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Microplastics in Blue Mussels (*Mytilus edulis*): Does mussel size and distribution affect the number of Microplastic particles found in the mussels?

Abul Fazal M Shahriar
Ecology

Preface

This thesis stands as the final part of my Master's degree in Ecology at the Norwegian University of Life Sciences (NMBU) on spring 2020. I want to express my gratitude to Norwegian Institute for Water Research (NIVA, Oslo) for the opportunity to facilitate this research project by providing access to lab facilities, materials and lab-spaces.

Very special thanks to my supervisor, Dr. Susanne Claudia Schneider, for continuous support and guidance all the way. It is important to have fast and constructive feedback, guidance during the writing process and help with statistical analysis.

I want to thank Dr. Amy Lusher and all the rest of "team microplastics" at NIVA, 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. Thanks to Nina Buenaventura for all the help down at the lab. It was a great time with you all.

Norwegian University of Life Sciences Ås, 01.06.2020

Abstract

Microplastic particles present ubiquitously throughout the marine environment. To assess the widespread environmental risk of the microplastic pollution in aquatic environment, a better understanding of the distribution and accumulation is needed. Blue mussels have been used as sentinel species to monitor the microplastic pollution.

Total of 213 blue mussels were collected from three different sites of Oslo fjord. Microplastics-mussels interaction was determined on the basis of the length of mussels. The number of MPs found in individuals were compared among three size groups-5-6 cm, 6-7 cm and 7-8 cm. The effect of mussel size on MP consumptions were studied and compared among the mussels collected from two substrates- water column and sediment.

Microplastic particles were found in all the individuals with an average of 5.09 microplastics per individual. Positive correlation was found between the length and the number of microplastic particles in individuals from two sites. It was also recorded that the samples collected from the sediments had less number of microplastic particles than those from the water column in two sample sites.

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Introduction:

1.1 History, definition and production of plastic

The invention of first plastic material in early twentieth century, paved the way for polymer science and the development of plastic materials as we see them today. In the 1950's mass production of plastic started, and the global production of plastics increased approximately 10-fold from 1950 to 2020 (PlasticEurope 2018). Plastic materials are used in almost every industrial area like packaging, agriculture, automobile, electrical and electronic, building, construction etc. and even in renewable energy sectors (PlasticEurope 2018). These products are still used in daily lives and have a vital role in present market for different purposes (Shashoua 2008). The physio-chemical properties of plastics like- less dense, durability, resistance to degradation, low manufacturing cost and wide spread manufacture make plastic substances more useable and more accessible to all people all over the world. But plastics are now a global environmental threat as all types of plastics are widely present in aquatic ecosystems as debris (Bergman *et al.* 2015; Wagner *et al*. 2018; Zeng 2018).

359 million tons of plastic were produced globally in 2018, which was 348 million tons in the previous year 2017 (PlasticEurope 2018). The most common type of plastics manufactured in Europe were- polypropylene (PP), polyethylene (PE, in different densities), polyvinyl chloride (PVC) etc. (PlasticsEurope 2018). It has been estimated that, 32.5% of plastics were recycled in 2018, 42.6% was used for the recovery of the energy, and 24.9% used for landfills (PlasticsEurope 2018). Due to long degradation time (estimated between hundreds to thousands of years) and improper disposition, these can assemble in the environment (Barnes *et al*. 2009).

Plastic is an extensive family of different material. The term "plastic" cannot be defined universally and as having different definitions. The most common one is- petroleum-based man-made synthetic polymers (UNEP 2015). According to IUPAC (International Union of Pure and Applied Chemistry), it is defined 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). It includes both natural polymers like cellulose and chitin etc. and synthetic polymers like PP and PVC. It also includes some bio-based semi-synthetic materials like rayon which is cellulose based but artificially produced. Fig. 1.1 describes the different sources of the plastic.

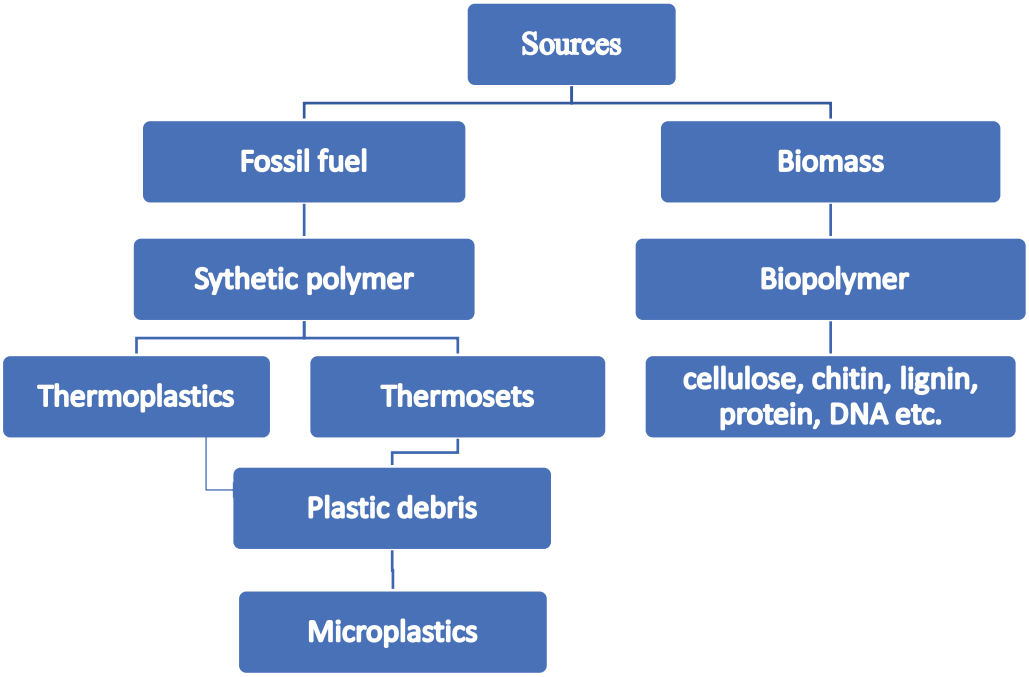


Fig. 1.1 Schematic presentation of plastic source. Adapted from GESAMP, 2015.

Remy *et al.* 2015, Wesch *et al*. 2016a, and Salvador Cesa *et al*. 2017 excluded natural and semi-synthetic polymers from plastic, while Lusher *et al.* 2013; Woodall *et al.* 2014; Neves *et al.* 2015; Li *et al*. 2016 included semi-synthetic polymers. This leads to confusion in reporting of plastics and comparing related results became inconsistent. According to GESAMP (2016), plastic can be divided into three categories- category one is bioplastics like cellulose and chitin and these can be obtained from bio sources; category two is called bio-derived like rayon etc., and these can be obtained from biomass then making derivative from these biomass (may be also called semi-synthetic);category three is called bio-based plastics like bio-polythene, monomers used for this type are obtained from the biomass (GESAMP 2016).

1.2 Plastics to microplastic

In 2004, a renowned marine biologist, Richard Thompson, Professor at University of Plymouth, used the term 'Microplastic' for the first time and he used the term 'microplastic garbage'. Since then this came to an influential attention to the scientific community and its impact on the global environment started to get attention (Cole *et al.* 2011).

Different studies used different ranges to define 'microplastic', but most of them agreed that the plastic particles <5 mm in size, can be termed as microplastics (Arthur *et al*. 2009).

According to GESAMP (2016), microplastics are to be less than 5mm and this was used in this study. The abbreviated form of microplastics- 'MP' would be used hereafter in this study.
Different countries of the world may have different methods of defining and classifying microplastic particles according to their own environmental protocols. The types and shapes of microplastic material are also important as these can determine the properties which affect the environment. MPs are of many different shapes and often used to group into a general category by which sources can be determined (Helm 2017). Between 4 and 7 different types of MPs are grouped on the basis of shapes- fiber, fiber bundle, fragment, sphere (or bead), pellet, film, and foam and these shapes also helps to find the source identification (Roschman *et al.* 2019). For instances, fibers and fiber bundles are originated generally from clothing or even from carpet, spheres from the personal care products etc. But, some researchers (for instance, Sundt *et al.* 2014) grouped fibres and tire fragments under the category of 'MP'.

1.2.1 Route of MPs to the marine environment

Microplastics can be grouped into two categories-primary (produced in smaller size) and secondary (produced from primary by the interaction with UVB, cold haline conditions, high availability of oxygen, and direct exposure of sunlight in the aquatic environment) (Cole *et al.* 2011). Primary MPs are produced in less than 5 mm size on purpose to use in different industries like- cosmetics, industrial scrubbers, further production of plastic products etc. (Talvitie *et al.* 2017). According to the study by Lusher *et al.* (2017b), secondary MPs are divided into two groups-one group originated from use like fibres from clothings, fragments of tires etc. and another group originated from the breakdown of larger plastics like plastic bags, fishing gear etc.
Widespread transport and distribution of MPs to the larger distances are done by currents and are thought to be contributed by the smaller size (< 5 mm) along with low density of the MPs (Eerkes-Medrano *et al.* 2015). The route of MPs to the environment can be observed by studying of fragmentation and degradation of larger plastics (Browne 2015). The degradation and fragmentation are classified on the basis of the cause of the degradation. There can be five different ways of degradation- (i) caused by living organisms-biodegradation, (ii) caused by higher temperature- thermodegradation, (iii) caused by light-photodegradation, (iv) caused by reacting with water-hydrolysis and (v) oxidative reaction with the help of temperature-thermooxidative degradation (Andrady 2011). Terrestrial environment contributes approximately 80% of all marine plastic litters (Andrady 2011). MPs found in the marine

environment are likely to be originated in the beach area for the marine environment (Andrady 2011).

Tourism, fish processing units, and aquaculture in coastal areas, contributes directly as source of plastics to the marine environment and these can be further degraded to MPs and added to marine environment. (Cole *et al*. 2011). The conceivable routes of MPs from terrestrial environment to the marine environment are- rivers, storms and natural disasters, untreated sewage, road run offs, agricultural sludge, wastewater treatment plants (Browne 2015; Dris *et al.* 2016; Duis & Coors 2016; Magnusson *et al.* 2016; Salvador Cesa *et al*. 2017; Schmidt *et al.* 2017).

Macroplastics (>5 mm) which are dumped into the shoreline can be degraded by adverse weather condition and can be another source for MPs and the route for these MPs are through the sea recycling ports and landfills (Auta *et al.* 2017). Sewage sludge transported to the aquatic environment, is another type of possible potential source of MPs as it contains more MPs than the effluent (Leslie *et al.* 2012, Alomar *et al.* 2016).

