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Microplastics in blue mussels (*Mytilus edulis*) from the marine environment of coastal Norway

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Abstract

Plastic has in recent decades become a globally ubiquitous material, and accumulation of plastic waste in the environment is causing concern both among the public and in the scientific community. Especially plastic ending up in the oceans are receiving considerable attention. Microplastics are most often described as plastic particles <5 mm in size and have been reported found in marine environments all over the world. It has been shown that microplastics are ingested by wild organisms, but the extent and effects remain uncertain. One reason for this uncertainty is the lack of globally standardized research methodologies. This thesis aims to contribute with empirical data on microplastic occurrence in Norwegian marine biota, as well as to the method development in the field of microplastic research.

Blue mussels (*Mytilus edulis*) were collected in 2016 and 2017 from 15 sites along the Norwegian coast, spanning from the Oslo fjord to the Barents Sea (N = 332). All tissue and other organic material was dissolved using a solution of 10% potassium hydroxide (KOH), incubating for 24 hours at 60°C. The remaining homogenate was filtered, and visual analysis was performed to identify suspected plastic particles. Subsamples of particles from sites in the Oslo fjord were then subject to chemical analysis by Fourier Transform Infrared Spectroscopy (μFTIR) in transmission mode, for verification. In addition, experimental testing targeting the identification accuracy for natural and synthetic materials by both transmission μFTIR and Attenuated Total Reflectance (ATR) FTIR were carried out.

Suspected plastic particles were found in 56% of the individuals. The average number of particles per individual was 1.52 (±2.34), and the average number of particles per gram (mussel wet weight) was 0.98 (±2.66). Significant differences in the number of particles occurred between sites, and Akershuskaia (Oslo fjord) and Skallneset (Barents Sea) had the highest numbers of particles per individual, being 6.8 (±4.00) and 3.6 (±3.05), respectively. The particles were mainly fibres (84%), followed by fragments (16%), and most (71%) were <1 mm. In the Oslo fjord, 11 polymer groups were identified, with cellophane being the most abundant (62%), followed by “parking lot tar” (21%), polyesters (10%) and acrylics (3%). Experimental testing demonstrated that the particles identified as cellophane (a cellulose-based, semi-synthetic plastic) could possibly be natural, cellulosic materials of anthropogenic origin, such as cotton, paper or linen, in addition to other semi-synthetics such as viscose rayon. This shows that transmission μFTIR, a method which is commonly used for identifying microplastic particles, misidentifies some materials, and that some particles reported as cellophane in the literature may in reality have been natural materials such as cotton or paper. Future studies should work on improving the detection methods.

Preface

This thesis marks the end of my master's degree in natural resource management at the Norwegian University of Life Sciences (NMBU), spring 2018. I want to thank the Norwegian Institute for Water Research (NIVA) for the opportunity to carry out this project, through provision of materials and lab-space.

To my main supervisor, Susanne Claudia Schneider, thank you for all the support and guidance along the way, I could never have done any of this without it. I also want to give a special thank you to Inger Lise Nerland Bråte, who has not only been an excellent assistant supervisor, but also a great lab companion and conversation partner throughout the whole process. You have made this experience a lot more inspiring!

I want to thank Amy Lusher and all the rest of "team microplastics" at NIVA, for taking me in and sharing so much of your knowledge, it's been great working with you all! And to Bjørnar Beylich, thank you for bringing me along in the field and bearing with me as a newbie mussel picker. Calin Steindal from the Museum of Cultural History, University of Oslo also deserves a huge thank you for all the time spent solving problems in front of the FTIR – we would never have managed without you.

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Abbreviations

Plastic polymers

LDPE	Low Density Polyethylene
PA-66	Polyamide 66
PAN	Polyacrylonitrile
PE	Polyethylene
PET	Polyethylene terephtalate
PMMA	Polymethyl methacrylate
PP	Polypropylene
PS	Polystyrene
PUR	Polyurethane
PVC	Polyvinyl chloride

Other

(µ)FTIR	(µ)Fourier transform infrared spectroscopy
ANOVA	Analysis of variance
ATR	Attenuated total reflection
BPA	Bisphenol A
FPA	Focal plane array
GESAMP	Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
IUAPC	International Union of Pure and Applied Chemistry
KOH	Potassium hydroxide
MILKYS	Miljøgifter i norske kystområder (Programme for monitoring contaminants in coastal waters of Norway)
MP(s)	Microplastic(s)
NIVA	Norwegian Institute for Water Research
OSPAR	Commission for protecting and conserving the North-East Atlantic and its resources
POP(s)	Persistent organic pollutant(s)
Pyrolysis-GC/MS	Pyrolysis–gas chromatography–mass spectrometry
RO-water	Reverse osmosis-water
Rpm	Revolutions per minute
UNEP	United Nations Environment Programme
VEAS	Vestfjorden Avløpsselskap
W.W.	Wet weight
W/v	Weight to volume
WWTP	Wastewater treatment plant

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1 Introduction

1.1 Plastic production, and definitions of plastic

Since the start of its mass production in the 1950's, plastic has become a ubiquitous material which has enabled innovation in a countless number of areas (including packaging, building and construction, and renewable energy). The progression away from natural products (of wood, iron, cotton etc.) to plastic items has much to do with the low weight and low production cost of plastics, as well as the durability. In 2016, 335 million tons of plastic were produced globally, with the most common plastic types, based on demand in Europe, being polypropylene (PP), polyethylene (PE, in different densities), polyvinyl chloride (PVC), polyurethanes (PUR), polystyrene (PS) and polyethylene terephthalate (PET; PlasticsEurope 2017). It is estimated that 31% of plastics were recycled in 2016, while 42% was used for energy recovery, and 27% ended up in landfills (PlasticsEurope 2017). However, total degradation of plastic products is estimated to require somewhere between hundreds and thousands of years, and if not disposed of properly, plastics can thus accumulate in the environment (Barnes et al. 2009).

The term "plastic" does not have one universal definition, which can lead to confusion. Probably the most common use of the term is when referring to synthetic (man-made) polymers, and often those that are petroleum-based (UNEP 2015). However, IUPAC (International Union of Pure and Applied Chemistry) defines plastic as a *"generic term used in the case of polymeric material that may contain other substances to improve performance and/or reduce costs"* (Vert et al. 2012). By this definition, plastic includes both natural polymers (e.g. cellulose and natural rubber) and synthetic polymers such as PP and PVC, as well as semi-synthetic bio-based materials including the cellulose-based, artificially produced viscose rayon. Unfortunately, there are inconsistencies in the use of the term "plastic" among researchers as well. For example, several exclude the natural and semi-synthetic polymers when referring to plastics (Remy et al. 2015; Wesch et al. 2016a; Salvador Cesa et al. 2017), while others include the semi-synthetic polymers (Lusher et al. 2013; Woodall et al. 2014; Neves et al. 2015; Li et al. 2016). As a result, this leads to inconsistencies in reporting and the inability to compare results. A report carried out by GESAMP (2016) specifies that plastic can be made of biomass in addition to fossil resources and divides this type of plastic in three categories. First are *biopolymers* or *bioplastics*, which are plastic extracted directly from biomass, like cellulose and

chitin. Next are the *bio-derived* plastics, where polymers are extracted from biomass and then regenerated to make the wanted plastic material, like viscose rayon, cellophane or cellulose acetate (also called semi-synthetics). Last, we have the *bio-based* plastics, which are synthetic polymers made from monomers derived from biomass, an example being bio-polyethylene (GESAMP 2016).

1.2 Microplastic

In recent years, increasing attention and concern has been focused on the issue of "microplastics" (hereafter referred to as MPs) and their potential impact on the environment (Cole et al. 2011). Numerous definition ranges for MPs have been used in different studies, but the most agreed on is the distinction of MPs as plastic particles < 5 mm in size (Barnes et al. 2009; Andrady 2015; Anderson et al. 2016; Bråte et al. 2016). It has been argued that the definition should be < 1 mm, to meet the SI units (Claessens et al. 2013; Browne 2015). In this study, however, the size classes set by GESAMP (2016) are adopted, being microplastic < 5 mm, mesoplastic 5– 25 mm, and macroplastic > 25 mm.

1.2.1 Sources and pathways of MPs to the marine environment

MPs are usually divided into two classes, primary and secondary, based on their source. Primary MPs are those that are intentionally produced in small sizes (< 5 mm), designed either for use in e.g. cosmetics and as industrial scrubbers, or as virgin resin pellets for further production of plastic products (Talvitie et al. 2017). Secondary MPs are on the other hand a result of fragmentation and degradation of larger pieces of plastic material, like textiles, tires and paint. This can either happen during use or after the plastics has ended up in the environment as waste. However, inconsistencies arrive when researchers classify fibres and tire fragments under primary MPs (e.g. Sundt et al. 2014). Therefore, Lusher et al (2017) has gone further and describes three different classes, where the secondary MPs are divided into those generated from use (e.g. fibres from clothes, fragments from tires) and those generated from breakdown of discarded products in the environment (e.g. fishing gear, plastic bags).

The degradation of larger plastics can be viewed as a pathway for MPs to the environment (Browne 2015), and can happen in several ways, generally classified in line with the cause of the degradation. Andrady (2011) names five ways of degradation: biodegradation (done by living organisms), photodegradation (by light), thermooxidative degradation (oxidative

breakdown in moderate temperatures), thermal degradation (action of high temperatures) and hydrolysis (reaction with water). Plastics lying on beach surfaces are quickly affected by solar UV radiation, and starts to degrade quite rapidly (photodegradation). Whilst in water, however, the temperature and oxygen concentration are lower, thus retarding the degradation severely. It is therefore likely that the beach is the most common site for generation of MPs in the marine environment (Andrady 2011).

Approximately 80% of the marine plastic litter originates from terrestrial sources (Andrady 2011). Possible pathways of MPs from terrestrial sources to the marine environment are thought to include wastewater treatment plants (WWTPs), rivers, storms and natural disasters, spreading of sewage sludge (as fertilizer) and atmospheric fallout (Browne 2015; Dris et al. 2016; Duis & Coors 2016; Magnusson et al. 2016; Salvador Cesa et al. 2017; Schmidt et al. 2017). In addition, fishing industry, aquaculture and coastal tourism are all direct sources of plastic to the marine environment, that may generate MPs through long-term degradation (Cole et al. 2011).

1.2.2 Occurrence of microplastics in the marine environment

As previously mentioned, the improper disposal of plastics, as well as accidental loss of plastics to the environment, is problematic and can lead to accumulation of plastics in the environment. It is estimated that about 50% of all produced plastics are disposed after just one use (mainly packaging; Mathalon & Hill 2014). In addition, even with a positive development across Europe, with the proportion of plastics being recycled in 2016 overcoming the proportion being landfilled (PlasticsEurope 2017), most of the globally disposed plastics still ends up in landfill sites and can – if not buried properly – resurface and end up as debris (Barnes et al. 2009). Plastic litter dominates marine debris (e.g., Barnes & Milner 2005), and the proportion of plastic in total marine debris is said to be somewhere between 60% and 80% (Derraik 2002). Eriksen et al. (2014) estimated that over 250,000 tons of plastic particles may be floating on the ocean surfaces worldwide. And this value does not include all the plastic that sinks to deeper waters or the sediments. Different types of plastic polymers have different densities: some might float while others sink to the mid water column or the sediment (Andrady 2015). The density of the plastics can, however, change due to weathering and biofouling, and can also be altered at production by the adding of fillers and other additives (Galgani et al. 2015). In addition, the distribution and accumulation rates of MPs are affected by wind and ocean currents, and by

factors such as proximity to shores, urban activities and coastal uses (Duis & Coors 2016; Li et al. 2016).

1.2.3 Effects of microplastics on marine biota

It is evident that macroplastics (> 25 mm) have an effect on marine wildlife, with ingestion and entanglement in marine birds, turtles and mammals receiving the most attention (Kühn et al. 2015), but the effects of MPs are still largely unknown. However, controlled laboratory exposure studies exposing biota to MPs have demonstrated some adverse effects. For example, Wright et al. (2013) demonstrated that MP ingestion by marine worms can cause suppressed feeding activity, inflammation and reduced energy reserves. Zooplankton has been found to ingest MPs (Andrady 2011; Cole et al. 2013) and studies have demonstrated that transfer of MPs between trophic levels is possible (Farrell & Nelson 2013; Setälä et al. 2014). Sussarellu et al. (2016) studied oysters under exposure to PS microparticles, and found that energy uptake and allocation, reproduction, and offspring performance were all significantly affected.

The toxicity associated with plastics has also been subject to several studies (Teuten et al. 2007; Avio et al. 2015; Hermabessiere et al. 2017), and can come from either residual monomers or additives from manufacturing (e.g. bisphenol A [BPA]), intermediates formed during partial degradation (e.g. styrene from burnt polystyrene), or the ability of plastics to absorb and thus concentrate persistent organic pollutants (POPs) present in sea water (Andrady 2011). In addition, plastics have been demonstrated to accumulate metals from the water, presenting another risk for organisms that ingest it (Ashton et al. 2010). Concerning POPs however, it may seem that plastic is not a substantial carrier to organisms, compared to e.g. natural prey (Koelmans et al. 2013; Herzke et al. 2016).

Several studies have been carried out to investigate MP presence and effect on blue mussels (*Mytilus edulis*) (e.g. Farrell & Nelson 2013; Vandermeersch et al. 2015). In fact, they are the most common species used for studying the fate and toxic effects of MPs in laboratory (Li et al. 2016). Von Moos et al. (2012) demonstrated that blue mussels which had ingested MP particles showed a strong inflammatory response after only three hours of exposure, and that the particles were taken up into the mussels' cells. In a study carried out by Van Cauwenberghe et al. (2015), blue mussels ingested MPs in the field, but no significant effect was detected on the total cellular energy allocation. Browne et al. (2008) showed that blue mussels both ingested

plastic particles, and that they had particles translocated into the circulatory system in just three days, which then persisted there for over 48 days. However, no significant effects were found in their evaluated endpoints concerning the mussels' fitness (Browne et al. 2008).

1.3 Methods for investigating microplastics in marine organisms

There are many different methods used to investigate MPs in marine organisms, most of which are not standardized across research groups on a global scale. This is a problem especially for comparative purposes. Several reviews are available which critically assess methods used (Löder & Gerdts 2015; Wesch et al. 2016b; Lusher et al. 2017b), and method development is considered a priority in the field of MP research (Cole et al. 2011; Tagg et al. 2015).

1.3.1 Sampling of biota

Several organisms have been used for investigations on MPs. Studies on vertebrates are rare as they require substantial efforts and involve ethical considerations, but fish and stranded carcasses (e.g. birds and seals) have been used (Löder & Gerdts 2015). More common is the use of smaller invertebrate organisms like worms, mussels and snails, as they can be directly collected in the field (Besseling et al. 2013; Claessens et al. 2013). The collection technique depends largely on the organism targeted and the research questions, but traps, trawling, nets, shovels and collection by hand are some methods used (Wesch et al. 2016b; Lusher et al. 2017b).

Blue mussels have been suggested by OSPAR as a possible sentinel species for water-borne MP contamination in marine environments (OSPAR 2015), and have been used for monitoring of other hazardous coastal contaminants in Norway since 1981 (Green et al. 2017). Beyer et al. (2017) and Wesch et al. (2016b) have thoroughly reviewed why blue mussels are appropriate in regards to environmental monitoring. In short, they are filter-feeders and abundant around the globe, their biology is well understood, they are sessile and can therefore provide location specific information, they are medium sized (easy to sample with still enough tissue for analysis) and grow together often in large numbers, and they are hardy creatures that can be investigated in laboratory exposure studies in addition to in the field. Lastly, they are ecologically important to other species, including humans. One drawback is that it is possible that not all plastic polymers are bioavailable to mussels, due to the plastics' difference in buoyancy (Wesch et al. 2016b; Beyer et al. 2017).

1.3.2 Extraction of MPs from samples

To be able to detect the MPs ingested by biota, different techniques have been applied and tested, including dissection, depuration, homogenisation and digestion of tissues with chemicals or enzymes (Lusher et al. 2017b). Claessens et al. (2013) tested different methods for extracting MPs from biota through digestion (acid, base and oxidizer) and found that hot acid digestion gave the best results of purification, although this seemed to degrade some of the plastic polymers. Karlsson et al. (2017), found that an enzymatic approach was better than the use of acid. Even more promising is the use of a 10% potassium hydroxide (KOH) solution, tested in several studies, both lab-based (Dehaut et al. 2016; Kühn et al. 2017), and field-based (Foekema et al. 2013; Phuong et al. 2017), with positive results. After the organic material is digested, MPs can be separated from the liquid matrix by density separation (Claessens et al. 2013; Dehaut et al. 2016), sieving (Foekema et al. 2013) or filtering (Vandermeersch et al. 2015; Karlsson et al. 2017; Phuong et al. 2017), with the latter being most used on biota, usually aided by vacuum (Hidalgo-Ruz et al. 2012).

1.3.3 Quantification and qualification of microplastics

To analyse the amount and composition of MPs in biota, visual identification (either by the naked eye, or with a light microscope) is an obligatory step (Hidalgo-Ruz et al. 2012). Here, using morphological characteristics of the particles such as size, shape and colour, the plastics can be separated from other materials, such as organic debris and items like glass (Bråte et al. 2017). The probability of misidentification by visual analysis of small particles is, however, very high, and it is recommended to conduct chemical analyses as well (Hidalgo-Ruz et al. 2012). This can be done in several ways, with Raman and Fourier Transform Infrared (FTIR) spectroscopy being the most commonly used (Foekema et al. 2013; Dehaut et al. 2016; Jung et al. 2018). FTIR can be used in attenuated total reflectance (ATR), transmittance or reflection mode, and with or without a connected microscope, with different advantages and disadvantages for each technique. For extensive review on these techniques, see Löder and Gerdts (2015) and Wesch et al. (2016b).

