IVM Institute for Environmental Studies

Microplastic survey of the Dutch environment

Novel data set of microplastics in North Sea sediments, treated wastewater effluents and marine biota

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Contents

Abbreviations

dw	Dry weight
GES	Good Environmental Status
IR	Infrared (as in infrared techniques Fourier Transform IR and Raman)
LIMS	Laboratory Information Management System
MBR	Membrane reactor
MSFD	European Marine Strategy Framework Directive
MWTL	Dutch National Monitoring Program (*Monitoring Waterstaatkundige Toestand des Lands*)
NaCl	Sodium chloride
NaOH	Sodium hydroxide
OS	Oosterschelde Neeltje Jan outside
RE	Rhine Estuary
RWS	Rijkswaterstaat
SVHC	Substances of very high concern
TNS	Ter Heide North Sea coast
UNEP	United Nations Environment Programme
WWTP	Wastewater treatment plant

Summary

Environmental contamination with microsized synthetic plastic particles, known as 'microplastics', is widely recognised as a threat to the marine ecosystems and the economies and societal wellbeing that depend on these ecosystems. Microplastics are addressed under descriptor 10 of Good Environmental Status in the Marine Strategy Framework Directive (MSFD).

The Ministry of Infrastructure and the Environment commissioned an exploratory survey of microplastics in the Dutch environment. The matrices selected for analysis included marine sediments (coastal, offshore and estuaries), wastewater treatment plant effluents and marine biota.

We report microplastic concentrations from 100 (min.) up to 3600 (max.) particles per kg dry sediment, median 500 particles/kg dw, mean 840 particles/kg dry weight (dw) sediment, collected at 15 Dutch National Monitoring Program (MWTL) and estuary locations representing a range of coastal and offshore North Sea waters. The average number of microplastics in sediment from the 12 sampling sites in the North Sea was 440 particles/kg dw sediment; in a Wadden Sea sediment 770 particles/kg dw sediment were found, and the average for the Rhine estuary locations was 3300 particles/kg dw sediment. These areas were identified as relative 'hotspots'. In a Belgian study that examined a more limited size fraction (38 -1000 µm) of microplastics in sediment than the present study, fewer microplastics were counted in Belgian harbours (by about an order of magnitude).

In individual wastewater treatment plant effluents from sites that discharge effluents to the North Sea, the Oude Maas River or the North Sea Canal, we found between 9 and 91 particles/L treated effluent with mean concentrations across three different plants at about 52 particles/L treated effluent. This provides further evidence that not all microplastics are captured in sewage sludge of wastewater treatment plants (WWTPs).

In field collected biota from three locations along the Dutch coast, microplastics were also detected in four of the five species investigated (analysis of pooled samples). Filter feeders such as oysters and mussels had higher concentrations than common periwinkles and amphipods. In pooled crab samples, no microplastics were detected in this survey. With the exception of the crabs samples, the number of particles ranged from 11 particles per g dw (amphipods) to 105 particles per g dw (blue mussels). Both of these pooled samples were collected from the same area, the Oosterschelde. The filter feeders with microplastic body residues in this study (oysters, mussels) are common species for human consumption.

At present, there is no known way to accurately trace the origins of the tiny microplastic particles detected in environmental samples from the field. In contrast, when plastic fragments are large, they sometimes identifiable as the original object, in which case more information can be collected regarding the origin of the material. The polymer type of plastics gathered in the field can be identified in some cases, but there are limits to the current techniques that need to be addressed, particularly when the fragments are in the low mm range, as found in this study.

This exploratory survey of microplastics in different environmental matrices shows the ubiquity of these contaminants and represents a unique data set for the Dutch (marine) environment, which can be used to further prioritize research and support future monitoring program design.

This report briefly discusses the huge potential for analytical method development in the emerging field of environmental microplastic research. Further improvements may still be made in the extraction steps, but also in the analytical techniques to identify plastic polymers, which currently work best for large microplastics made of the one of the most common polymers (copolymer blends are more difficult due to the large number of reference materials needed).

The current extraction and analysis of microplastics is quite labour intensive. There is room to improve microplastics method selectivity, sensitivity as well as infrastructure for quality control and quality assessment of environmental microplastics data (tools, training, interlaboratory studies, reference materials, etc.). These improvements would not only help advance the scientific knowledge of microplastics in the environment but they also have practical applications in environmental monitoring. The European Technical Subgroup on Marine Litter is disseminating guidance based on current state-of-the-art knowledge and monitoring methods with the recent online publication of the draft document entitled 'Monitoring Guidance for Marine Litter in European Seas', available to all Member States and other interested groups. CleanSea[1], the European research project on marine litter led by IVM that runs from 2013-2015, is also contributing to method development for microplastics research, monitoring and impact assessments as well as policy options.

Future developments are expected to lead to identification of appropriate indicators for marine microplastics and a better understanding of the toxicological consequences of exposure to microplastics for both marine organisms, but also for human health. Besides the potential impact of particle toxicity from microplastics there is concern for the chemicals associated with plastics (e.g. toxic additives, residual monomers and sorbed contaminants). The implementation of the EU MSFD brings with it the incentive for researchers and Member States to work towards developing indicators for (marine) microplastics, to evaluate the ecological, social and economic harm of microplastics in the marine environment, and to devise programmes of measures to mitigate emissions.