Another possible explanation for the abundance of MPs is the melting of snow. One of the important source to the road dust particles is melting snow, as large amounts of such MPs are found in snow from the roads (Iversen, 2018). Pathway for MPs to the marine environment can be the rivers and rivers are considered as potentially important source (Claessens *et al.* 2011; Duis & Coors 2016). Rain water run offs from the roads goes into the rivers and possibly another source of MPs in the fjord water.

1.2.2 MPs in the marine environment

The availability of microplastics is a major threat to the coastal and marine environment (UNEP 2016). The demand and usage for plastic products are increasing with time and the amount of the microplastic particles in the ocean is huge and in numbers it is some million metric tons per year (Leberton *et al.* 2017). Abundance and availability of microplastic are tremendous. MPs 92% of all the marine plastic debris is MPs and MP are considered as a major global environmental threat (Eriksen *et al*. 2014).

Improper plastic littering, inappropriate disposition, and obvious adding of plastics to the environment always enormous and contributes to the accumulation of plastics in the marine environment. Approximately 50% of all produced plastics are disposed after just single use which is mainly coming from plastics used for packaging (Mathalon & Hill 2014). In Europe,

more amount of plastics is recycled than plastics used for landfilling in 2018. But, globally the scenario is totally opposite mostly in China (PlasticsEurope 2018). As the plastics are used globally for landfilling which is been done inappropriately, in most of the cases, plastics are freed from the landfill site and becomes floating plastic litter in aquatic and marine environment (Barnes *et al*. 2009). Microplastic particles are found from the surface water of to every water column of the ocean, even in sediments of ocean beds, marine biota, and other different consumables sources (Wesch *et al.* 2016).

Due to different densities, some plastics float others may sink (Andrady 2015). Weathering and biofouling can change the density of the plastic (Galgani *et al*. 2015). Accumulation and distribution of plastics in marine environment are also affected by natural events like wind, water current etc. and by anthropogenic activities like-urban activities, coastal usage etc. (Duis & Coors 2016; Li *et al*. 2016). Water current, water temperature and site location could be another type of factors to consider.

Up to 80 per cent or sometimes more of the marine accumulated waste is plastic (Barnes *et al.* 2009). It has been estimated that, this proportion is in between 60-80% (Derraik 2002). It was also estimated that, all ocean surface has over 0.25 million tons of plastic particles floated (Eriksen *et al.* 2014) and this estimation didn't include all plastics present in the depth of water column and sediments.

1.2.3 Microplastics in marine biota and effect

The microplastic particles has been found in many species from zooplanktons to mammals, affects different trophic levels of over 220 different species which ingest microplastic particles with foods, and it is predicted that within 2050, 99% of all seabird species will ingest microplastic particles with their food (Ter Halle *et al*. 2017). By ingesting microplastics, marine organism may speed up the transference of microplastics in water columns present in the sea, sea bed sediments and even the trophic level through their egestion or excretion (Santana *et al*. 2017). Microplastics may also accelerate the colonization of different microorganisms and invertebrates, helps long-range transportation for alien invasive species, becomes the medium for pathogen transmission, which may increase the pollution risk factors for marine and freshwater organisms and ecosystem (GESAMP 2016).

How the biota interact with microplastics, is not been studied enough but from the laboratory experiments, we may suggest that the exposure to microplastics may have a collection of negative health effects on marine biota. The size and shape play an important role in the

dispersion of MPs in any environment. For example, in marine aquatic systems, denser plastics are more common in sediments than lighter plastics are expected to float. Denser particles are less likely to be picked up by wind and cannot dispersed easily (Rochman *et al*. 2019). Other factors like size and presence of a biofilm may also change the fate of MPs in the respective environment (Oberbeckmann *et al.* 2015). How micro-plastics are transported is dependent on the size of the MPs. MPs can be ingested by all animals- zooplankton to fish and birds in the higher trophic level. Smaller sized MPs (<150mm) may able to leave the alimentary canal gut and may enter into cells (Lusher *et al*. 2017). the lesser the size of the MPs the higher the chances to be in the cells. This can make MPS easier to be bio accumulated and or biomagnificationed in food webs. Sizes of the MPs plays an important role in terms of staying inside the biota, for example fibers tends to retained in the alimentary canal for the longer period of time (Rochman *et al.* 2019).

The toxicity of the MPs on the biota depends on different characteristics of the MPs like- type of the polymer, sizes, shape, and definitely the chemicals it would be interact with (Bråte *et al.* 2018). There is some polymer which are considered as more harmful than other types because of their chemical constituents and or the additives within the polymer (Lithner *et al.* 2011).

Surface area to volume ratio of the MPs is another important are to consider. The larger the ratio, the higher the sorption capacity of the MPs to the other harmful chemicals and this may leas easier ingestion easier for the organisms (Rochman 2015). Lusher *et al.* (2017) suggested that smaller microplastics are real concern for toxicity as their potential ability to transfer between the tissues and cells of organisms (Lusher *et al*. 2017).

Some other studies on oyster, suggested higher immune response, less intake of food, impaired growth rate, reduced wright, less energy release, apoptosis, higher stress level and improper repairing pathways and definite adverse impacts on offspring (Sussarellu *et al*. 2016).

Controlled laboratory experiments demonstrated some adverse effects of MPs on biota. It has been observed, MP affects feeding activity, induce inflammation and reduce the energy reserves in marine worms (Wright et al. 2013). MPs could be transferred between trophic levels (Farrell & Nelson 2013; Setälä *et al.* 2014).

The plastic toxicity to biota is also a concern and several studies been done (Teuten *et al.* 2007; Avio *et al*. 2015; Hermabessiere *et al*. 2017). The toxicity may come from the residual monomers of the plastics, or additives, or the intermediates formed during the degradation process, or absorbance ability of the plastics towards POP already present in water (Andrady

2011). Plastics can also accumulate metals present in sea water, which is another type of threat to organism that can ingest it (Ashton *et al*. 2010).

Biota always potentially interact with microplastic particles in the aquatic environment. More than 230 different marine species from all trophic levels take up microplastics, and the exposure and interaction may occur in different ways (Lusher *et al*. 2017a). Being smaller in size and the ability to be present in both presence both pelagic and benthic ecosystems, allow them to be easily available for ingestion (Auta *et al*. 2017). Some studies show that zooplankton, bivalves, mussels, fish, shrimps, oysters, lugworms and whales ingest microplastic (Auta *et al.* 2017; GESAMP 2015; Van Cauwenberghe *et al*. 2015a). Species like mussels, fish etc. are being commercially harvested and make microplastics a potential part of the human food. It has been reported that the storm sewers, wind and even the current can bring MPs into the aquatic environment (Zalasiewicz *et al.* 2016, Murphy *et al.* 2016). Runoff can transport out some MPs to the marine environment through runoff (Cole *et al.* 2011).

1.2.4 Distribution of MPs

Distribution of the MPs is affected by wind flow which helps in the redistribution of MPs in the layers of the water column (Collignon *et al*. 2012). The distribution may also determine by different oceanographic factors which has been observed in the Mediterranean (Lusher *et al.* 2013). MPs (including beads and pellets) are reported in different sedimentary habitats in European Seas and for instances, MPs were found in the sediment samples collected from the Norderney, North Sea (Dekiff *et al*. 2014; Fries *et al*. 2013).

Higher abundance of MPs was reported in the areas with low hydrodynamics such as samples collected from the lagoon in Venice (Vianello *et al*. 2013). Higher concentrations of MPs were identified in sediments from Belgian harbors and it was due to the reduced water movement in the harbor area (Claessens *et al.* 2011). MPs were even found in deep offshore sediments (Van Cauwenberghe *et al.* 2013; Fischer *et al*. 2015) and indicated that the deep seafloor can act like sink for MPs. Deep seafloor was found as a major sink for MPs by some other researches like Woodall *et al.* 2014 (Woodall *et al*. 2014).

1.3 Using Blue Mussels

Biomonitoring has been used to find the level of impact of microplastics on ecosystem and in the organisms involved in that ecosystem (Wesch *et al*. 2016). An ideal bioindicator must- be widely distributed, have adequate knowledge about all biological systems, can predict pollution alert pretty much early, have a specific function in the ecosystem, produce proper response to the specific concentration of the pollutants and the degree of pollution, and have the ability to detect the toxic effects of specific pollutant (Goodsell *et al.* 2009). Seabirds and sea turtles are used as bioindicators to monitor the plastic debris which are of less than 1 mm with their ingested foods to find out the for the interaction between the land and the sea. As an example, under OSPAR convention, in Northern Europe, a bird species, fulmar (*Fulmarus glacialis*) is used as bioindicator, and to detect the plastic pollution, the digestive contents of this bird species are used (Van Franeker *et al*. 2011).

Blue mussels fulfilled all conditions to be used as ideal bioindicator. Firstly, they are globally distributed, they are tolerant with all environmental parameters like- oxygen, salinity, temperature food availability (Bayne, 1976; O'Conor, 1998). Secondly, in laboratory condition, they can accumulate chemical pollutants and provide the concentration and bioavailability of the pollutants (Beyer *et al.* 2017). Third, Mussels can be used as food and habitat for other species. Fourth, Mussels also transport route of pollutants to the higher trophic level in the marine food chain (Strand and Jacobsen 2005). Fifth, as food, mussels have been serving humans for thousands of years (Beyer *et al.* 2017). So, mussels are getting concerned in regard to assess human health risks due to marine pollution (UNEP, 2016). And lastly, mussels are commonly used in many environmental monitoring programs, like- US musses watch project, MEDPOL, OSPAR (Beyer *et al.* 2017).

Mussels have seven subspecies, they can interbreed and form different subspecies which can be widely distributed around the world (Beyer *et al*. 2017). Different species of mussels have different genetics and different genetic expressions for which they have different ways to deal with microplastic materials as stressor (Wright *et al.* 2013). In this investigation we use blue mussels (*Mytilus edulis)* which is commonly used in laboratory investigations to detect microplastic particles in ingested form.

Blue mussels (*Mytilus edulis*) have been used to investigate the presence of MPs and effects of it on the organism (e.g. Farrell & Nelson 2013; Vandermeersch *et al.* 2015). Blue mussels are also commonly used for observing the toxicity and route of MPs (Li *et al*. 2016). It has been observed that after few hours of exposure, blue mussels showed inflammatory response and

particles were also taken up by the cells within that time (Von Moos *et al*. 2012). In another study, MPs were translocated in their circulatory system in 72 hours and then stayed there for more than 48 days but no significant change in their overall fitness (Browne *et al*. 2008).