1.4 Microplastic in the Norwegian marine environment

There are limited studies investigating MP pollution in Norwegian marine environments, but Strand et al. (2015) provide an overview of the data available on marine litter in the Nordic

environment. Bråte et al. (2017) give a review of plastic in marine species in Nordic water, and in a report from Sundt et al. (2014), sources of MP pollution to the marine environment with a focus on Norway are assessed. The latter states that an estimated 8,000 metric tons of MP are emitted from Norwegian primary sources (which in this context comprises both primary MPs and MPs generated from use, such as tire wear) each year, and a significant proportion of this could reach water bodies and the ocean. They also report that the largest source of MPs is most likely the abrasion from tires and road markings, followed by dust and particles from plastic-based paint, city storm water effluent and road runoff (Sundt et al. 2014). Another report, on possible measures to deal with these major MP pollution sources, was published in 2016 (Sundt et al. 2016). Magnusson (2014) demonstrated that wastewater treatment plants (WWTPs) are a possible source or pathway of MP to the ocean when studying three WWTPs in Norway (VEAS, Tønsberg and Fuglevik). The study showed that many MP particles were released with the effluent water in all three WWTPs, but more from VEAS than the other two (~35 million particles per hour). However, it was also observed that what was emitted from VEAS was a smaller proportion of the total that came in with the influent water, and that the retention efficiency (90-99%) thus was higher than for the other two plants (Magnusson 2014).

Other studies that have investigated MP occurrence in the Norwegian marine environment includes Lusher et al. (2015), who sampled surface and subsurface water south and southwest of Svalbard, and found MPs in more than 90% of the samples, being the first to identify MPs in Norwegian marine waters. The finds were mainly fibres (95%), and although no source could be determined, this suggests that the MPs are from the breakdown of larger products and has most likely been transported over long distances (Lusher et al. 2015). Herzke et al. (2016) investigated northern fulmars (*Fulmaris glacialis*) from Norwegian waters caught as by-catch by fishermen, and out of 75 birds, 81% had visible plastic in their stomach, with 106 pieces in one bird at most. A study performed on Atlantic cod from the Norwegian coast (Bråte et al. 2016) demonstrated that cod ingests MPs as MPs were found in the cod stomachs from two out of six locations, with three percent of the total individual stomachs containing MPs. Nine different polymers were identified using FTIR, including polyester, PP and PVC, and Bergen City Harbour was identified as being a hot spot (Bråte et al. 2016). At the time when the work of this thesis was initiated, no studies had been carried out on MPs in Norwegian blue mussels.

1.5 Aims of the study

The aim of this study was to contribute with empirical data on MP occurrence in Norwegian marine biota, considering the significant knowledge gap that exists in this area. This overall aim was divided into three objectives:

1) To test and develop methods used to investigate MPs in marine biota, using blue mussels (*Mytilus edulis*) as test organism.

2) To quantify the number of MPs present in blue mussels along the Norwegian coast, and to some extent look at qualitative traits.

3) To investigate MPs in blue mussels in the Oslo fjord in more depth, by looking at qualitative traits, spatial trends and local differences.

To reach these aims, blue mussels were collected from different sites along the coast of Norway, with several sites located in the Oslo fjord.

2 Materials and methods

2.1 Sampling sites and collection of blue mussels

Blue mussels (*Mytilus edulis*) from 15 sites were analysed. Seven sites were located in the Oslo fjord, where one (O7) was from the outer Oslo fjord, and the remaining six (O1-O6) from the inner fjord. The other eight sites (N1-N8) were located along the coast of Norway, from Finnmark in the north to Hordaland in the southwest (Fig. 1). Four of the sites (N5 – N8) were situated relatively close to each other, all located in Hordaland and three in the Hardanger fjord. Thirteen sites were sampled in 2016, whereas two (O1 and O5) were sampled also in 2017, and finally two sites (N5 and O3) were sampled only in 2017.

All the samples were collected according to a standardized NIVA procedure for "Sampling of marine biological material for chemical analysis of environmental pollutants and biological effect parameters". In brief, between 20 and 30 representative mussels were collected from each site, and only mussels that were not visibly damaged were included. The technique for sampling depended on the position of the mussels and the substrate they grew on (Table 1). The mussels, all closed, were roughly rinsed for fouling and washed 2-3 times with seawater before packed in freezer plastic bags. These were labelled and transported in cooler boxes until they were frozen (-20°C). Three of the sites (O1, O3 and O5, from 2017) were sampled by myself, the rest by NIVA in connection with the MILKYS monitoring programme. Details about each site, including collection method are presented in Table 1.

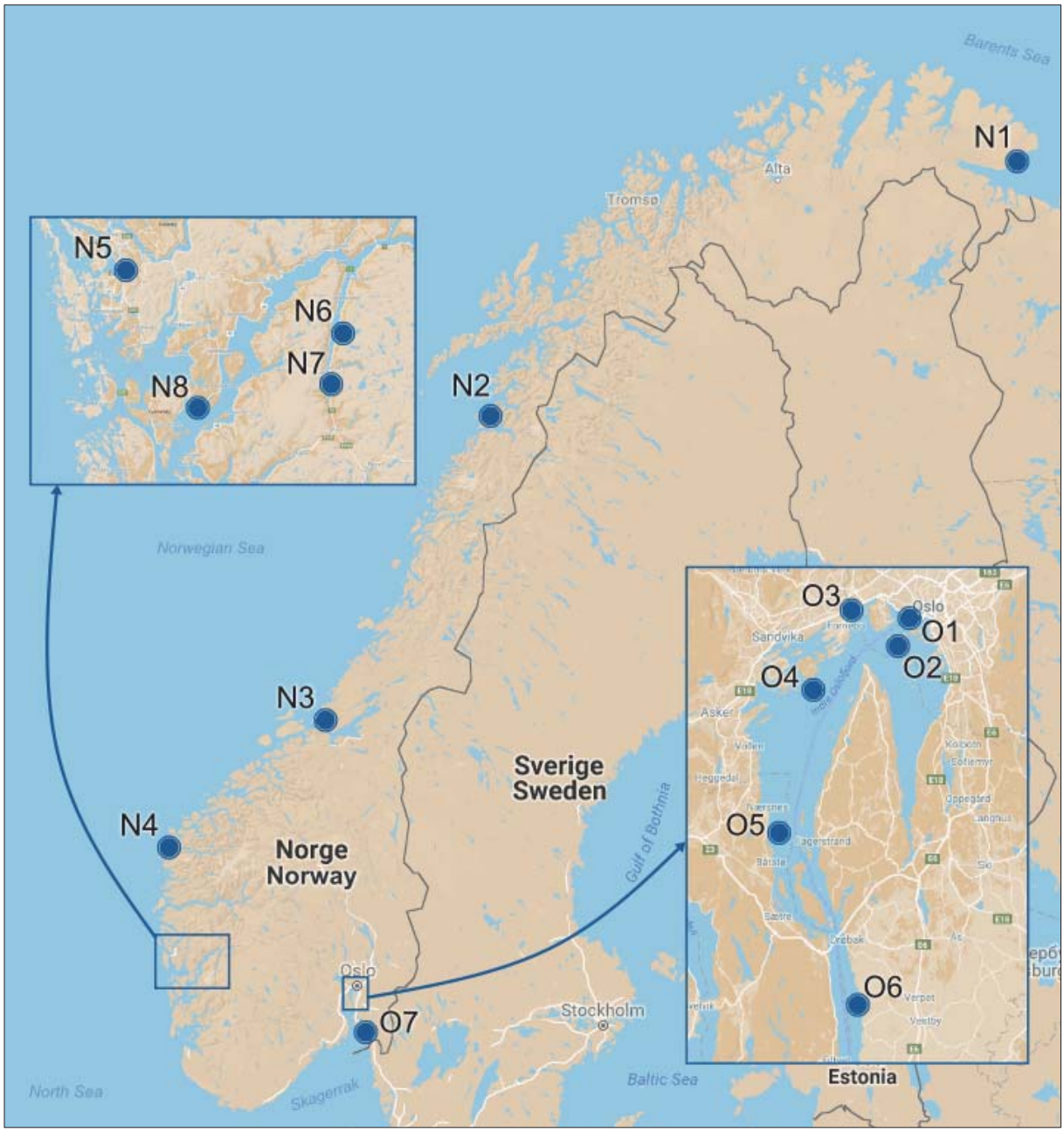

Fig. 1 Map of the sample sites, marked with blue circles and station code. N1 to N8 are locations outside the Oslo fjord, while O1 to O7 are the locations in the Oslo fjord, which were studied in more depth. Station codes correspond to Table 2. Map modified from Google Maps.

Table 1 Sample stations sorted from north to south, with key details of each site. Year is the year the samples were collected, n the number of individuals analysed. The samples collected in 2017 are marked with b. FW = freshwater. WWTP = Wastewater treatment plant. Table based on Lusher et al. (2017a), with additions.

Site	Name	Year	Location (GPS)	n	Position (depth in m)	Substrate	Collection method	Comment
N1	Skallneset	2016	70.1372, 30.34175	20	Shoreline, intertidal (0)	Rock	Hand	Near national park. Very exposed to the sea.
N2	Bodø Havn	2016	67.41271, 14.62193	20	Subsurface (0-1)	Concrete pier	Hand	Exposed area. Some rope and plastic surfaces. 20 km from Bodø port.
N3	Ørland	2016	63.65186, 9.56386	20	Shoreline, intertidal (0)	Rock and sand	Hand	Close to airport, urban and rural areas, boat harbour.
N4	Måløy	2016	61.93098, 5.05241	20	Subsurface (0.2-1.2)	Pontoon	Hand	Exposed area.
N5b	Bergen	2017	60.40080, 5.30352	20	Shoreline, intertidal (0)	Rock	Hand	Harbour with boat traffic.
N6	Kvalnes	2016	60.22050, 6.60200	20	Intertidal, subsurface (0-1)	Rock and sand	Snorkelling	Metal and plastic industry. FW from high rain and river flushing.
N7	Byrkjenes	2016	60.08383, 6.55050	20	Subsurface, possible exposure (0-1)	Attached to submerged branch	Snorkelling	Metal and plastic industry. Large FW influence from river.
N8	Lille Terøy	2016	59.98400, 5.75450	20	Subsurface (0-0.5)	Pontoon	Hand	Mouth of Hardanger fjord. FW from high rain and river flushing.

O1	Akershuskaia	2016	59.90533, 10.73633	20	Subsurface (0-1)	Quayside	Metal rake with net	Harbour with high boat traffic. Near outlet of Alna river and Akers river.
O1b	Akershuskaia	2017	59.90533, 10.73633	20	Subsurface (0-1)	Quayside	Metal rake with net	Near deposit of snow.
O2	Gressholmen	2016	59.883837, 10.711940	20	Subsurface (0-0.5)	Sandy shore	Hand	Nature reserve. About 3 km west of Bekkelaget WWTP.
O3b	Lysaker	2017	59.911553, 10.645526	20	Subsurface (0-1)	Concrete pier	Metal rake with net	About 0.5 km north of the overflow outlet from VEAS. Close to outlet of Lysaker river.
O4	Gåsøya	2016	59.851357, 10.588807	20	Subsurface (0-1)	Rock	Snorkelling	5-6 km northeast of VEAS WWTP
O5	Ramtonholmen	2016	59.74450, 10.52283	12	Subsurface (1-2)	Rock and sand	Snorkelling	About 5 km south of VEAS WWTP
O5b	Ramtonholmen	2017	59.74450, 10.52283	20	Subsurface (1-2)	Rock and sand	Snorkelling	
O6	Solbergstrand	2016	59.61550, 10.65150	20	Intertidal (0-1)	Sand and rock	Hand	Mouth of inner Oslo fjord. FW stream.
O7	Singlekalven	2016	59.09500, 11.13667	20	Subsurface (0.5-1.5)	Sandy bottom, some rocks	Snorkelling	Close to national park. Near outlet of river Glomma.

2.2 Description of main study area - The Oslo fjord

Seven of the 15 sampling sites were located in the Oslo fjord, and the samples from these were subjected to a more detailed analysis than the rest, including chemical analysis by FTIR.

The Oslo fjord extends from the city of Oslo in the North to Skagerrak in the South between Norway, Denmark and Sweden. It is divided into the inner Oslo fjord and the outer Oslo fjord, separated by the Drøbak sound. Here, the depth is only 27 m and the width of the sound only about 1 km at minimum. This, as well as hilly seabed topography, makes the water exchange and circulation low in the inner fjord. In the inner fjord, we find the two deep basins the Vestfjord and the Bunnefjord (Arnesen 2001; Fig. 2).

Norway's two most water rich rivers, the Glomma and the Drammens river empty into the outer Oslo fjord (Thorsnes 2017), while several smaller rivers like Akers river, Lysaker river, Alna river and Sandviks river, run into the inner fjord (Askheim 2013; Fig. 2). The Oslo fjord also has Norway's highest traffic of boats and is the country's most used recreational area at sea (Askheim 2013).

Most of the pollution to the fjord comes from municipal and industrial wastewater from especially the municipalities Bærum and Oslo (Thorsnes 2017). There are two operative wastewater treatment plants (WWTP's) in the inner Oslo fjord, VEAS in Røyken (located in the Vestfjord) and Bekkelaget in Oslo (located in the Bunnefjord; Fig. 2; Arnesen 2001). VEAS is the biggest wastewater treatment plant in Norway and treats wastewater from more than 600.000 citizens (VEAS 2017).

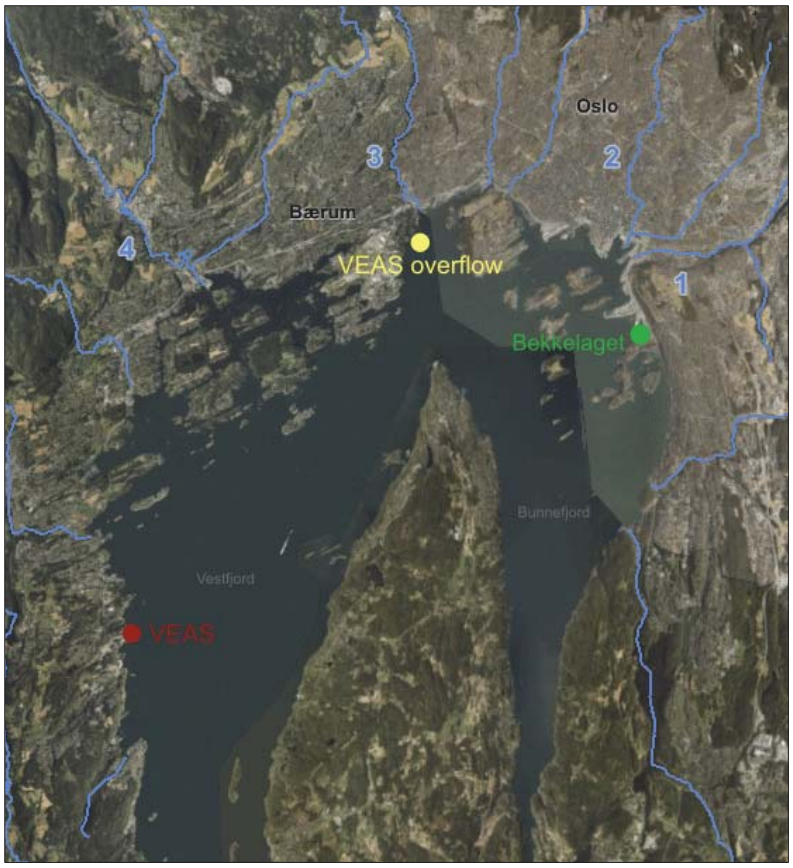

Fig. 2 Map over part of the inner Oslo fjord, with the rivers discharging to the fjord drawn in blue. Numbered are 1) Alna river, 2) Akers river, 3) Lysaker river and 4) Sandviks river. The red circle marks VEAS WWTP, the yellow the place where VEAS' stormwater overflow discharges, and the green Bekkelaget WWTP. Map modified from NVE Atlas 3.0.

2.3 Digestion of mussels using 10% KOH

In order to be able to analyse the number of MPs in the blue mussels, all organic material needed to be broken down without damaging the plastic. To do so, 20 randomly selected individuals from each site were first taken from the freezer and thawed before their shell length was measured using a caliper. Then the soft tissue was excised from the shells using scalpel and forceps. All of the biological material was included, except for the muscular foot, as this was considered to possibly be more resistant to the solvent, and as it was seen as highly unlikely to contain MPs, because it is not part of the digestive or filtering system.

Each individual mussel was put in a 100-mL glass beaker cleaned with filtered (0.22 μm) reverse osmosis (RO) water and covered with aluminium foil, before weighing (wet weight). Then, a filtered solution of 10% KOH (w/v) was added to each beaker. The volume of the added

KOH was determined by the weight of the mussel (Table 2). Previously performed tests (unpublished data) showed that 1 g of mussel tissue corresponded to approximately 1 mL volume. The choice of 10% KOH as solution for digestion was based on studies done by Dehaut et al. (2016), Kühn et al. (2017) and Foekema et al. (2013). As for the volume added, it was decided on approximately ten times the volume of the mussel, even though the mentioned studies had shown that three times the volume was sufficient. This was due to the relatively big size of the beakers used in this study, to reduce the volume to surface ratio and thereby the risk of evaporation ruining the sample, and also ensuring that all the mussel tissue was submerged.

To ensure that this modification in volume did not affect the plastics, a recovery test was conducted. Reference beads of polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), PA-66 (nylon) and low-density polyethylene (LDPE) were each put in 70 mL of 10% KOH and underwent the exact same process as the mussel samples. The beads were visually identified in the microscope before and after being exposed to the treatment. All beads were recovered, and no degradation was found.

When the weight of a mussel was > 6 grams, a 250-mL glass beaker was used instead of a 100-mL beaker, as a precaution against spills. As negative controls, three clean 100-mL beakers containing only 10% KOH (60 mL) and no mussels were prepared at the same time. All the beakers were then incubated at 60°C, 140 rpm, for 24 hours in an incubator shaker (New Brunswick™ Innova® 44). These settings were chosen based on Dehaut et al. (2016).

Table 2 Volume of 10% KOH added to the beakers with mussel tissue, depending on the weight of the mussel. g. w.w. = gram wet weight.