[1] www.cleansea-project.eu

1 Introduction

The EU Marine Strategy Framework Directive (MSFD) came into effect on 15 July 2008 and is being implemented in the Netherlands by the Ministry of Infrastructure and the Environment. The MSFD aims to achieve good environmental status (GES) by 2020 in European seas and coastal waters. Under the MSFD there are eleven descriptors for GES, one of which being that marine litter has no detrimental effects on the environment (Descriptor 10).

Marine litter consists of large pieces of plastic, rubber, wood and other materials, but also of microsized particles, including "microplastics" (synthetic polymer particles <5 mm). The items reach the environment due to deliberate discards or unintentional losses to the sea either directly or via wind and river transport. Microplastics can be emitted to the environment from various sources but may also arise from macroplastic that fragments into smaller pieces when the polymeric material weakens from ultraviolet light exposure, mechanical stress and/or leaching of additives.

Member States of the European Union will be measuring various indicators of Descriptor 10 as part of the MSFD implementation. Programs of measures due in 2015 are being currently prepared and represent one of the key aspects of MSFD implementation. In the Netherlands preparations for selecting measures began in 2012. Monitoring programs are being prepared for the MSFD by Member States and are due in 2014. Monitoring programs may be used to measure spatial and temporal trends of marine litter concentrations in the environment and to evaluate if certain measures taken are effective for reducing marine litter during a given time frame. The European Technical Subgroup on Marine Litter has just published an online draft of their Monitoring Guidance for Marine Litter in European Seas (2013), to be finalized later in 2013.

The Ministry of Infrastructure and Environment has an interest in generating knowledge about the presence and distribution of microplastics in the Dutch part of the North Sea. In cooperation with Deltares, IVM has worked on these issues, producing a report on the state of knowledge on microplastics in the North Sea (Leslie *et al.* 2011). To gain empirical evidence of microplastics in the Dutch marine environment, this exploratory study examines levels of microplastic marine litter in coastal, offshore and estuarine sediments, effluents from wastewater treatment plants, and from marine biota species collected along the Dutch coast.

2 Sampling

2.1 Overview

Marine sediments from 15 locations, wastewater treatment plant effluents from three locations, and five marine biota species from three coastal locations were sampled for analysis of microplastics (Figure 2.1).

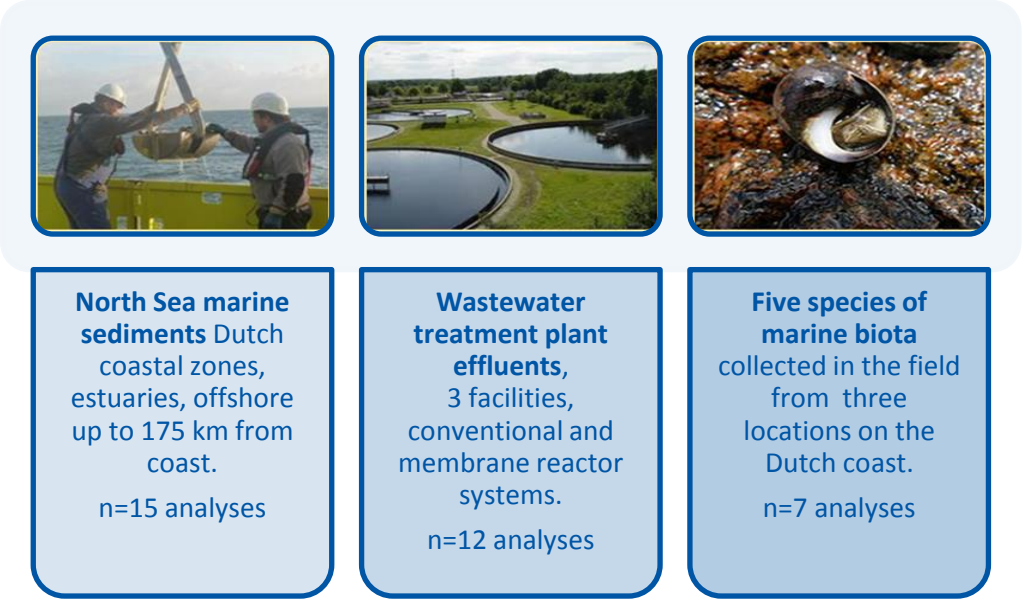

Figure 2.1 Overview of the matrices sampled for this study.

2.2 Marine sediments

Sediment samples were collected in July 2012 as part of the Dutch National Monitoring Program[2] (Bogaart-Scholte *et al.* 2010). Each sample consists of a homogenized pool of five individual sediment grab samples of surface sediments. A total of 15 locations were sampled, (Table 2.1) representing a range of proximities to possible microplastics sources and including some ecologically sensitive areas such as in the Wadden Sea (Lindenboom *et al.* 2008). The samples were stored in glass sample jars in the dark at 4 ºC until analysis.

2.3 Wastewater treatment plant effluents

Three WWTP locations were selected, Houtrust (The Hague municipality), Amsterdam West and Heenvliet. The sample set included both conventionally treated effluent (all locations) and membrane reactor treated effluent (Heenvliet). The selected effluents come from locations that discharge directly to the North Sea, to the Oude Maas River and to the North Sea Canal (Table 2.2). Each sample was taken in triplicate, making a total of 12 samples for analyses. Wastewater treatment plant (WWTP) effluents were collected in glass jars and stored cool and dark until analysis.