1.4 MPs in the Norwegian marine environment

In 2014, MPs on surface water was first reported in the marine environment of Nordics between Tromsø and Svalbard (70 – 78 °N) in 2014 (Lusher *et al.* 2015). It has been estimated that, annually 8000 tonnes (approximately) of primary MPs are added to the Norwegian environment and the biggest source of secondary microplastics in Norwegian environment is the torn tires and road marking which is estimated approximately 5 000 tonnes annually (Sundt *et al.* 2014). To minimize these MP pollution different steps were suggested in a report which was published in 2016 (Sundt *et al.* 2016). Another report indicated that, one of the important source of MPs in Norway was Waste Water Treatment Plants; and in that report, the routes of MPS to the ocean from WWTP were demonstrated (Magnusson *et al.* 2016).

In 2015, a major investigation on the presence of MPs in the Norwegian marine environment was done by Lusher *et al.* (2015). They collected samples from Svalbard rom both surface and subsurface water and reported that more than 90% samples were found with MPs. This research is first one to detect the MPs in Norwegian marine waters. This research also suggested that, even though the sources of MPs were not confirmed, but they are originated from the degradation of the larger plastics and can be transported a long distance (Lusher *et al.* 2015). In another investigation, 81% of Northern Fulmars (*Fulmaris glacialis*) from Norwegian waters were detected to have plastics in their stomach; highest number of plastics recorded was 106 in one individual (Herzke *et al.* 2016). MPs were reported in the fishes of Norwegian coast. In the stomach of Atlantic cod demonstrated MPs were present (Bråte *et al.* 2016). Blue mussels have been used a bioindicator species to detect MPs in several studies in Norway. But, status of the numbers of MPs with the increased size of mussels were not done at the time of this work was initiated.

1.5 Potential sources of MPs in Oslo fjord (study area)

Study areas are situated in the Oslo fjords which is surrounded by urban environment. The areas are close to the different potential sources- both land and sea based sources of MPs with anthropogenic impacts. Among the important sources of MPs to the fjord or to the ocean, there

are-Rivers, wastewater treatment plants (WWTP), shipping industry and harbours (GESAMP 2016; Jambeck et al. 2015; Lusher *et al.* 2017b).

Rivers which flow through residential and industry areas, offices, parks and roads are main contributors to the MPs into the fjord. The water of the river Alnaelva, which flows into the fjord, contains different types of MPs (Bottolfsen 2016). Another river, Akerselva, also run off MPs with its water (Buenaventura 2017).

The outflow of the Wastewater treatment plants (WWTP) are frequent source of MPs to the marine aquatic environment (Carr *et al*. 2016; Sun *et al*. 2019). In case of heavy rainfall, overflows are occurred in WWTPs are exceeded their capacity. Untreated or moderately treated runoffs are also an important source of MPs to the aquatic environment (Magnusson *et al.* 2016). Few large WWTPs of Oslo are connected to the fjord water- Bekkelaget, Ormsund and VEAS. Almost 36 million MPs of ≥ 20 µm and 0.35 million of MPs with ≥ 300µm are released with runoff water from VEAS per hour with a retention of 97 – 99% of all particles (Magnusson & Norén, 2014) and as the rest of the smaller MPs cannot be retained by the WWTPs, those directly pass into the fjords or into oceans (Lusher *et al*. 2017b).

The study area has lot of water traffics- ferries and boats. According to the GESAMP 2016, the shipping industry is also considered an important source of MPs into the aquatic environment (GESAMP 2016). Boat maintenance and additives used in boats, different anthropogenic activities in harbor and recreational activities are also an important source of MPs which can be considered to be the reasons of higher MPs in sediments of the fjord. 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. Tourist littering can also be another source of MPs (Syakti *et al*. 2017).

In the aquatic environment, MPs are distributed at among the beaches, water surface, water column and in the biota (Lusher *et al.* 2017a). Blue mussels (*Mytilus edulis*) collected from the water column and from the sediments of the Oslo fjord are used to investigate presence and abundance of MPs in the biota positioned in water column and sediments. MPs are found both in blue mussels (*Mytilus edulis)* and the samples from sediments (Besley *et al.* 2017; Hengstmann *et al.* 2018; Lusher *et al*. 2017a; Van Cauwenberghe *et al*. 2015a). Sediments are considered as 'final destination' of MPs. Blue mussels are also considered as the 'sentinel'

species to study the abundance of MPs in the aquatic environment (Lusher *et al.* 2017a; Mathiesen *et al.* 2017). The habitat of the blue mussels is both in water column and in the sediment (Lusher *et al.* 2017a). Being a bivalve filter feeder, they also fed from both the positions and so it should provide a better scenario of the abundance of MPs in Oslo fjord.

This study investigated whether or not a positive correlation between the size of blue mussels and the number of MPs found in them. Larger sized mussels are ingesting for food compared to the smaller sized mussels. Larger mussels are considered to contain more MPs compared than smaller the sized mussels (Bråte *et al.* 2018). It has also been reported that, larger sized mussels are more efficient to ingest and egest the MPs compared to the smaller ones (Bråte *et al*. 2018 and Catarino *et al*. 2018). Van Cauwenberghe *et al*. (2015) reported that mussels of 4 cm can efficiently ingest and remove MPs compared to smaller ones. The ingestion of MPs by blue mussels depends on- size, shape and density of the MPs and that implies where would be the position of MPs-whether in the water column or in the sediments (Van Cauwenberghe *et al.* 2015). Generally, MPs with low densities would float in the water column and MPs with high-density has a tendency to sink and accumulate in the sediment, which allow them to be available for the filter feeders like blue mussels (Browne *et al.* 2011).

Are there more MPs in blue mussels collected from the water column or from the sediment? Finding from this investigation would allow us to determine that which size of the mussels would be eaten with caution along with from which position of the habitat they were collected from. This type of investigation on the correlation between size of mussels and number of MPs would be a monitoring parameter which can help to mitigate the MP pollution and this type of analyses is needed to have better understanding on the occurrence and distribution of MPs in Oslo fjord.

1.6 Aims of the study

The aim of this study was to contribute with empirical data on MP occurrence in Norwegian marine biota, by using blue mussels as indicator species. Larger mussels filter a larger amount of water, so we expected that, larger mussels would contain more microplastic materials. MPs with low densities are expected to float in the water column while MPs with high-densities have a tendency to sink and accumulate in the sediment. It is, therefore expected that mussels collected from the water column and the sediment would contain different numbers of MPs.
The aims of the study therefore where:

1) to quantify the occurrence of microplastics in blue mussels from three sites in the Oslo fjord

2) To investigate if the mussel size affects the numbers of microplastic particles found per individual

3) To investigate if the amount of microplastics in mussels collected from the water column differs from mussels collected from the sediment.

From Oslo fjord, total 215 samples were collected from two different positions- water column and sediments. Three size groups were selected to investigate with-5-6 cm, 6-7 cm and 7-8 cm.

Microplastics -mussels interaction was addressed on the basis of the length of the mussels in this study. The number of MPs found in samples among three size groups were compared. As long as it was not known that the effect of mussel size on microplastic consumptions, the comparison among the different sized mussels collected from different sites cannot be said as site-wise different. Mussels of different sizes were collected, and analysed for the number of MPs in each individual. Then number of MPs found in the large mussels were compared with number of MPs found smaller mussels and tried to find if there are differences in the numbers of microplastic ingestion in the mussels.
Blue mussels are distributed among different spatial zones of the aquatic environment. And the concentration of the microplastics are varied among the mussels collected from different zones of the aquatic environment.

2. Materials and methods:

2.1 Description of sampling area - Oslo fjord

The Oslofjord is a fjord that extends from the Skagerrak in a roughly northerly direction to Oslo. Outer Oslofjord goes from the Færder lighthouse in the south to Hurumlandet, where the fjord divides into the Drammensfjord and the inner Oslofjord.The outer part is 10-20 kilometers wide, and south of the Fulehuk lighthouse outside Nøtterøy it is almost an ocean piece. Inner Oslofjord, within the one-kilometer wide strait at Drøbak, the fjord is only three to five kilometers wide. The fjord's length from Færder to Oslo is approx. 100 km. From Færder to the innermost part of the Bunnefjord, its innermost southward arm, approx. 120 km. From Fulehuk lighthouse to the bottom of the Bunnefjord the length is 100 km. The country's two most water-rich watercourses, Glomma and Drammenselva (Drammensvassdraget), culminate in the outer Oslofjord. The rivers with estuary in the inner part of the fjord are comparatively

shorter and with correspondingly smaller water flow. (Available at: https://snl.no/Indre_Oslofjord Store norske accessed: 10.02.2020).

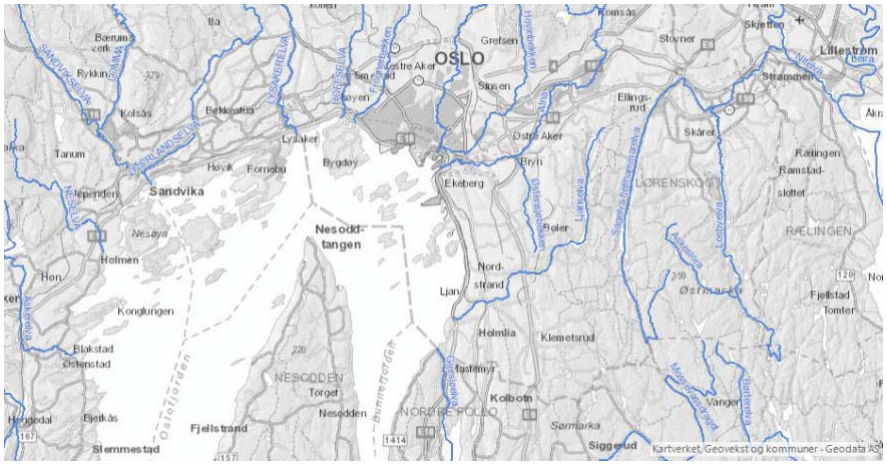


Fig. 2.1 Map of Oslo fjord (inner), with different rivers discharging into fjord drawn in blue. (Map modified from NVE Atlas 3.0).

2.2 Sampling sites and collection of blue mussels

There were three sample site and sites were in the Oslo fjord, and the samples from these areas were collected both from sediments and water column. Three sites on Oslo fjord were selected for sample collection. Blue mussels (*Mytilus edulis*) from these sites were collected and then analysed. Samples were collected from these sites in August 2019 as a part of one of the NIVA's long-term microplastic monitoring program. NIVA is using blue mussels as an indicator of environmental pollutants and the sampling sites were chosen on the basis of basic focus on high probable chances of being polluted. As the distribution of the MPs in Oslo fjord environment is not fully understood, the selection of sites was with lot of uncertainties about different human influences on the environment.