Mussel weight (g. w.w.)	KOH volume (mL)
< 2	20
2-3	30
3-4	40
4-5	50
5-6	60
> 6	70

After incubation, the glass beakers were cooled at room temperature before filtration. The filtration was carried out using a Millipore® vacuum filtering assembly (Millipore Corporation, Billerica, MA, USA), an aquarium pump and a glass microfiber filter (Whatman® GF/D, 2.7 µm). After filtration, each filter was removed from the filtering system with forceps and put

into a small petri dish with a lid, and marked with the sample ID. These were then stacked and stored in boxes until analysis. A schematic representation of the process from excision to filtering is shown in Fig. 3.

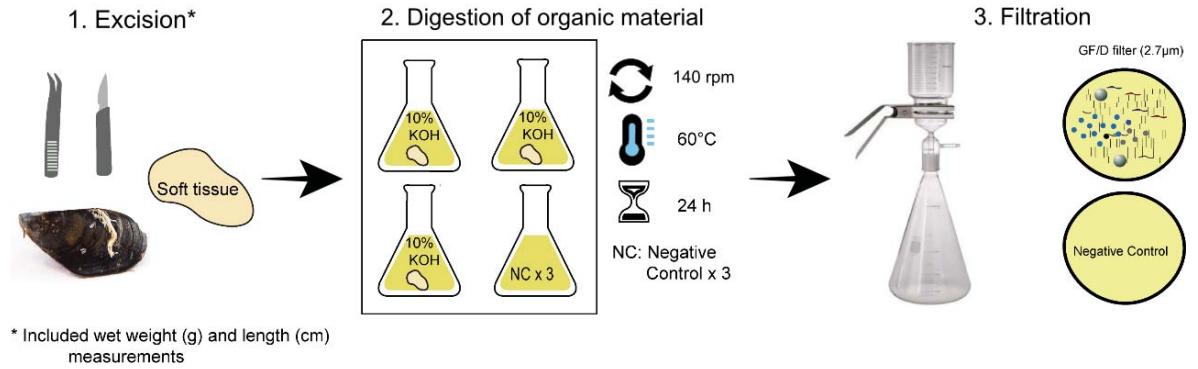

Fig. 3 A schematic representation of the process of excision, digestion and filtration of blue mussel samples. Reprinted (and modified) with permission, from Lusher et al. (2017a).

2.4 Visual analysis using microscope

Each of the individual samples underwent visual inspection with the help of a stereo microscope (Nikon SMZ745T) with an attached Infinity 1-3C camera and the image analysis software Infinity Analyse version 6.3.1. All particles suspected of being plastic were circled and numbered with a soft pencil on the filter, as well as measured at largest cross-section and photographed. The characteristics (shape, colour and size) of each particle were recorded. The shape was characterized as either fibre/filamentous, fragment or bead/spherical, where fragments included fragments of film, foam and other more undefinable shapes. Inspection of the samples was principally conducted with the lid of the petri dish kept on, to avoid contamination. However, as the characteristics of a particle sometimes can be easier recognized through manipulation with probe or forceps, the lid was at times taken off. This was also the case when the markings were made. A control with a clean filter was therefore exposed simultaneously with the sample and checked for airborne contamination between each sample analysis.

2.5 Chemical analysis using µFTIR

Chemical analysis was conducted to identify the plastic particles based on their polymeric identity. As there was a large number of particles found, this made identification of all particles impossible, therefore, particles were grouped based on similar shape, colour and size (based on

the pictures and recorded characteristics of each particle). From each group, or subsample, 1-13 particles (depending on the size of the group) were analysed by a Fourier transform infrared spectroscopy (FTIR) device in transmission mode with an associated microscope (μFTIR; ThermoScientific Nicolet iS50 FT-IR). Particles with distinctive traits were not placed in groups, but all tested individually. A diamond compression cell was used for flattening each sample and holding it in place before exposing it to a beam of infrared light (4000-400 cm^{-1}). The infrared transmittance spectrum was recorded with the settings set on 32 scans and resolution 4. After being converted to an absorbance spectrum, it was automatically compared against spectra of standard substances saved in a series of libraries, to obtain the chemical characterisation of the sample. The results were recorded as "percentage match". When specified in studies, the confidence threshold for spectra matches is often set between 60 and 75% (Lusher et al. 2013; Phuong et al. 2017). In this study, a 60% threshold was used. This was done in order to include all the particles that, when analysed visually, had characteristics clearly suggesting plastic constituents, despite the lower match (60-70%). The identity of the rest of the particles in each subsample was then derived from the subsample results.

2.6 Experimental testing of known materials

FTIR-analyses revealed a large proportion of cellophane among the MP particles tested, and as this was considered to be unlikely, experimental tests were performed to understand what caused the unexpected results. To test whether the KOH-treatment or other parts of the process affected the final spectra of the materials obtained through FTIR, samples of known materials were collected and put through different treatments and analysis methods, including the ones performed on the actual mussel samples. Samples of cotton, viscose rayon, paper, hemp, linen, cellulose acetate, polyester, acrylic fibre, polyamide, and wool and silk were collected and placed in 100 mL beakers with 60 mL solution of filtered 10% KOH. These were then incubated and filtered following the same method as for the blue mussel samples, described in section 2.3. In addition, two more treatments were applied to the cotton material: H_2O only and drying at 80 °C after treated with KOH. This was carried out to investigate whether another part of the process (soaking or drying) rather than the KOH-treatment itself had an effect on the final particle spectra. Also, untreated pieces of all materials (except cellulose acetate) were put in clean petri dishes. The materials were all tested with the same method of μFTIR as described in section 2.5. Additionally, the chemical characterisation of the materials was obtained by using attenuated total reflectance (ATR) FTIR, where the samples were not flattened

beforehand. This was carried out to test whether different instruments would give different results. The infrared absorption spectrum was recorded with 32 scans and resolution 4, and then compared against spectra in the same libraries as before (section 2.5).

2.7 Measures to minimize contamination

As MPs and especially fibres from clothes are easily spread through air, several measures were taken to avoid contamination. First, the processing and analysis were carried out in clean and separated lab-spaces where only one or two people were present, and the door was closed to avoid unnecessary air draft. Second, all researchers wore cotton laboratory coats and the laboratory was regularly dusted and cleaned. The laboratory coats were also regularly cleaned with lint rollers.

As far as possible, glassware was used instead of plastics, and plastic gloves were not used when in direct contact with the samples. All glassware was rinsed thoroughly with filtered (0.22 μm) RO-water beforehand and between the processing of each sample, and the solution of KOH was also filtered before adding to the samples. Aluminium foil was used to cover the mussel samples during thawing until filtering, and also as lids for the glass beakers. All filter papers were inspected for contamination under a microscope prior to filtering. After filtering, the filter papers were kept in petri dishes with lids, where the ID markings were made underneath the dish so that the lid could be kept on during visual inspection.

2.8 Corrections

The number of MP particles found in the mussel samples was corrected for particles appearing both in procedural controls (Table 3) and on the clean filters (blanks) exposed during analysis (Table 4). The number of particles in the procedural controls were subtracted from the samples processed the same day, separated by shape (fibres and fragments). The average number of fibres registered in the three replicates ranged from 0 to 4, while the average number of fragments ranged from 0 to 1. Two of the sites (N1 and O5A) had no controls run the same day due to processing errors, so the average of all the other controls were used (I). Contamination was registered on the clean filter papers (blanks) during analysis of 11 samples, with 1-2 fibres occurring in each, a total of 13 fibres, and no fragments (Table 4). The fibres ranged in size from 200 - 4740 μm. The number of fibres in table 4 were subtracted from the fibres found in the corresponding individual samples.

Table 3 Average number of particles found in procedural controls, separated by shape. Three replicates were conducted for each day.

Control	A	B	C	D	E	F*	G	H	I**
Samples processed the same day	N2, N4	N6, O1	N8, O6	N5, O3	N7, O4	O2	O1b, O5b	N3, O7	N1, O5
Av. fibres (St.dev.)	4.00 (3.61)	2.00 (1.73)	0.33 (0.58)	0.00 (0)	1.67 (0.58)	1.50 (0.71)	0.67 (1.16)	4.00 (1)	1.77 (1.17)
Av. fragments (St.dev.)	0.67 (0.58)	1.00 (0.58)	0.33 (0.58)	0.00 (0)	0.33 (0.58)	0.00 (0)	0.00 (0)	0.00 (0)	0.29 (0.43)

** Only two replicates, because one was lost*
*** Did not run blanks, used mean of all blanks*

Table 4 Number of fibres and fragments appearing in blanks exposed during analysis of samples. Each sample ID represents a single individual of blue mussel.

	Contamination in blank	
Sample ID	*Fibres*	*Fragments*
O1_9	1	0
O1b_12	1	0
O1b_18	1	0
O2_8	2	0
O2_10	1	0
O2_15	1	0
O4_8	1	0
N1_8	1	0
N1_10	1	0
N2_8	1	0
N4_6	2	0
Total	**13**	**0**

2.9 Data analysis

All data handling, statistical analyses and charts were executed in Microsoft Excel for Mac version 16.11, with the add-in XLSTAT version 2018.1. A Shapiro-Wilk test was conducted on all data to test for normality, as well as Levene's test and Bartlett's test for assessing the equality of variances. As the data were not normally distributed and with unequal variances, a non-parametric ANOVA, Kruskal-Wallis, was performed to test for statistical differences in the data. To see where the differences lay, Dunn's procedure for multiple comparisons were used post-hoc. Two multiple linear regression analyses were performed to test for the effect of different explanatory variables on the results. In all statistical tests, the significance level, α, was set to be 0.05. Standard deviations (SD) were calculated for the data as a measure of variation.

3 Results

3.1 Weight of the blue mussels

The weight (g. w.w.) of the individual mussels ranged from 0.14 in Måløy (N4) to 16.30 in Ramtonholmen 2017 (O5b; Fig. 4), with the total average being 3.46 (± 2.64). Mussels at Skallneset in the north (N1) were the smallest mussels by weight, with the least variation as well. In contrast, Ramtonholmen 2017 (O1b) had the largest mussels and also the widest size range. Overall, mussels from the inner part of the Oslo fjord (O1-O5) were relatively large (Fig. 4). The mussels from the two stations further out in the fjord (O6 and O7) were considerably smaller.

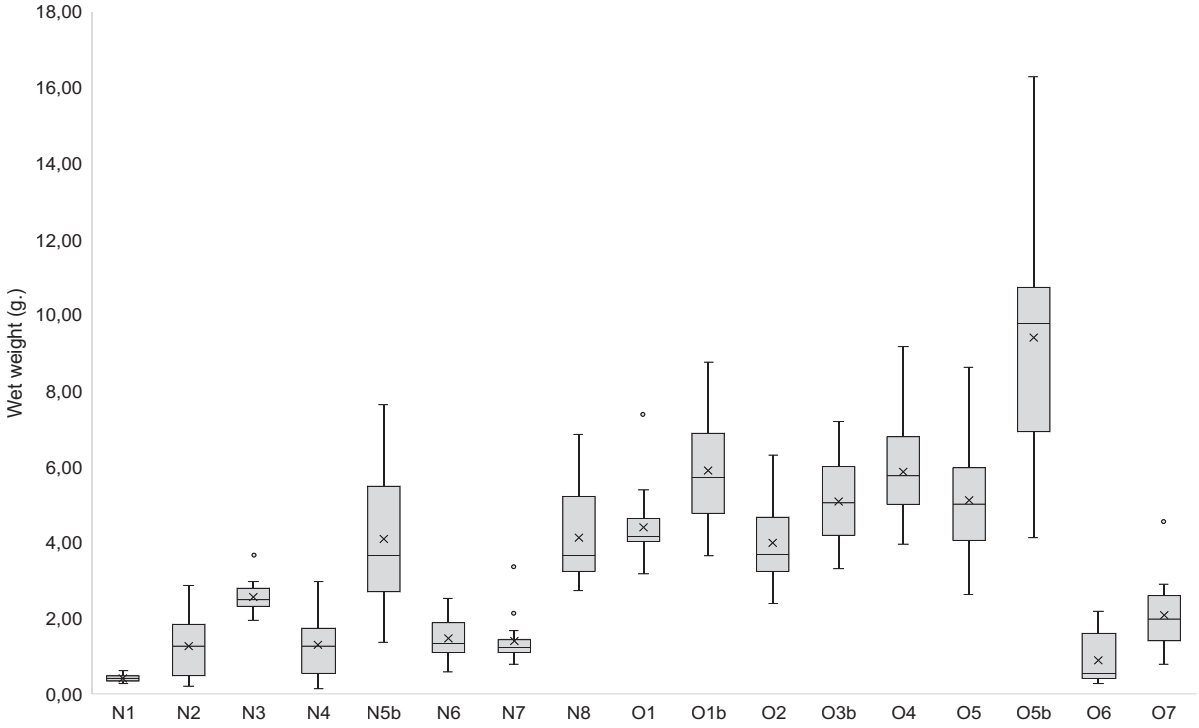

Fig. 4 Weight (g. w.w.) of blue mussels (*Mytilus edulis*) from each site. The plot shows average (x), median (horizontal line), interquartile range (box), maximum and minimum value (whiskers) and outliers (circles) for each site.

There was a significant positive correlation between number of suspected plastic particles per individual, and mussel weight (Spearman correlation coefficient = 0.21, $p < 0.0001$). This means the number of MPs found could possibly be partly explained by the mussels' weight, with larger mussels containing more particles. In the following, the results are therefore presented both as MPs per individual and MPs per gram, to account for this possibility.

3.2 Visual analysis of all sites (Quantitative and qualitative data)

In total, 507 particles were suspected of being plastic after visual analysis (after correction), and 56% of all the mussels analysed had ingested suspected plastic particles (N = 332). Prior to correction, the number of suspected particles was 894 (81% ingestion). Average number of particles found per individual was 1.52 (±2.34), and the average number of particles per gram w.w. was 0.98 (±2.66). Particles < 1 mm were most common (71%), with the size class 0.25 to 0.5 mm accounting for the highest proportion of total (25%; Fig. 5a). Ten particles > 5 mm in size and by that strictly defined as mesoparticles, were also found and included in the results. Fibres were the most abundant particles, accounting for 84% of the visually identified particles, while the remaining 16% were fragments (including fragments of films and others with more undefinable shapes; Fig. 5b). No beads or other particles of spherical shape were found. The representation of size and shape is based on numbers prior to correction, as these variables were not accounted for in corrections.

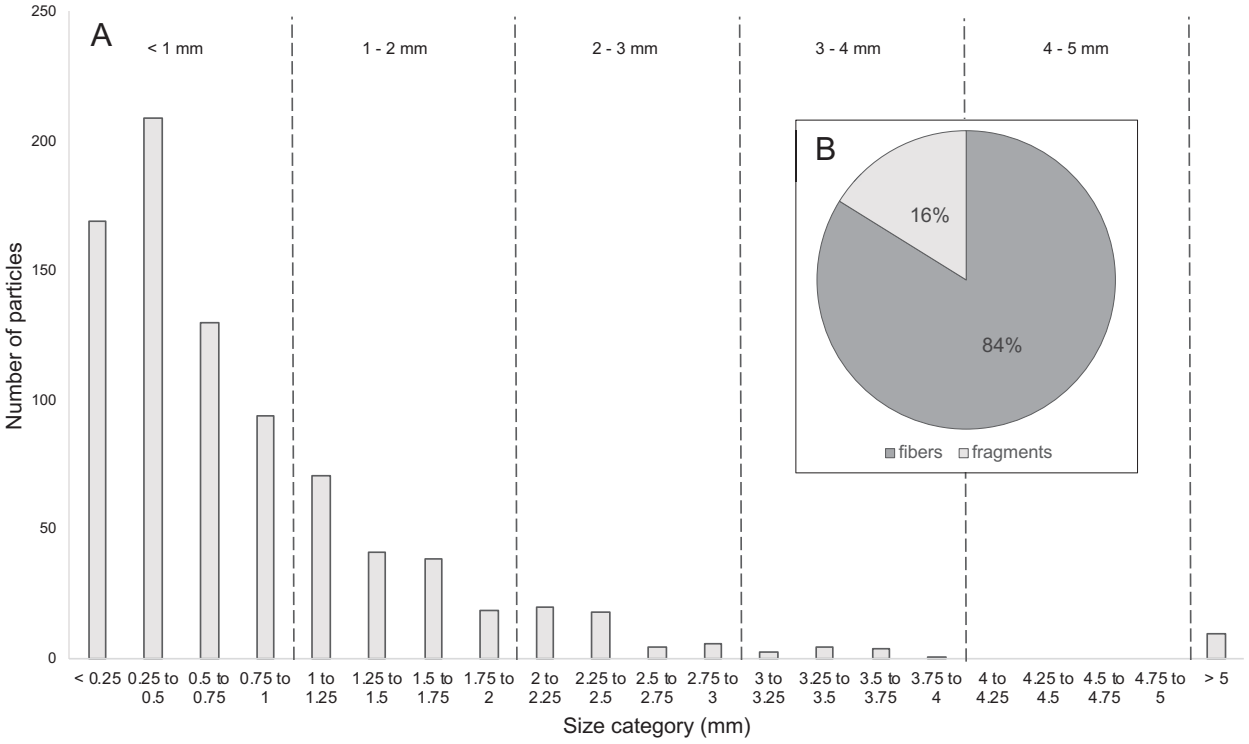

Fig. 5 Distribution of **a)** size and **b)** shape for suspected plastic particles found in blue mussels (*Mytilus edulis*) from 15 sites along the Norwegian coast.

Significant differences occurred between the number of suspected plastic particles in the mussels among sites, both when presented per individual and per gram (Kruskal-Wallis, $p < 0.0001$, for both representations). When presented as particles per individual, Ørland (N3) was

the only site where no individuals had ingested plastic, all other sites had at least one individual containing suspected plastic particles (Fig. 6a). The highest count of particles in one individual was 20, found in Akershuskaia 2017 (O1b), which was also the only site where all individuals had ingested suspected plastic. Akershuskaia 2017 was the site with the highest average ingested particles per individual (6.8 ±4.0), significantly higher than all other sites except Skallneset (N1; Dunn's Multiple Comparison test, see appendix A for p-values). When comparing the two sampling years in Akershuskaia (O1 and O1b), the number of particles in 2017 was significantly higher than in 2016 (Dunn's test, $p < 0.0001$). No significant difference was however found between the two samples from Ramtonholmen (O5 and O5b; Dunn's test, $p = 0.19$). The mussels collected from Skallneset (N1) had a significantly higher number of ingested particles (average 3.6 ±3.05) than 11 of the other 16 sites (Dunn's test, see appendix A for p-values).