[2] Monitoring Waterstaatkundige Toestand des Lands (MWTL)

2.4 Marine invertebrate biota

Biota were collected from three littoral zone locations along the Dutch coast in March 2013 and were preserved either frozen or in 70% isopropanol and (stored cool and dark) until analysis.

Five species were selected for this first screening of microplastics in field collected invertebrates from the Dutch marine environment (Table 2.3). Samples were pooled for analysis.

Table 2.1 Sediment sampling locations on the Dutch coast, estuaries and offshore for microplastic analysis. Codes and coordinates supplied by Rijkswaterstaat (RWS). Coordinates: E50, European Datum 1950; RD, 'Rijksdriekhoeks' coordinates.

Description	RWS Location code	IVM LIMS code 12/	Coordinates (x,y)	Comments
North Sea				
Walcheren, 2 km from coast	WALCRN2	1143	003°24′39″, 051°32′56″ (E50)	Mouth of Western Scheldt River
Voordelta, Goeree 6 km from coast	GOERE6	1144	003°52′25″, 051°52′11″ (E50)	SPM transport from W. Scheldt; south of mouth of River Meuse
Voordelta, Haringvliet 1 km from coast	HARVT1	1145	004°00′54″, 051°51′18″ (E50)	SPM transport from W. Scheldt; south of mouth of River Meuse
Dutch coast, Ter Heide 10 km from coast	TERHDE10	1137	65950, 458662 (RD)	Loswal Noord is a former sewage sludge dumping site, possible hotspot
Dutch coast, Ter Heide 1 km from coast	TERHDE1	1138	71406, 451521 (RD)	North of mouth of River Rhine
Dutch coast, Noordwijk 2 km from coast	NOORDWK2	1139	004°24′22″, 052°15′41″ (E50)	North of Rotterdam Harbour, close to coastline and possible land emission sources of microplastics
Dutch coast, Noordwijk 10 km from coast	NOORDWK10	1136	004°18′09″, 052°18′08″ (E50)	North of Rotterdam Harbour, 9 km further offshore (gradient)
Breeveertien, Noordwijk 50 km from coast	NOORDWK50	1135	003°47′12″, 052°28′51″ (E50)	North of Rotterdam Harbour, but 40 km further offshore (gradient)
Terschelling, 10 km from coast	TERSLG10	1146	005°06′03″, 053°27′40″ (E50)	Oestergronden is a sedimentation zone for SPM
Oestergronden, Terschelling 100 km from coast	TERSLG100	1141	004°20′31″, 054°08′58″ (E50)	Sedimentation zone for SPM; high density of benthic marine species
Oestergronden, Terschelling 135 km from coast	TERSLG135	1140	004°02′28″, 054°24′56″ (E50)	Sedimentation zone for SPM; high density of benthic marine species
Oestergronden, Terschelling 175 km from coast	TERSLG175	1142	003°41′30″, 054°43′09″ (E50)	Sedimentation zone for SPM; high density of benthic marine species
Wadden Sea				
Waddenzee Dantziggat Zuid	DANTZGZD	1149	177014, 601488 (RD)	Large sedimentation zone south of Ameland Island with high biodiversity and fish nursery grounds
Estuaries				
Rhine Estuary 1	RHNEST1	1147	n/a	Large river estuary, possible hotspot
Rhine Estuary 2	RHNEST2	1148	n/a	Large river estuary, possible hotspot

Table 2.2 Wastewater treatment facilities selected for effluent sampling for microplastics analysis.

WWTP	Samples (n)	Water body receiving effluents	Hydraulic capacity (m^3/h)
Houtrust (The Hague)	3	North Sea	13900
Amsterdam West	3	North Sea Canal	30000
Heenvliet (conventional)	3	Oude Maas River	3000
Heenvliet (membrane reactor)	3	Oude Maas River	3000

Table 2.3 The five species of marine invertebrates sampled for this study. [a]samples preserved in alcohol; [f]samples preserved at -20ºC.

IVM LIMS code	Species name	English common name	Dutch common name	Sampling location	Number of individuals pooled (n)
13/0144	*Littorina littorea* [a]	Common periwinkle	Alikruik	Oosterschelde, Neeltje Jans	10
13/0145	*Gammarus* sp.[a]	Amphipod	Vlokreeft	Oosterschelde, Neeltje Jans	16
13/0146	*Crassostrea gigas*[f]	Pacific oyster	Japanse Oester	Oosterschelde, Neeltje Jans (outside)	3
13/0147	*Mytilus edulis*[f]	Blue mussel	Mossel	Oosterschelde, Neeltje Jans (outside)	10
13/0148	*Carcinus maenas*[a]	Common littoral crab	Strandkrab	Rhine estuary	9
13/0149	*Crassostrea gigas*[f]	Pacific oyster	Japanse Oester	Rhine estuary	3
13/0150	*Mytilus edulis*[f]	Blue mussel	Mossel	Ter Heide, North Sea coast	10

3 Analytical method

3.1 Sediments

Sediment samples were homogenized at the laboratory before taking subsamples for extraction and analysis and for dry weight determination so that concentrations could be expressed as number of particles per kg dry sediment.