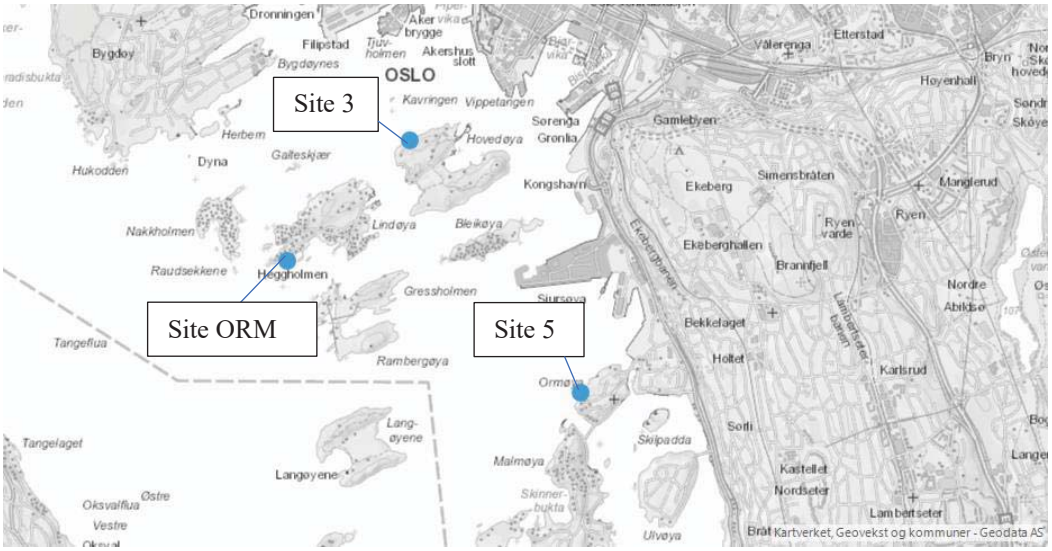


Fig. 2.2 GPS location of three sample sites.

There are many different methodologies tested, developed and modified accordingly to perform proper monitoring of the presence and abundance of microplastics in aquatic environment. Blue mussels are sampled, chemically digested, filtered the dissolved tissue material, then identifying the MPs through visual identification.

Fig. 2.3 Flow chart of the method.

Sampling was doing according to the NIVA procedure which was developed accordance to the method developed by Lusher *et al.* 2017b. Total 213 individuals were collected from all designated sites from two positions at each of the three sites– water column and sediments. Blue mussels do not grow in water columns. The samples from the water column were collected from the lining of the permanent anchors of the jetties, bottom of the pier stations and from the lower surface of the landing ports and the samples from the sediments were collected by trawling, nets, and hand collection from shore (Lusher *et al.* 2017b).

From each sample sites and from each location, 10-18 samples were collected. Sampling technique was dependent on the site location, the substrate they grew on and the position of the mussels. Mussels which were close to the shoreline were collected by hand and mussels which were submerged under water were collected by snorkeling.

Just after the collection, at the sampling spot, sizes were measured and the collected in a big bucket. Mussels were finally selected on two basic criterions- not visibly damaged and closure of the shells. Selected mussels were rinsed and then rinsed with field water 3-4 times. The samples were sorted according to three different sized groups-50-59 mm, 60-69 mm and 70-79 mm for the analysis. Mussels were then measured with plastic calipers, roughly and collected into plastic bags which were marked with sample size, position of the samples collected and the site marked on them. Each group of samples was then sorted and put in plastics bags and then into a cooling box to prevent clumping. After collection of samples, boxes were transported to NIVA for further freezing at -20°C. Table 1 represents the sites, positions and numbers of the mussels collected.

Table 1

Site	year	Location (GPS)	No. of mussels	Position (water column /sediment)	How it was collected
ORM	Aug, 2019	Lat: 59.88708$_0$ N Lon:10.70553$_0$ E	82	both	Snorkeling and hand
Site-3	Aug 2019	Lat: 59.89773$_0$ N Lon:10.72593$_0$ E	68	Both	Snorkeling and hand
Site-5	Aug 2019	Lat: 59.87673$_0$ N Lon:10.75627$_0$ E	63	Both	Snorkeling and hand

2.3. Dissection

Selected individuals were first taken out of the freezer and allowed those to be defrosted. Then again, the length of the individuals was measured with a slide caliper. After defrost, soft tissue was collected from the shells by opening the valves with scalpel and forceps. Except for the tough 'foot' muscles, all living tissues were extracted as the tough muscles are not easy to be digested in the chemical and there is a less possibility for present microplastic particles there. These muscles are not even part of their digestive system or the part of the filtering system.

After each collection, forceps and scalpel were rinsed with distilled water to avoid contamination. Soft tissues of mussels were collected in 100ml pre-cleaned glass beaker which were rinsed 3 times with distilled water and then were covered with aluminum foil. Then the mass of the muscles was weighed which was basically the wet weight. On top of the cover foil, each beaker with soft tissues were marked properly.

2.4 preparing and adding KOH

Filtered 10% KOH (w/v) solution was then added to each beaker with the soft tissue. 10% KOH is ranged between 1.5-1.8 molar, with pH of 14 and was prepared at the temperature of 20_0 C (Dehaut *et al.* 2016). The volume of the KOH in each beaker is dependent on the mass of each mussel's muscles. KOH has lower risks to health and safety issues; cost efficient and enables a high throughput of samples (Bråte *et al.* 2018). Amount of KOH is ten times than the soft tissue weight, for example- for 3-4 gm weight of soft tissues, 40 ml of KOH was added; for 4-5 gm weight of soft tissues, 50 ml of KOH was added; for 5-6 gm weight of soft tissues, 60 ml of KOH was added and so on.

10% KOH is used for digestion as it is based on the research article by Dehaut *et al.* 2016. It was ensured that all the tissues were submerged under KOH solution and to minimize the evaporation foil cover was always present. As control, four clean 100-ml beakers containing only 10% KOH (60 ml) were prepared.

2.5 Incubation

The weight of the sampled mussels was varied. So, bigger sized beaker was used when the weight of the mussels were > 6 grams so that there was no spill inside the incubator. For each batch to be incubated, four beakers with only 50 ml of 10% KOH were used as negative control. The beakers were then left in incubator (New Brunswick ^{TM} Innova® 44).) This setting was guided by the standard NIVA procedure which was based on Dehaut *et al.* (2016).

2.6 Filtration

Each sample in the beaker was expected to be digested after 24 hours (Dehaut *et al.* 2016). To isolate the MP particles for analysis, the samples were filtered. A vacuum filter was used and the samples were passed through 47 mm Whatman® GF fibre filter papers. Before use, each filter paper was examined under microscope for any other particles. Filter funnel was rinsed with distilled water 3 times to make sure that any adhering particles were washed away onto the filter paper. Filter paper was also rinsed with a further spray of filtered distilled water to wash through any residual on the side of the funnel. A glass dish was used to keep the top of the filter funnel covered as much as possible to avoid contamination. The filter paper was

immediately put into a petri dish with lid on it and left for drying and further analysis. Filter funnel was thoroughly rinsed with filtered distilled water for 3-4 times at the time of each filtration to prevent cross contamination.

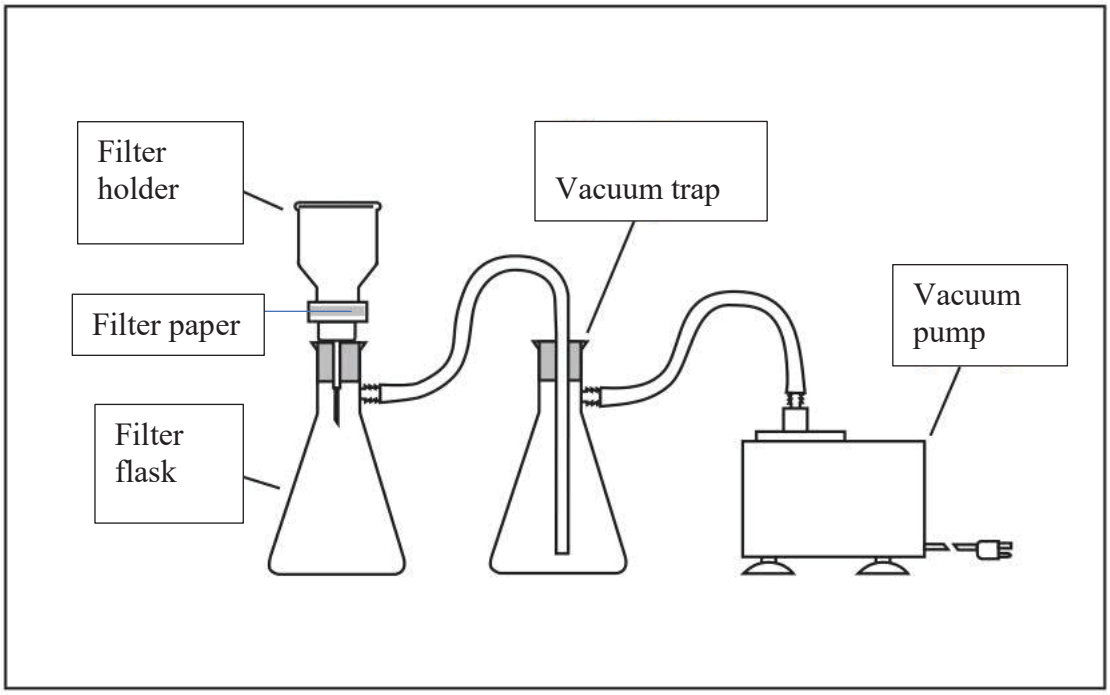


Fig. 2.5 Schematic diagram of vacuum filtration with a pump.

2.7 Visual identification using microscope

Each filter paper was put under a stereo microscope (Nikon SMZ745T). Filter paper was kept inside the petri dish while identifying MPs, and the lid of the petri dish was closed at the time of identification so that the chances of contamination was minimized. Boxes that designated sections of the petri dish were drawn on the lid top of each petri dish using a fine marker. The boxes helped to isolate areas of the filter paper for easier visual analysis. Any suspected MPs were circled on the filter with a soft pencil and then counted.

The morphological characteristics (shape and size) of each particle were recorded. The shape was considered as either fibre, pellet and other common shape like filaments, beads. At times, the petri dish lid was opened to verify the presence of MPs, if there was visual uncertainty. At the time of markings, it was also done. For each time of opening the lid a control was used which was exposed simultaneously. It was done as the precaution of any airborne MPs.

