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Microplastics in blue mussels and beach sediments - A study of Hovedøya in the inner Oslo Fjord

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Natural Resource Management

Preface

This thesis is written as the final work of my master's degree in natural resource management at the Norwegian University of Life Sciences (NMBU). A special thanks to the Norwegian Institute for Water Research (NIVA) who gave me the opportunity to write about microplastic with access to facilities, materials and lab-space. To see what is going on at NIVA has been enlightening and a great experience.

A special thanks to my main supervisor, Susanne Claudia Schneider, for support and guidance through this project. Fast and constructive feedback, guidance during the writing process and help with statistical analysis has been important. I also want to give a special thanks to Amy Lusher, my assistant supervisor, for guidance in the start-up, with lab-analysis and in the writing process. Thanks to Bjørnar Beylich for help throughout the fieldwork and some great days out in the fjord. And thanks to Nina Buenaventura for all the help down at the lab.

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Abstract

The quality and durability of plastics combined with improper waste management leads to contamination on land, in freshwater and in the marine environment. An increasing scientific concern is that microplastic (plastic particles <5 mm) pose a threat to biota and marine wildlife. There will be more plastic than fish in the ocean by 2050 (by weight) if we continue on the same path as today.

This study investigates the microplastic abundance at Hovedøya in the inner Oslo Fjord. Blue mussels (*Mytilus spp)* and beach sediments were collected from six different beaches facing out in different cardinal directions to represent the whole island. Microplastics were extracted from blue mussels by dissolving organic material with 10% potassium hydroxide (KOH), and beach sediments with a saturated sodium iodide (NaI) density solution. All samples underwent visual identification followed by chemical confirmation with a Fourier transform infrared spectrometer (FTIR).

Microplastics were found at every beach, in blue mussels and in beach sediments. 51.7% of the blue mussels analysed had ingested microplastics, with an average of 0.70 microplastics per individual and correspondingly 0.17 microplastics per gram wet weight. The overall average in beach sediments was 117.29 MP/m^2, while the number of macroplastic items were 1.34 items/m^2. Two potential correlations were investigated in this study. Firstly, the correlation between microplastic in blue mussels and sediment from the beach. Secondly, the number of microplastics and macroplastics (plastic items >5 mm) at the same beach. No significant correlation was found.

Sammendrag

Plast er et materiale med høy slitestyrke og god holdbarhet, og i kombinasjon med dårlig avfallshåndtering resulterer det i plastforurensing på land, i ferskvann og marine miljøer. En økende vitenskapelig bekymring er at mikroplast (plastpartikler <5 mm) utgjør en trussel mot biota og marint dyreliv. Hvis utviklingen fortsetter som i dag vil det være mer plastavfall enn fisk i havet innen 2050, målt i vekt.

Denne studien undersøker forekomsten av mikroplastpartikler på Hovedøya i indre Oslofjord. Blåskjell og sediment fra strender er samlet og analysert fra seks forskjellige lokaliteter som ble valgt for å representere hele øya. Mikroplast i blåskjell ble separert ved å bryte ned organisk materiale med 10% kaliumhydroksid (KOH), mens en mettet løsning med natriumjodid (NaI) ble brukt for å separere mikroplast og sedimenter. Potensiell plast ble funnet gjennom visuell identifikasjon, og verifisert gjennom kjemisk identifisering (Fourier transform infrared spectroscopy).

Mikroplast ble påvist i både blåskjell og sedimenter på hver lokalitet. Det ble funnet mikroplast i 51,7% av alle blåskjellene, med et gjennomsnitt på 0,7 mikroplastpartikler per blåskjell og tilsvarende 0,17 mikroplastpartikler per gram målt i vekt. I sedimentene ble det funnet 117,29 mikroplastpartikler per kvadratmeter og 1,34 makroplastbiter per kvadratmeter. Det ble også undersøkt om det er en potensiell sammenheng mellom mikroplast i blåskjell og sedimenter fra samme strand, og mikroplast og makroplast (>5 mm) på en strand. Det ble ikke funnet en signifikant sammenheng.

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1 Introduction

1.1 Plastics and plastic production

Plastics are synthetic polymers made from a wide range of chemical compounds with different characteristics (Hidalgo-Ruz et al. 2012). It is a basic element in many products and solutions and an important contributor to the global society. It is used in almost all aspects of daily life such as packaging, transportation, building materials, construction and fishing. Its durability, versality and high resource efficiency exceeds many other materials (PlasticsEurope 2017). Europe has initiated a transition from a linear economy towards a more resource efficient and circular economy. Plastic is a key material in this transition as they can help to preserve resources in every single step of a product (PlasticsEurope 2017).

348 million tonnes of plastic was produced in 2017 (PlasticsEurope 2018) and 64.4 million of these were produced in Europe. According to MacArthur et al. (2016), 15 million tonnes were produced in 1964 which means that the production has increased with more than 20 times in approximately 50 years. The report also conveys that plastic production is expected to double over the next 20 years.

Different types of plastics are manufactured to give optimal properties for different applications. According to GESAMP (2015), six classes of plastics dominates the plastic market (Table 1). Plastics are mostly manufactured from fossil fuels, but biomass such as maize and plant oils are increasingly being used (UNEP 2016).

Table 1 Examples of application and abbreviations for general plastic types (Kershaw & Rochman 2015; Sundt et al. 2014)

Type of plastic	Abbreviation	Examples of common applications
Polyethylene	PE	Plastic bags, bottles, six-pack rings
Polyethylene terephthalate	PET	Bottles, strapping
Polypropylene	PP	Rope, bottle caps, gear, strapping
Polyvinyl chloride	PVC	Film, pipe, containers, buoys
Polystyrene	PS	Utensils, containers, packaging
Polyurethane	PUR	Insulation

The quality and durability of plastics combined with improper waste management leads to contamination on land, in freshwater and in the marine environment (Lusher et al. 2017b), resulting in a global environmental problem (UNEP 2016). Optimally, plastics should be a part of the circular economy where it gets recycled and reused. Today, 72% of plastic packaging is not recovered at all. The global flow of plastic packaging has 32% leakage to the environment, 40% ends up in landfills and only 14% is collected for recycling (World Economic Forum 2016). For the last decade, plastics leaking out of the system has arised as a considerable global concern due to the risk of environmental and human health issues. Plastic products will degrade slowly over time leading to fragmentation of the material into smaller plastic particles called microplastics (GESAMP 2015; Lusher et al. 2017b).

1.2 Microplastic

Particularly sunlight (ultraviolet radiation) and high temperatures, including movement and "wear and tear" leads to the fragmentation of plastics into smaller pieces. However, there is currently no universally accepted definition regarding the size of microplastic (MP) (Van Cauwenberghe et al. 2015b). The definition of microplastics as plastic particles smaller than 5 millimetres (mm) is commonly accepted (Cole et al. 2011; GESAMP 2015; Lusher et al. 2017a) and therefor used in this study. Microplastics can furthermore be divided into small microplastics (<1 mm) and large microplastics (1-5mm) (Lusher et al. 2017a).

Microplastics are separated into primary and secondary microplastics to easier indicate potential sources and the different ways microplastic enter the marine environment (GESAMP 2015). Primary microplastics are manufactured plastic particles designed for specific applications. It has been produced to be in that size and is directly released into the environment as microplastics. For example, the microbeads in products such as cosmetics (e.g. shower gels) or from the abrasion of synthetic textiles during washing (Boucher & Friot 2017). Secondary microplastics originates from larger plastic items degraded into microplastics by fragmentation and weathering. For example, discarded plastic bags or fishing nets. What type of plastic it is has a large impact on the rate where the plastics degrade into microplastics. GESAMP (2015) states that weathering and fragmentation rates are relatively high on beaches. Plastics flowing in water, the mid-water column or in the marine sediments will degrade slower than plastics on beaches. Higher mechanical abrasion or higher UV radiation could be factors enhancing the processes (GESAMP 2015).

1.3 Plastics in the ocean

Jambeck et al. (2015) estimated that 4.8 to 12.7 million metric tons (MT) of plastic waste entered the ocean in 2010. This calculation is based on a generation of 275 million MT of plastic waste by 6.4 billion people living in 192 coastal countries in 2010. FOUNDATION (2016) states that at least 8 million tonnes of plastics leaks into the ocean every year. This amount of plastics equals one garbage truck dumping its content in the ocean every minute. Plastics can remain in the ocean for hundreds of years and current research estimates that there are 150 million tonnes of plastic waste in the ocean today (FOUNDATION 2016).

Both formation and distribution of plastic waste in the ocean is influenced by a combination of type of plastic and environmental factors (UNEP 2016). It is challenging to estimate the distribution considering that it relies on whether it is primary or secondary microplastics, accurate information about the distributions of macroplastics (plastic items >5 mm) and the degradation process (GESAMP 2015). Auta et al. (2017) states that microplastics in the ocean usually comes from a mix of different sources, originates from different locations and are emitted at different times. For example, macroplastic will easier be transported by winds than microplastics because of a larger surface. The density of plastics relative to the density of seawater will influence the behaviour whether it floats or sinks (GESAMP 2015). An unbroken, closed plastic bottle will float due to the air inside, but the plastic density will determine whether it floats or sinks after fragmentation. However, Wabnitz and Nichols (2010) state that plastic has a light weight and that approximately 50% of plastics are buoyant. Therefore, they can easily be transported by driving forces such as inland waterways, wastewater outflows, rivers, waves, tides and ocean currents, and discharged into the ocean. Both sea and land-based activities are responsible for the ongoing input of plastics to the ocean (Lusher et al. 2017b). Examples of marine sources are losses and illegal dumping from fishing activities, sea-vessels or other marine industries such as aquaculture (Lusher et al. 2017b). Nevertheless, Cole et al. (2011) states that terrestrial sources contributes with approximately 80% of all plastic waste found in marine litter.

An increasing scientific concern is that microplastic pose a threat to biota and marine wildlife (Cole et al. 2011). According to FOUNDATION (2016), it will be more plastic than fish in the ocean by 2050 (by weight) if we continue on the same path as today.

1.4 The impacts of microplastic on marine life

All organisms have the potential to interact with microplastics in the marine environment. More than 230 different marine species across trophic levels have been found to take up plastics, and there are a number of different ways by which exposure and interaction may occur (Lusher et al. 2017a). The small size of microplastics combined with their presence in both pelagic and benthic ecosystems makes them easily available for ingestion (Auta et al. 2017). Studies show that zooplankton, bivalves, mussels, fish, shrimps, oysters, lugworms and whales have been reported to ingest microplastic (Auta et al. 2017; GESAMP 2015; Van Cauwenberghe et al. 2015a). Some of these (e.g. mussel and fish) are species being harvested commercially, making microplastic a potential part of the human food chain.

Plastic are often supplied with plastic additives (also called plasticisers) during manufacture to provide resistance to heat, oxidative damage or microbial degradation which result in a longer life span (Cole et al. 2011). However, these additives makes plastic a source of toxic chemicals (Engler 2012). Some plastics will leach toxins that have been added during manufacture into the environment. Some types of plastics can also absorb toxic chemicals present in the environment, only to release them at a later stage (Teuten et al. 2009). Furthermore, wildlife that ingest plastics may accumulate toxins in their bodies and suffer from chemical contamination (Engler 2012). Chemicals accumulating in marine life, may be transferred up the food chain and into seafood ingested by humans. This means that there is a potential risk of toxic chemicals from marine harvest in human diets.

There is a lack of quantitative and empirical data on the occurrence of microplastics in the environment (GESAMP 2015; IUCN 2014; Lusher et al. 2017a). This study will investigate the microplastic abundance at Hovedøya in the inner Oslo Fjord. There is an urgent need to increase public awareness about the adverse effects of plastic pollution and the impacts on marine organisms, in order to foster a sense of individual responsibility and encourage government action (IUCN 2014). Hovedøya is a popular recreational area for the inhabitants in Oslo and using Hovedøya as study area could potentially enhance the sense of individual responsibility and awareness.

1.5 Potential sources of microplastics to Hovedøya

Hovedøya is situated in the inner bay of Oslo in an urban environment, close to several potential sources with both land- and sea-based sources of macro- and microplastics and anthropogenic influence. Rivers, wastewater treatment plants (WWTP), shipping industry and harbours are among the most important sources of plastic to the ocean (GESAMP 2016; Jambeck et al. 2015; Lusher et al. 2017b).