For the extraction of microplastics the method of Thompson *et al.* (2004) was followed, in a slightly adapted, miniaturized form. Briefly, 25 g sediment was added to an Erlenmeyer with pure analytical grade water (milliQ) to which NaCl had been added (saturated solution, 1.2 kg NaCl/L). The sediment was allowed to settle to the bottom of the Erlenmeyer flask while the particles which were less dense than saturated salt solution were allowed to float at the top of the water layer. The top water layer was filtered over a 0.7µm Whatman glass filter and observed by light microscopy. The number of microplastic particles was counted and corrected for the low and stable blank (control chart mean 2 microplastic particles per analysis). The concentrations were expressed as number of particles per kg dry sediment. Particles were classified into two broad size categories: particles between 1 and 300 µm and those between 300 and 5000 µm. (The latter corresponds to particle sizes commonly targeted in seawater surface microplastic sampling).

3.2 Wastewater treatment plant effluents

The wastewater treatment plant effluents were analysed in triplicate, and four different types of samples were measured. The effluents were filtered similarly to the sediment samples. The samples were well mixed immediately prior to taking aliquots from each effluent sample for extraction, since microplastics are not in solution and most can be assumed not to be neutrally buoyant.

3.3 Biota

The soft tissues of invertebrate biota species were dissected out of the shell or exoskeleton (with the exception of the very small amphipods). The material was freeze dried and then homogenized to a powder form. Between 3 and 16 individuals were pooled for the analysis (Table 2.3). An aliquot (100 mg) of the pooled freeze-dried sample then underwent a nitric acid and microwave destruction under conditions of high temperature and pressure in closed Teflon vessels, according to a standard operating procedure at IVM (Van der Horst, 2013). Analytical grade water (milliQ) was used for making solutions or for rinsing the filter. The extract was then neutralized with NaOH solution, treated with hydrogen peroxide (30%), rinsed with MilliQ, and then examined by light microscopy on the same 0.7 µm glass filters as for sediments.

4 Results and discussion

4.1 Microplastics in marine sediments

Microplastics were detected in sediment samples from all 15 sediment sampling locations (Table 4.1). Procedural blanks, which are part of the quality control for background contamination during sample preparation, extraction and analysis, were low and stable; when calculating the total number of microplastics in a sample the background level was corrected for. The duplicate sample gave identical results.

Over half of the particles were smaller (or shorter) than 300 µm (ca. 65 % of all microplastics counted were in the size category <300 µm). The largest particle detected was not readily visible to the naked eye (fibres could be as long as a mm). Spheres, fibres and fragments were among the shapes detected (Fig 4.1); fibres were dominant in most samples. The sediments with the highest numbers of microplastic particles were from the Rhine Estuary (12/1147 and 12/1148). The next highest concentration was detected in the Wadden Sea at the Dantziggat (12/1149).

The remaining North Sea locations averaged 440 particles/kg dw sediment. The location 50 km offshore from the municipality of Noordwijk (12/1135) had the lowest number of particles and here only particles > 300 µm were detected. 100 km offshore of the island of Terschelling (12/1141) we found the highest percentages of particles < 300 µm (Table 4.1). To visualize the variation in microplastics concentrations in sediment in relation to the geographical location see Figure 4.2.

The microplastics concentrations found in these sediments are of a similar magnitude as those reported by Claessens *et al.* (2011), who measured a variety of sediments in the coastal and offshore zones of Belgium, although the estuarine hotspots in the present study exceed levels reported by Claessens *et al.* (2011). It is difficult to compare our measured concentrations to those generated for estuarine and shoreline sediments in recent UK studies, since they report on a wet weight basis of sediment (Thompson *et al.* 2004; Browne *et al.* 2010; 2011).

That synthetic fibres are prevalent in sewage sludge has been known for quite some time. The presence of synthetic fibres has been used as an indicator of municipal wastewater sludge materials deposited at sea (Habib *et al.* 1996) and on agricultural lands (Zubris *et al.* 2005). The sediment sampling location 10 km off the Dutch coast at Ter Heide (12/1137) is in the vicinity of a historical sewage sludge dumping site. The number of microplastics detected in sediment from this site was somewhat higher than 10 of the other 15 sites examined in this study, although this cannot provide conclusive evidence of the origin of the microplastics. Pinpointing the origin or source of field-collected, microplastic fragments is for the most part impossible to do accurately.

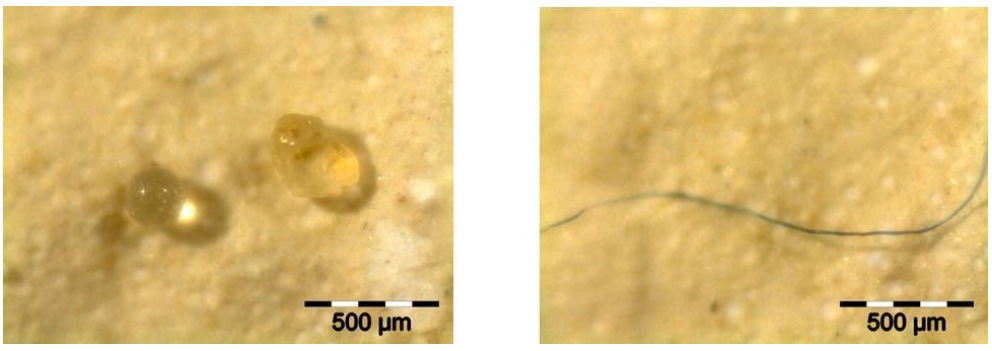

Figure 4.1 Examples of microplastics detected in North Sea marine sediments.