2.8 Measures to minimize contamination

MPs are everywhere and these can spread very easily through air, water. To minimize the contamination, several measures were taken in each step of the procedure.

During the processing of the soft tissue material, separated laboratory space was used. The lab coat was always worn and it was rubbed against the lint roller with sticky paper to get rid of any fibre. Laboratory was regularly dusted before the procedure. Door was always closed during the procedure to minimize airborne fibres. Gloves were worn all the time frequently washed to avoid contamination. During thawing, the samples were covered with aluminum foil and they remained like that till filtration.

At the time of dissection, after each dissection, scalpel and forceps were rinsed with distilled water. Glassware were used for measuring the soft tissue and incubation. Before use, glassware was thoroughly rinsed with filtered (0.22 μm) RO (Reverse Osmosised)- water. 10% KOH solution was filtered before use. Beaker lid was covered with aluminum foil till the soft tissue measurement and it was covered again immediately. After pouring KOH solution the lid was again back to minimize the airborne contamination.

To minimize contamination during filtration, before filtration, each filter paper was examined under microscope for the presence of any particles. After filtration, each filter paper was kept in a petri dish with lid on and the petri dish was marked properly with specific sample ID number. Marking was made at the periphery of the petri dish so for uninterrupted visual inspection. The filter papers were kept in petri dishes with lids, where the ID markings were made on the side (circumference) of the dish so that the lid could be kept on during visual inspection.

Lids of the petri dish were only open when it was required to minimize the contamination. Every time the lids opened, a blank control was used as per the same time duration for any airborne microplastic contamination. Usually the number of particles found in the blank control is subtracted from the number of particles found in the sample. For example, if four fibres were found in the sample, and at the time of identification, if one fibre was found in the blank control, the number of fibres in sample was recorded as three.

2.9 Data Analysis

In this study, data analysis was performed on the length, weight and no of MPs in the blue mussel's individuals separately. To increase the comparability with other studies of similar trends, having standard reporting units is important (Lusher *et al*. 2017a). In this study, results are presented as number of MPs individual^{-1} to increase the comparability with other studies. Weight was used as another indicator for the size which is similar to normal procedure to monitor environmental contaminants using blue. mussels (Bråte *et al.* 2018).

Data handling, making charts and graphs, and statistical analysis were performed in Microsoft Excel along with SPSS. To identify the normal distribution of data, Shapiro-Wilk test was performed (see Appendix). As most of the data were not in normal distribution, Kruskal-Wallis test was performed to find the statistical differences in the dataset. To find the correlation between the data, bivariate correlation analysis was done with spearman's correlation coefficient. Dunn's procedure was conducted for multiple comparisons with post-hoc where significant differences were observed. Spearman's correlation coefficient was used to investigate potential correlations. The significance level for correlation analyses was set to 95% ($p< 0.05$). Mann-Whitney-U test ($p<0.05$) was performed to find out whether the MPs collected from water column and sediments were having the same distribution.

3. Result

MPs found in the blue mussels are resulted and discussed site wise. The finding of the visual identification is corrected for the blank and there was no MPs were recorded in the blanks. The results are discussed based site wise, on MPs individual^{-1}, relation between increased number of MPs with larger size, relation between length and weight and samples from which position (either from water column or from sediment) has more MPs individual^{-1}. As the samples collected from three different sites from two different positions and are grouped into three class sizes from, the correlation between each size class with their weight and the number of MPs found in each individual would be described. Three size classes- 51-60 mm, 61-70 mm and 71-80 mm would be annotated as size class A, B and C respectively.

3.1 Site ORM:

3.1.1 Samples from water column.

3.1.1.1 Length and the weight of the blue mussels:

The mean length of the groups A, B and C is 55.45 mm (± 2.70), 66.05 (± 2.23) mm and 75.31 mm (±2.83) mm and mean weight 5.44g (±1.50), 8.65g (±1.31) and 7.60 g (±1.32) respectively. Fig. 3.1 describes the correlation between the length of the samples from water column of the site-ORM.

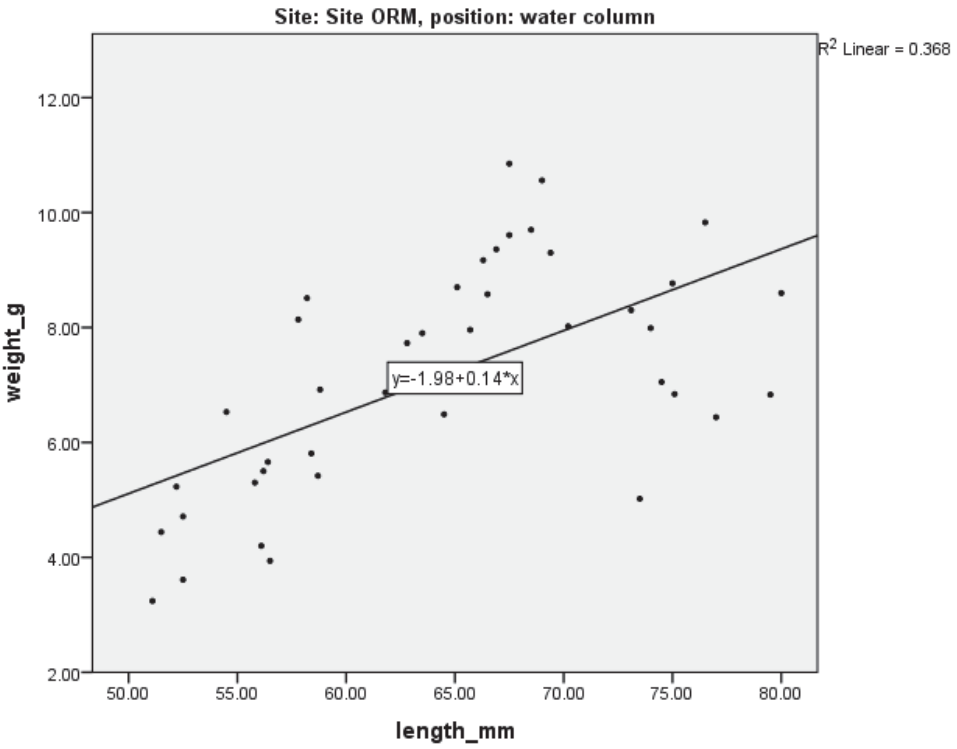


Fig. 3.1 Relation between the length and weight of blue mussels (*Mytilus edulis*).

There was a significant correlation between the length and the weight of mussel (Spearman's rho 0.618, $p<0.05$). Kruskal-Wallis test showed the distribution of weight is significantly different across the size groups. Dunn's test showed the significant difference between the groups A and B and between A and C.

3.1.1.2 MPs in sampled blue mussels:

MP was found present in each of the individual at this site. Total 209 MPs was identified in 42 samples through the visual identification, with the highest number of MPs was 20 in a single individual and lowest was 1 which were found in Size class C and size class A respectively. With the least number of samples in size class C, has the highest number of MPs per individual- 9.18 (±4.16) was also found that the number of MPs is increasing with the size of the blue mussels.

The samples from this site and position, the mean number of MPs per individual was 4.97 (±3.74). During the identification, MPs were grouped into three-fibres, pellets and other MPs. In total, 46 fibres were found which was 22% of the total number of MPs, and 46 black colored pellets were present with 22% of the total number of MPs (Fig. 3.2).

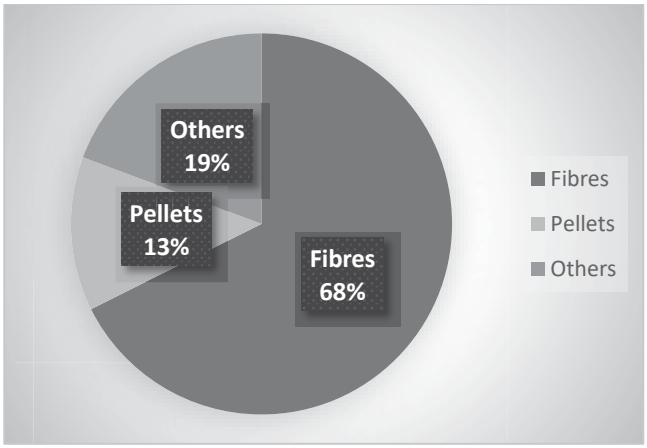


Fig. 3.2 Distribution of different types of MPs in the samples.

3.1.1.3 Length and MPs of the blue mussels:

The mean length of the groups A, B and C is 55.45 mm (± 2.70), 66.05 (± 2.23) mm and 75.31 mm (±2.83) mm and mean number of MPs is 2.75(±1.94), 4.26 (±2.12) and 9.18 (±4.16) per individuals in size groups A, B and C respectively (Fig. 3.3).

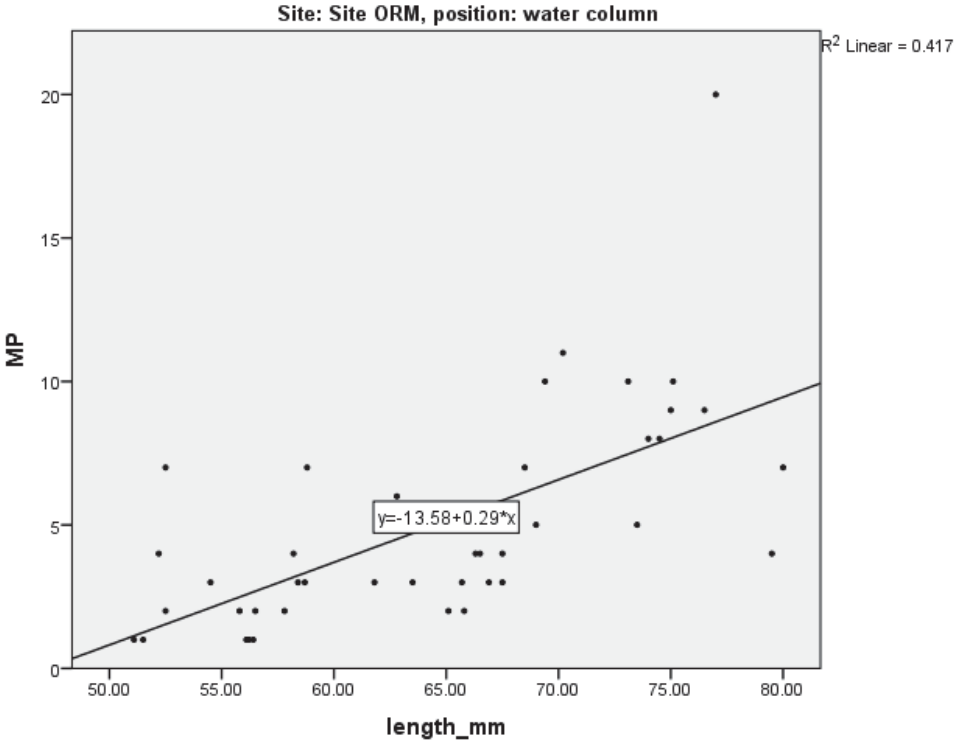


Fig.3.3 Relation between the length and number of MPs in blue mussels (*Mytilus edulis*).