Svein Taksdal from the Norwegian Water Resources and Energy Directorate (NVE) (personal communication, 07.02.2019) informs that the average total runoff from rivers from Nesodden, Oslo, Bærum and parts of Asker to the inner Oslo Fjord is 27.64 m^3/s, calculated from the years 2000 – 2017. The rivers Alnaelva and Akerselva are the two largest river systems in Oslo, both with outlets in Oslo harbour (Ranneklev et al. 2009). The river Akerselva has an average runoff of 3.31 m^3/s (Taksdal 07.02.2019) and a drain catchment of 250 km^2 which makes it the largest river system in Oslo. The river Alnaelva is the longest river with an average runoff of 1.54 m^3/s (Taksdal 07.02.2019). Both rivers flow through residential and industry areas, offices, parks and roads. Research by Bottolfsen (2016) on microplastic in the river Alnaelva, indicates that microplastic are present and that PE, PET, PUR, PP and PS are dominating among the plastic particles. In the river Akerselva, research by Buenaventura (2017) indicates that microplastic are present and that PUR followed by PE, PVA and acrylics were the most common plastic types.

Municipal wastewater treatment plants are frequently suspected as a significant point source of microplastics to the environment (Carr et al. 2016; Sun et al. 2019). Microplastics can enter the ocean through the wastewater outflow. When plastics are too small for retention by the wastewater plant, it will pass directly into the oceans (Lusher et al. 2017b). Events with heavy rainfall can also lead to overflow when wastewater plants exceed their capacity. The result is runoff of untreated or moderately treated wastewater that may transport both macro- and microplastics directly into the ocean (Magnusson et al. 2016). Bekkelaget is one of two wastewater treatment plants in Oslo and the second largest wastewater plant in Norway (Karstensen 2015). The outlet of the wastewater plant is in Ormsund southeast of Sjursøya, approximately 2.5 kilometres from Hovedøya. The second wastewater plant in Oslo is VEAS, which is the biggest wastewater plant in Norway (VEAS n.d.). VEAS is located in Bjerkås in Asker, approximately 17 kilometres away from Hovedøya. A study by Magnusson (2014)

states that almost 36 million plastic particles ≥ 20 µm and 350 000 plastic particles ≥ 300µm is released with effluent water from VEAS every hour. However, the study also states that 97 – 99% of all particles coming into the waste water plant with the influent water were retained.

The ocean surrounding Hovedøya is heavy frequented by ferries and boat traffic. The shipping industry is also considered an important source of microplastics according to (GESAMP 2016). Maintenance and boat additives, harbour activities and recreational boating activities are considered to be reasons why high level of microplastics have been found in harbour sediments. The Port of Oslo has 50 to 70 ships with goods or passengers arriving each week (Oslo Havn KF 2011), with a distance to Hovedøya ranging from 500 to 1500 metres. A boat harbour is located in a bay at the northeast side of Hovedøya. The harbour consists of different boat associations with room for approximately 650 boats. Furthermore, littering from day tourists needs to be considered (Syakti et al. 2017). Up to 15 000 people visit the island each day during summertime, increasing the potential contamination.

Microplastics entering the marine environment will be distributed at e.g. beaches, the water surface, the water column and biota (Lusher et al. 2017a). In order to investigate microplastic abundance at Hovedøya, blue mussels and beach sediments are used as monitoring parameters. Studies reveals that microplastic are found in both blue mussels and beach sediments (Besley et al. 2017; Hengstmann et al. 2018; Lusher et al. 2017a; Van Cauwenberghe et al. 2015a) and that microplastic potentially accumulates in these (GESAMP 2015; Karlsson et al. 2017). Blue mussels (*Mytilus spp)* are frequently used as environmental indicators, are semi-sessile, have a relatively long life span and are widely distributed in coastal regions (Lusher et al. 2017a; Mathiesen et al. 2017). Sediments are proposed as the final destination of microplastics in the environment, and monitoring of blue mussels in combination with sediments gives a broader picture of microplastic pollution (Lusher et al. 2017a).

This study will also investigate whether or not there is a correlation between the amount of microplastics in blue mussels and beach sediments gathered at the same site. Are there more microplastics in blue mussels when the amount of microplastics in beach sediments from the same site are high? If this research finds that there is indeed a positive correlation, this will indicate that blue mussels from contaminated beaches most likely contains microplastics and

should be eaten with care. To my knowledge, there has not earlier been conducted research of this kind on the relationship between the abundance of microplastic in blue mussels and beach sediments. Additionally, there is a lack of data comparing the abundance of microplastic and macroplastics at local scales (GESAMP 2015). Research on the correlation between different microplastics monitoring parameters could reduce the amount of analyses needed to understand the occurrence and distribution of microplastic in the marine environment.

1.6 Aims of the study

The aim of this study was to investigate the occurrence of microplastics at Hovedøya in the Oslo Fjord. Furthermore, it will contribute with quantitative data on microplastic occurrence in the Norwegian environment. This aim is divided into three objectives:

1) To quantify the amount of microplastics in blue mussels and beach sediments from beaches at Hovedøya.
2) To investigate if there is a correlation between microplastics in blue mussels and sediments at a specific beach.
3) To investigate if there is a correlation between the amount of microplastics and macroplastics at specific beach.

2 Materials and methods

2.1 Study area

Hovedøya is an island located in the Inner Oslo Fjord, close to the city of Oslo, Norway (Fig.1). It is about 469 000 square meters in size (Vestreng 2018), with a maximum elevation of 46 meter above sea level (Thuesen 1984). It was bought by the City of Oslo for four million NOK in 1958 and is currently managed by Bymiljøetaten (Vestreng 2018). Hovedøya is a popular recreational area with a lot of visitors especially during summer. The closeness to Oslo city, combined with a long cultural history and a rich flora and fauna diversity, make Hovedøya the most visited island in the Oslo Fjord. According to Busterud (2018), as many as 15 000 people may visit the island in a single day during the summer. Beach activities, walking, boating, exploring of the cultural heritage and photographing among others are common activities. Consequently, Hovedøya is an island in an urban environment.

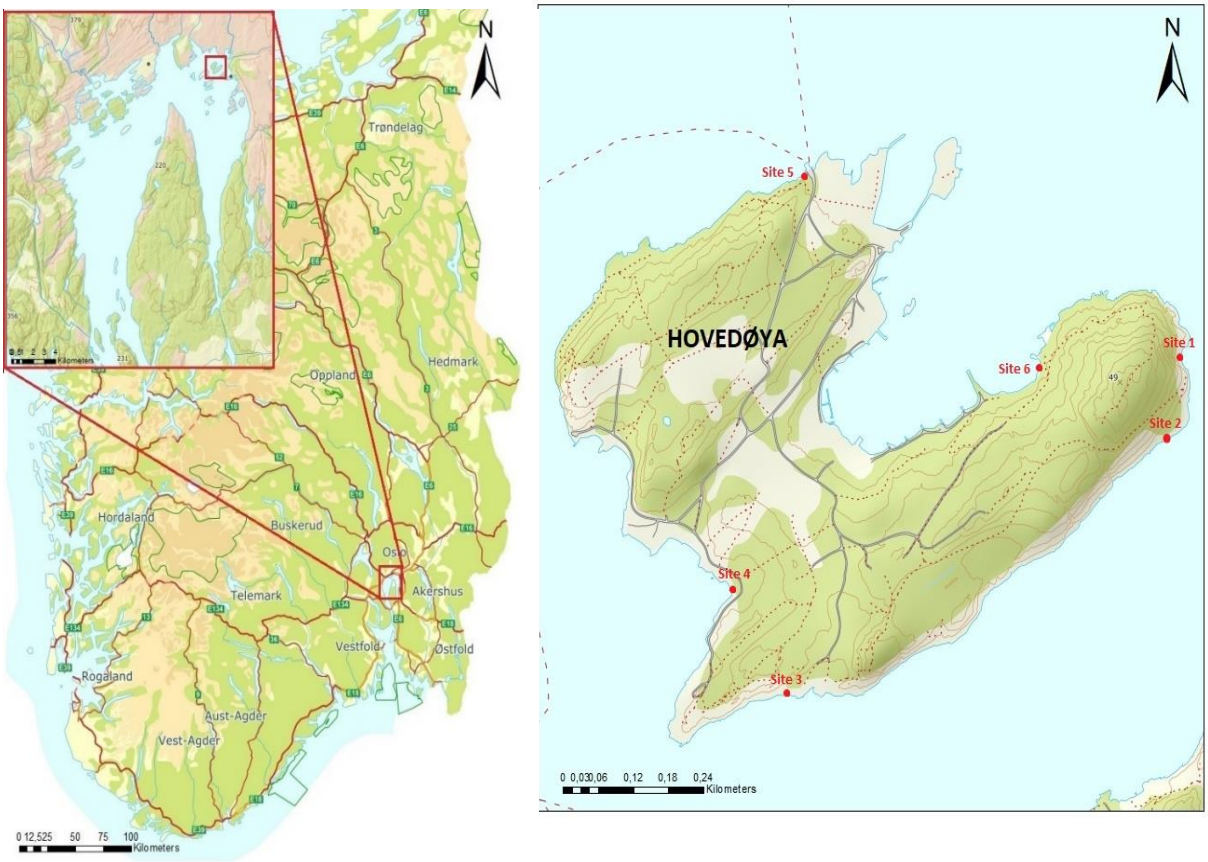

Figure 1 Map of Hovedøya, situated within the inner Oslo Fjord. Sampling sites are marked numerically. Sites were chosen to represent different cardinal directions.

2.2 Sampling of blue mussels, sediments and macroplastic

Six sampling sites were established around Hovedøya (Table 2, Fig. 1). The sites are located at beaches spread around at Hovedøya, facing out in different cardinal directions to represent the whole island.

Mussels were collected between July 31st and September 26th, 2018. 20 – 30 mussels per site were collected by hand, either by wading or snorkelling, approximately at 0.5 meters depth. Only living individuals with no visible signs of damage were collected. All samples were rinsed for fouling and washed properly with seawater. All the mussels were closed during this process. The mussels were stored in a freezer (-20ºC) right after the sampling.

Sediments were sampled at the same sites as mussels at Hovedøya between September 3-4th, 2018. Sediment samples were taken from 5 sub-sites at each site. In order to get a representative picture of an entire beach, the five sub-sites were approximately evenly distributed along the beach. All samples were taken close to, but under the high tide line. The top layer with larger sediments (>1 cm) was removed before taking the sample from a sampling quadrat of 20 x 20 cm from each sub-site. The depth was not registered but was approximately 1-2 cm. Approximately 200 grams of sediments were sampled with a spoon and stored in marked plastic bags. The sediment samples were taken to the laboratory and dried (40 degrees, 24 hours) before further processing.

The registration of macroplastic (plastic particles >5 mm) followed the method described in NOAA Marine Debris Shoreline Survey Field Guide (Opfer et al. 2012). The method is developed as a standardized marine shoreline survey by NOAA Marine Debris Program. A standing-stock study provides information on the amount and types of debris on the shoreline and was used during the registration with some modifications. Independent of the length of the beaches (which ranged from eight to 36m), a 50% coverage of each beach was analysed. Every beach was divided in two meter wide transects, where the number of transects varied depending on the beach length (Fig. 2). To randomly select which transects to analyse to get a 50% coverage, a function called "*sample"* in R studio was used. In this study, macroplastic are plastic items >5 mm.

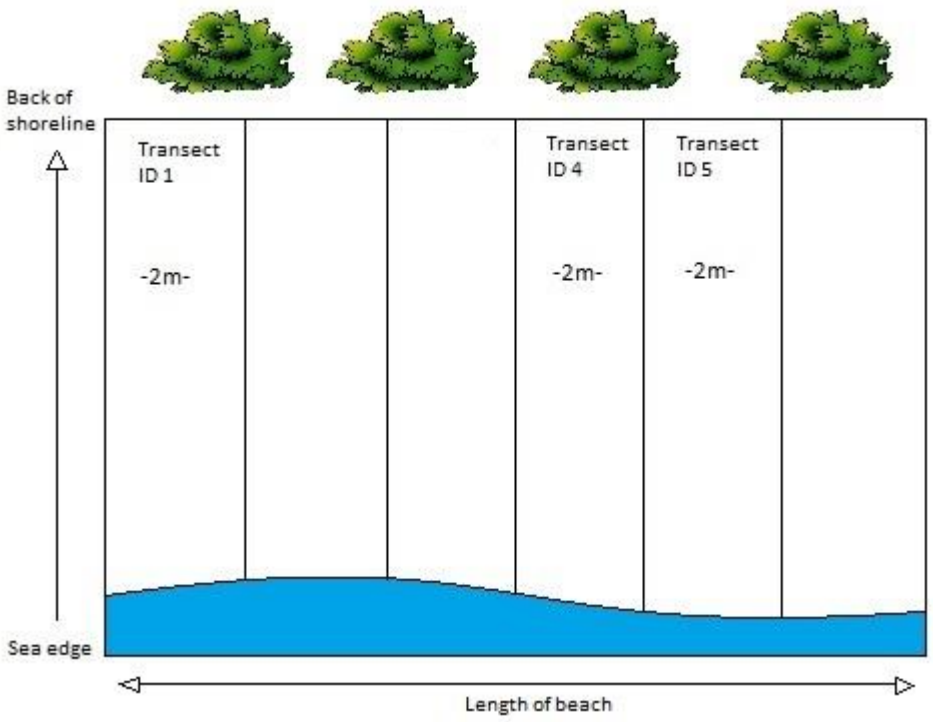

Figure 2 Example of a shoreline section. Width of the beach determines number of transects. Figure adapted for Hovedøya based on (Opfer et al. 2012)

All plastic litter (>5 mm) from the sea edge to the highest strandline in all selected transects were counted. Each plastic item was counted and placed in a similar category to the NOAA marine debris shoreline survey field guide, during registration. These categories include e.g. food wrappers, bottle or container caps and plastic ropes/small net pieces. Each plastic item was counted and placed in a similar category during registration.