Table 4.1 Microplastic particle concentrations in sediments from 15 Dutch coastal, offshore and estuarine locations incl. average and standard deviation (s.d.)

IVM LIMS code	RWS location code	Total particles per kg dry wt	% particles >300 µm	% particles <300 µm
North Sea				
12/1135	NOORDWK50	100	100	0
12/1136	NOORDWK10	500	50	50
12/1137	TERHDE10	560	91	9
12/1138	TERHDE1	440	56	44
12/1139	NOORDWK2	240	40	60
12/1140	TERSLG135	520	70	30
12/1141	TERSLG100	440	25	75
12/1142	TERSLG175	470	44	56
12/1143	WALCRN2	410	38	63
12/1144	GOERE6	330	29	71
12/1145	HARVT1	520	30	70
12/1146	TERSLG10	720	80	20
	Average (s.d.)	440 (160)		
Wadden Sea				
12/1149	DANTZGZD	770	31	69
Estuaries				
12/1147	RHNEST1	3600	14	86
12/1148	RHNEST2	3010	26	74
	Average (s.d.)	3300 (420)		

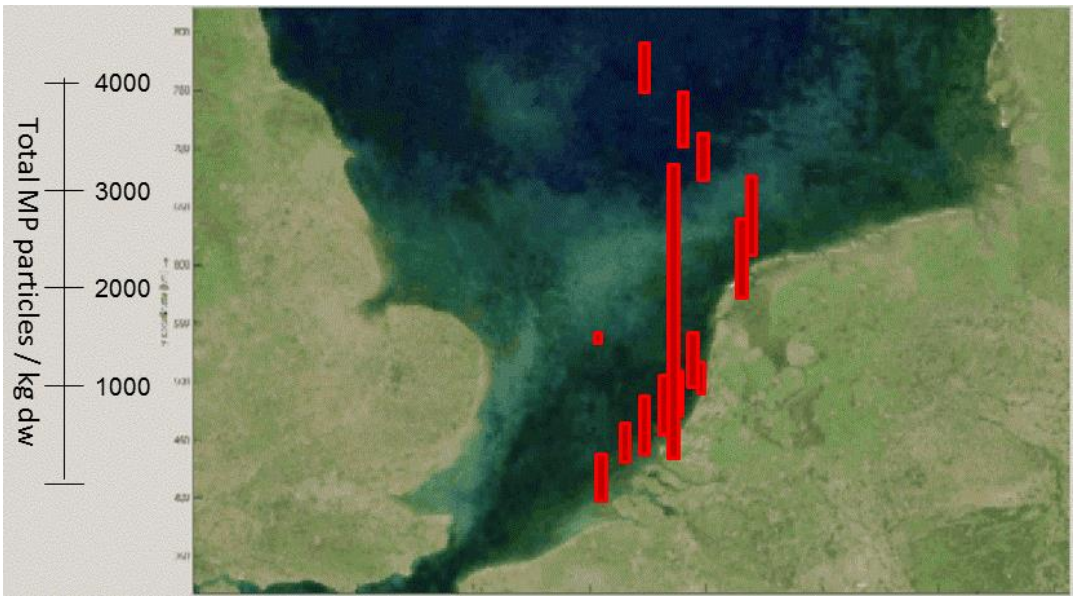

Figure 4.2 Visualisation of relative microplastics concentrations per kg dry sediment in zones along the Dutch North Sea coast, offshore and estuaries selected for sampling and analysis. (Sample point located at the lower end of each red concentration bar.)

4.2 Microplastic in treated wastewater effluents

Microplastics were detected in all effluents (Table 4.2), with significant variation among the three samples taken on the same day, possibly suggesting not all effluents samples are representative of the bulk (therefore multiple samples or pooling is recommended). The concentrations in individual samples ranged from 9 particles/L (min.) to 91 particles/L (max.) with a mean and median of all samples of 52 particles/L. Fibres, spheres and fragments were detected (Figure 4.3). The data shown here do not demonstrate any additional removal of microplastics if effluents are treated in the membrane reactor (MBR) that is located at Heenvliet. In fact, when looking at the means of all WWTP locations, they all emitted similar numbers of microplastic particles/L treated effluent.

Table 4.2 Measured concentrations of microplastics in WWTP effluents (triplicate samples per site). Average and standard deviation (s.d.) given per location and for Heenvliet, for both effluent types (conventional and membrane reactor, MBR).