When presented as particles per gram (Fig. 6b), the results looked somewhat different from when presented as particles per individual. Skallneset (N1) in the north clearly stood out as the site with most particles per gram and had a significantly higher number of ingested particles per gram than all other sites (Dunn's test, see appendix A for p-values), with an average of 7.9 (±6.6). Even though it looked like Solbergstrand (O6) in the Oslo fjord had relatively high numbers of plastic, it was not significantly higher than any other site except Ørland (N3) (Dunn's test, see appendix A for p-values). Akershuskaia 2017 (O1b) still stood out by having a significantly higher number of ingested particles per gram than all other sites except Skallneset (N1; Dunn's test, see appendix A for p-values). As when presented per individual, the number of particles per gram from Akershuskaia was significantly higher in 2017 (O1b) than in 2016 (O1; $p < 0.0001$), and no significant difference was found between the two samples from Ramtonholmen (O5 and O5b; $p = 0.74$).

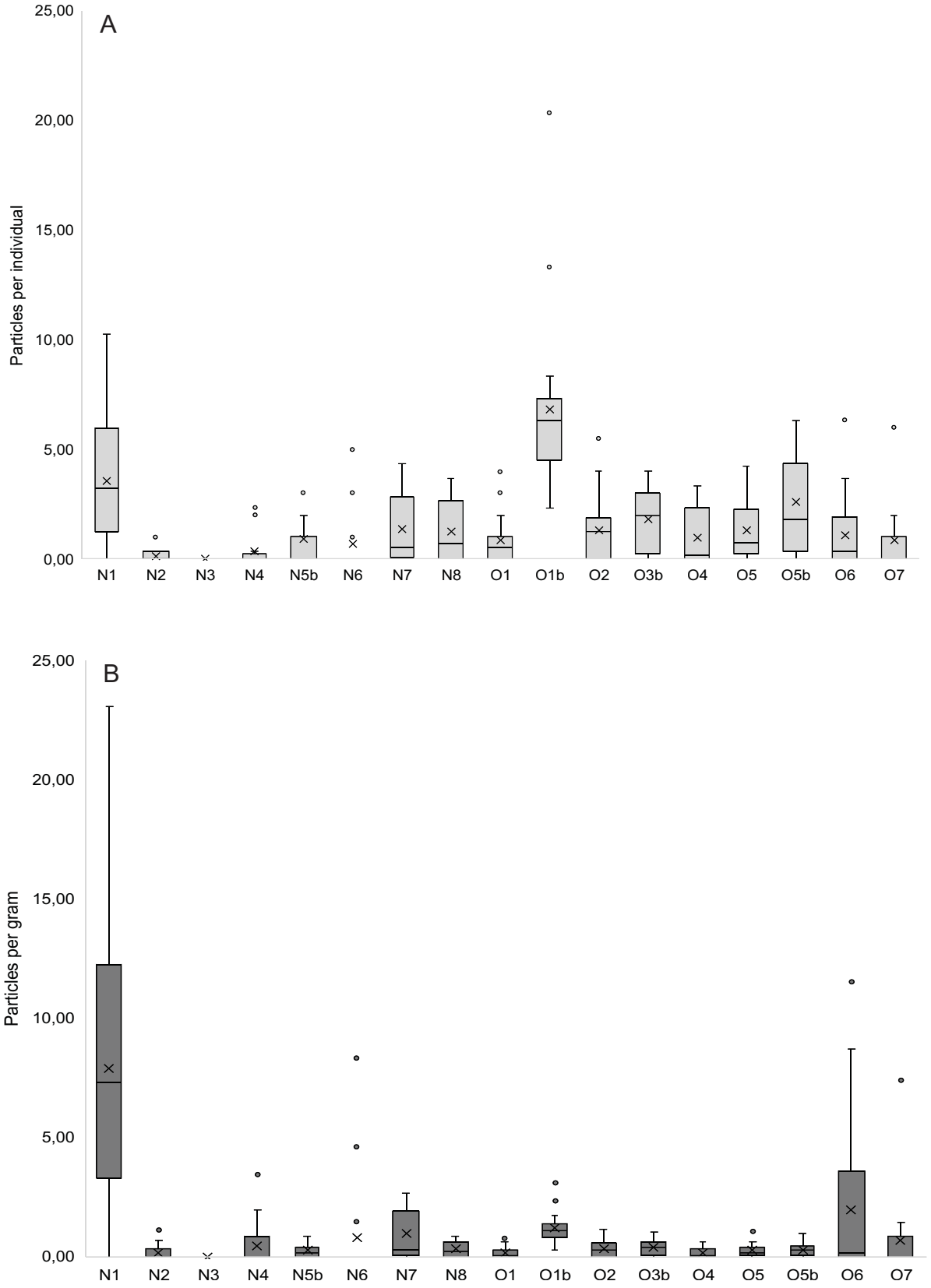

Fig. 6 Suspected plastic particles visually identified per **a)** individual and **b)** gram wet weight, represented by site. Samples marked with b were collected in 2017, all other in 2016. The plots show average (x), median (horizontal line), interquartile range (box), maximum and minimum value (whiskers) and outliers (circles) for each site.

3.3 Chemical analysis of the samples collected in the Oslo fjord (Qualitative data)

Out of the nine sites (including both 2016 and 2017) from the Oslo fjord, eight underwent chemical analysis. O5 from 2016 was left out of the detailed analysis due to lack of time and resources. Subsamples of representative particles from the eight sites were run through µFTIR to determine their chemical characterization. Out of the total 499 particles (uncorrected) suspected of being plastic in these sites after visual analysis, 194 were tested by FTIR, a fraction of 39%. The identity of the remaining particles was derived from the subsample results, as described in section 2.5. From this, a total of 438 particles were confirmed as plastic (the remaining 61 being minerals or organic material).

Of the total 438 particles that were confirmed as plastic, most (76%) were smaller than 1 mm (See Appendix B for size distribution). 120 particles (27%) were below 0.25 mm in size, being the highest count in the size categories. As in the results from all sites, fibres were considerably more abundant than fragments in the Oslo fjord sites as well, accounting for 76% of the total (Appendix B).

There was identified 11 different polymers in the samples from the Oslo fjord, including cellophane, “parking lot tar”, polyester, acrylic, and polyethylene (Fig. 7). Akershuskaia 2016 (O1) had the widest range of polymers, with seven different polymer types identified. The most abundant polymer over all was cellophane (62% of confirmed plastic), which was also the most abundant in each single site except for O1b, where “parking lot tar” was more prevalent. Following cellophane, the most abundant polymers overall were “parking lot tar” (21%), polyester (10%) and acrylic (3%). Examples of FTIR spectra for these four polymers are shown in appendix C.

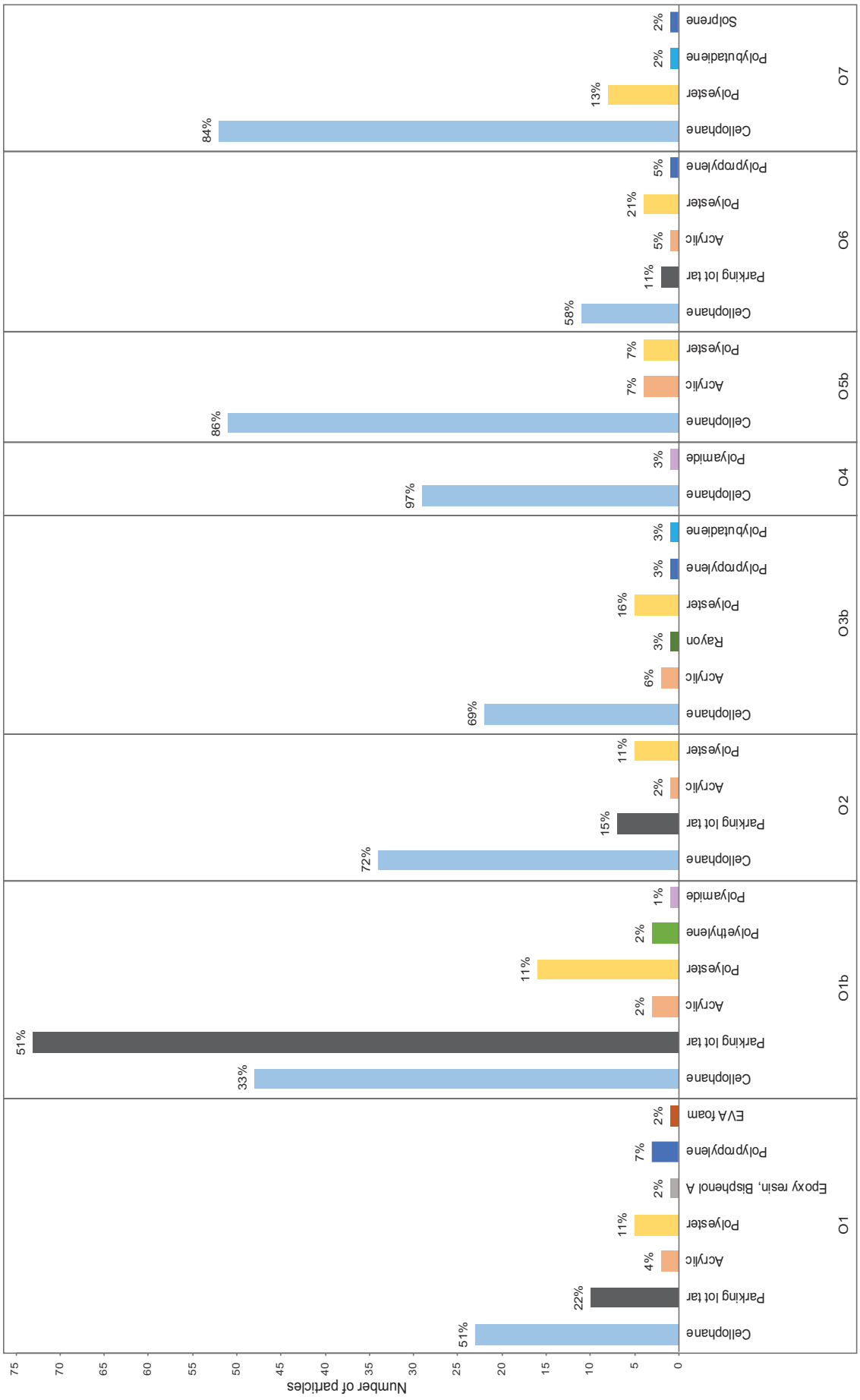

Fig. 7 Plastic polymers identified through transmission μFTIR, across the stations O1-O7 in the Oslo fjord. Stations marked with b had samples collected in 2017, all other in 2016. The percentage of the total particles identified per site is given for each polymer above the bar.

The particles identified as cellophane were exclusively fibres, but often very different in size and colour (Fig. 8). Many were transparent and not easy to detect against the white filters, so there is reason to believe there could be more of these than what was quantified. Several of the fibres also seemed to have lost colour either during the treatment or while in the environment (Fig. 8 c, d and e).

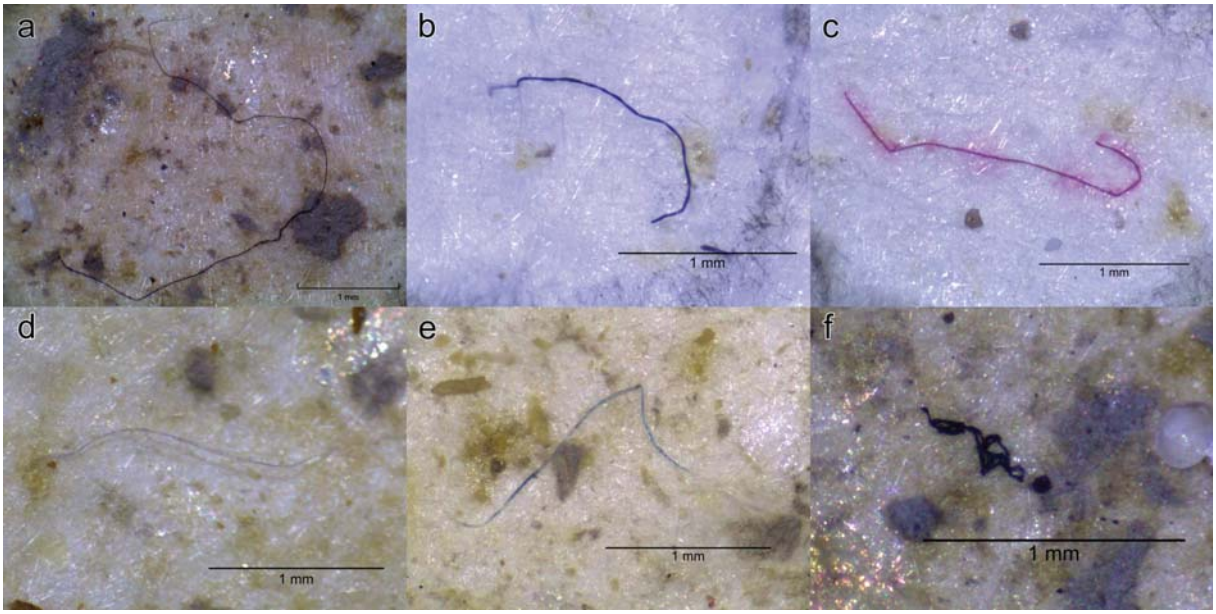

Fig. 8 A representative selection of fibres identified as cellophane through transmission μFTIR, from sites **a)** O3, **b), c) and d)** O7**, e)** O4, and **f)** O1. In **c)**, red colour has leaked out onto the filter. Picture **d)** shows an almost transparent fibre, and **e)** a blue fibre with loss of colour in places.

The particles identified as "parking lot tar" all looked similar, being black and rubbery, and often with an elongated and tapered shape (Fig. 9). There were many more particles (>100 in some individuals) observed in the samples from Akershuskaia (O1 and O1b) that resembled these, but they were smaller than the limit for secure handling (~70 μm) and couldn't be confirmed through visual or chemical identification. It would thus be reasonable to believe that the number of these particles were highly underestimated in the results. Some of these smaller particles can be seen in Fig. 9 c and d.

In Fig. 10, a selection of other plastic polymers identified through μFTIR is shown. These are all from Akershuskaia (O1 and O1b), as these were the samples with the widest range of polymers, as well as with some of the most distinctive-looking particles.

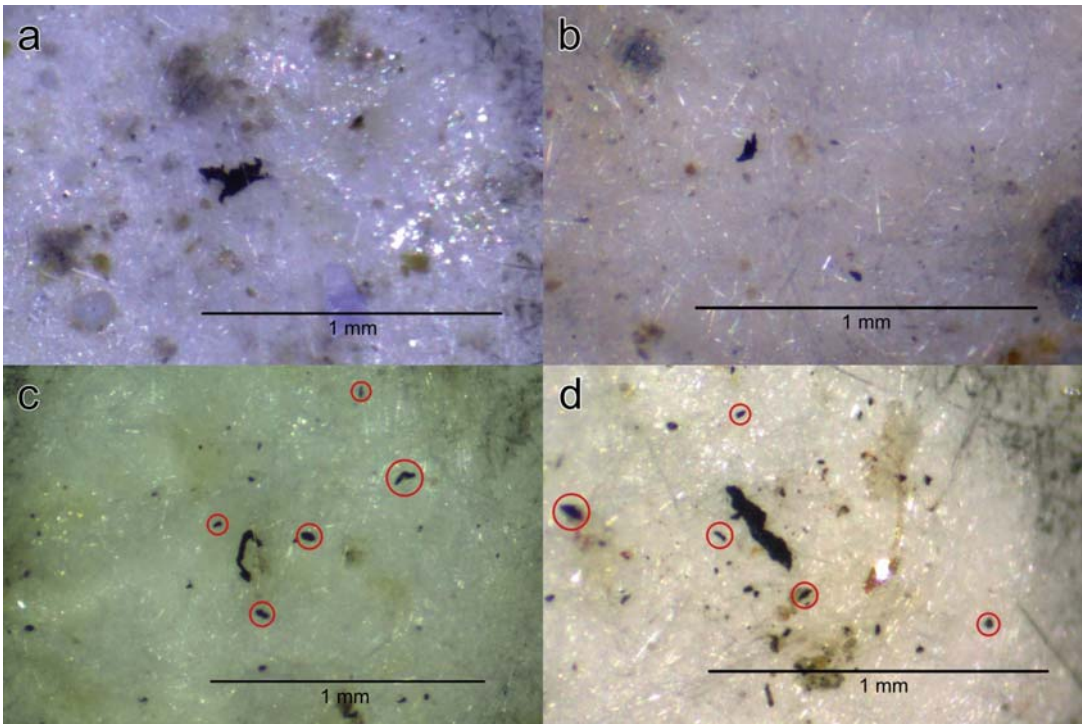

Fig. 9 A representative selection of fragments identified as "parking lot tar" through transmission μFTIR, from sites **a)** O1, **b)** O2, **c) and d)** O1b. In **c) and d)**, some smaller particles of similar character, but not included in the results, are circled in red.