Table 2 Characteristics of the sampling sites at Hovedøya. Substrate uniformity = percent coverage of the main substrate type

Site	1 (S1)	2 (S2)	3 (S3)	4 (S4)	5 (S5)	6 (S6)
Location (GPS coordinates)	N 59º 57.770 E 10º 44.522	N 59º 53.699 E 10º 44.488	N 59º 53.502 E 10º 43.787	N 59º 53.591 E 10º 43.687	N 59º 53.924 E 10º 43.844	N 59º 53.763 E 10º 44.260
Substrate and substrate uniformity	Coarse gravel (100%)	Coarse gravel (100%)	Sand and gravel (50%)	Sand (100%)	Gravel and sand (80%)	Gravel and sand (70%)
Collection method	Hand	Hand	Hand	Hand	Snorkelling	Hand
Beach direction	East	Southeast	South/southeast	South/southwest	Northwest	North/Northwest
Beach length (m)	22	36	32	28	8	30
Comment	Some kind of hardened oil spill. Close to industrial area and Oslo		Partly crowded beach during summer	Shallow. Crowded beach during summer	Ferry stop	Shallow. Boat harbour

2.3 Methods for extraction of microplastics

2.3.1 Extraction of microplastics in blue mussels

Blue mussels processing followed the method presented in Bråte et al. (2018). 20 mussels were thawed and the length of each individual was registered with a calliper. Subsequently, scalpel and forceps were used to open the shells and to extract the soft tissue into a 50 ml plastic beaker (Falcon tube) (Fig. 3). Excess water was discharged. Each mussel was weighted in grams wet weight. The muscular foot and the byssus filaments are not a part of the digestive or filtering system and were therefore excluded from the analyses. Before putting the soft tissue in a beaker, each beaker was cleaned with Reverse Osmosis (RO) water. RO water is water that has gone through a process where dissolved inorganic solids like microplastics or salts have been removed (Products 2018). To clean everything with RO water is important to reduce the risk of contamination from the working environment.

Figure 3 Extraction of the soft tissue (to the left) and beakers in the incubator for 24 hours (to the right)

10% potassium hydroxide (KOH) was used to remove and degrade the natural organic matter without degrading plastics. KOH has low health and safety risks, is cost efficient and enables a high throughput of samples (Bråte et al. 2018). 100 grams of KOH pellets were put in a glass jar and mixed with 900 ml of RO water. Based on a visual estimation, approximately three to four times the volume of the mussels of KOH was added in a 50 ml beaker. Additionally, approximately 25 ml of 10% KOH was added in three empty beakers without mussels as procedural controls. The beakers were then placed in an incubator (New Brunswick Scientific™ Innova®44) for 24 hours, at 120/140rpm and 60ºC (Fig. 3). After incubation, samples were allowed to cool to room temperature.

Before filtration, glass microfibre filters (Whatman® GF/D, pore size 2.7µm) were checked in the microscope to make sure they were clean with no contamination. Clean filters were stored in petri dishes. A Millipore® vacuum filtering assembly and an aquarium pump was used to filter the digestate (Fig. 4). Each filter was put back in the petri dishes after filtration, marked with the sample ID.

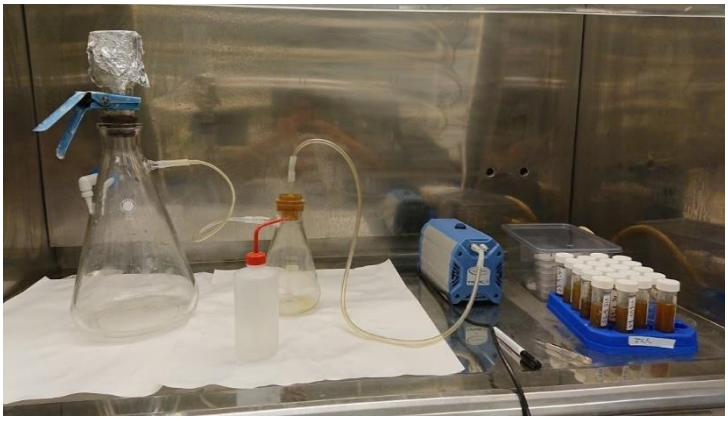

Figure 4 Vacuum filtering assembly and aquarium pump

2.3.2 Extraction of microplastics from beach sediments

The sediments were dried at 40 degrees for 24 hours, to easier process the samples. A one-millimetre (mm) sieve was used to separate the sediments into particles <1 mm and >1 mm. The sediments <1 mm were put in marked plastic bags, while the sediments >1 mm were put in marked glass jars. Weight was registered for all sub sites.

All of the sediments underwent density separation. It is used to separate sediments and plastic particles based on the difference in density (GESAMP 2016), where potential plastic will float and sediments will sink. Firstly, sediment >1 mm from each sub-site was mixed in a glass jar. Approximately 1 litre of a saturated sodium iodide (NaI) density solution was prepared in a glass bottle (10 dl). Saturated NaI solution has a density of 1.8 g/cm^3. 500 ml of the NaI solution was poured in another open glass measuring jar and mixed with the sediments from one site. The NaI solution was inserted first to make the sediments sink through the solution. This creates a higher circulation and potential plastics have a better chance to float. Some more NaI solution was added before stirring the sediments with a thin spoon. All floating particles were collected and sieved through a 38 µm sieve and placed in a box. The sunken sediments together with the NaI solution were filtered with a 500 µm sieve, where the

sediments were taken back in the glass beakers. As much as possible of the NaI solution was recovered and reused.

Of the sediments <1 mm, 25 grams (+/- 0.5g) from each subsite were put in 50 ml plastic beakers. 6 beakers without sediments were rinsed and filled with 35 ml of NaI solution as procedural blanks. Approximately 35 ml was also filled in each beaker containing sediments and shaken until all sediments were loosened from the beaker wall or floating in the solution. A “syringe” was used to wash the lid for sediments and refill to the top. The beakers stood for three days to “settle”. With a rolling movement, the solution was poured in the filter system, to collect the floating particles on a filter paper. The solution went through, while the sediments were kept in the beakers. The beakers were processed in order, and the filter system was washed after each site to reduce the risk of cross contamination. The sediments underwent two extractions to collect as much potential plastic/floating particles as possible. Additional NaI solution was added to the remaining material in the beakers for the second density extraction. Filter papers were left to dry at room temperature in a closed petri dish.

2.4 Verification of plastics

All samples underwent visual identification followed by chemical confirmation of the polymer material. The visual identification of plastic particles on the filters was carried out with a stereomicroscope (NIKON SMZ745T), where particles assumed to be plastics were registered. The method followed “Visual identification for plastic analysis” (NIVA 2018). The particles assumed to be plastic were sorted into fragments and fibres at the side of the filter paper, for easy identification prior to chemical characterization. Every particle was photographed and measured, using an INFINITY 1-3C camera with the image analysis software INFINITY ANALYZE and CAPTURE attached to the microscope (Fig. 5). Type (fibre, fragment or pellet), colour, and length of long and short axis were registered using the measuring equipment on the software.

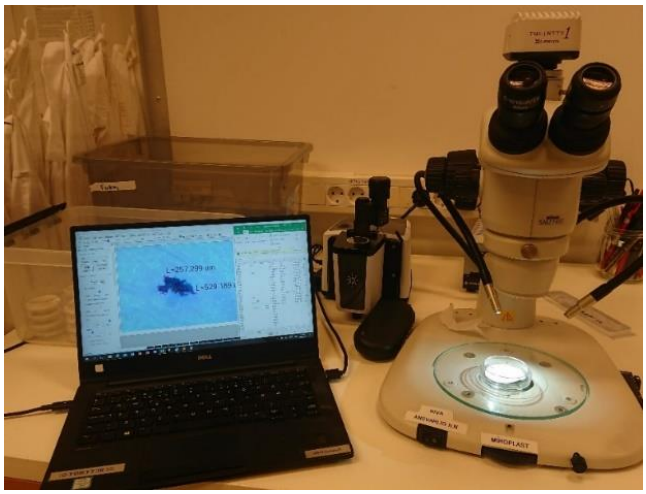

Figure 5 Stereo microscope with attached camera and analyse software

After visual identification, chemical classification was conducted to confirm the polymeric material. All of the particles analysed through visual ID, except lost particles, underwent chemical classification. A Fourier transform infrared spectrometer (FTIR) was used to classify the particles based on their polymeric identity. Each fibre was placed on a diamond compression cell, flattened and transferred to a PerkinElmer FT-IR Microscope Spotlight 400 and exposed to infrared light (4000-400 cm^{-1}). Transmission spectral data were recorded using 32 scans, a resolution of 4 and an optical velocity of 1.8988 (Bråte et al. 2018). The spectra were automatically compared against reference spectra libraries, resulting in a percentage match (Fig. 6).

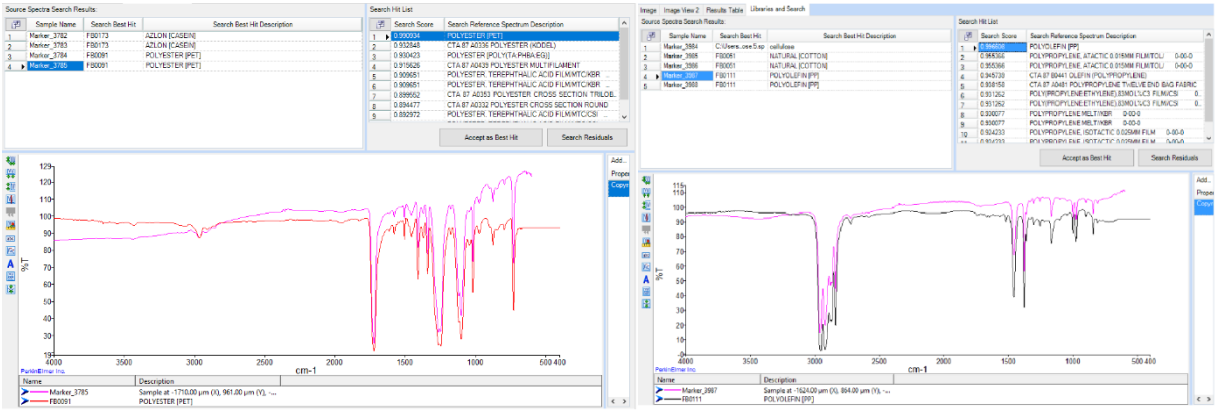

Figure 6 Spectra for polyester (to the left) and polyolefin (to the right)

2.5 Contamination precautions

Precautions taken to avoid contamination were carried out during extraction and analysis. A pre-brushed cotton laboratory coat was used, and extraction was done in a sterile cabinet with a fan to reduce the contamination from the working environment. RO-water was used to rinse the glassware and other equipment thoroughly before and after processing.

The petri dishes were open during processing and analysis. Fibres occur commonly in air (Gasperi et al. 2018), such that contamination from air needs to be considered. Three beakers per site with KOH as negative controls (procedural blanks) were processed in the same way as the samples to correct for this. When plastic particles were found in the procedural blanks, the average number of particles found on the procedural blanks per day (separately for particles of different shape), was subtracted from the results of the corresponding samples. Blue mussels at site 3 did not have any procedural blanks, so the blanks were calculated from the mean of all replicates across all sites to account for contamination. 12 sediment blanks from the extractions underwent the same process.