There was a significant correlation between the length and the number of MPs mussel (Spearman's rho 0.732, $p<0.05$). Kruskal-Wallis test showed the distribution of MPs is

significantly different across the size groups. Dunn's test showed the significant difference between the groups A and C and between B and C.

3.1.1.4 Weight and the no of MPs in the blue mussels:

The mean weight was 5.44 g (±1.50), 8.65 g (±1.31) and 7.60 g (±1.32) in size class of A, B and C respectively with the MPs is 2.75(±1.95), 4.26 (±2.15) and 9.18 (±4.16) respectively (Fig. 3.4).

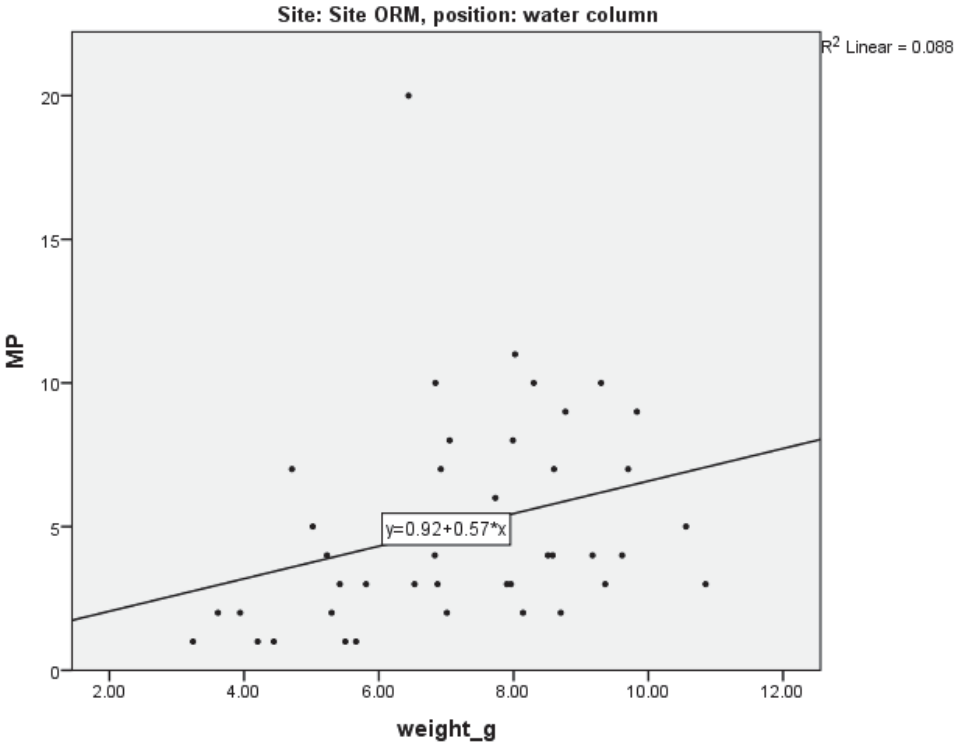


Fig.3.4 Relation between the length and number of MPs in blue mussels (*Mytilus edulis*).

There was a significant correlation between the weight and the number of MPs mussel (Spearman's rho 0.437, $p<0.05$). Kruskal-Wallis test showed the distribution of MPs is not significantly different across the size groups.

3.1.2 Samples from sediment.

3.1.2.1 Length and the weight of the blue mussels:

The mean lengths of the groups A, B and C is 54.95 mm (± 2.03), 64.05 (± 2.01) mm and 73.88 mm (±3.20) and mean weight 5.25 g (±1.50), 7.68 g (±1.26) and 7.74 g (±1.82) respectively. Fig. 3.5 describes the correlation between the length of the samples from sediment of the site-ORM.

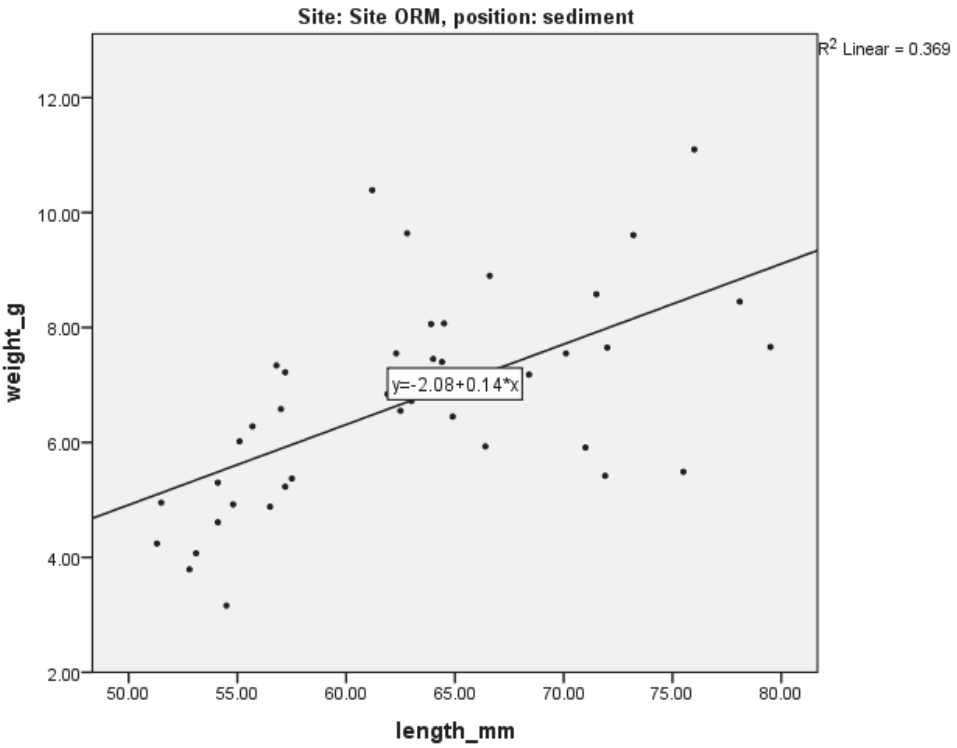


Fig. 3.5 Relation between the length and weight of blue mussels (*Mytilus edulis*).

There was a significant correlation between the length and the weight of MPs mussel (Spearman's rho 0.673, $p<0.05$). Kruskal-Wallis test showed the distribution of weight is significantly different across the size groups. Dunn's test showed the significant difference between the groups A and B and between A and C.

3.1.2.2 MPs in sampled blue mussels:

MPs were found present in each of the individual at this site. Total 206 MPs were identified in 40 samples through the visual identification, with the highest number of MPs was 12 in a single individual and lowest was 1 which were found in size group C and A respectively. With the least number of samples in size class C, has the highest number of MPs per individual-7.8 (±2.85) and was also found that the number of MPs is increasing with the size of the blue mussels.

The total number of MPs across the size groups was 206 with a mean number of MPs per individual was 5.15(±2.78). During the identification, MPs were grouped into three-fibres, pellets and other MPs. In total, 45 fibres were found which was 21.8% of the total number of

MPs, and 45 black colored pellets were present with 21.8% of the total number of MPs (Fig. 3.6).

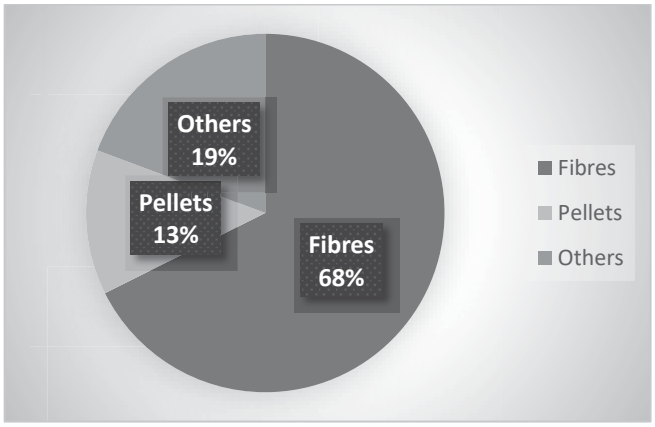


Fig. 3.6 Distribution of different types of MPs in the samples.

3.1.2.3 Length and MPs of the blue mussels:

The mean lengths of the groups A, B and C is 54.95 mm (± 2.03), 64.05 (± 2.01) mm and 73.88 mm (±3.20 and the mean number of MPs is 4.5(±2.44), 4 (±1.83) and 7.8 (±2.82) per individuals in size groups A, B and C (Fig. 3.7).

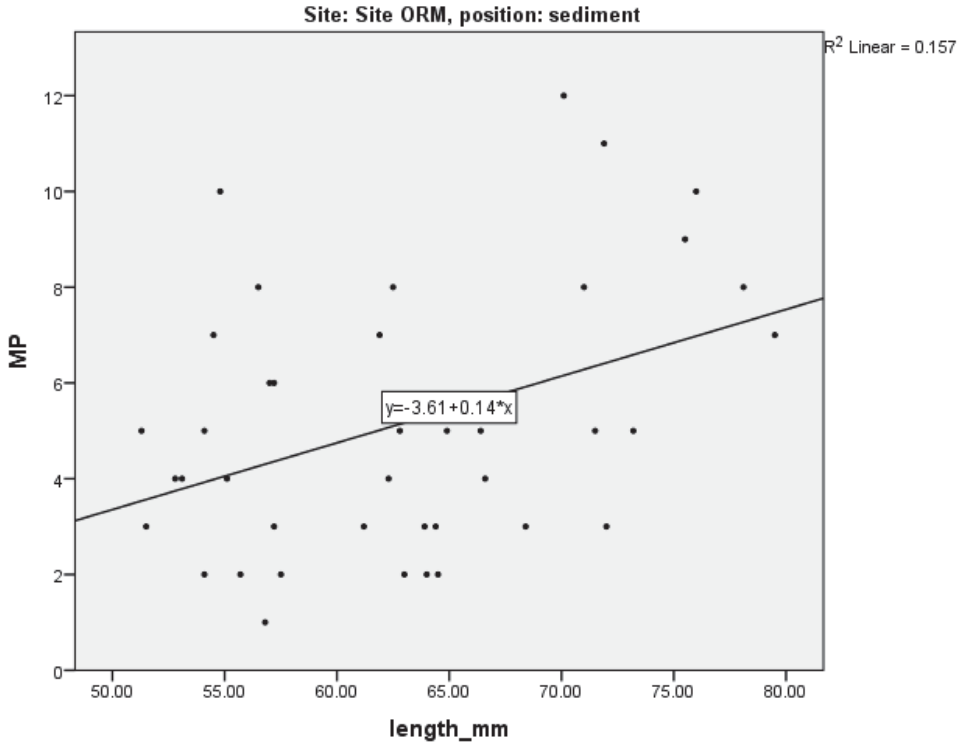


Fig.3.7 Relation between the length and number of MPs in blue mussels (*Mytilus edulis*).