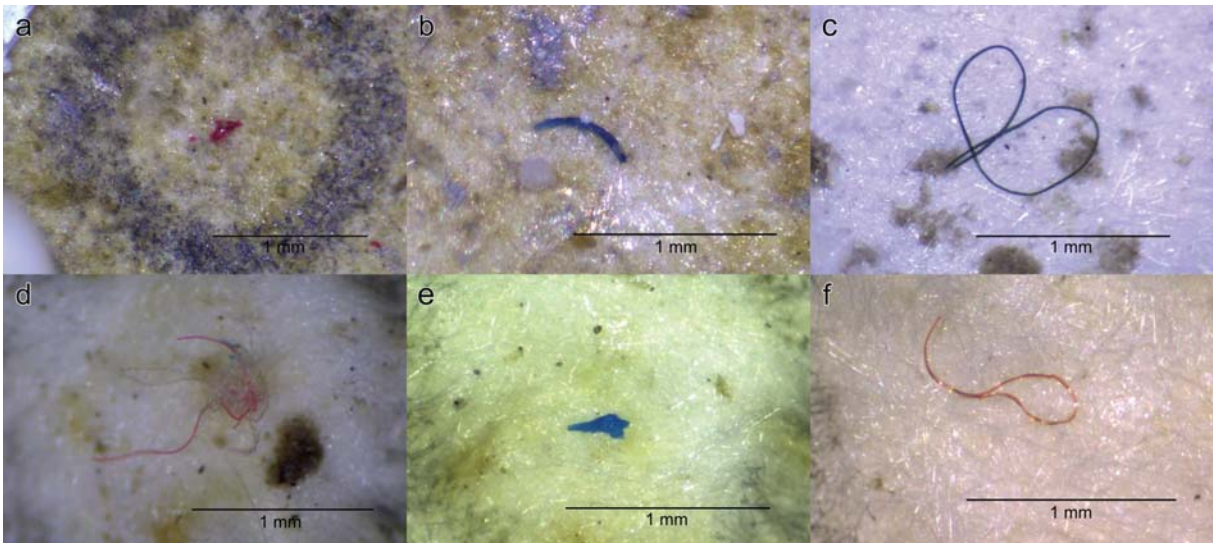

Fig. 10 A selection of different plastic polymers identified through transmission μFTIR. **a)** Epoxy resin, BPA from O1, **b)** Polypropylene from O1, **c)** Acrylic fibre from O1, **d)** A knot of cellophane (transparent) and polyester (red) from O1b, **e)** Polyethylene from O1b and **f)** PET from O1b.

3.4 Results of experimental testing

Experimental tests on different materials were conducted in order to understand why cellophane amounted such a big proportion of the results. A range of materials were put through different treatments and tested with both μFTIR in transmission mode, and ATR FTIR. The complete test results with details are presented in Appendix D.

The synthetic plastic polymers, polyamide and polyester, proved no difficulties for identification in either of the FTIR methods, while wool and silk were completely degraded when put through KOH-treatment, as would be expected of organic material, and no FTIR tests were run on these treated materials. Acrylic fibre was also correctly identified. These materials were therefore of no further concern, considering they were not likely to contribute to any false results.

However, the identification by FTIR of the cellulose-based materials cotton, viscose rayon, hemp, linen, paper and cellulose acetate, gave variable results. In Fig. 11, the fraction of cotton correctly identified by the two FTIR methods (transmission μFTIR and ATR FTIR) is illustrated, separated by treatment (none, H_2O, dried, KOH). Half of the untreated samples were correctly identified when put through μFTIR, whilst all of the treated (H_2O, dried, KOH) and the other half of the untreated were wrongly identified as cellophane. In contrast, all cotton samples were correctly identified by the ATR FTIR. Similar results occurred for paper, viscose rayon, hemp and linen: all samples were correctly identified using the ATR FTIR, whilst none were correctly identified using transmission μFTIR (Appendix D). Cellulose acetate was not tested in any other way than with KOH-treatment and μFTIR, but this as well was identified as cellophane.

Another discovery worth mentioning is that the KOH-treatment made the fibres leak colorant or dyes, often making them entirely transparent. It was also discovered by a coincidence that a cotton fibre that was not properly flattened before running it through transmission μFTIR, was correctly identified. This was unlike the properly flattened part of the exact same fibre, which was identified as cellophane.

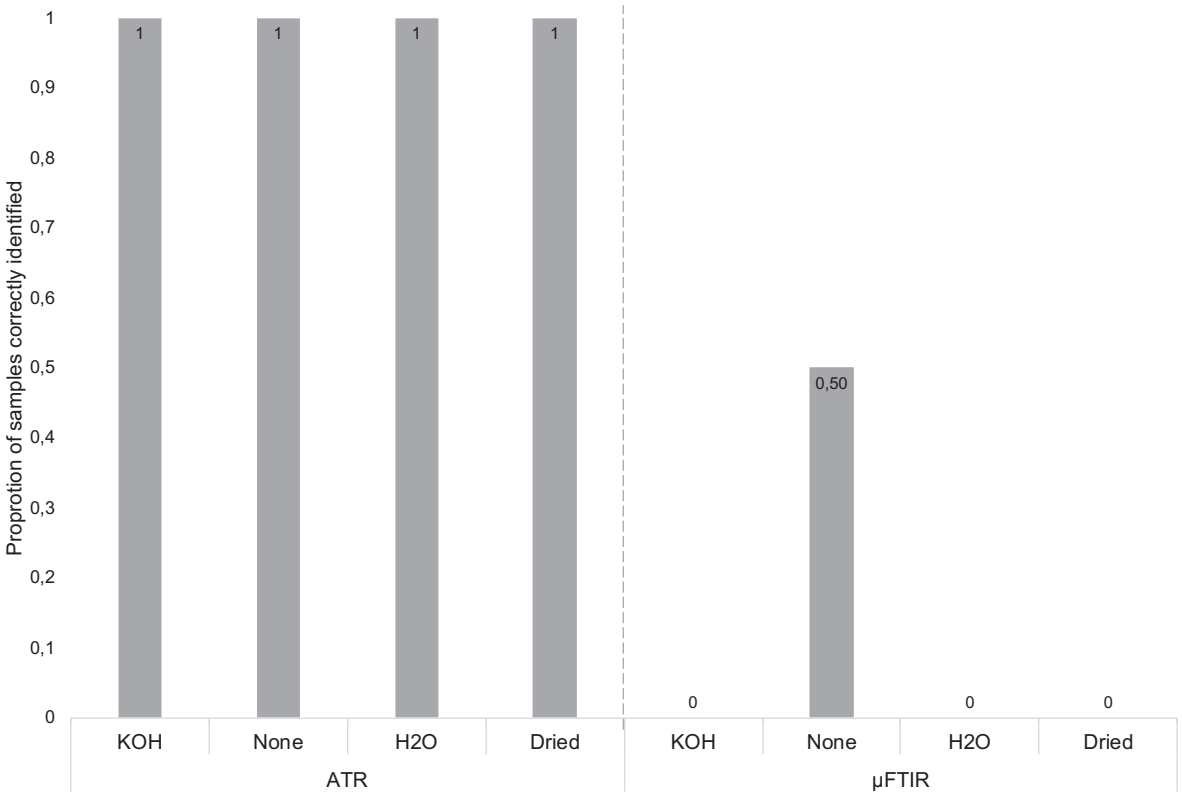

Fig. 11 Proportion of FTIR-tests that gave correct identification when conducted on cotton material. Each sample underwent one of four different treatments (KOH, None, H_2O, Dried), and were tested in either transmission µFTIR (µFTIR) or ATR FTIR (ATR). When incorrect identification, the match was for all samples cellophane.

3.5 Adjusted quantitative results for the samples collected in the Oslo fjord

The results from the experimental testing indicated that some part of the particles chemically identified as cellophane may not have been plastic (semi-synthetic like viscose rayon), but organic material, although still most likely anthropogenic. This called for a different representation of the original results, where this uncertainty was eliminated. This is represented for the Oslo fjord sites (O1-O7, O5 from 2016 being excluded) in Fig. 12, where all particles identified either as cellophane or as non-plastic (e.g. minerals, tobacco or chitin) have been subtracted from the total and presented next to the original representation. Here, the results are presented only as particles per individual, for simplicity. In total, the number of particles definitely not being plastic (i.e. not including cellophane, mainly minerals) was 27 out of 499, a fraction of 5%. The number of particles identified as cellophane was 270, accounting for 54% of the visual analysis results. The fractions in each site being cellophane and non-plastics, and thereby the fractions being subtracted, ranged from 35 to 85% between the sites.

When eliminating the particles identified as cellophane and non-plastic, the total number of particles in the analysed Oslo fjord samples was 141. This was after the original corrections based on controls and blanks were carried out. Akershuskaia 2017 (O1b) still had the highest count of particles in one individual, 13, and no individuals with 0 ingested particles. The total average particles per individual of all sites in the Oslo fjord was 0.88 (±1.65). There still were significant differences among the sites in the adjusted data (Kruskal-Wallis, $p < 0.0001$), also when excluding Akershuskaia 2017 (O1b; Kruskal-Wallis, $p = 0.01$). Akershuskaia 2017 (O1b) had as before significantly higher numbers of particles ingested per individual than all the other Oslo fjord sites (Dunn's test, $p < 0.0001$ for all comparisons; See appendix B).

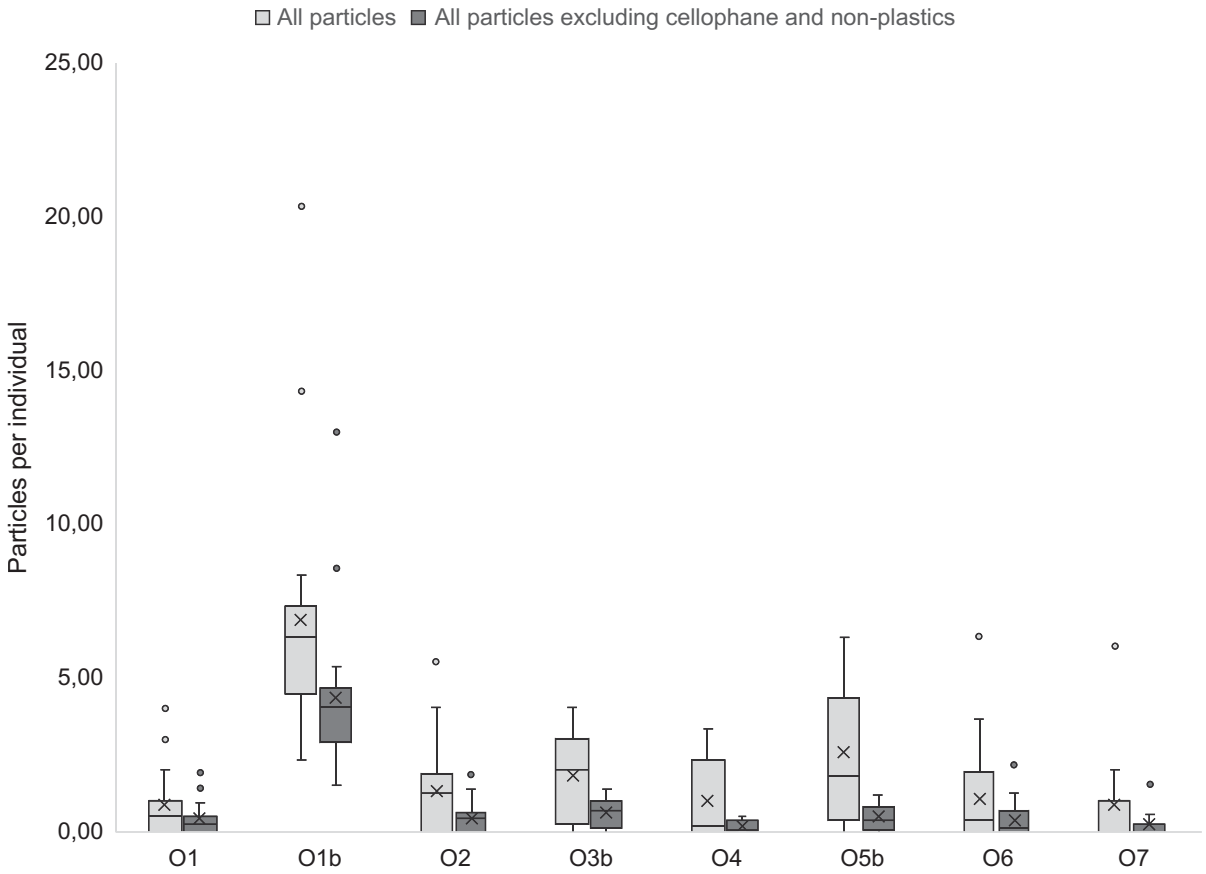

Fig. 12 Particles per individual for each site in the Oslo fjord that underwent μFTIR analysis. Light grey boxes represent all visually identified particles, dark grey boxes all particles excluding the ones identified through μFTIR as non-plastic or cellophane (adjusted results). Samples marked with b were collected in 2017, all other in 2016. The plots show average (x), median (horizontal line), interquartile range (box), maximum and minimum value (whiskers) and outliers (circles) for each site.

3.6 Potential pathways of microplastics to blue mussels in the Oslo fjord

To understand why there were differences between the sites in the Oslo fjord, two multiple linear regression analyses were performed. First, particles per individual (average per site) was set as the dependent variable, and the following as possible explanatory variables: distance from city harbour, distance from nearest WWTP, and distance from nearest large river outlet, all in kilometres (Table 5), the model formula being the following:

Average particles per individual ~ City harbour + WWTP + River outlet

The exact same analysis was performed a second time, only this time with average number of particles per gram as dependent variable (Table 5).

Table 5 Variables used in multiple linear regression, with average particles per individual/per gram as dependent variable, and distance (km) from city harbour, wastewater treatment plant (WWTP) and large river outlet as explanatory variables.

			Distance from (km)		
Site	**Av. particles ind^{-1}**	**Av. particles g^{-1}**	**City harbour**	**WWTP**	**River outlet**
O1	0,85	0,19	0	3,08	0,89
O1b	6,83	1,19	0	3,08	0,89
O2	1,33	0,34	2,75	2,98	3,07
O3b	1,80	0,39	5,12	7,38	0,15
O4	0,97	0,15	10,2	8,45	5,24
O5b	2,60	0,31	21,5	5,35	4,61
O6	1,05	1,97	32,6	21,3	12,3
O7	0,85	0,70	93	85,5	12,9

None of the models computed were significant (Table 6), i.e. distance from neither city harbour, wastewater treatment plant or river outlet significantly explained the abundance of MPs in blue mussels in the Oslo fjord. Results from sum of squares analysis for both models are provided in appendix E.

Table 6 Analysis of variance table for the two linear regression models, with average particles per individual and average particles per gram as dependent variables, and km from city harbour, WWTP and river outlet as explanatory variables.

Source	DF	Sum of squares	Mean squares	F	Pr > F
Particles ind^{-1}					
Model	3	5,102	1,701	0,287	0,834
Error	4	23,717	5,929		
Corrected Total	7	28,819			
Particles gram^{-1}					
Model	3	1,225	0,408	1,058	0,460
Error	4	1,544	0,386		
Corrected Total	7	2,769			

Computed against model Y=Mean(Y)

4 Discussion

4.1 Assessment of methods

4.1.1 Sampling and extraction of microplastics

No specific issues were encountered considering the sampling of the blue mussels in this study, but there are nevertheless unanswered questions considering the best procedure for sampling. The mussels sampled were rinsed for fouling before being put into plastic bags, and the question of whether the mussels rid themselves of any ingested plastic through gut clearance during this process arises. However, all the mussels were closed when picked and apparently stayed firmly shut during both rinsing and transport, which may suggest that no such clearance occurred. Karlsson et al. (2017) preserved their mussel samples in ethanol on site, in order to avoid stress causing an increase in output of faeces, but no studies have been conducted on the actual effect of the different sampling protocols. Another possible issue, is that it is considered likely that the depth from which the mussels are collected affects the results, due to exposure to airborne plastics as well as waterborne (Lusher et al. 2017a). This constitutes thus another source of error in the current study, as the mussels were collected from different depth zones. As for the packing of mussels in plastic bags, this raises a question of contamination from the bags. However, none of the particles found in this study appeared to originate from the bags used. This is in accordance with the results of Phuong et al. (2017), who – through running blanks – did not find any contamination from the plastic bags they used for storage.

In this study, the mussels' soft tissue was weighted without drying first, i.e. in wet weight. Some studies (e.g. Karlsson et al. 2017) have used the dry weight, but it has not been explicitly investigated whether using dry weight or wet weight is the best approach for MP studies. It is, however, important to minimize the number of sample processing steps, in order to avoid introducing airborne contamination. Beyer et al. (2017) point out that using wet weight can be advantageous since the mussel tissue then can be used for other analyses after weighing, but that it is less reliable than dry weight, as it is dependent on the sampling method (e.g. draining of water). It has also been pointed out that dry weight might be better for comparisons between studies, as the variance is lower than for wet weight (Karlsson et al. 2017). On the other hand, it is unknown what effect the drying of tissue might have on the MPs, it might make it more brittle and harder to identify. This uncertainty, in addition to the shorter process, lower

contamination risk, and that wet weight is more widely used in the literature (Van Cauwenberghe et al. 2015; Li et al. 2016; Phuong et al. 2017), were the reasons why wet weight was used in this study.

A 10% KOH solution was used to dissolve the mussel tissue and other organic material present in the mussels, so that it would be possible to quantify the presence of MPs. The method was adapted mainly from Dehaut et al. (2016), who found this to be the best suited out of six different protocols. This protocol was efficient in the digestion of organic material also in the current study, and no issues with clogging of filters (pore size 2.7 μm) occurred. There was however, to varying degrees (seemingly associated with the size of the mussels), some organic material left on the filters, which made identification of plastic particles more difficult. This both in terms of possible misidentification of organic material as plastic and vice versa, and in terms of organic material possibly concealing the presence of MPs – thus leading to underestimation. Another case of underestimation might occur due to the loss of colour in some fibres, as these were transparent/white and therefore difficult to spot on the white background of the filter. As discovered in the experimental testing, this may in some cases be due to dye leakage resulting from the KOH-treatment. On the other hand, it may also be due to weathering of the plastics in the environment and thus inevitable, regardless of digestion protocol.

Previous studies have had issues with the degradation of some plastics using 10% KOH, and especially cellulose acetate (Dehaut et al. 2016; Kühn et al. 2017). The recovery test conducted in this study did not include this polymer, but in the experimental tests later performed, cellulose acetate was included, and did not visibly degrade. The degradation was however not investigated by weighing to check for loss of mass, or other more thorough inspections. In addition, the cellulose acetate used in the experimental tests was collected from a fresh cigarette and was – unlike the particles present in the blue mussels – unaffected by such as UV radiation, sea water and other degrading factors that could alter the structure and chemistry of the material (Andrady 2011). It is thus possible that the presence of this material has been underestimated in the results.