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on the whole study (not pr. site), to give a measure of the uncertainties that comes with the data. These uncertainties can be determined by a statistical approach based on measuring replicate blanks (Armbruster et al. 1994). LOD defines the point at which the analysis becomes just feasible, while LOQ presents a greater probability that a value at the LOQ is true and not a random fluctuation of the blank. LOD is calculated as the "Mean + (St.dev * 3)" and LOQ as the "Mean + (St.dev * 10) (Armbruster et al. 1994).

2.6 Data analysis

Data analysis was conducted on biota, beach sediments and macroplastic separately. It is important to have standard reporting units to increase the comparability with other studies (Lusher et al. 2017a). This study presents the results for blue mussels as both microplastic per gram wet weight (w.w) and number of microplastics per individual (MP/ind.) to increase the comparability with other studies. Weight is used as an indicator of size similar to normal procedure when monitoring contaminants using mussels (Bråte et al. 2018). The beach sediments are presented as MP/m² and macroplastic as items/m^2.

ArcGIS (ArcMap 10) was used to create maps, while data handling and some charts were executed in Microsoft Excel for Office 365 in Windows. Statistical analyses, included some charts, were carried out using R-studio (version 1.1.419 – © 2009-2018 RStudio, Inc).

A Shapiro-Wilk test was used to test for normality. Because most data were not normally distributed, a Kruskal-Wallis test was used to test for differences in micro and macroplastic abundance among sites. Dunn's procedure for multiple comparisons was used post-hoc where significant differences were observed. Spearman rank correlation was used to investigate potential correlations between plastic particles in blue mussels and beach sediments, and with beach sediments and macroplastics at a beach. The significance level for all analyses was set to 95% ($p = 0.05$).

3 Results

3.1 Corrections

Between zero and two fibres were found on the procedural controls (Table 3). Blue mussels at site 3 did not have any procedural blanks. In order to correct for the "usual" occurrence of microplastics in the air, the procedural control for this sample was calculated from the mean of all replicates across all sites. 12 sediment blanks from the extractions underwent the same process, and between zero and one fibre was found in the blanks (Table 4).

Table 3 Particles found in blank samples for blue mussels, with limit of detection (LOD) and limit of quantitation (LOQ). (*) is calculated from the mean of all replicates across all sites

Procedural controls

Only fibres - no fragments were found

		Site1	Site2	Site3(*)	Site4	Site5	Site6
Blue mussels	**Replicate 1**	1	0		0	0	0
	Replicate 2	1	0		0	0	0
	Replicate 3	0	0		0	0	2
	Mean	0,67	0	0,27	0	0	0,67
	St.dev	0,58	0	0,59	0	0	1,15
	Site LOD	2,40	0	2,05	0	0	4,13
	Site LOQ	6,44	0	6,20	0	0	12,21

Table 4 Particles found in blank samples for sediments. 6 blanks were extracted two times and are presented as 12 replicates, with limit of detection (LOD) and limit of quantitation (LOQ)

Prosedural controls - SEDIMENT	
Only fibres - no fragments were found	
	Extraction 1 and 2
Replicate 1	0
Replicate 2	1
Replicate 3	1
Replicate 4	0
Replicate 5	0
Replicate 6	0
Replicate 7	0
Replicate 8	0
Replicate 9	1
Replicate 10	0
Replicate 11	0
Replicate 12	0
Mean	0,25
St.dev	0,45
Site LOD	1,61
Site LOQ	4,77

At sites S1 and S6 the number of plastic particles found in blue mussels exceeded the detection limit (LOD), two individuals at S1 (4 and 3 MP/ind. respectively) and one individual at S6 (5 MP/ind.) (see appendix A). In regards of the sediment samples, all sites had microplastic levels exceeding the LOD with at least one sub-site. Three of the sites (S1, S2 and S6) had sub-sites with microplastic values exceeding the limit of quantification (LOQ). See appendix A.

3.2 Occurrence of microplastics in blue mussels

3.2.1 Size and shape of particles from all sites

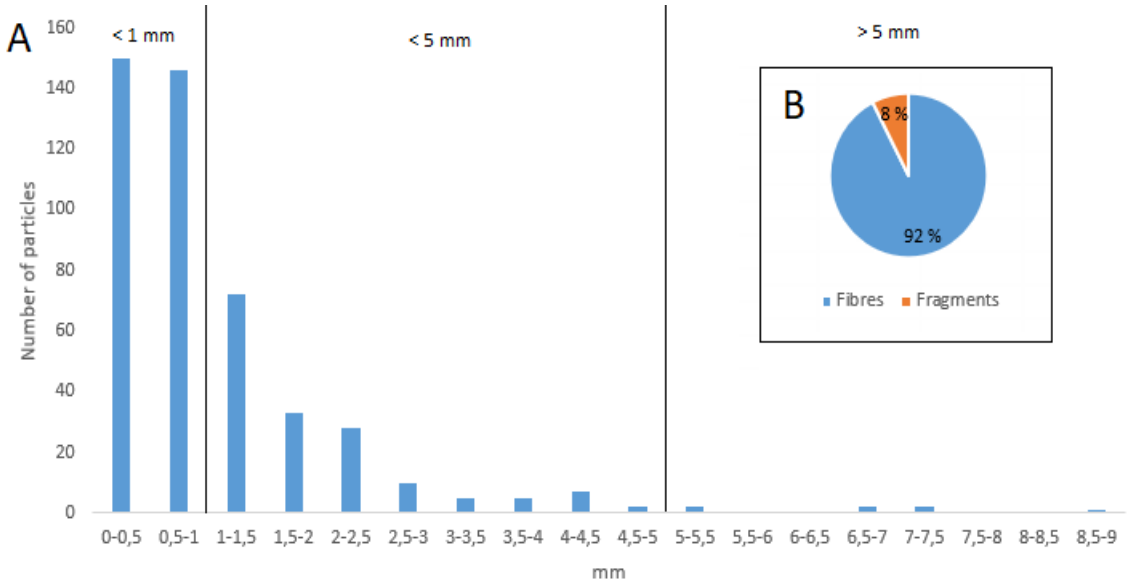

Figure 7 Distribution of size (A) and shape (B) for particles extracted from blue mussels at Hovedøya (n = 465)

465 particles suspected for being plastic were identified from blue mussels at Hovedøya. 64% of the particles found were particles <1 mm, and particles between 1 – 5 mm contributed with 35%. The remaining two percent were 7 particles between 5 – 9 mm (Fig. 7A). Fibres were more abundant than fragments at all beaches; respectively 92% fibres and 8% fragments in total (Fig. 7B). The distribution of size and shape was calculated based on the results from visual identification (n = 465) and prior to correction where the procedural controls were subtracted from the results.

3.2.2 Number of microplastic particles per individual

Of the 465 particles suspected for being plastics, 106 plastic particles (22.8%) were identified as plastics prior to correction where the procedural controls were subtracted from the results. The rest were identified among others as natural cotton, cellulose, or natural wool (See appendix B). 83 plastic particles (18%) were found after correction.

62 out of 120 analysed blue mussels had ingested microplastics. This corresponds to 51.7% of the individual blue mussels analysed. At least one individual contained microplastic at each site.

No significant difference was found in the number of microplastic per individual among sites (Kruskal-Wallis, p = 0.1996). The average number of plastics per individual was 0.70 (± 1.23 SD) with a range from 0 – 8 particles (Fig. 8). The highest number of microplastics per site was found at site 6 with 20.27 plastic particles and an average of 1.01 plastic particles per individual. 17 plastic particles were found at site 4 with an average of 0.85 plastic particles per individual. One individual with 8 ingested plastic particles was found at site 5, which is the highest number per individual (Fig. 8). Blue mussels at site 3 were least contaminated, with an average of 0.38 plastic particles per individual.

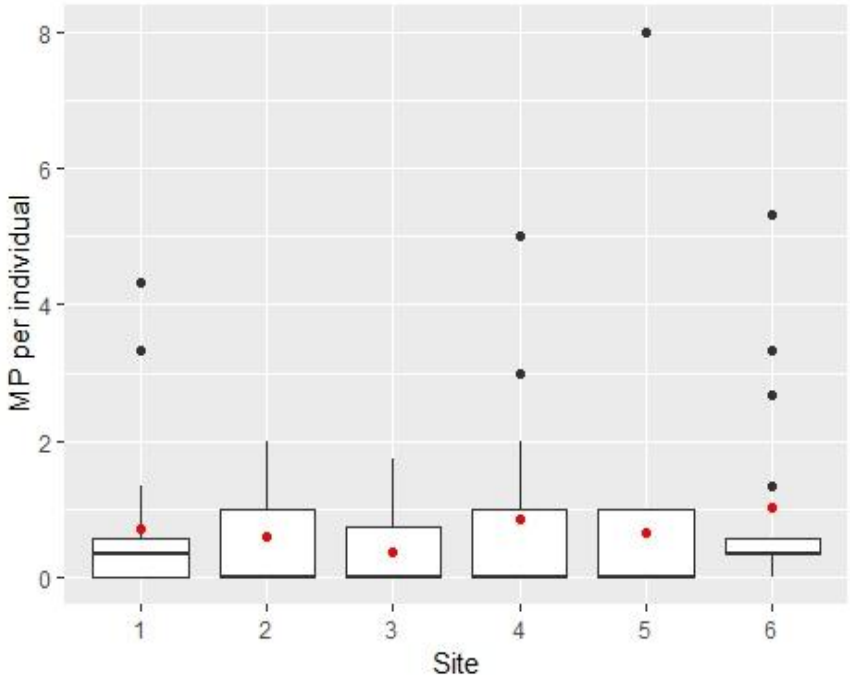

Figure 8 MP per individual. The boxplot shows the median (horizontal line), mean value (red dot), interquartile range (box), maximum and minimum value (vertical stripes) and outliers (dots).

3.2.3 Number of microplastic particles per gram mussel weight

The mussel wet weight varied from 1.30 (site 4) to 10.80 (site 2) grams. Site 2 stands out with relatively large mussels (average of 7.07g) and site 4 with relatively small mussels (average of 2.88g) (Fig. 9).

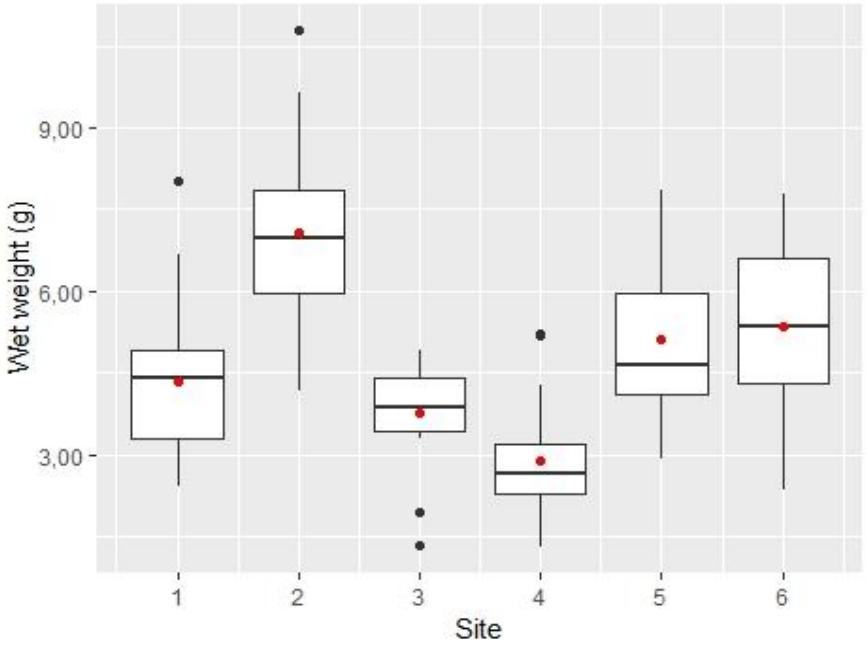

Figure 9 Wet weight (g. w.w) of blue mussels from each site. The boxplot shows the median (horizontal line), mean weight (red dot), interquartile range (box), maximum and minimum value (vertical stripes) and outliers (dots).

No significant difference was found in the number of microplastic per gram among sites (Kruskal-Wallis, p = 0.1627). The average number of plastic particles per gram wet weight was 0.17 (± 0.32 SD), with a range from 0 – 1.99 plastic particles per gram (Fig. 10). Site 4 had the highest level of ingestion with an average of 0.34 (± 0.51 SD) plastic particles per gram w.w. The lowest level of ingestion was found at site 2 with an average of 0.09 (± 0.13 SD) plastic particles per gram w.w. Site 6 had the highest level of ingestion after site 4 (0.19 ± 0.23 SD).