IVM LIMS code	Location	Particles/L effluent	% particles >300 µm	% particles <300 µm
11/1347B	Heenvliet Conv.	57	100	0
11/1347C	Heenvliet Conv.	67	78	22
11/1347D	Heenvliet Conv.	19	0	100
	Average (s.d.)	48 (25)		
11/1352A	Heenvliet MBR	42	23	77
11/1352B	Heenvliet MBR	43	31	69
11/1352C	Heenvliet MBR	67	26	74
	Average (s.d.)	51 (14)		
12/0710	Amsterdam W	63	78	22
12/0711	Amsterdam W	9	33	67
12/0712	Amsterdam W	91	58	42
	Average (s.d.)	54 (42)		
13/0214A	Houtrust	72	80	20
13/0214B	Houtrust	47	86	14
13/0214C	Houtrust	45	71	29
	Average (s.d.)	55 (15)		

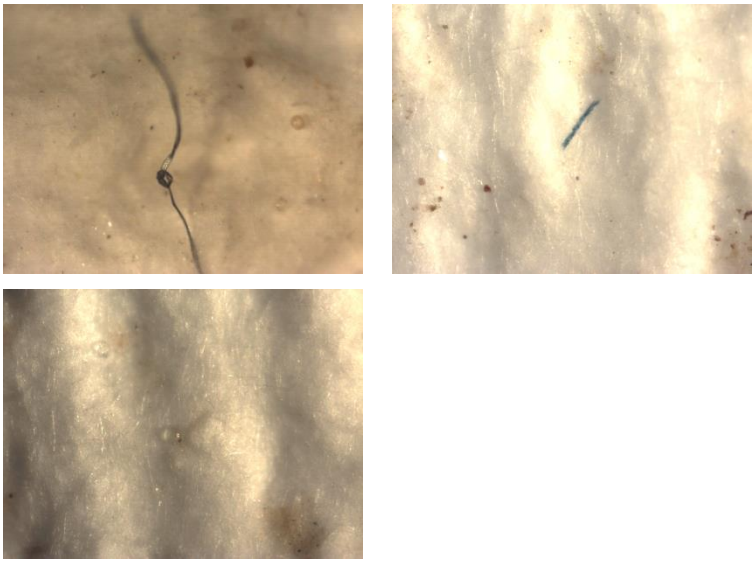

Figure 4.3 Examples of particles (fibres, spheres) detected in treated wastewater effluents.

4.3 Microplastic in marine biota

The microplastics were measured in soft tissues of the biota (including gut contents) and in whole bodies of the amphipods (Figure 4.4). In four of the species investigated, microplastics were detected at concentrations between 11 and 105 particles/g d.w. (Table 4.2). In the crabs, no microplastic particles were detected at all, despite the location of their habitat in the Rhine Estuary, where the highest number of microplastics in sediment was detected in a composite sediment sample (Table 4.1).

Many of the particles detected in the four other species were fibres (Figure 4.5). The highest concentration was found in the blue mussel (105 particles/g d.w.). The higher concentrations observed were in species that are filter feeders (oysters, mussels) and which were sampled from the Oosterschelde (Eastern Scheldt). Oysters from the Rhine Estuary had fewer particles than oysters from the Oosterschelde (Table 4.3).

The sizes of the microplastics detected were largely in the range of 1-300 µm. This size range is typically not investigated in the stomach content analysis of Northern Fulmar seabirds, where only the 1-5 mm range of microplastics is recorded (Van Franeker *et al*. 2011). In laboratory exposure experiments, Brown *et al.* (2008) previously demonstrated that mussels are capable of ingesting plastic particles between 3 and 10 µm in size that they filter from the water phase. Von Moos *et al.* (2012) showed in laboratory exposures that amorphic microplastic particles between 1 and 80 µm in size were taken up within hours through the gills of blue mussels, ingested, and transported to the digestive gland where they accumulated in vacuoles. Microplastic exposure had pathological effects on exposed animals and gave rise to a strong inflammatory response. The field-collected mussels from the Oosterschelde (13/0147) had the highest microplastics concentration of all samples in the current study and contained more of the smaller size range of particles (1-300 µm) than the larger size range (300-5000 µm) (Table 4.3).

Figure 4.4 *Impression of sample preparation stage of marine biota (Clockwise from upper left: blue mussel, sand crab, Pacific oyster, common periwinkle).*

Table 4.3 *Microplastics concentrations detected in five marine invertebrate species collected from the Dutch coast at Oosterschelde Neeltje Jan outside (OS); Rhine Estuary (RE), Ter Heide North Sea coast (TNS). Concentrations in number of particles per gram of dry tissue.*

IVM LIMS code	Species (location)	Dry weight (%)	Total particles particles/g d.w.	Particle size 1-300 µm (%)	Particle size 300 -5000 µm (%)
13/0144	Periwinkle (OS)	33	20	25	75
13/0145	Amphipod (OS)	10	11	67	33
13/0146	Pacific oyster (OS)	14	87	75	25
13/0147	Blue mussel (OS)	13	105	82	18
13/0148	Sand crab (RE)	24	0	0	0
13/0149	Pacific oyster (RE)	8	30	80	20
13/0150	Blue mussel (TNS)	19	19	50	50

Figure 4.5 Examples of fibres detected in extracts from marine biota.