4.1.2 Visual and chemical analysis

After sample collection and preparation, the first step to quantify the number of MPs present in Norwegian blue mussels was visual analysis using a microscope. This method requires a trained

eye to be able to distinguish between plastics and non-plastics and is widely discussed due to its subjectivity and labour-intensive process. There is a general agreement that it should be accompanied by more objective methods and be simply the first step of identification (Hidalgo-Ruz et al. 2012; Song et al. 2015; Phuong et al. 2017). This is due to the high probability of overestimating MP presence, considering the small sizes of the particles and the difficulties in distinguishing plastic from organic materials or minerals, using only visual characteristics. Hidalgo-Ruz et al. (2012) states that as much as 70% of particles visually identified as plastic is not confirmed as plastics in later chemical analyses, and in the study by Phuong et al. (2017), only 6% of the chemically tested particles were identified as plastic.

In the current study, the proportion of particles visually identified as plastics and then confirmed as not plastic (minerals or organic material like leaf, tobacco etc.) via FTIR was as low as 5%, meaning that the visual identification had an accuracy of 95%. This is when including the cellophane fibres in the results. The visual distinction of different anthropogenic fibres (e.g. cotton vs. viscose rayon vs. polyester) was considered to be next to impossible, and this accuracy could thus be considered as the accuracy of separating anthropogenic, man-made particles from the natural. To try anything else through only visual identification is not recommended based on the experiences of this study, as it will probably lead to underestimation, and this distinction should rather be carried out through other steps. When considering the accuracy of 95%, it can be argued that the quantification of MP (or anthropogenic particles) presence in blue mussels along the Norwegian coast is highly accurate. Then again, this is results based on sub-samples, and to be perfectly sure of the accuracy, all particles should be tested. This is however not feasible with a large number of samples, and as Song et al. (2015) recommends, a combination of visual and chemical identification should be used when dealing with many samples or large sample sizes. With the number of particles visually identified in this study being so high (894 before correction), dividing it into sub-samples was the most practical solution.

When testing the visually identified particles with transmission μFTIR, 54% of the particles were identified as cellophane, all in the shape of fibres. As cellophane is a cellulosic material produced as sheets, and not fibres, these results became subject to suspicion. Further testing showed that, when using transmission μFTIR for chemical identification as in this study, it is nearly impossible to distinguish between natural cellulosic fibres like cotton and linen, and

semi-synthetic, manmade cellulosic fibres like viscose rayon. They were all identified as cellophane. This observation was also made by Comnea-Stancu et al. (2017), as they could not distinguish between natural and man-made fibres when using transmission μFTIR. The identification of viscose rayon as cellophane is not that far from correct, as both cellophane and viscose rayon is made from the same liquid solution, viscose, only viscose rayon is when it's made into fibres and cellophane is when it's made into sheets. Both are products of regenerated cellulose and will thus have similar chemistry, but different structures (Klemm et al. 2005). Also cotton, paper and other cellulosic fibres is chemically very similar to viscose rayon and cellophane, due to them all being based on cellulose (Cook 1984).

It appears that the identification of cotton was to a certain degree affected by the treatment it underwent before testing, as half of the untreated samples were correctly identified. However, the other half was still incorrectly identified as cellophane, and the treatment cannot thus be the full explanation. It is important to mention that there was no difference in the results from cotton treated with only 10% KOH, and from those dried after or treated with only water. This indicates that any form of external influence will affect the identification accuracy, and that the use of KOH is not by itself a problem. This could be one explanation for the difficulties with the samples collected from mussels, as these likely have been exposed to a range of environmental factors affecting the structure and chemistry of the particles.

Another explanation for the incorrect identification is the choice of instrument and mode of detection. The experimental testing showed that when using ATR FTIR, 100% of the particles tested were correctly identified, regardless of treatment, unlike when using the μFTIR in transmission mode, where only 25% were correctly identified. Nevertheless, in the case of studying as small particles as those identified in the blue mussel samples, the ATR FTIR was inadequate. The handling and placing of the particles was impossible without the use of a microscope and something to hold them in place, and none of the two methods tested were thus optimal for the purpose of this study. An important aspect, is that the spectra libraries used for comparisons to standard substances were acquired through ATR mode, which might explain some of these difficulties when using transmission mode. This is also brought up by Comnea-Stancu et al. (2017), who experienced that the quality of identification was lower for transmission spectra than ATR spectra, and explains that this might be due to the library spectra being acquired by ATR. As ATR spectra are created from the surface information of the particle

through reflectance, and transmission FTIR spectra are created from the bulk information through transmission (Comnea-Stancu et al. 2017), it is reasonable that these spectra are different and not perfectly comparable. It should be tested how accurate the identification is when the spectra library is acquired using transmission FTIR. Furthermore, building spectra libraries solely for the purpose of identifying MP particles which has been subject to weathering and digestion treatments might be an interesting experiment as well.

Interestingly, it was discovered that a non-flattened fibre of cotton was identified as cotton by the μFTIR, while the properly flattened part was identified as cellophane. The reason for this is unknown, but it could indicate some difficulties when it comes to the physical structure of the material. The flattening is however necessary for this method, but a method without the need for flattening the sample might be a solution for future research. ATR FTIR is such a method, but as mentioned, it is still inadequate. There are other methods that could be suitable, such as Focal Plane Array (FPA)-based μFTIR (Tagg et al. 2015), Raman spectrometry (Van Cauwenberghe et al. 2015) and Pyrolysis-GC/MS (Dehaut et al. 2016), but further testing and development of methods is necessary to find the most appropriate and reliable for MP identification.

4.2 MPs found in blue mussels along the Norwegian coast

Out of all the mussels analysed, 56% had ingested suspected plastic. This proves that mussels in the Norwegian marine environment are exposed to MPs, in addition to the fact that blue mussels are capable of ingesting MPs, as previously demonstrated by several studies (e.g. Mathalon & Hill 2014; Van Cauwenberghe et al. 2015; Li et al. 2016). The average number of MPs ingested per individual was 1.52, and per g. wet weight (w.w.) it was 0.98. In comparison, Van Cauwenberghe et al. (2015) identified 0.2 particles per g. w.w., while Phuong et al. (2017) found 0.23 MP per g. w.w. and 0.6 MP per individual. However, the methods used in studies for quantifying MPs in mussels are many and varied, and direct comparisons between the quantitative results of this study and others are thus inexpedient. Studies differ greatly in the protocols used, both for sampling, extraction of MPs and the identification and quantification of MPs, and standardized procedures needs to be developed to enable comparisons. Another difference is in how the results are presented, either as MPs per individual, or per gram (either wet weight or dry weight). In this study, the data were given in both particles per individual, and particles per gram, as also suggested by Bråte et al. (2017). As the number of particles

identified was significantly positively correlated to the mussels' weight, and the data appeared to be different for the two representations, this is considered a reasonable recommendation for further research in the MPs field. This is also supported by Li et al. (2015), who found that the variation in the data was less when presented as "MPs per gram wet weight", compared to "MPs per individual". Previous studies also report that water pumping rates and clearance rates of blue mussels are dependent on the mussel size (Riisgård & Møhlenberg 1979; Jacobs et al. 2015). As long as the relationship between size or biology of the mussels and MP occurrence is not fully understood, both representations should currently be given.

When looking at differences between sites in number of particles both per individual and per gram, it was Ørland (N3), Skallneset (N1) and Akershuskaia 2017 (O1b) that stood out. Ørland was the only site where no mussels had ingested any MPs. The reason for this is unknown, especially since this site is relatively close to both a harbour and an airport. It could be expected to see higher levels of MP in an area with relatively high anthropogenic influence. The high levels of MPs found at Skallneset were also surprising, as this is a rural area close to a national park. Although there is no obvious explanation, it could be speculated that the smaller size of the mussels from this site led to a more accurate estimate of particles, as there was less undigested organic material to possibly conceal the MP particles. However, a more likely explanation is that ocean currents play a major role and that these particles have been transported a long way. It has previously been suggested that buoyant marine debris may be transported long distances in the same sense as water, nutrients and biota, aided by wind and the major ocean currents (Van Sebille et al. 2012; Lusher et al. 2015).

Akershuskaia 2017 (O1b) stood out by having a high number of particles both per individual and per gram and seems to be a hot-spot for MP. This site is close to the city and highly urbanized, as well as being a short distance from the outlet of two of Oslo's largest rivers, the Akers river and the Alna river. As this site was subject to a more detailed analysis together with the other Oslo fjord sites, the findings from this site will be discussed in more detail below (section 4.3).

As the different protocols used in MP studies would not be expected to have much effect on the types of particles found (shape and size), some comparisons between the qualitative data from the Norwegian coast in the current study, and other studies, can to some degree be conducted.

In this study, the particles visually identified were 84% fibres and 16% fragments, and no beads or other spherical shapes were found, even though Van Cauwenberghe et al. (2015) have demonstrated that mussels can ingest plastic beads of size 10-90 μm. The results concur well with previous field studies on blue mussels, that also found fibres to be the most abundant type of particles ingested (Gustafsson 2016; Li et al. 2016). Interestingly, the study by Phuong et al. (2017) – the study that probably resembles the present one the most in regards to methodologies – found 82% fragments with the remaining being fibres, quite the opposite of what was demonstrated here. In addition, most of the particles identified by Phuong et al. (2017) were smaller than 100μm, and the largest was only 400μm. This differs from the findings of the current study, where the largest proportion of particles identified was between 250 and 500 μm in size, and even particles larger than 5 mm were found. The reasons for this discrepancy in both shape and size can be many, as it is not known how the measurements of particles was performed by Phuong et al. (2017), and the process of choosing which particles (visual analysis) to test by FTIR is not described. It is not unlikely that the differences in size is a result of the differences in shape, however, as fragments ingested by blue mussels has been observed in the current study to be smaller than fibres ingested (when measuring largest cross-section). It could further be speculated that the differences are a result of the different locations (Norway and France), and that there are spatial differences in occurrence of MP, but more information is required in this case for this statement to hold. Considering the mesoparticles (> 5 mm) included in this study, these were considered to be of equal interest as the MPs found, and it was decided not to exclude them even though they are strictly not included in the MP definition used in this study.

The reasons for fibres being the most abundant shape of particles identified in the mussels investigated, could possibly be related to the bioavailability of particles to the mussels. Fibres have a shape and often density that will allow them to stay afloat in the water-phase over time, and by that be available to the filtering mussels, especially when considering the currents in the ocean as well. Many fragments and beads have a shape that are less easily carried with the flow of water and might more often sink to the seafloor and sediments, or in some cases float on the surface, all depending on the density. However, studies investigating MP in sediments (Browne et al. 2011; Claessens et al. 2011; Sundet et al. 2015) and fish (Lusher et al. 2013; Jabeen et al. 2017) have also found fibres to be the primary type of MP, which demonstrates that this might be a trend for the marine environment in total.

4.3 MPs found in blue mussels from the Oslo fjord

In total, the most abundant polymer in the Oslo fjord samples was “cellophane”. As discussed in section 4.1.2, this might not all be plastic (in the form of semi-synthetics like viscose rayon), a fraction may be natural fibres like cotton or linen. It is therefore not possible to say much about the origin of these fibres, other than that it is most likely anthropogenic. Furthermore, the difference in origin among these particles is the most likely explanation to why this group accounts for such a high proportion of the results, and possible sources could be clothing and other textiles. Some fibres may also originate from e.g. disposed cotton wool, paper and cigarette filters (cellulose acetate). And although these natural materials may break down in the environment significantly faster than traditional, petroleum-based plastic, they were still present in the mussels sampled, and did not break down during digestion using KOH. This means they still have the potential to affect the mussels or other biota, especially if they contain dyes and other additives from production, as also discussed by Remy et al. (2015) and Salvador Cesa et al. (2017). In a study performed by Park et al. (2004) it was found that cotton was less biodegradable than viscose rayon. This raises a question of where to draw the line when investigating anthropogenic microparticles in the environment, as the persistence and effects in the environment among the types may be different from what is commonly assumed. Further investigations on the effects that different anthropogenic particles (e.g. cotton vs. viscose rayon vs. synthetic plastics) have on biota need to be performed to be able to know what findings are of significance, and how to lay out future research.

The findings of cellophane are in accordance with other studies, such as Li et al. (2016) who found cellophane to be the most common polymer (41%) in blue mussels in China. However, they did not discuss these results. The same is true for the study by Jabeen et al. (2017), who found 49% cellophane in fish, without any further notes on the reasons for these findings. Both studies used μFTIR in transmission mode for the identification, as in the current study, which indicates that the cellophane found might represent misidentified particles of natural materials such as cotton. Furthermore, Lusher et al. (2013) found viscose rayon to be the most common polymer in their study of fish from the English Channel. They also used μFTIR, but the mode (transmission, reflectance or ATR) is not specified.

The second most abundant polymer found in mussels from the Oslo fjord, was what was identified by the FTIR as “parking lot tar”. This is an uncertain and somehow vague identification, as “parking lot tar” is not a singularly defined material, and the percentage matches were sometimes as low as 60%. The reason for this is thought to be that the particles found consisted of several materials coming from a variety of sources, as is typical for what is commonly called “road dust” – wear and tear from tires, asphalt and road markings (Magnusson et al. 2016). In addition, the particles were a deep black colour, possibly due to carbon black, which is added as a filler in tires to make them more UV-resistant (Kole et al. 2017). The black colour might have contributed to making identification of the particles more difficult in the FTIR – as it likely absorbs much of the light – and might be one reason for the low matches. Sundt et al. (2014) remark that these types of particles have not previously been included in the count of MPs, but that they probably should be, as a substantial portion of the constituents are plastic polymers originating from e.g. tires and building materials. Another important reason to include these particles, is the additives they may contain, which may be hazardous to biota. Stephensen et al. (2003) demonstrated that exposure to rubber tires in the water resulted in harmful effects in rainbow trout, due to the leaching of toxic substances. Kole et al. (2017) also reports that toxic effects from substances leaching from tire wear and tear have been documented in green algae, water fleas and frogs.

The sample station at Akershuskaia clearly stood out considering the “parking lot tar” particles, especially in the 2017 samples (O1b). Here, 51% of the particles were identified as “parking lot tar”, and even more were present but not possible to quantify due to the high number and small size of the particles. It is estimated that the relative contribution of tire wear and tear to the amount of plastics ending up in the ocean in Norway is about 32% (Kole et al. 2017), and according to Sundt et al. (2014), it is the most important source of MP to the Norwegian environment. When considering this, the findings of high proportions of “parking lot tar” at Akershuskaia are not surprising. The exact source of the “parking lot tar” particles is currently unknown, but some suggestions can be made. First, the site at Akershuskaia is located at the city harbour, where there is high anthropogenic activity including cars driving right past the sampling site. However, there was found no particles of “parking lot tar” at Lysaker (O3b), which also is a place of high anthropogenic activity and in addition has a busy highway close by. It is not possible to say why there were no such particles identified at Lysaker, but it could possibly indicate that the high levels at Akershuskaia has a more complex explanation than just

high anthropogenic activity. Interestingly, the mussels from Akershuskaia in 2017 were collected from right underneath the tractor tires which are placed on the dock side as pier cushions for big ships coming in. This might be part of the explanation for the findings of the black particles. However, it might be expected that fragments from such tires would not be identified as “parking lot tar”, but rather some sort of rubber. Rubber granulates from artificial football turfs have also been considered as a source of MPs to the environment, including the ocean (Sundt et al. 2014; Kole et al. 2017). Magnusson et al. (2016) explains that these particles can be removed from the football field via snow clearance, run-off water and rivers, and in clothes and shoes. In Norway, 90% of such granulates are made out of recycled tires, which is mostly styrene butadiene rubber (SBR; Magnusson et al. 2016). This could possibly be another source of the black rubbery particles identified as “parking lot tar”, but it is considered less likely, as it could be expected that such particles would have been identified as other than “parking lot tar”, as in the case of the tractor tires. However, as the identification method used in this study has proved to be somewhat inaccurate for some materials, this might be the case for others as well.

A fourth possible explanation for the high amount of these particles, is the melting of snow that is conducted in this precise area in winter. Large amounts of snow that is removed from the city streets are being collected by trucks and dumped on a barge, where a treatment plant constructed for melting snow is located. The melted snow gets filtered and purified before the water is released into the harbour (Fylkesmannen i Oslo og Akershus 2015). The final report of the trial period for the plant reveals, however, that the plant has been exceeding the limit of release set for suspended solids (Fylkesmannen i Oslo og Akershus 2015). It also concludes that the releases from the plant have not had any noticed effect on the blue mussels in the area. Still, it is not implausible that the snow melting is one source to the road dust particles found here, as snow that are scraped off the roads usually contains large amounts of such particles (Ranneklev 2016).

Following cellophane and “parking lot tar”, the most abundant polymers found in the Oslo fjord were polyesters (10%) and acrylics (3%), which both are actually families of plastics including several polymers (for example is PET the most common polyester, and both PMMA and PAN are types of acrylics). In comparison, Browne et al. (2011) observed that polyester and acrylic fibres, respectively, were the most common MPs in sediments, both on shorelines around the

globe and in previous sewage disposal sites. They also saw the same trend in sewage effluents. Catarino et al. (2018) found polyester to be the most abundant polymer in mussels investigated, and in the study by Li et al. (2016), polyester was the second most abundant polymer, following cellophane. Polyester was also the most common polymer found in the study by Bråte et al 2016, investigating Atlantic cod from the Norwegian coast. According to PlasticsEurope (2017), polyester in the form of PET is the sixth most common plastic polymer type produced, and it is thus not surprising to find high numbers of this polymer in the ocean. Both polyester and acrylic are materials commonly used in clothing and other textiles, and this could be considered as possible sources of the fibres found.

One finding of perhaps special concern, was the identification of a particle as epoxy resin with bisphenol A (BPA), from site O1b. This due to the fact that BPA is a known endocrine disruptor in fish, crustaceans and invertebrates (Talsness et al. 2009), and may by that do harm in biota that ingests it. This was only one particle, but it indicates that the possibility for MPs to harm biota is present. The origin of this particle is at this point impossible to establish, as epoxy resins have a wide range of applications including adhesives, coatings, electronic materials and biomedical systems (Jin et al. 2015).