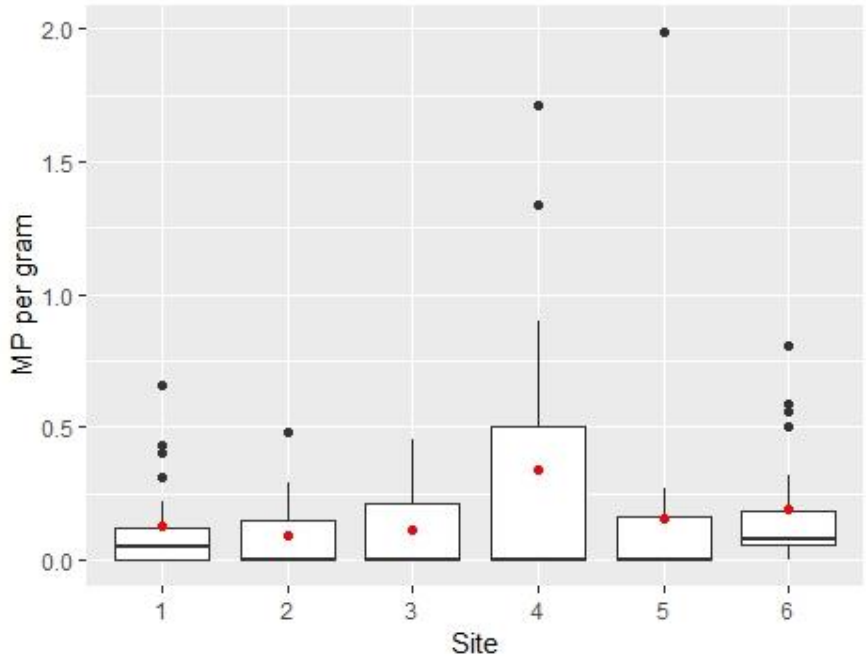

Figure 10 Microplastic per gram. The boxplot shows the median (horizontal line), mean weight (red dot), interquartile range (box), maximum and minimum value (vertical stripes) and outliers (dots).

3.3 Occurrence of microplastics in beach sediments

3.3.1 Size and shape of particles from all sites

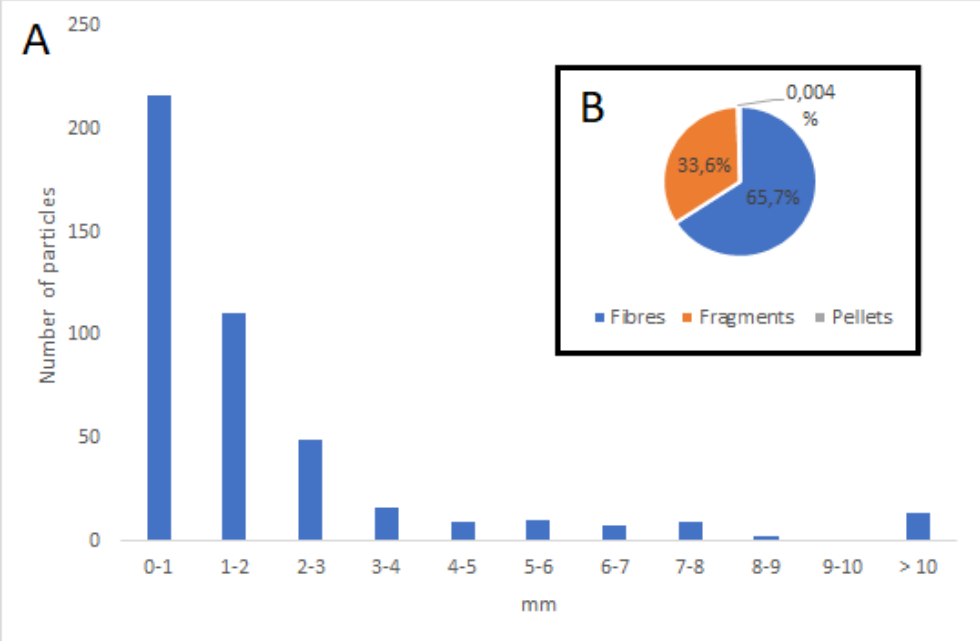

Figure 11 Size (A) and shape (B) distribution of suspected plastic particles found in beach sediments across all sites (n=446)

446 particles from six different beaches at Hovedøya were suspected for being plastics after the visual ID. 48% of the suspected plastic particles were between 0 – 1 mm and 25% between 1 – 2 mm (Fig. 11A). 65.7% of all the plastic particles found at Hovedøya were fibres. Fragments accounted with 34%, while 2 pellets accounted with 0.004% (Fig. 11B).

3.3.2 Number of plastic particles

Out of the 446 particles suspected of being plastics, 150 particles were verified as plastics after FTIR which corresponds to 33.6% of the suspected plastic particles. The rest were identified as among others natural cotton, azlon, natural wool, and cellulose (see appendix C). 141 plastic particles were found after corrections (31.6%). Microplastic particles were identified across all sites at Hovedøya.

Table 5 shows the results when sediments <1 mm and >1 mm are merged to get a representative picture of the whole beach. On average, 117.29 MP/m^2 were found across beaches (range 46.25 – 213.75 MP/m^2, Table 5). Most microplastics were found at site 1 with 213.75 MP/m^2. Site 4 and 6 had the second and third highest abundance with 173.75 and 118.75 MP/m^2 respectively. Site 5 had the lowest amount with 46.25 MP/m^2.

Table 5 Sediments <1 mm and >1 mm merged across all sites.

	Total number of MP	Area (m^2)	Number of MP/m^2
Site 1	42,75	0,2	213,75
Site 2	16,5	0,2	82,50
Site 3	13,75	0,2	68,75
Site 4	34,75	0,2	173,75
Site 5	9,25	0,2	46,25
Site 6	23,75	0,2	118,75

The average abundance in sediments <1 mm across all beaches was 73.1 MP/m^2 (range 26.2 – 193.75 MP/m^2, Fig. 12). The highest abundance of MP was found at site 1 with 193.8 MP/m^2 (± 177.8 SD). Site 6 comes out with the second highest abundance with an average of 93.8 MP/m^2 (± 57.6 SD). There was a significant difference in number of MP among sites for the sediments <1 mm (Kruskal-Wallis, $p = 0.02$). Dunn's post hoc test revealed that the number

of particles from site 1 was significantly higher than from site 5 (Dunn's test, p = 0.03). Note different scales at the y-axis between the Fig. 12 and 13.

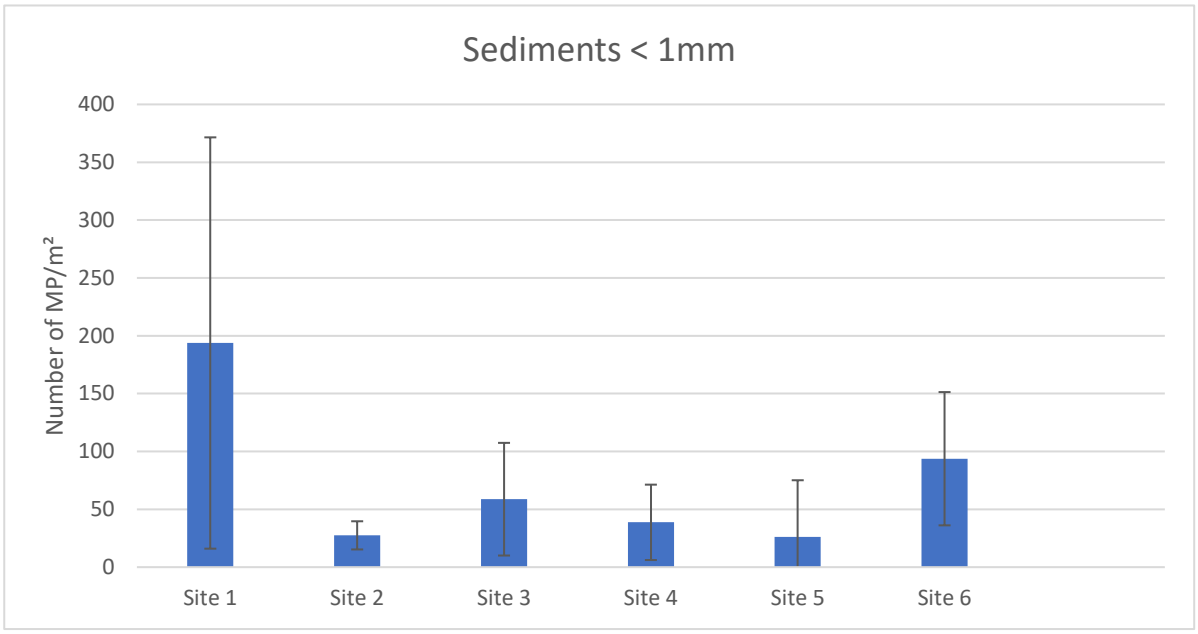

Figure 12 Number of MP/m2 in beach sediments <1 mm. SD marked as vertical lines

The average abundance in sediments > 1mm was 44 MP/m^2 with a variation from 10 – 135 MP/m^2 (Fig. 13). Site 4 showed the highest contamination by microplastics with 135 MP/m^2 followed by the site 2 with 55 MP/m^2. Site 3 showed the least contamination with 10 MP/m^2. SD data is not available for sediments >1 mm because all subsamples were merged and analysed in a single procedure.

Figure 13 Number of MP/m² found in beach sediments >1 mm. Each sub-site was merged during analyse for sediments >1 mm, and SD are not available.

3.4 Chemical classification

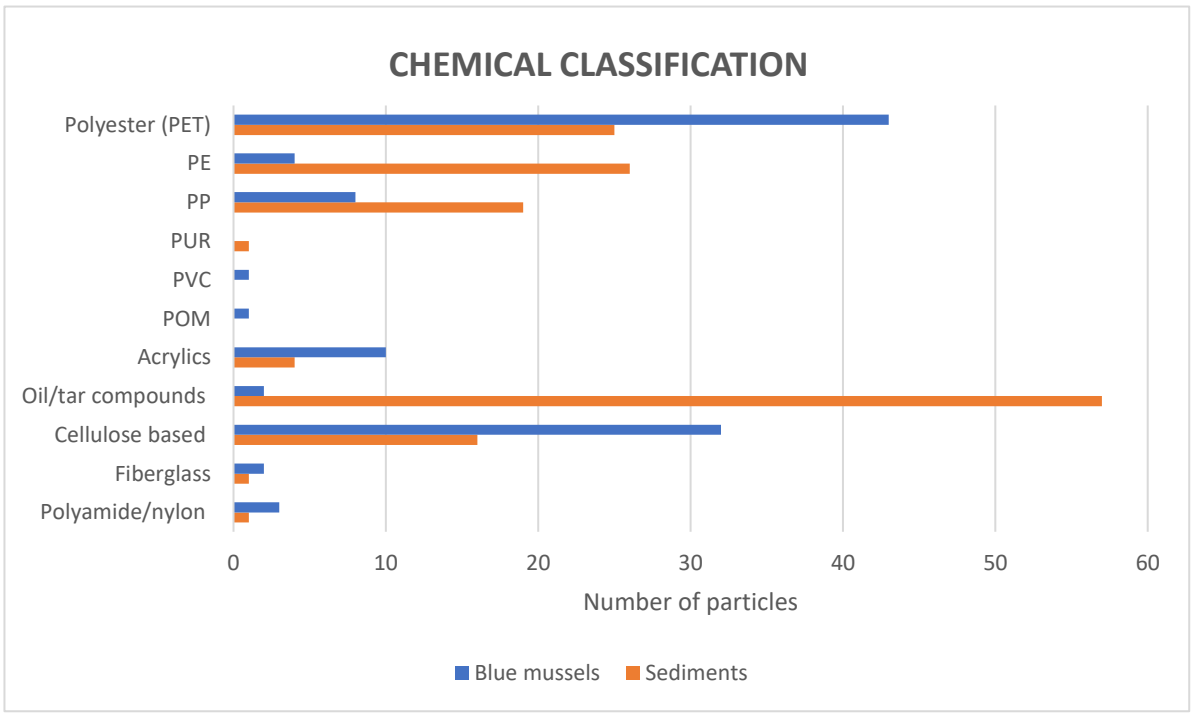

Figure 14 A comparison of plastic types found in blue mussels and beach sediments prior to correction

Every suspected plastic particle found in blue mussels and sediments underwent chemical classification. 256 plastic particles were found prior to correction where the procedural controls were subtracted from the results. Polyester and cellulose-based polymers dominated

in the blue mussels, while oil/tar compounds dominated in the sediment samples (Fig. 14). Polyester accounted for 27% of all plastic particles found in both blue mussels and beach sediments and was the most abundant polymer at Hovedøya. Following polyester, oil/tar compounds (23%) and cellulose based polymers (19%) were the most abundant. PE and PP accounted for respectively 12% and 11%.