There was no significant correlation between the length and the number of MPs found in each mussel individuals (Spearman's rho 0.302, p=0.058). Kruskal-Wallis test showed the distribution of MPs is significantly different across the size groups. Dunn's test showed the significant difference between the groups A and C and between B and C.

3.1.2.4 Weight and the number of MPs in the blue mussels:

The mean weight 5.25 g (±1.50), 7.68 g (±1.26) and 7.74 g (±1.82) in size class of A, B and C respectively with the MPs is 4.5(±2.44), 4 (±1.83) and 7.8 (±2.82) per individuals in size groups A, B and C (Fig. 3.8).

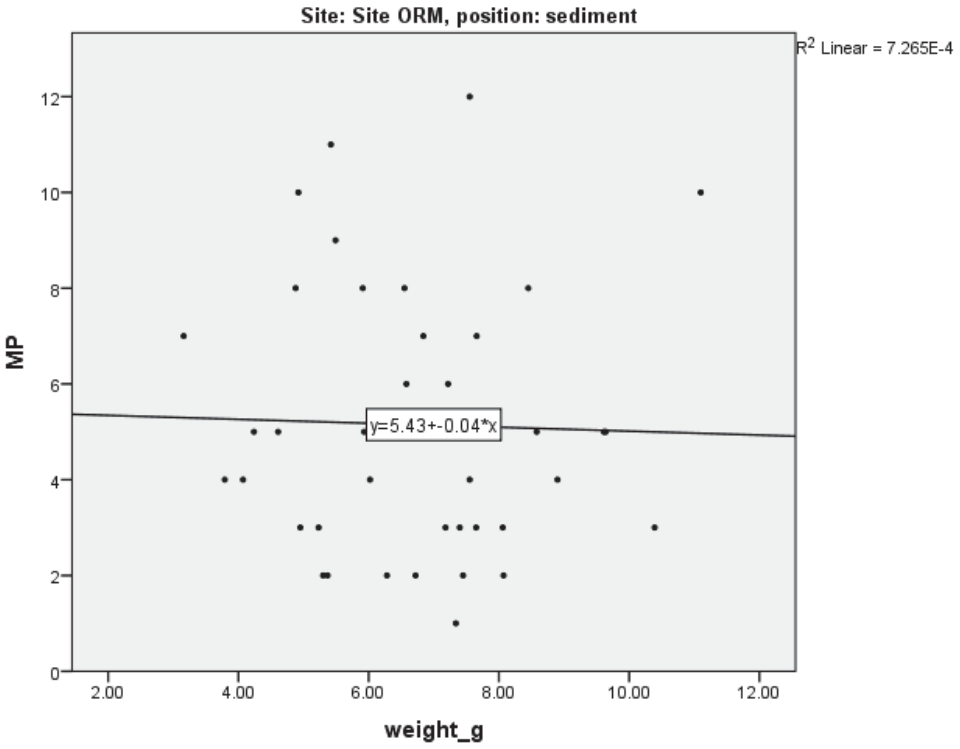
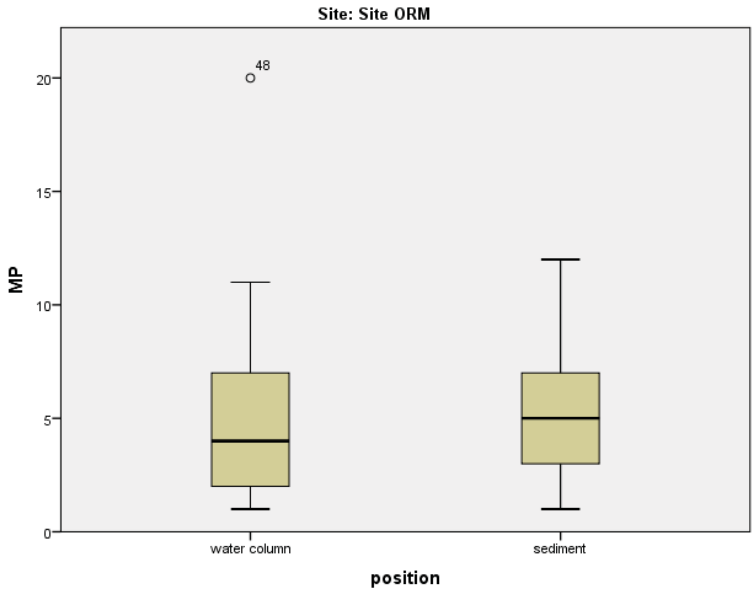


Fig.3.8 Relation between the weight and number of MPs in blue mussels (*Mytilus edulis*).

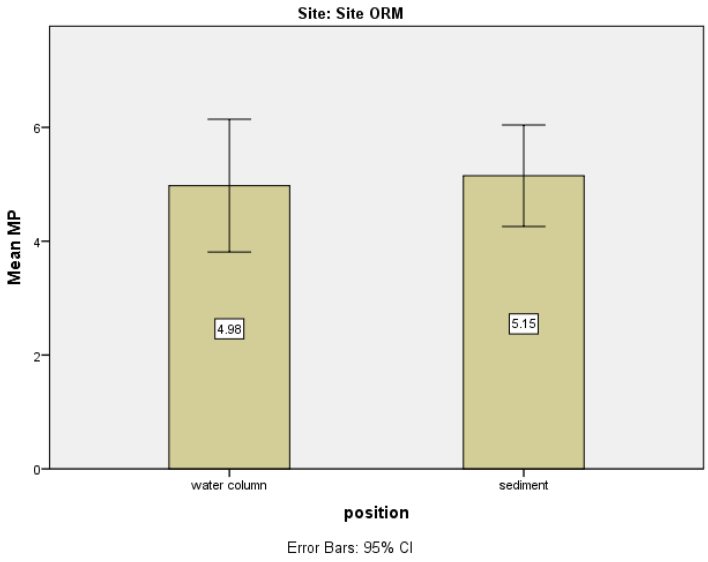
There was no significant correlation between the weight and the number of MPs found in each mussel individuals (Spearman's rho -0.66, p=0.686). Kruskal-Wallis test showed the distribution of MPs is not significantly different across the size groups.

3.1.3. Comparing number of MPs per individuals collected from water column and sediments.

The mean number of MPs per individual is 4.97(±3.74) and 5.15 (±2.78) in the samples collected from water column and those from sediment respectively (Fig. 3.9a) and Fig. 3.9 b shows the mean number of MPs from the site.



(a)



(b)

Fig.3.9 (a) Distribution of MPs in samples, (b) mean number of MPs.

Mussels collected from the sediment contained significantly more MP particles than in the mussels collected from the water column. The distribution of the MPs is the same across the position (Mann-Whitney-U test, $p<0.05$).

3.2 Site 3:

3.2.1 Samples from water column.

3.2.1.1 Length and the weight of the blue mussels:

The mean lengths of the groups A, B and C is 55.60 mm (± 2.09), 65.05 (± 2.66) mm and 74.04 mm (±2.38) and mean weight 5.36 g (±1.03), 6.18 g (±1.28) and 8.41 g (±1.35) respectively. Fig. 3.10 describes the correlation between the length of the samples from water column of the site-3.

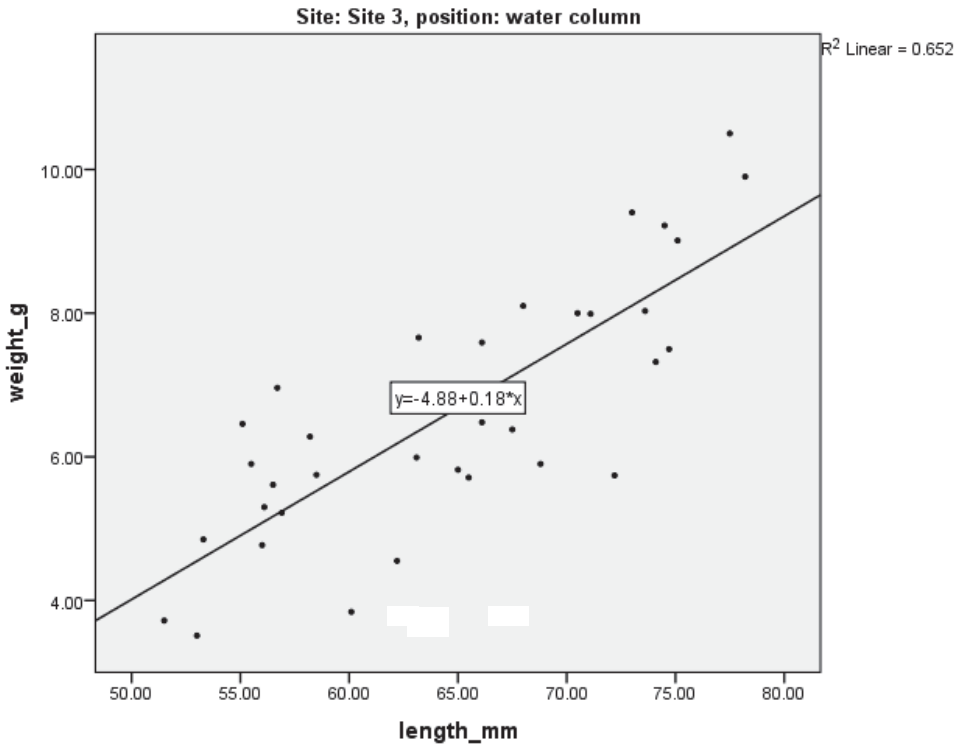


Fig. 3.10 Relation between the length and weight of blue mussels (*Mytilus edulis*).

There was a significant correlation between the length and the weight mussel (Spearman's rho 0.784, $p<0.05$). Kruskal-Wallis test showed the distribution of weight is significantly different across the size groups. Dunn's test showed the significant difference between the groups A and C and between B and C.