5 Concluding remarks

This exploratory study generated unique data for the Dutch marine environment through the analysis and detection of microplastics in field collected invertebrates and sediments. In addition, this study provided the opportunity to investigate the concentrations of microplastics in treated wastewater effluents, already signalled in the summer of 2012 as a possible emission route to surface waters and eventually to the marine environment (Leslie *et al*. 2012). The methods applied have been recently developed and/or adapted from existing methods. This is a relatively new field and we recognize that there is still work to be done on improving the data quality and comparability of measured microplastic concentration data between laboratories. The quality will be easier to assure and control in the future once e.g. reference materials, interlaboratory studies, proficiency testing and training initiatives become available.

For spatial or temporal comparisons, it is useful to express concentrations in sediments based on the dry weight of the sediments (as the wet weights vary more than the dry weights). The Belgian group, Claessens *et al.* (2011) published sediment data for locations a few hundred km south of the locations investigated in this study, and also found high amounts of microplastics which they expressed as number of particles per mass of dry sediment. These reporting units are more useful for comparing between sample sites than the number of particles per 50 ml wet sediment expressed in earlier studies.

To make the data even more comparable in the future, and to properly interpret spatial and/or temporal trends, there would be a need to measure or estimate the mass of the microplastic materials in the sample as well. The current state-of-the-art approach is to count the particles in a given amount of sediment. The particles belong to a wide variety of sizes, from potentially 10 nm up to 5 mm and potentially (further) fragment in time. If a piece of plastic breaks in two fragments, it means a doubling of the number of microplastics counted, while the amount of plastic remains constant. This can potentially give a false impression that there is more plastic material mass present at a given sampling location or time point when there is actually only a larger number of relatively small plastic particles present. Solving this issue will require investments in more method development in this area. Collecting data on size, number and total mass of microplastic particles in sediments would provide for a powerful dataset.

Also more polymer identification method development is required to measure the very small particles regularly encountered in this study. The infrared (IR)-based techniques are of limited use when the particle size is very small. With light microscopy as an identification technique it is important to exclude as many non-plastic particles as possible. This can be achieved through improving the extraction methods, which put the characteristic properties of plastic to use. Destruction of organic matter through methods that only plastic can withstand (e.g. homogenization, acid, microwave, hydrogen peroxide treatments) is an option for biota, for instance.

Density separation of plastics from aqueous samples, slurries or suspensions in water may be improved for abiotic matrices. Other techniques, which may or may not be applicable to the analysis of field collected samples, could be interesting to further develop for laboratory exposure experiments (e.g. Claessens *et al.* 2013). Many methods will be operationally defined in nature, as it will be difficult to find a single analytical method that extracts and quantifies all plastic types with equal selectivity and sensitivity. The extraction of microplastic from environmental matrices is an area with huge potential for further method development.

The relatively high measured concentrations of microplastics in sediment compared to various studies of the water column concentrations (e.g. Norén 2008 but see also overview in Leslie *et al.* 2011), lends support to the suggestion that sediments act as sinks for microplastics and would be a suitable matrix for monitoring. Common methods for sampling microplastics from seawater

often rely on nets with 333 µm mesh sizes, limiting the catch to larger size categories than are targeted when applying the sediment methods used here. This means the method for microplastic extraction from sediment targets a broader size range of particles than most seawater extraction methods. The levels of microplastics is high enough in North Sea sediments that should a future increase or decrease occur at a given location, it is expected that there could be sufficient statistical power built into a study design and sufficient power of analytical detection to observe such a trend.

The detection of microplastics from WWTPs provided additional evidence for the inability of WWTPs to retain all microplastics in the sludge, as in every treated effluent large numbers of microplastics were observed. The data presented here suggests that membrane reactor systems are not better at retaining microplastics in effluents than conventional WWTP system effluents. Especially during heavy rainfall, municipalities may exceed their sewage system capacity and emit untreated wastewater with microplastics.

Besides the emissions via wastewater effluents, sewage sludge is another possible emission route. While incineration of sewage sludge is common practise in the Netherlands, many EU Member States do not burn their sewage sludge. More than a third of sewage sludge in the EU is applied as biosolid fertilizer to agricultural land. Other sewage sludge goes to landfills. Sewage sludge dumping at sea was commonly practised by many EU Member States till the 1990's. Run-off surface water from agricultural fields treated with sewage sludge may bring microplastics to rivers and eventually to the sea.

While improvements in wastewater treatment have been considerable since the 1980's, a significant percentage of EU citizens are not served by wastewater collection, particularly in Southern and Eastern Europe, although there are also northern and central regions without wastewater treatment (European Environment Agency, 2013). Considering the WWTP situation in the EU and the evidence of emissions emerging from this present study, it would be advisable to investigate and promote solutions to keep microplastics out of this waste stream. Besides potential end-of-pipe solutions it would be recommended to seriously consider cleaner production solutions, since the latter are immediately effective not only in regions with the capacity to invest in advanced end-of-pipe treatment technologies, but also in less developed regions where such advanced technologies are not readily available.

The detection of microplastics in field collected biota from the Netherlands coast has been demonstrated in this study. Particularly the smaller size fraction was dominant in several of the samples, with the exception of periwinkles. The physical impacts of microplastic body residues are of concern but are only beginning to be studied in marine invertebrates (Moos *et al*. 2012; Besseling *et al.* 2012; Wright *et al*. 2013). One hypothesis that arises from the results presented here is that filter feeders may, through their feeding strategy, be more exposed to microplastics than species with other feeding strategies (such as scavenging or grazing).