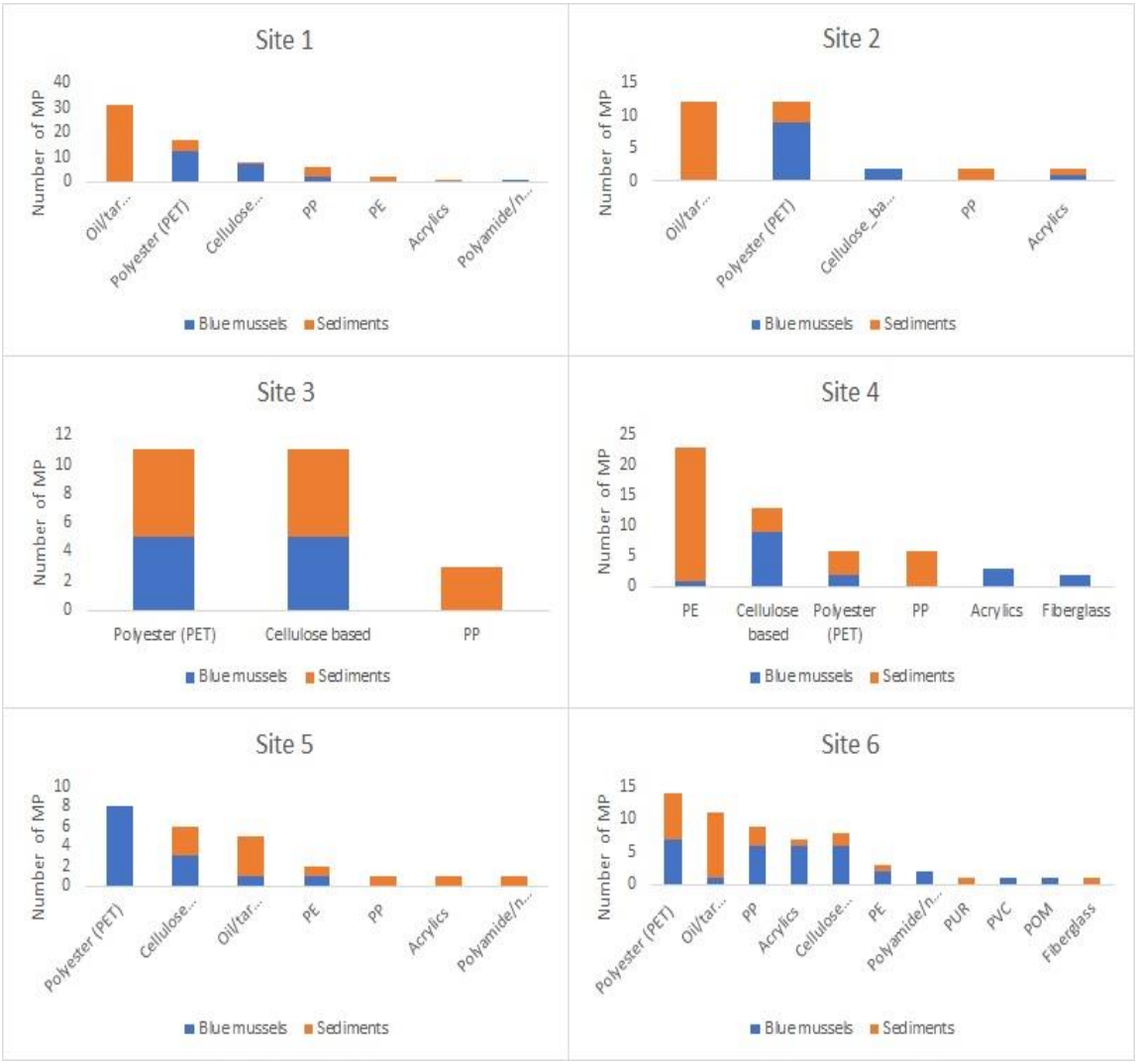

Figure 15 Chemical classification distributed among each site. Note different value at y-axis. N = 256 particles

Oil/tar compounds, polyester (PET) and particles based on cellulose were types dominating at Hovedøya (Fig, 14, Fig. 15), while polyester (PET), particles based on cellulose (rayon and cellophane) and PP were found at all sites (Fig. 15).

3.5 Macroplastic

A total of 602 plastic items were counted and registered at six sites at Hovedøya. The number of items per beach ranged from 41 items at site 5 to 157 items at site 1 (Fig. 16).

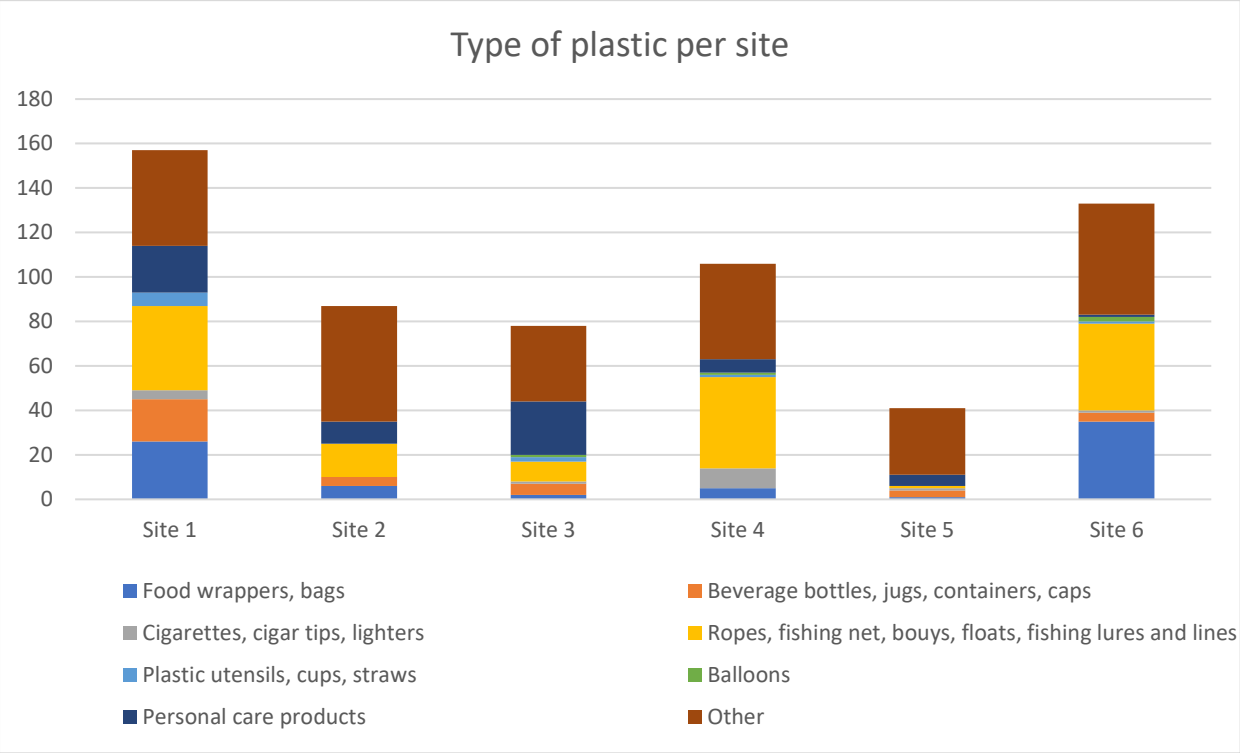

Figure 16 Type of plastic per site. Plastic particles categorized as "Other" are types not included in the NOAA Marine Debris Shoreline Survey Field Guide or items hard to identify.

The highest amount of macroplastic was registered at site 1 followed by site 6, with respectively 26% and 22% of all the plastic items found at all sites (Fig. 16). Sites 1 and 6 also had the highest density of plastic items (Table 6). Plastic particles categorized as "Other" accounted for 42% of all macroplastic found. Ropes, fishing net, buoys, floats, fishing lures and lines accounted for 24% and most were found at sites 1, 4 and 6 (Fig. 16). 12% of the macroplastic was food wrappers and bags, and most of it were found at sites 1 and 6. 82% of the personal care products registered were found at site 1, 2 and 3, which is located at the southeast side of Hovedøya. A large amount of the personal care products were Q-tips.

Table 6 – Plastic debris density among sites. Stated area is equal to 50% of the beach due to sampling method.

	Total number (items)	Total area (m²)	Density (items/m²)
Site 1	157	66	2,38
Site 2	87	126	0,69
Site 3	78	144	0,54
Site 4	106	210	0,50
Site 5	41	24	1,71
Site 6	133	60	2,22
Average:			1,34

Plastic debris density ranged from 0.50 to 2.38 items/m^2 between the sites with an average of 1.34 items/m^2 (Table 6).

3.6 Correlations between plastic particles in blue mussels and beach sediments and with microplastic and macroplastic at a beach

Table 7 Correlation test with rho and p-value results

Correlation test	Unit	rho	p-value
MP per ind. → MP in sediment	MP/ind. → MP/m²	0,60	0,242
MP per gram → MP in sediment	MP/gram → MP/m²	0,31	0,564
MP in sediment → Macroplastic	MP/m² → Items/m²	0,26	0,658
MP per ind. → Macroplastic	MP/ind. → Items/m²	0,31	0,564
MP per gram → Macroplastic	MP/gram → Items/m²	-0,08	0,919

No significant correlation was found between any of the measured parameters. However, the lowest p-value and highest Spearman rho was found between the number of microplastics per individual blue mussel and number of microplastics in sediment (Table 7, Fig. 18).

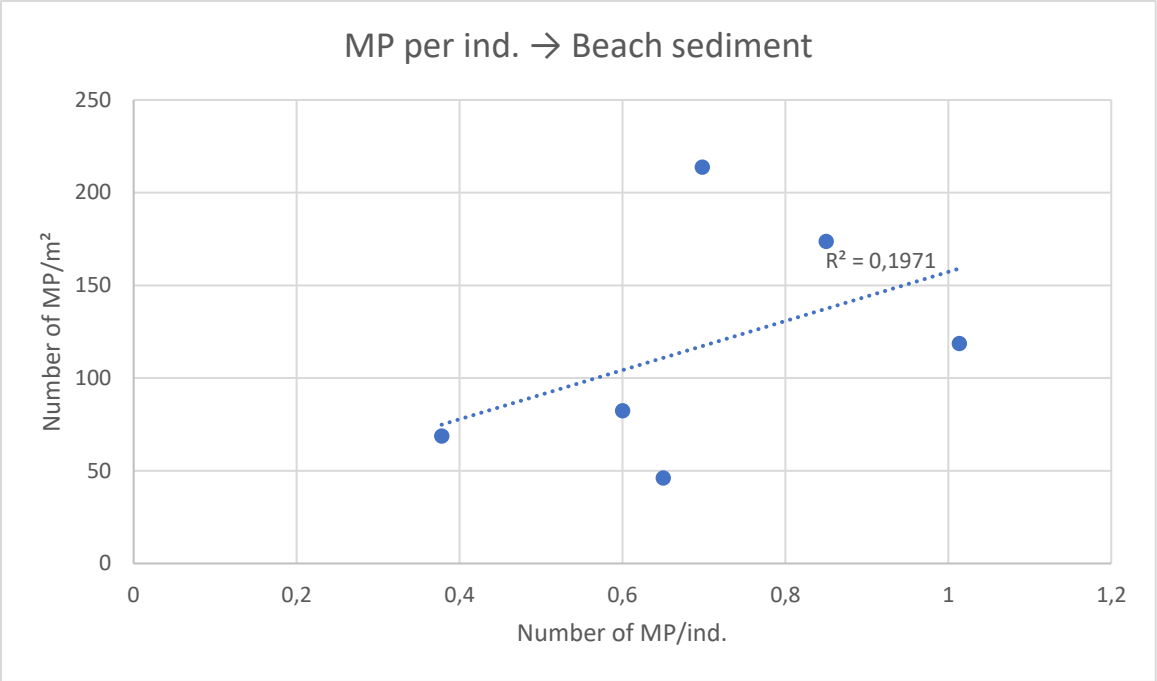

Figure 17 An example of the comparison between number of MP found in individual blue mussels and beach sediments, with R-squared value

4 Discussion

The aim of this study was to investigate the occurrence of microplastics at Hovedøya in the Oslo Fjord. Microplastics were found at every beach, in blue mussels and in beach sediments. 51.7% of the blue mussels analysed had ingested microplastics, with at least one individual containing microplastics from all sites at Hovedøya. The overall average in beach sediments was 117.29 MP/m^2, while the number of macroplastic items were 1.34 items/m^2. The study also investigated if there is a correlation between microplastic in blue mussels and sediments from the same location, and number of microplastics and macroplastic at a beach. No significant correlation was found. However, the lowest p-value and highest Spearman rho was found between the MP per individual blue mussel and MP in sediments.