Two multiple linear regression analyses were performed to see if the distance from large river outlets, WWTPs or the city harbour could explain the differences in number of MPs in blue mussels among sites in the Oslo fjord. However, none of these variables showed a significant relationship with the number of MPs found in the mussels, neither per individual nor per gram. This does not necessarily mean that these three factors do not have any effect on the quantity of MPs found, the effects may have simply been overridden or masked by other important factors not included in this model. Possibly important factors include ocean currents (Barnes et al. 2009), smaller streams or stormwater outlets (Cole et al. 2011), atmospheric fallout (Dris et al. 2016) and activities such as fishing and recreation at sea (Andrady 2011). The relationship between all these factors is probably too complex to be identified by the methodologies used here.

Considering the proportion of fibres in the results, it is not implausible that domestic waste water (e.g. from washing machines) and by that WWTPs is an essential pathway to the ocean for the particles identified in this study. Previous studies have demonstrated that fibres from

clothing are released during washing (Browne et al. 2011) and that these are not all retained by the facilities in WWTPs (e.g. Anderson et al. 2016; Mason et al. 2016). An estimated 35 million MP particles are leaving with the effluent water of VEAS every hour (Magnusson 2014), and as these are released into the environment, it is likely they are partly incorporated by the filter feeding mussels. In addition to WWTPs, atmospheric fallout may be a significant pathway for microplastic fibres to the marine environment, as fibres are easily spread through air. Results by Dris et al. (2015) suggest that atmospheric fallout could be a significant source of fibres in freshwater environments, which could indicate that it is a possible source for marine environment as well, but this has not been specifically investigated. Furthermore, Cai et al. (2017) found that 73% of the fibres in the atmospheric fallout they investigated were cellulose-based, which agrees with the results of this study. Rivers may also be a pathway for MPs to the marine environment, as several studies identify these as potentially important in this sense (Claessens et al. 2011; Duis & Coors 2016). Rain water and rivers might combined bring e.g. tossed cigarette filters of cellulose acetate to the marine environment, which could in turn explain part of the findings of “cellophane”.

In the same way that many variables are likely to have contributed to the differences between sites, there are several possible explanations for the differences between the two sampling years in Akershuskaia (O1 and O1b) as well. It could be that in 2017 (O1b) there was higher traffic by both cars and boats, or it could be that more snow was dumped in the area that year than in 2016 (O1). As currents and weather may play a role as well, it is impossible to say anything concrete about the differences just from this study. When considering that no differences were detected between the two years in Ramtonholmen (O5 and O5b), it could be speculated that the differences seen in Akershuskaia might be due to this being an area highly influenced by several variables, and that this leads to higher variation between samples as well.

5 Conclusion

Through this study, it has been demonstrated that digestion of organic material by using 10% KOH can be an appropriate protocol for extracting MP particles from marine biota, specifically from blue mussels. No apparent damages of tested plastics occurred, except discolouring, and the protocol was found to efficiently remove most of the organic material from the mussel samples. Furthermore, based on the experiences of this study, it is recommended to combine visual identification of MPs with more objective methods such as FTIR or other methods of chemical identification. The main reasons for including this additional step are that overestimation as a result of the subjectivity of visual identification is avoided, that useful additional information about polymeric composition is obtained, and that it will make comparisons between studies easier. Other methodological aspects have also been highlighted through this study, such as the importance of including contamination controls and the correction of results accordingly.

The study also reveals that there are unresolved issues concerning chemical identification using transmission μFTIR, as it was discovered that differences among cellulosic materials (e.g. viscose rayon and cotton) were not detected, and they were all identified as cellophane. This finding is of importance as previous studies have indiscriminately included cellophane as MP, and thus might have overestimated the quantities of MPs, while other studies have excluded these cellulosic particles, and thus possibly underestimated the quantities. There is a strong need both for further research targeting the effects different anthropogenic materials have on biota, and for defining what to include when investigating MP presence in the environment. More research is also required to understand where specifically the difficulties of identification lies, and what methodologies of identification are best suited for MP investigations. It is apparent that both over- and underestimations of quantitative levels of MPs are likely with the methods currently practiced, therefore quantitative results should be interpreted with caution.

Despite the methodological challenges, the results of this study show that MPs are present in Norwegian mussels, which, before this work was initiated, had not yet been demonstrated. It was also found that there are significant differences in MP quantities among sites, with hot-spots in the Barents Sea (Skallneset) and in the Oslo fjord (Akershuskaia). The reasons for these

differences are currently not understood, and further research should be conducted aiming to identify sources and pathways for MPs to the Norwegian marine environment. In the Oslo fjord, 11 different polymers were identified, with cellophane and “parking lot tar” being the most abundant, and these findings can give some indication on common sources and pathways for MPs to the Norwegian marine environment.

The successful extraction, quantification and qualification of MPs from blue mussels in this study indicates that blue mussel is a promising sentinel species for water-borne MP contamination in marine environments. Nonetheless, further investigations on the interactions between the biology of the mussel (such as size) and the occurrence of MPs should be carried out in order to be able to standardize quantitative findings. Standardizations of protocols and representations of results should also be established, so that comparisons among studies may be possible. In the process of achieving such standardizations, studies such as this one, are of great importance.

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Appendix

Appendix A – Tests of significant differences in quantitative data between sites

Table A 1 P-values of multiple pairwise comparisons using Dunn's procedure, following Kruskal-Wallis significance test of differences between sites (in particles per individual). Grey cells indicate values <0.05 (significance level), while bold text indicates values <0.0004 (Bonferroni corrected significance level).

	N1	N2	N3	N4	N5b	N6	N7	N8	O1	O1b	O2	O3b	O4	O5	O5b	O6	O7
N1	1	**< 0,0001**	**< 0,0001**	**< 0,0001**	**0,000**	**< 0,0001**	0,086	0,038	0,002	0,016	0,053	0,223	0,004	0,116	0,767	0,003	0,001
N2	**< 0,0001**	1	0,343	0,836	0,304	0,691	0,003	0,008	0,106	**< 0,0001**	0,005	0,000	0,070	0,012	**< 0,0001**	0,080	0,170
N3	**< 0,0001**	0,343	1	0,248	0,048	0,178	**< 0,0001**	**0,000**	0,010	**< 0,0001**	**0,000**	**< 0,0001**	0,006	0,001	**< 0,0001**	0,007	0,020
N4	**< 0,0001**	0,836	0,248	1	0,411	0,849	0,005	0,015	0,159	**< 0,0001**	0,010	0,001	0,109	0,019	**< 0,0001**	0,122	0,243
N5b	**0,000**	0,304	0,048	0,411	1	0,528	0,048	0,106	0,558	**< 0,0001**	0,078	0,013	0,435	0,104	0,001	0,469	0,730
N6	**< 0,0001**	0,691	0,178	0,849	0,528	1	0,009	0,024	0,223	**< 0,0001**	0,017	0,002	0,158	0,030	**< 0,0001**	0,176	0,329
N7	0,086	0,003	**< 0,0001**	0,005	0,048	0,009	1	0,721	0,165	**< 0,0001**	0,830	0,617	0,232	0,934	0,155	0,211	0,103
N8	0,038	0,008	**0,000**	0,015	0,106	0,024	0,721	1	0,302	**< 0,0001**	0,887	0,391	0,402	0,821	0,075	0,371	0,203
O1	0,002	0,106	0,010	0,159	0,558	0,223	0,165	0,302	1	**< 0,0001**	0,240	0,059	0,846	0,263	0,005	0,891	0,809
O1b	0,016	**< 0,0001**	**< 0,0001**	**< 0,0001**	**< 0,0001**	**< 0,0001**	**< 0,0001**	**< 0,0001**	**< 0,0001**	1	**< 0,0001**	**0,000**	**< 0,0001**	**0,000**	0,007	**< 0,0001**	**< 0,0001**
O2	0,053	0,005	**0,000**	0,010	0,078	0,017	0,830	0,887	0,240	**< 0,0001**	1	0,475	0,327	0,918	0,101	0,300	0,157
O3b	0,223	0,000	**< 0,0001**	0,001	0,013	0,002	0,617	0,391	0,059	**0,000**	0,475	1	0,090	0,606	0,356	0,080	0,033
O4	0,004	0,070	0,006	0,109	0,435	0,158	0,232	0,402	0,846	**< 0,0001**	0,327	0,090	1	0,341	0,009	0,955	0,663
O5	0,116	0,012	0,001	0,019	0,104	0,030	0,934	0,821	0,263	**0,000**	0,918	0,606	0,341	1	0,188	0,317	0,184
O5b	0,767	**< 0,0001**	**< 0,0001**	**< 0,0001**	0,001	**< 0,0001**	0,155	0,075	0,005	0,007	0,101	0,356	0,009	0,188	1	0,007	0,002
O6	0,003	0,080	0,007	0,122	0,469	0,176	0,211	0,371	0,891	**< 0,0001**	0,300	0,080	0,955	0,317	0,007	1	0,705
O7	0,001	0,170	0,020	0,243	0,730	0,329	0,103	0,203	0,809	**< 0,0001**	0,157	0,033	0,663	0,184	0,002	0,705	1

Table A 2 P-values of multiple pairwise comparisons using Dunn's procedure, following Kruskal-Wallis significance test of differences between sites (in particles per gram). Grey cells indicate values <0.05 (significance level), while bold text indicates values <0.0004 (Bonferroni corrected significance level).

	N1	N2	N3	N4	N5b	N6	N7	N8	O1	O1b	O2	O3b	O4	O5	O5b	O6	O7
N1	1	**< 0,0001**	**< 0,0001**	**< 0,0001**	**< 0,0001**	**< 0,0001**	0,029	0,000	**< 0,0001**	0,967	0,001	0,003	**< 0,0001**	0,003	0,002	0,001	**< 0,0001**
N2	**< 0,0001**	1	0,110	0,759	0,202	0,997	0,002	0,084	0,542	**< 0,0001**	0,068	0,025	0,645	0,116	0,027	0,057	0,190
N3	**< 0,0001**	0,110	1	0,057	0,004	0,110	**< 0,0001**	0,001	0,027	**< 0,0001**	0,001	**0,000**	0,039	0,003	**0,000**	0,000	0,004
N4	**< 0,0001**	0,759	0,057	1	0,332	0,757	0,006	0,156	0,762	**< 0,0001**	0,128	0,053	0,877	0,191	0,058	0,110	0,315
N5b	**< 0,0001**	0,202	0,004	0,332	1	0,201	0,074	0,653	0,505	**< 0,0001**	0,582	0,334	0,415	0,640	0,352	0,531	0,973
N6	**< 0,0001**	0,997	0,110	0,757	0,201	1	0,002	0,084	0,540	**< 0,0001**	0,067	0,025	0,642	0,115	0,027	0,057	0,189
N7	0,029	0,002	**< 0,0001**	0,006	0,074	0,002	1	0,182	0,014	0,033	0,217	0,412	0,009	0,281	0,392	0,247	0,080
N8	0,000	0,084	0,001	0,156	0,653	0,084	0,182	1	0,264	0,001	0,920	0,606	0,206	0,938	0,631	0,859	0,678
O1	**< 0,0001**	0,542	0,027	0,762	0,505	0,540	0,014	0,264	1	**< 0,0001**	0,224	0,103	0,882	0,296	0,110	0,196	0,483
O1b	0,967	**< 0,0001**	**< 0,0001**	**< 0,0001**	**< 0,0001**	**< 0,0001**	0,033	0,001	**< 0,0001**	1	0,001	0,003	**< 0,0001**	0,003	0,003	0,001	**0,000**
O2	0,001	0,068	0,001	0,128	0,582	0,067	0,217	0,920	0,224	0,001	1	0,678	0,172	0,992	0,704	0,939	0,606
O3b	0,003	0,025	**0,000**	0,053	0,334	0,025	0,412	0,606	0,103	0,003	0,678	1	0,075	0,712	0,972	0,735	0,352
O4	**< 0,0001**	0,645	0,039	0,877	0,415	0,642	0,009	0,206	0,882	**< 0,0001**	0,172	0,075	1	0,241	0,081	0,149	0,396
O5	0,003	0,116	0,003	0,191	0,640	0,115	0,281	0,938	0,296	0,003	0,992	0,712	0,241	1	0,735	0,939	0,662
O5b	0,002	0,027	**0,000**	0,058	0,352	0,027	0,392	0,631	0,110	0,003	0,704	0,972	0,081	0,735	1	0,762	0,371
O6	0,001	0,057	0,000	0,110	0,531	0,057	0,247	0,859	0,196	0,001	0,939	0,735	0,149	0,939	0,762	1	0,553
O7	**< 0,0001**	0,190	0,004	0,315	0,973	0,189	0,080	0,678	0,483	**0,000**	0,606	0,352	0,396	0,662	0,371	0,553	1

Table A 3 P-values of multiple pairwise comparisons using Dunn's procedure, following Kruskal-Wallis significance test of differences between sites in the Oslo fjord (in particles per individual). Grey cells indicate values <0.05 (significance level), while bold text indicates values <0.0018 (Bonferroni corrected significance level).

	O1	O1b	O2	O3b	O4	O5b	O6	O7
O1	1	**< 0,0001**	0,462	0,103	0,313	0,176	0,723	0,474
O1b	**< 0,0001**	1	**< 0,0001**	**< 0,0001**	**< 0,0001**	**< 0,0001**	**< 0,0001**	**< 0,0001**
O2	0,462	**< 0,0001**	1	0,372	0,081	0,537	0,275	0,147
O3b	0,103	**< 0,0001**	0,372	1	0,008	0,783	0,047	0,019
O4	0,313	**< 0,0001**	0,081	0,008	1	0,018	0,513	0,769
O5b	0,176	**< 0,0001**	0,537	0,783	0,018	1	0,087	0,039
O6	0,723	**< 0,0001**	0,275	0,047	0,513	0,087	1	0,719
O7	0,474	**< 0,0001**	0,147	0,019	0,769	0,039	0,719	1

Appendix B – Size and shape of plastic particles identified in the Oslo fjord

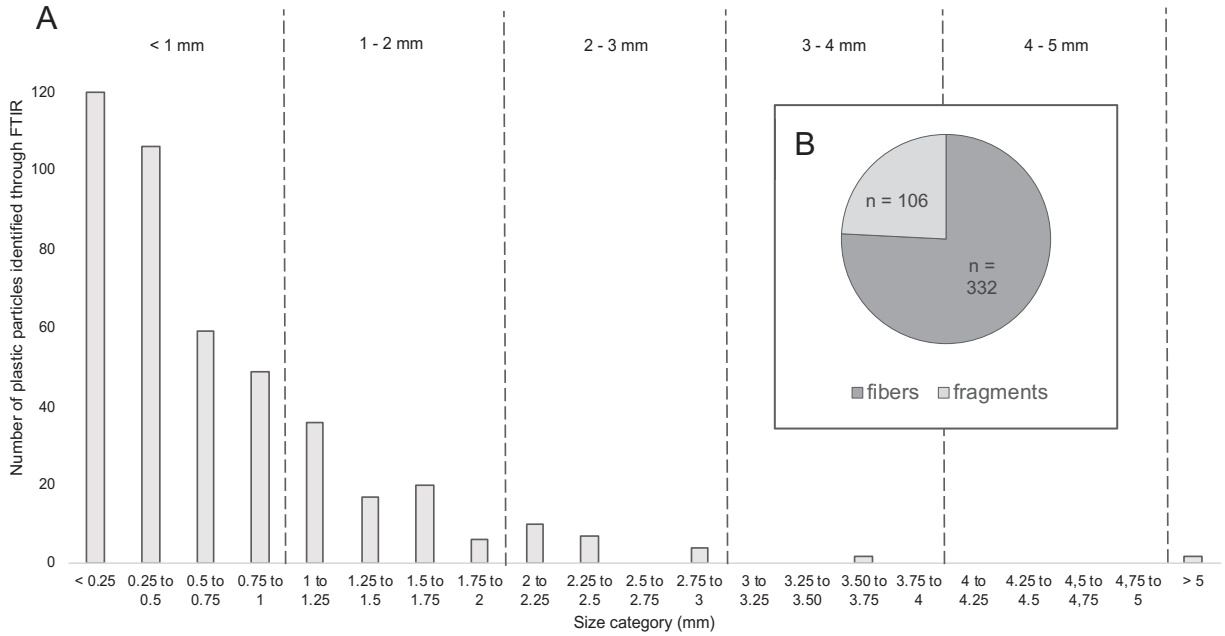

Fig. B 1 Distribution of **a)** size and **b)** shape for plastic particles found in blue mussels (*Mytilus edulis*) from seven sites in the Oslo fjord in Norway.

Appendix C – Example FTIR spectra of most abundant polymers

Cellophane

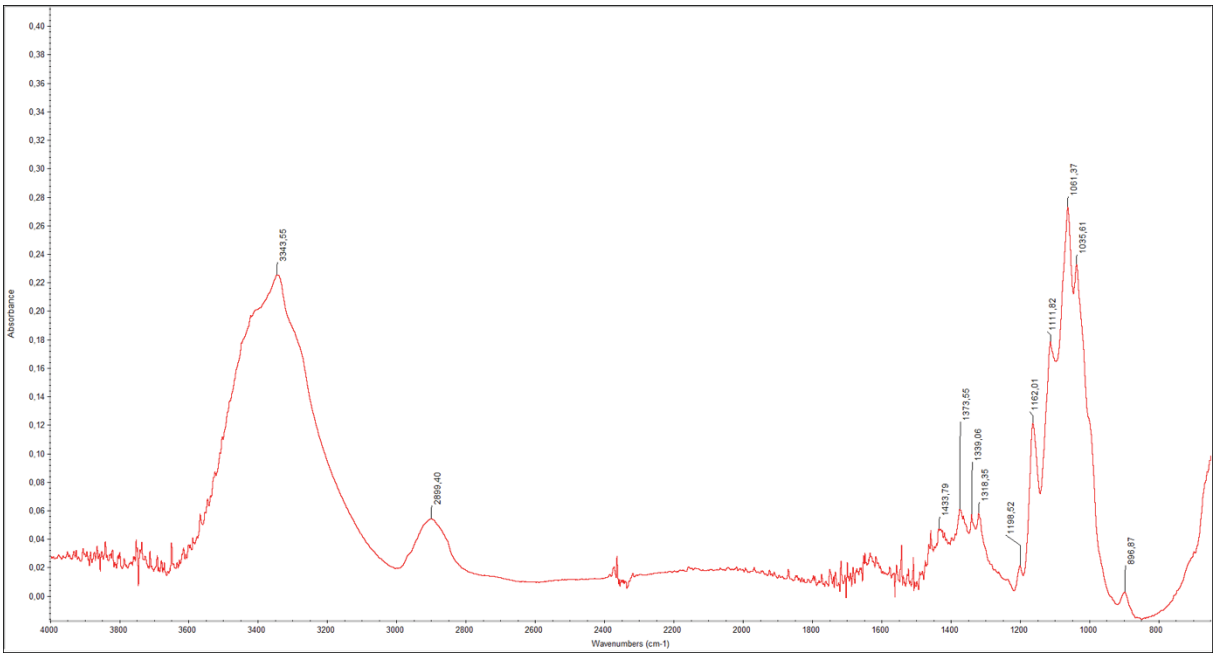

Fig. C 1 Absorbance spectrum recorded with transmission μFTIR from particle 30A_17_4, found in a mussel from site O2 (Gressholmen). Library match: 84% Cellophane. The spectrum also matched with library spectra of paper (lower than 84%). X-axis: Wavenumbers (cm^{-1}). Y-axis: Absorbance.