There remains much more to learn about which species are more susceptible to microplastics exposure and if there are certain habitats where species are at higher risk than others. It should be noted that that marine species preferred for human consumption include some filter feeders, e.g. mussels, oysters. When new environmental contaminants are detected in biota, food chain transfer studies are recommended to investigate whether secondary poisoning is taking place. When prey organisms are consumed whole, the entire body residue of the prey is consumed by the predator.

It was beyond the scope of this study to examine toxicological consequences of microplastic exposure on the species selected for this study, although this is a key area of novel environmental microplastics research (see examples given in Leslie *et al*. 2011). Besides the potential impact of particle toxicity from microplastics, there is concern for the chemicals associated with plastics (e.g. toxic additives, residual monomers and sorbed contaminants). According to the Global

Chemicals Outlook Report (UNEP, 2012) there are relatively high numbers of substances of very high concern (SVHCs) contained in plastics and product categories which are dominated by plastic materials such as textile and clothing, and home and office articles.

References

Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J. & Koelmans, A.A. (2013). Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). *Environmental Science & Technology 47,* 593-600.

Bogaart-Scholte, M.W.M., van der Weijden, M.H., Naber, A., Wetsteijn, L.P.M.J. & Roos, M. (2010). MWTL Meetplan 2010. [*Dutch National Monitoring Program Plan 2010*], 390 pp. Waterdienst, Rijkswaterstaat, Ministry of Infrastructure and Environment, The Netherlands. In Dutch.

Brown, M.A., Crump, P., Niven, S.J., Teuten E.L., Tonkin, A., Galloway T. & Thompson, R.C. (2011). Accumulations of microplastic on shorelines worldwide: sources and sinks. *Environmental Science & Technology, 45*, 9175-9179.

Brown, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M. & Thompson, R.C. (2008). Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L). *Environmental Science & Technology, 42,* 5026-5031.

Brown, M.A., Galloway, T.S. & Thompson, R.C. (2010). Spatial patterns of plastic debris along estuarine shorelines. *Environmental Science & Technology, 44*, 3404-3409.

Claessens, M., de Meester, S., van Landuyt, L., de Clerck, K. & Janssen, C.R. (2011). Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Marine Pollution Bulletin, 62,* 2199-2204.

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B. & Janssen, C.R. (2013). New techniques for the detection of microplastics in sediments and field collected organisms. *Marine Pollution Bulletin, 70,* 227-233.

European Environment Agency. (2013). Urban waste water treatment (CSI 024) – Assessment.

Habib, D., Locke, D.C. & Cannone, L.J. (1998). Synthetic fibers as indicators of municipal sewage sludge, sludge products, and sewage treatment plant effluents. *Water, Air & Soil Pollution, 103,* 1-8.

Leslie, H.A., Moester, M., de Kreuk, M. & Vethaak, A.D. (2012). Verkennende studie naar lozing van microplastics door rwzi's. [*Pilot study on emissions of microplastics from wastewater treatment plants*]. *H2O, 14/15*, 45-47. In Dutch.

Leslie, H.A., van der Meulen, M.D., Kleissens, F.M. & Vethaak, A.D. (2011). *Microplastic litter in the Dutch Marine Environment*. Deltares-IVM Report 1203772-000. The Netherlands, 85 pp.

Lindeboom, H.J., Witbaard, R., Bos, O.G. & Meesters, H.W.G. (2008). Gebiedsbescherming Noordzee: Habitattypen, instandhoudingsdoelen en beheersmaatregelen. [*Conservation of the North Sea: Habitat types, conservation goals and management measures*] Wageningen, Wettelijke Onderzoekstaken Natuur & Milieu, WO Report 114, 33 pp. In Dutch.

Monitoring Guidance for Marine Litter in European Seas. MSFD GES Technical Subgroup on Marine Litter (TSG-ML). Draft Report. July 2013.

Norén, F. (2008). Small plastic particles in coastal Swedish waters. N-Research report, commissioned by KIMO Sweden. 11 pp.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D. & Russell, A.E. (2004). Lost at sea: Where is all the plastic? *Science, 304,* 838-838.

UNEP. (2012). Global Chemicals Outlook: Towards sound management of chemicals. Report No. DTI/1543/GE, 44 pp.

Van der Horst, A. (2013). Destructie van vetarm dierlijk en plantaardig materiaal m.b.v. een micro-golfoven. [*Destruction of non-fatty animal and plant material by microwave*], IVM Standard protocol W-DE-001, Institute for Environmental Studies, VU University Amsterdam, The Netherlands, 7 pp. In Dutch.

Van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen P-L., Heubeck, M., Jensen, J-K., & Le Guillou, G. (2011). Monitoring plastic ingestion by the Northern fulmar *Fulmarus glacialis* in the North Sea. *Environmental Pollution, 159*, 2609-2015.

Von Moos, N., Burkhardt-Holm, P. & Köhler, A. (2012). Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. *Environmental Science & Technology 46*, 11327-11335.

Wright, S.L., Thompson, R.C. & Galloway, T.S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution 178*, 483-492.

Zubris, K.A.V. & Richards, B.K. (2005). Synthetic fibers as an indicator of land application of sludge. *Environmental Pollution, 138*, 201-211.