Hovedøya constitutes with a small fraction of the coastline in the Oslo Fjord. The results carried out in this study, combined with other studies as Bråte et al. (2018), indicates that microplastic pollution is widespread in the Oslo Fjord. There are several islands located in the inner Oslo Fjord, e.g. Lindøya, Gressholmen-Rambergøya and Bleikøya, that are influenced by the same factors and may be contaminated in the same degree as Hovedøya.

4.1 Methodology and sources of error

The width of the beaches was measured from the water's edge to back of shoreline at the widest point, although it varied across the length. At some beaches (e.g. site 3), the widest point is in the middle and decreasing out to the sides. The result is a larger calculated area than the actual total area.

Some aspects of the approach may have affected the data sampling, the results and the accuracy, e.g. visual identification and the chemical classification. Notable issues include:

- Filter papers from sediment samples contained in some instance's large quantities of organic matter after the extraction. This made it harder to separate the organic matter and identify potential microplastics on the filter paper. Consequently, some particles may have been overlooked. Extraction of mussels left some organic matter on the filter papers, but in small amounts compared to some of the filter papers after extraction of sediments. Especially samples from site 1 and 6 contained large amounts of organic material after extraction of sediments.

- Separation of plastic and non-plastic particles is demanding and the accuracy improves with experience, e.g. distinguishing plastic from natural particles based on only visual inspection. However, plastic particles are softer than rock or mineral which can be detected with use of needles and forceps (Buenaventura 2017), as a supplement to visual identification.
- Treatment of KOH or weathering of plastics in the environment can lead to loss of colours in some fibres (Iversen 2018; Lusher et al. 2017a). Transparent or white particles are harder to visually recognise on a white background of the filter paper.
- Some particles were lost during transport. All suspected plastic particles were gathered during visual ID before chemical classification. Some particles were lost inside the petri dishes, most likely changing position when the petri dishes were transported. Some particles were also lost under transport from the filter paper to the diamond compression cell. The particles are small, hard to handle with forceps and difficult to locate if lost.
- The spectra were automatically compared against reference spectra libraries during chemical classification. The match was sometimes bad, resulting in interpretation based on the graph. Potentially wrong interpretation may have affected some of the results.

An organic matter removal step could ease the visual ID of particles after sediment extraction. One possible method is described in (Lusher et al. 2018) with Fenton's solution (30% H_2O_2 with Fe catalyst) where the number of removal attempts depends on the amount of organic matter. However, these steps demand additional time for the study overall, and may not be appropriate if time is an issue. 14% of the particles in blue mussels identified through visual ID were lost before chemical classification, in comparison with 7% in the sediment samples. Increased experience is believed to reduce the number of lost particles. Further testing of the methodology, not least the practical approach to sampling and handling, is recommended to find solutions to reduce the loss of particles.

4.2 Characterisation and comparison with other studies

There is a significant variation in methodology across microplastic studies for both mussels and beach sediments (Besley et al. 2017; Bråte et al. 2018; Hengstmann et al. 2018). Differentiating methods for sampling, extractions, identification, verification and estimating background contamination between this and other studies challenges the comparability with other studies.

The average abundance of microplastics in blue mussels in this study indicates that blue mussels at Hovedøya are not more contaminated than other blue mussels in Norway (Table 8). Bråte et al. (2018) collected blue mussels from different places in Norway, located along the Norwegian coast from the Oslo Fjord in the south to Finnmark in the north. A higher average abundance of microplastic was found in the study, compared to the average found at Hovedøya (Table 8). It should be logical to assume that their results are influenced by a greater variability of factors, due to a larger area investigated with larger variations in environmental conditions.

Overall, this study revealed that blue mussels from Hovedøya contained fewer microplastics than blue mussels in similar studies worldwide (Table 8). It is important to have in mind that the following studies investigate larger areas. Van Cauwenberghe et al. (2015a) found microplastic abundances relatively similar to Hovedøya where three of the sites were located close to coastal harbours where shipping and industrial activity is high. However, Mathalon and Hill (2014) found as much as 34 MP/ind. in Canada which is in comparison with this study, 48 times as much as the blue mussels from Hovedøya. Hovedøya is in similarity to the sampling sites in Canada, surrounded by potential contamination sources. The reasons determining these high differences are unknown and demand more research.

Table 8 A selection of microplastics studies performed on blue mussels worldwide

Sample Area	Average concentration	Comment	Reference
Hovedøya	0,7 MP/ind. and 0,17 MP/g	Range 0-8 MP/ind. and 0-1,99 MP/g	Current study
Norwegian coast	1,5 MP/ind. and 0,97 MP/g	Range 0-6,9 MP/ind. and 0-7,9 MP/g.	(Bråte et al. 2018)
French, Belgian and Dutch North Sea coast	0,2 (±0,3) MP/g	Found from 6 sampling stations along the coast in 2011	(Van Cauwenberghe et al. 2015a)
Belgian coast	0,37 MP/g (SD 0,22)		(De Witte et al. 2014)
China	2,2 MP/g (range 0,9 – 4,6)	2/3 of the coastline of mainland in China investigated	(Li et al. 2016)
United Kingdom	0,7 – 2,9 MP/g or 1,1 – 6,4 items/ind.	8 sites along the coastal waters of U.K investigated. Average not presented	(Li et al. 2018)
Canada	34 MP/ind.		(Mathalon & Hill 2014)

The microplastic abundances in beach sediments at Hovedøya revealed both abundances less and above similar studies worldwide. Hidalgo-Ruz and Thiel (2013) in Chile, Martins and Sobral (2011) in Portugal and Kaberi et al. (2013) in Greece shows quite similar abundance of microplastics in beach sediments (Table 9). Fok and Cheung (2015) states that Hong Kong is a hotspot of marine plastic pollution with a mean abundance almost 50 times higher than at Hovedøya. They found averages higher than international averages where the Pearl River was suggested as a potential source. Lee et al. (2013) found an average of 8205 MP/m^2 in South Korea, which is 70 times higher than Hovedøya. This high abundance is most likely explained by all the Styrofoam floats used to harvest oyster in Korea. Styrofoam was the type of plastic which dominated among the particles and may have originated from aquaculture facilities near the sampling sites.

Table 9 A selection of microplastics studies performed on beach sediments worldwide

Sample Area	**Average concentration**	**Comment**	**Reference**
Hovedøya	117,29 MP/m^2	Range 46,25 - 213,75 MP/m^2	Current study
Chile	27 items/m^2	Ranging from 1 - 805 items/m^2	(Hidalgo-Ruz & Thiel 2013)
Portugal	133,3 items/m^2		(Martins & Sobral 2011)
Greece	Ranging between 57-602 items/m^2 (size 21-2mm) and 10-575 items/m^2 (size 2-4mm)	Average not presented	(Kaberi et al. 2013)
Germany	2862 items/m^2	Sampled from the intertidal zone, high tide line and upon the plateau of the beach	(Hengstmann et al. 2018)
Hong Kong	5595 items/m^2		(Fok & Cheung 2015)
South Korea	8205 items/m^2	Size 1 - 5mm	(Lee et al. 2013)

The mentioned studies in table 9 shows the result in items/m^2 while there are several studies only showing the results in mass of plastic per kg dry weight of sediment. According to Besley et al. (2017), most studies (n = 22) presents the amount of microplastic per weight unit of sediment. To increase the comparability with other studies, new research should be presented as both microplastic per m^2 and per kg dry weight.

A comparative study of the different concentrations (items/m^2) of macroplastic found in other coastal areas worldwide shows that both higher and smaller densities of macroplastics are identified (Table 10). Syakti et al. (2017) shows greater magnitude of macroplastics than at Hovedøya. The study is conducted in Indonesia where the waste manage is relatively poor and a higher abundance of macroplastic than Hovedøya was expected. Burning, burying or throwing directly into the sea are normal ways to reduce visible litter and large quantity of plastic litter accumulates (Syakti et al. 2017). Macroplastics related to fishing were also found.

Table 10 A selection of microplastics studies performed on macroplastics worldwide

Sample Area	Average concentration	Comment	Reference
Hovedøya	1,34 items/m^2		Current study
California	0,083 items/m^2		(Leggett et al. 2014)
India	1,37 items/m^2		(Kumar et al. 2016)
Seychelles, Western Indian Ocean	4,7 items/m^2		(Duhec et al. 2015)
Indonesia	16,8-41,6 items/m^2	Average not reported	(Syakti et al. 2017)

Site 1 had a significantly higher number of microplastic particles in the sediments <1 mm than site 5. Site 5 is the smallest beach analysed in this study and the only beach located at the west side, which may impact the rate of accumulation. Polyester (PET) and cellulose based plastic particles were most abundant. Polyester has a density of 1.38 g/cm^3 and is likely to sink in the water column exposing organisms that are feeding subsurface (Lusher et al. 2017a). Polyester was not found in the beach sediments at site 5, only blue mussels. This could indicate stronger ocean currents at the west side of Hovedøya because no polyester has settled in the sediments. Oil/tar compounds dominates at site 1 and includes particles as e.g. ethylene propylene rubber (EPR), styrene-butadiene rubber (SBR), thermoplastic elastomers (TPE), hydrogenated nitrile butadiene rubber (HNBR), butyl rubber and parking lot tar. Synthetic rubber are mostly made from petroleum (Fang et al. 2001), has a density of around 0.82 – 0.92 g/cm^3 and floats in sea water (Lusher et al. 2017a). The oil/tar compounds found at Hovedøya could potentially originate from discarded tires, pipes, shoes and other products which are produced in rubber processes (Fang et al. 2001). Additionally, SBR from car tires and TPE are both used as small rubber granulates in artificial football turfs (Tandberg & B.Raabe 2017). Oil/tar compounds are only found at sites facing towards the mainland, closest to Oslo and river outlets (site 1,2,5 and 6). It could be speculated that artificial football turfs and degraded car tires have an impact on the distribution of oil/tar compounds found at Hovedøya. Though, further research should be conducted on sources and pathways determining the distribution of microplastics in the inner Oslo Fjord, to understand the difference among site 1 and 5.

It was only found a significant difference between the number of microplastic particles in sediments <1 mm between site 1 and 5. No other significant difference between the sites was found. All the sites are located at a relatively small island and this could be expected. The mean abundance of microplastic at site 6 was higher than the mean abundance both in blue mussels and sediments at Hovedøya, though no significant difference between site 6 and the other sites were found. Similarly high levels in harbours is in accordance with Claessens et al. (2011) and Stolte et al. (2015) who find higher microplastic concentrations in the harbours than other coastal areas. Harbour activities are also mentioned as a potential plastic source (GESAMP 2016; Lusher et al. 2017b). High concentrations could also be related to the geometry of the harbour, rather than harbour or boating activities (Claessens et al. 2011). The geometry of a bay could potentially lead to a low flushing rate, which makes the plastic debris or microplastic accumulate in the bay. Instead of floating back out, it will settle down in in the harbour. All the floating piers in the harbour could potentially contribute to a low flushing rate.

It must be stated that none of the differences among site 4 and other sites at Hovedøya was significant. However, why site 4 comes out with high microplastic amounts in this study can be impacted by several factors, and a few assumptions are made. The combination of fine sand and its close location to a walking track makes site 4 a popular and crowded beach during the summer. It is therefore logical to assume that anthropogenic debris at this beach could be higher than a non-crowded beach. Large quantities of PE were found in the sediments from the beach compared to other sites (Fig 15). Polyethylene (PE) is one of the most common polymers in terms of production (Lusher et al. 2017a). PE is used for plastic bags, bottles, clothing and other general use plastics (GESAMP 2015; Lusher et al. 2017a). Thus, it is not surprising to find high numbers of this polymer on a beach with high use-intensity by humans and in the ocean. On the other hand, low concentration of microplastic was found at site 4. Additionally, polyethylene has low density of 0.92 – 0.96 g/cm^3 (Hidalgo-Ruz et al. 2012) and float in sea water (Auta et al. 2017; Lusher et al. 2017a). It is therefore likely to assume that the plastic found at site 4 has been distributed by oceanic transport over large distances and originate from different sources.

