLIZ using micro-Fourier transform infrared spectroscopy (µFTIR, Spotlight 200i FT-IR microscope). Polytetrafluorethylene (PTFE) filters have been used.

7. Quality control

In parallel of sample analysis, positive controls and negative controls (=procedure blanks) are also analyzed. The positive controls consist of the spiking of a sample with microplastics of a fixed type, size and colour. The negative controls followed the same steps as the samples but without the actual sample. Then, the positive and negative control are analyzed with the same analysis procedure as the samples, thus allowing to determine respectively either the recovery efficiency or the potential contaminations.

8. Reporting

Based on the procedural blank samples, in case of a contamination, a particle average is calculated per type and colour and is subtracted from the number of microplastics observed in the corresponding samples.

9. References

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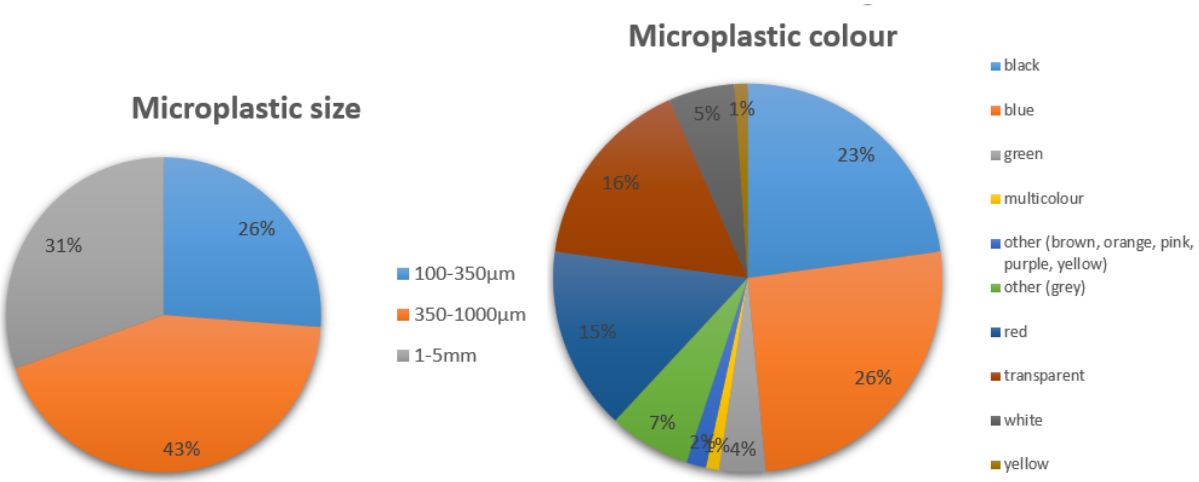
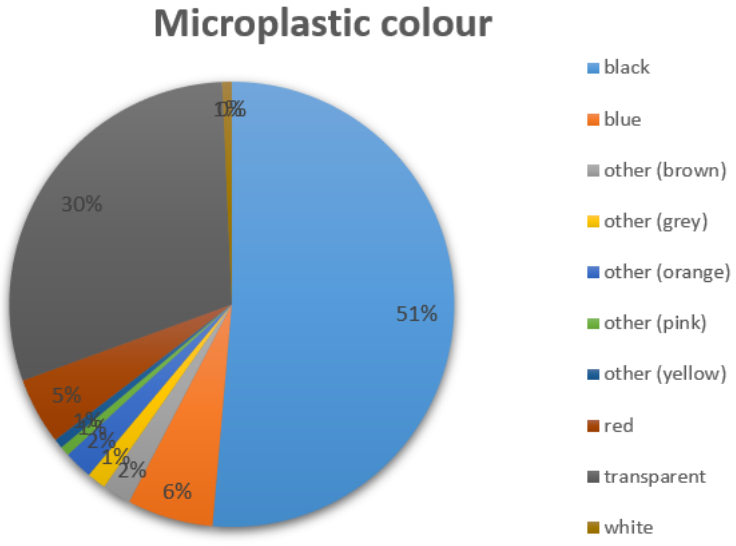
Annex 6: Microplastic size class and colour

Water results



Sediment results



Annex 7: WP4 annex data

Annex data inventory microplastics in abiotic environment

	number of stations	samples	particles	parameters	meta info
Seawater	5	13	549	size, type, colour, photoID, µFTIR validation (subset)	tide, volume sampled, sampling gear, time & position, analysis date
Sediment	2	6 samples x 3 subsamples	155	size (µm), type, colour, photo, µFTIR validation (subset)	tide, grainsize TOC dry weight sampling gear, time & position, analysis date
Total	5	31	704		