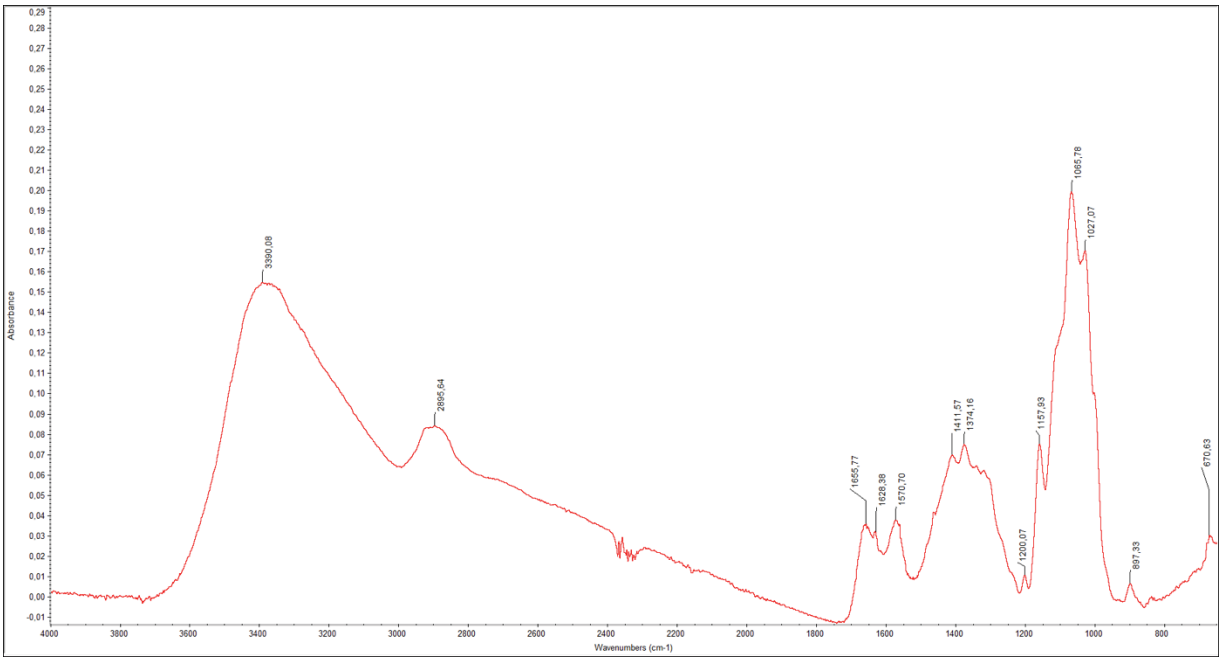

Fig. C 2 Absorbance spectrum recorded with transmission μFTIR from particle I307_2017_9_4, found in a sample from site O5b (Ramtonholmen 2017). Library match: 85% Cellophane. The spectrum also matched with library spectra of rayon (65%). X-axis: Wavenumbers (cm^{-1}). Y-axis: Absorbance.

Parking lot tar

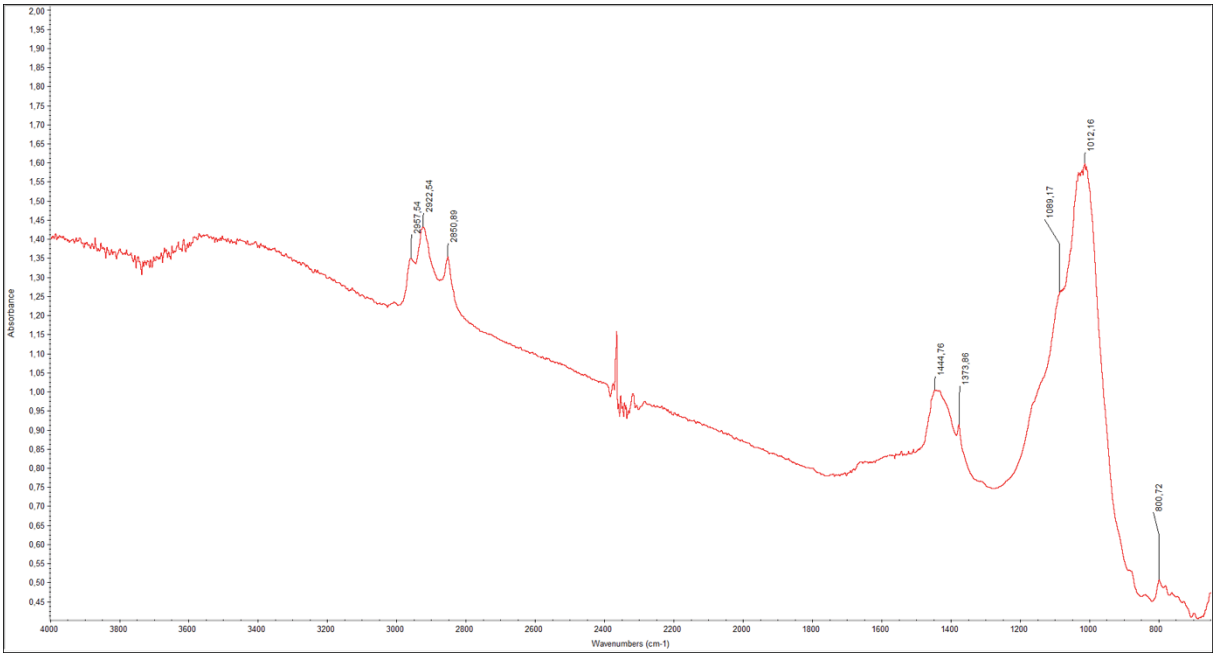

Fig. C 3 Absorbance spectrum recorded with transmission µFTIR from particle I301_2017_20_13, found in a sample from site O1b (Akershuskaia 2017). Library match: 70% Parking lot tar. X-axis: Wavenumbers (cm^{-1}). Y-axis: Absorbance.

Polyester

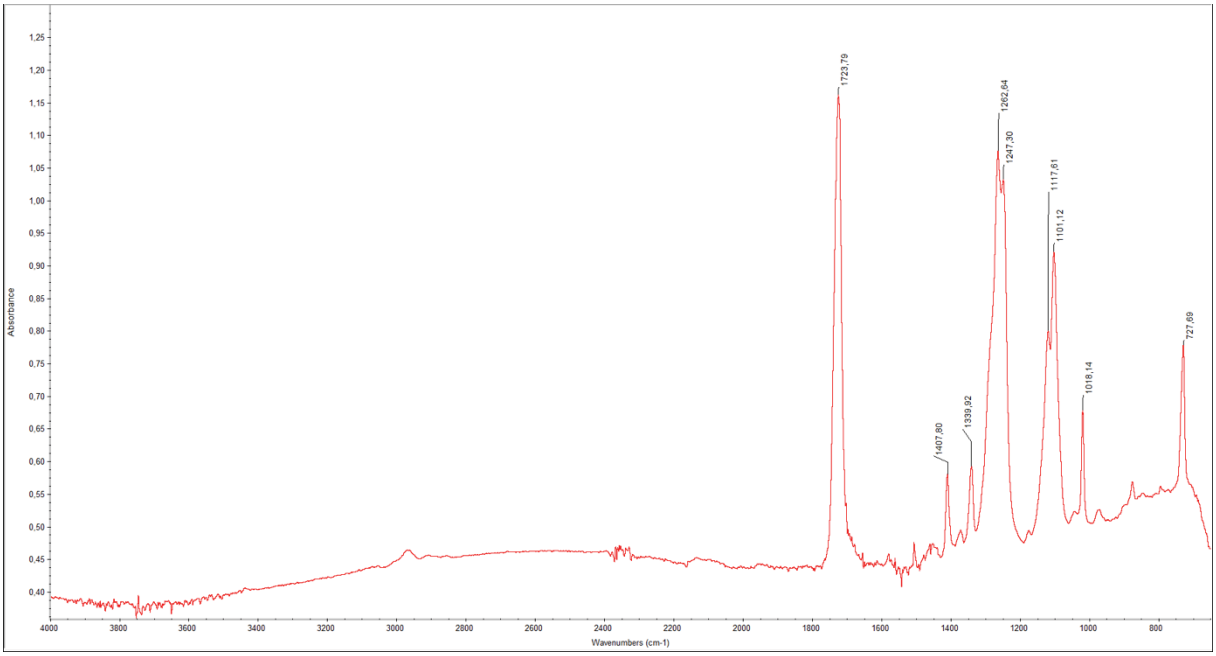

Fig. C 4 Absorbance spectrum recorded with transmission µFTIR from particle I023_12_1, found in a sample from site O7 (Singlekalven). Library match:89% Polyester. X-axis: Wavenumbers (cm^{-1}). Y-axis: Absorbance.

Acrylic

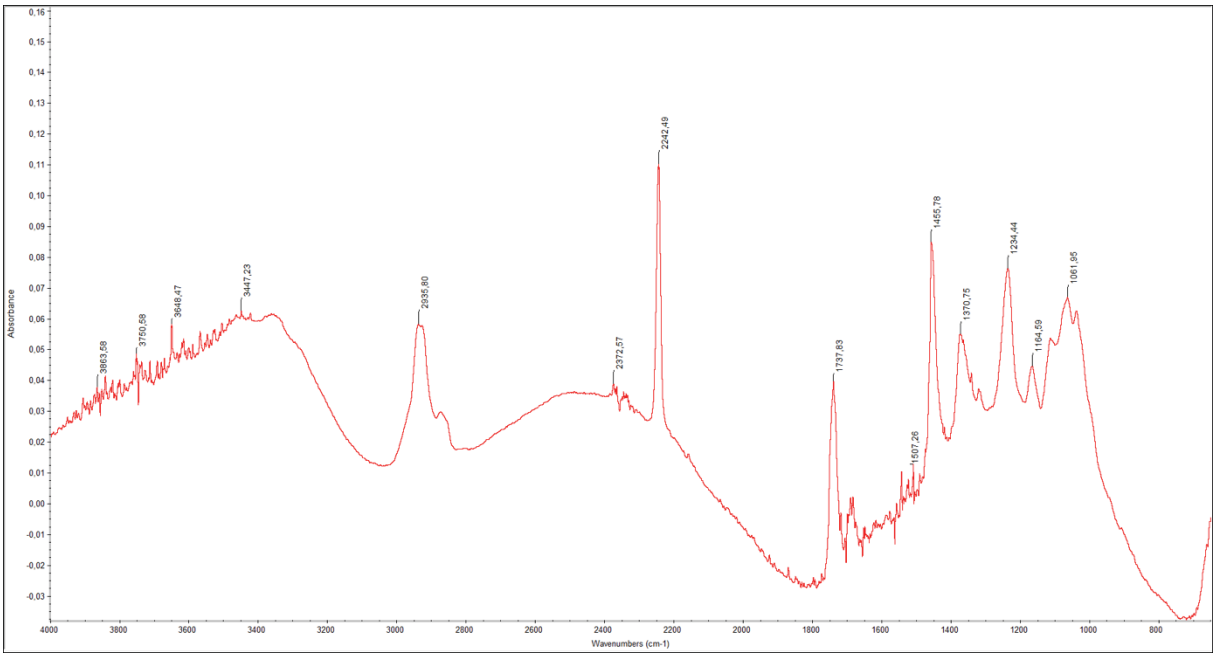

Fig. C 5 Absorbance spectrum recorded with transmission μFTIR from particle 30A_14_2, found in a sample from site O2 (Gressholmen). Library match: 89% Acrylic fibre. X-axis: Wavenumbers (cm^{-1}). Y-axis: Absorbance.

Appendix D – Results from experimental tests

Table D 1 Results from experimental tests conducted on different materials, with two different methods (transmission μFTIR and ATR FTIR). Treatment = the treatment the material underwent before chemical analysis. ID = Identification. Cells containing a hyphen (-) indicates no test was conducted.

			Transmission μFTIR			*ATR FTIR*		
Source	**Material**	**Treatment**	**% match**	**Polymer**	**Correct ID**	**% match**	**Polymer**	**Correct ID**
Organic wool	100% wool	None	92	Bacitracin	No	-	-	-
Wool and silk 1	70% wool and 30% silk	None	74	Wool	Yes	-	-	-
Wool and silk 2	70% wool and 30% silk	None	74	Silk	Yes	-	-	-
Acrylic scarf	100% acrylic fibre	None	89	Acrylic fibre	Yes	90	Acrylic fibre	Yes
Acrylic scarf	100% acrylic fibre	KOH	90	Acrylic fibre	Yes	55	Acrylic fibre	Yes
Fleece blanket	Polyester	None	84	PET	Yes	98	100% Polyester	Yes
Fleece blanket	Polyester	KOH	86	PET	Yes	97	100% Polyester	Yes
Nylon stockings	Polyamide	None	94	Nylon 6.6	Yes	98	Rip-stop nylon	Yes
Nylon stockings	Polyamide	KOH	96	Polyamide	Yes	98	Rip-stop nylon	Yes
Tights	Viscose	KOH	80	Cellophane	No	-	-	-
Bamboo sock 1	Viscose	KOH	93	Cellophane	No	-	-	-
Bamboo sock 1	Viscose	None	93	Cellophane	No	-	-	-
Bamboo sock 2	Viscose	None	93	Cellophane	No	-	-	-
Bamboo sock 2	Viscose	KOH	93	Cellophane	No	-	-	-
Modal	Viscose	None	91	Cellophane	No	96	100% Rayon	Yes
Modal	Viscose	KOH	88	Cellophane	No	90	100% Rayon	Yes
Viscose white	Viscose	None	92	Cellophane	No	98	100% Rayon	Yes
Viscose white	Viscose	KOH	82	Cellophane	No	91	100% Rayon	Yes
Viscose top	100% viscose	KOH	90	Cellophane	No	-	-	-
Cotton bandage	Cotton	KOH	82	Cellophane	No	-	-	-
Cotton cloth	Cotton	KOH	88	Cellophane	No	-	-	-
Cotton lab coat	100% cotton	KOH	86	Cellophane	No	-	-	-
Cotton sweater	100% cotton	KOH	76	Cellophane	No	98	100% cotton	Yes
Cotton sweater	100% cotton	None	74	100% Cotton	Yes	97	100% cotton	Yes
Cotton wool	100% cotton	KOH	84	Cellophane	No	-	-	-
Cotton lab coat	100% cotton	None	86	Cellophane	No	-	-	-
Cotton lab coat	100% cotton	KOH	80	Cellophane	No	97	100% cotton	Yes
Cotton sweater	100% cotton	None	74	Cotton	Yes	97	Cotton	-
Cotton sweater	100% cotton	KOH	76	Cellophane	No	98	Cotton	Yes

Cotton wool	100% cotton	None	83	Cellophane	No	-	-	-
Cotton wool	100% cotton	KOH	82	Cellophane	No	82	100% cotton	Yes
Organic cotton	100% cotton	None (Not flattened)	86	100% Cotton	Yes	97	100% Cotton	Yes
Organic cotton	100% cotton	None (Flattened)	83	Cellophane	No	-	-	-
Organic cotton	100% cotton	H20	82	Cellophane	No	96	100% Cotton	Yes
Organic cotton	100% cotton	KOH	81	Cellophane	No	94	100% Cotton	Yes
Organic cotton	100% cotton	Dried (80°C)	78	Cellophane	No	98	100% Cotton	Yes
Paper towel 1	Paper	KOH	86	Cellophane	No	-	-	-
Paper towel 2	Paper	None	82	Cellophane	No	97	Paper towel	Yes
Paper towel 2	Paper	KOH	62	Cellophane	No	92	paper towel	Yes
Hemp	Hemp	None	75	Cellophane	No	96	Burlap (100% jute)	Yes
Hemp	Hemp	KOH	80	Cellophane	No	91	Burlap (100% jute)	Yes
Linen	Linen	None	87	Cellophane	No	96	100% Linen	Yes
Linen	Linen	KOH	88	Cellophane	No	97	100% Linen	Yes
Cigarette filter	Cellulose Acetate	KOH	69	Cellophane	No	-	-	-
Total correct ID (fraction)					**0.25**			**1**

Appendix E – Results from sum of squares analysis for multiple linear regression models

Table E 1 Sum of squares analysis for the explanatory variables "City harbour", "WWTP" and "River outlet", with the dependent variable being "average particles ind^{-1}".

Source	DF	Sum of squares	Mean squares	F	Pr > F
City harbour	1	3,008	3,008	0,507	0,516
WWTP	1	0,117	0,117	0,020	0,895
River outlet	1	1,976	1,976	0,333	0,595

Table E 2 Sum of squares analysis for the explanatory variables "City harbour", "WWTP" and "River outlet", with the dependent variable being "average particles gram^{-1}".

Source	DF	Sum of squares	Mean squares	F	Pr > F
City harbour	1	0,150	0,150	0,388	0,567
WWTP	1	0,100	0,100	0,260	0,637
River outlet	1	0,975	0,975	2,526	0,187

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