A similarity between site 4 and 6 is the stretch of shallow area from the sea edge towards the sea. Erosion of the shore happens when the energy in waves reaches the shore, and available

sediment is suspended and carried away with the water (Andrade et al. 2019). This may impact the distribution of microplastic in sediments. If the shallow area decreases the potential of beach erosion, less microplastic will move from the beach and out in the sea. However, the sites at Hovedøya are different in terms of orientation and exposure to wind and waves, and more testing is needed to determine the factors contributing to plastic accumulation at these sites.

It was only found one significant difference among the sites at Hovedøya. This indicates that the assumed potential sources may not, or to a minimal degree, impact the distribution of microplastic in the inner Oslo Fjord. It has been challenging to find detailed information about the environmental factors in the inner Oslo Fjord. Generally, ocean currents, density and wind impact the distribution of microplastics (GESAMP 2015). According to Rustad (2010), the hydrodynamic conditions are controlled by temperature, salinity, wind and bathymetry, of which some are variables with hourly, daily and seasonal changes. It is also stated that the conditions in the inner Oslo Fjord differs from other Norwegian fjords, because the supply of fresh water comes from the outer Oslo Fjord and gets transported in. This is in accordance with a model showing ocean currents going north to the inner Oslo Fjord (FjordOs 2018). Additionally, Rustad (2010) states that the supply of water from the waterways with outlets in the inner Oslo Fjord is minimal. It is accordingly logical to assume that the ocean currents will have a larger impact on the accumulation and distribution of microplastic at Hovedøya, than nearby rivers and potential microplastic sources.

82% of the personal care products found during registration of macroplastic was distributed between site 1, 2 and 3. Site 1-3 are located at the southeast side of Hovedøya which is in the direction of the wastewater plant outlet. A large amount of the personal care products registered among the sites were Q-tips, and studies shows that Q-tips are some of the deposit found in or close to the WWTP (Andersson and Holmberg (2006); Diez et al. 2014). It is therefore likely to assume that the Q-tips registered at Hovedøya originate from the WWTP.

4.3 Correlations

Two potential correlations were investigated in this study. Firstly, the correlation between microplastic in blue mussels and sediment from the beach. Secondly, the correlation between microplastic and macroplastic at a beach.

A correlation between microplastics in blue mussels and sediments from the same beach was expected. Blue mussels and beach sediments are located at the same site and should be impacted by some shared variables. However, no significant correlation was found between microplastic in blue mussels and beach sediments from the same beach at Hovedøya. Additionally, no studies indicating a direct correlation have been identified. A reasonable assumption may be a sequential difference in the accumulation of plastics in sediment and blue mussels. Respectively, that microplastic in beach sediments accumulates faster than microplastic in blue mussels. According to Van Cauwenberghe et al. (2015a), a standard blue mussel has a filtration rate of 2 L/h, and microplastic remaining in the blue mussel are usually determined by intake and elimination (Qu et al. 2018). Mainly small microplastic particles are found in blue mussels (Bråte et al. 2018; Li et al. 2018; Qu et al. 2018), while sediments are proposed as the final destination of most microplastics in the environment (Lusher et al. 2017a). This results in a higher accumulation rate for microplastics in beach sediments than in blue mussels and reduces a potential correlation.

Another expected correlation was between micro- and macroplastic at a beach. It seems valid to assume that amounts of macroplastic gives more material to degrade, which results in more microplastics at the same site. Site 6 comes out as the site with the second highest density of macroplastic and includes high amounts of microplastic (118.75 MP/m^2). However, no significant correlation was found between the number of microplastics and macroplastics at a beach in this study. This indicates that microplastic at a beach do not originate from the degradation of macroplastic from the same beach. GESAMP (2015) states that it is unlikely that the abundance of micro- and macroplastic are closely correlated as large and small objects will be influenced by environmental processes to differing degrees. Other factors such as weather, wind, waves among more could have a large impact and distribute the plastics randomly. This is in accordance with Van Cauwenberghe et al. (2015b) who reports that there is a difference in macro- versus microplastic distribution. According to (Lusher et al. 2017a), rural locations had more microplastic than those from urban and industrial locations. This

may seem unreasonable, but it shows how much influence the environmental variables have and that microplastic can be distributed for long distances.

Microplastic per individual blue mussel and microplastic in beach sediments had the lowest p-value and highest Spearman rho, though no significant correlation was found. Both matrices are located close to each other, and microplastics in beach sediments washed up on shore from the surface or water column, are likely to drift close to the blue mussels. High amounts of microplastic washed up on shore, should if a correlation is present also mean high amounts of microplastic in blue mussels from the same site. A correlation between these matrices could mean that beach sediments and mussels get their microplastic partly from the same source. However, it must be stated that no significant correlation was found and only speculations can be made.

5 Conclusion

This study on microplastic pollution confirms that microplastic is abundant in both blue mussels and beach sediments at Hovedøya. 51.7% of the blue mussels analysed had ingested microplastics, with an average of 0.70 microplastics per individual and correspondingly 0.17 microplastics per gram wet weight. The overall average in beach sediments was 117.29 MP/m^2, while the number of macroplastic items were 1.34 items/m^2. It was also found that site 1 had a significant higher number of microplastic particles in the sediments <1 mm than site 5. The reason for this difference is currently not understood, and further research on sources and pathways of microplastic in the inner Oslo Fjord is needed.

This study predominantly revealed a smaller microplastic abundance in blue mussels compared to the abundance reported worldwide. Regarding microplastic in beach sediments and macroplastic, studies indicate greater variations in abundance worldwide with both lower and higher levels. Results from Hovedøya show low abundance in comparison to other studies investigated.

Two potential correlations were investigated in this study. Firstly, the correlation between microplastic in blue mussels and sediment from the beach, where no significant correlation was found. Microplastics are assumed to accumulate faster in sediment than blue mussels and could be the reason determining this result. Secondly, the correlation between the number of microplastic and macroplastic items at the beach, where no significant correlation was found. This could indicate that microplastic at a beach do not originate from the degradation of macroplastic from the same beach, because large and small objects are influenced by environmental processes to differing degrees. Factors such as wind, waves and currents can have a large impact, and might contribute to random distribution of plastics. More research is needed to better understand the distribution of microplastic and the relationship between different matrixes.

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Appendix

Appendix A

Table 3 Fractions of individuals with levels higher than limit of detection (LOD) and limit of quantification (LOQ) from each site., with numbers after correction. Only results with levels > LOD or LOQs are shown and the values >0, are in bold. Results presented after correction. *Sediments > 1mm.

	Site	Frac > LOD	Frac > LOQ
Blue mussels	S1_6	**1,93**	0,00
	S1_7	**0,93**	0,00
	S6_6	**1,20**	0,00
Sediment	S1*	**2,39**	0,00
	S2*	**9,39**	**6,23**
	S3*	**0,39**	0,00
	S4*	**25,39**	**22,23**
	S5*	**2,39**	0,00
	S6*	**3,39**	**0,23**
	1,1	**17,89**	**14,73**
	1,2	**7,89**	**4,73**
	1,3	**1,89**	0,00
	1,4	**1,14**	0,00
	1,5	**1,89**	0,00
	2,4	**0,14**	0,00
	3,1	**4,14**	**0,98**
	3,2	**0,14**	0,00
	3,4	**0,14**	0,00
	3,5	**0,14**	0,00
	4,2	**2,14**	0,00
	4,5	**0,14**	0,00
	5,2	**2,89**	0,00
	6,1	**1,89**	0,00
	6,3	**0,89**	0,00
	6,4	**4,89**	**1,73**
	6,5	**3,89**	**0,73**

Appendix B

Table 4 Number of different particle types found in blue mussels that are not plastic among sites

	Site 1	Site 2	Site 3	Site 4	Site 4	Site 6	Total
Natural (cotton)	44	17	20	17	17	23	138
Cellulose	9	11	5	9	4	7	45
Natural (wool)	5	3	3	0	1	1	13
Fibre hemp rought	1	0	0	0	0	0	1
Tomatine	3	0	0	0	0	0	3
Azlon (casein)	4	0	0	1	3	3	11
Chitin	1	0	2	0	1	0	4
Fibre flax	1	0	0	0	1	1	3
Fur cotton combers	0	4	0	1	0	2	7
Glass	0	1	0	0	0	0	1
Wild boar	1	0	0	0	0	0	1

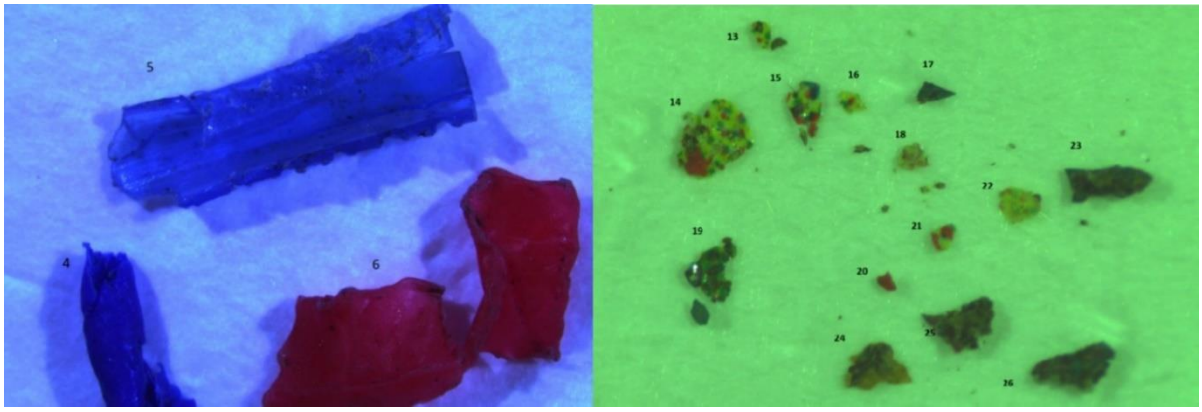
Appendix C

Table 5 Number of different particle types found in beach sediments that are not plastic among sites

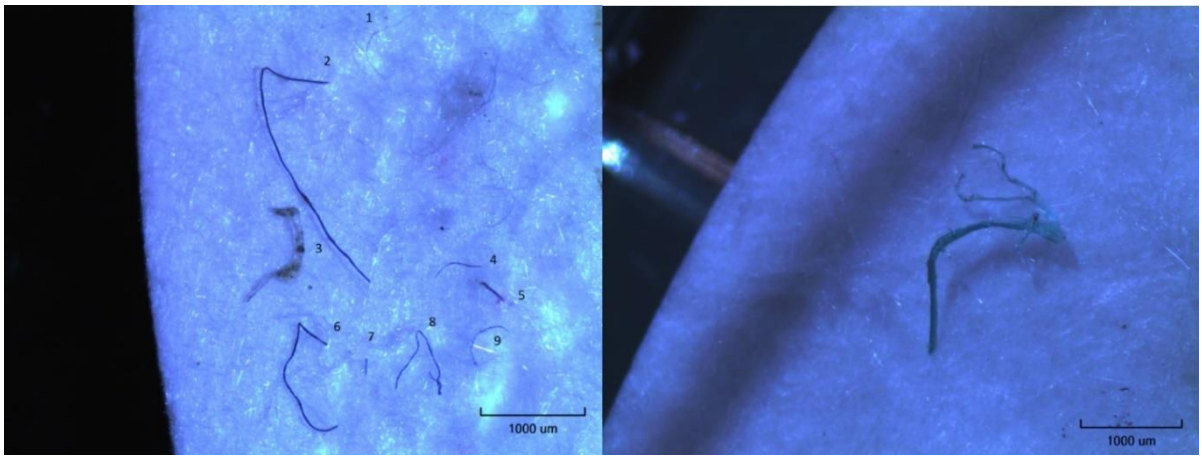
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Total
Natural (cotton)	4	8	32	28	34	11	117
Azlon	7	7	7	8	5	3	37
Natural (wool)	4	2	7	0	5	1	19
Cellulose	2	0	3	3	3	0	11
Glass	0	0	0	0	4	0	4
Fur cotton combers	1	0	0	0	2	0	3
Nisin kbr disc	2	0	0	0	0	0	2
Natural rubber	2	0	0	0	0	0	2
Wood	0	0	0	0	0	2	2
Xantan gum	0	0	0	0	0	1	1

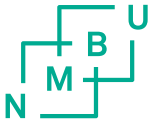
APPENDIX D

Example of microplastic found in sediments. Left: PE and PP from Site 4. Right: Neoprene, SBR and Ethyl-acrylate from Site 1



Example of mmicroplastics found in blue mussels. Left: S1_7 with PET, Rayon and natural (cotton) fibres. Right: S6_14 with PP fibre





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