3.2.1.2 MPs in sampled blue mussels:

MP was found present in each of the individual at this site. Total 165 MPs was identified in 34 samples through the visual identification, with the highest number of MPs was 12 in a single individual and lowest was 1 which were found in Size class C and size class A respectively. With the least number of samples in size class C, has the highest number of MPs were 7(±3.43) per individual and it was also found that the total number of MPs is increasing with the size of the blue mussels.

The total number of MPs across the size groups was 165 with a mean number of MPs per individual was 4.85 (±3.12). During the identification, MPs were grouped into three-fibres, pellets and other MPs. In total, 38 fibres were found which was 23% of the total number of MPs, and 34 black colored pellets were present with 21% of the total number of MPs (Fig. 3.11).

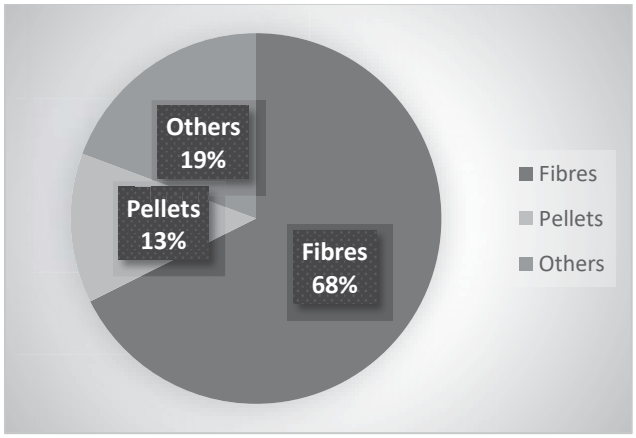


Fig. 3.11 Distribution of different types of MPs in the samples.

3.2.1.3 Length and MPs of the blue mussels:

The mean lengths of the groups A, B and C is 55.60 mm (± 2.09), 65.05 (± 2.66) mm and 74.04 mm (±2.38) and the mean number of MPs is 2.08(±0.99), 5.73 (±2.05) and 7 (±3.43) per individuals in size groups A, B and C (Fig.3.12).

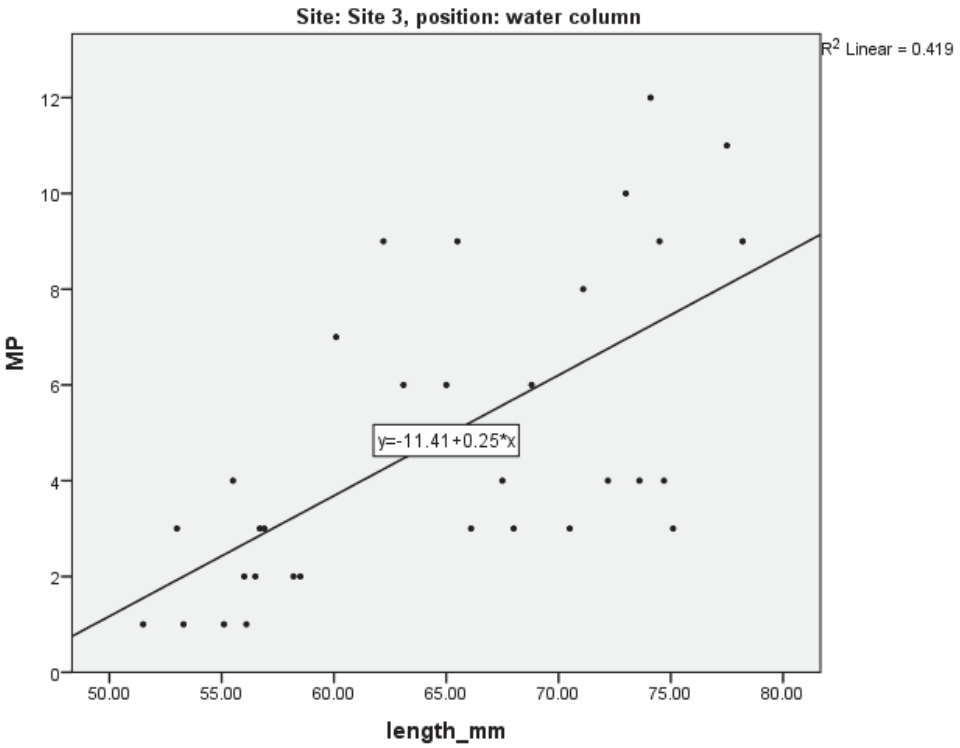


Fig.3.12 Relation between the length and number of MPs in blue mussels (*Mytilus edulis*).

There was a significant correlation between the length and the number of MPs found in each mussel individuals (Spearman's rho 0.664, $p<0.05$). Kruskal-Wallis test showed the distribution of MPs is significantly different across the size groups. Dunn's test showed the significant difference between the groups A and B and between A and C.

3.2.1.4 Weight and the no of MPs in the blue mussels:

The mean weight was 5.36 g (±1.03), 6.18 g (±1.28) and 8.41 g (±1.35) in size class of A, B and C respectively with the no of MPs 2.08 (±0.99), 5.73 (±2.05) and 7 (±3.43) per individuals in size groups A, B and C (Fig. 3.13).

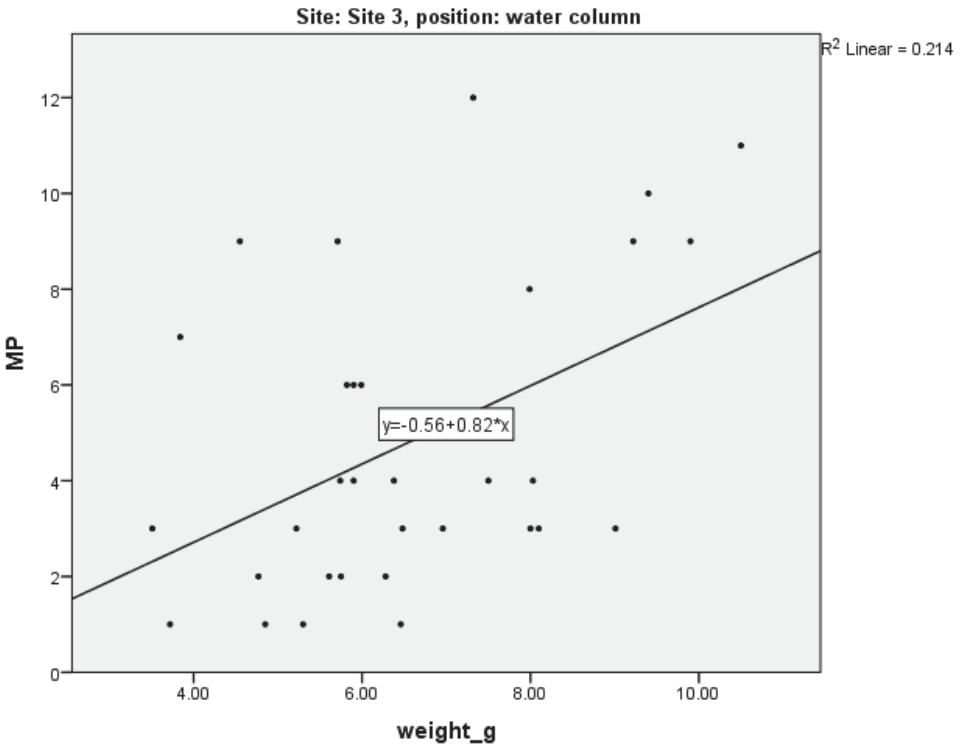


Fig.3.13 Relation between the weight and number of MPs in blue mussels (*Mytilus edulis*).

There was a significant correlation between the weight and the number of MPs found in each mussel individuals (Spearman's rho 0.415, $p<0.05$). Kruskal-Wallis test showed the distribution of MPs is not significantly different across the size groups.

3.2.2 Samples from sediment.

3.2.2.1 Length and the weight of the blue mussels:

The mean lengths of the groups A, B and C is 54.74 mm (± 2.54), 64.83 (± 1.85) mm and 72.73 mm (±3.11) and mean weight 4.30 g (±0.69), 5.01 g (±1.02) and 6.51 g (±1.76) respectively. Fig. 3.14 describes the correlation between the length of the samples from sediment of the site-3.

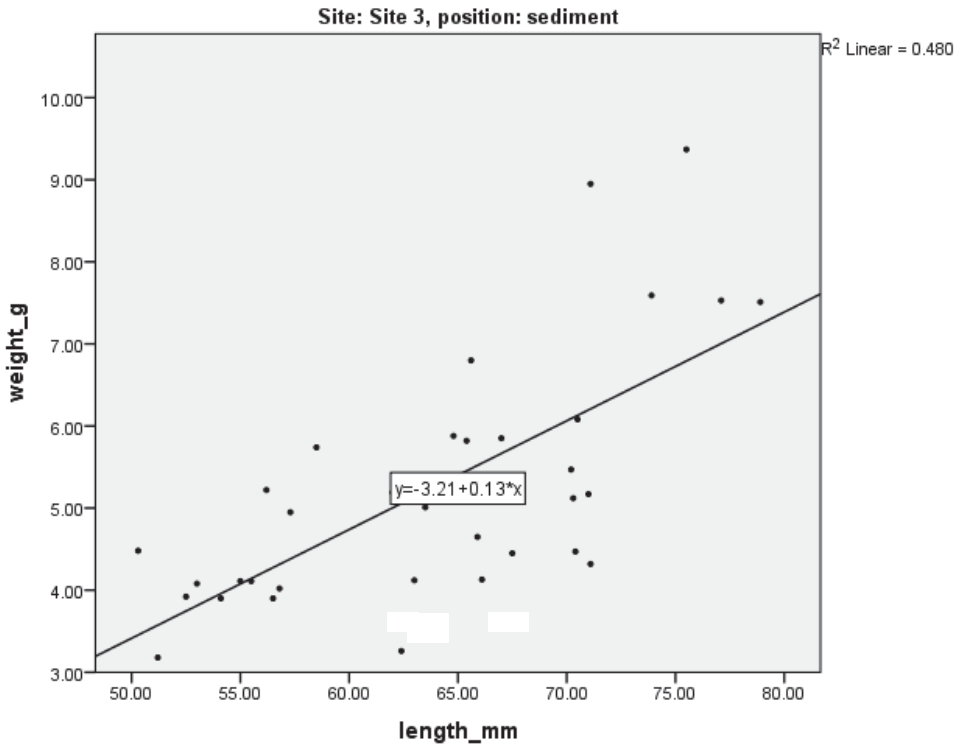


Fig. 3.14 Relation between the length and weight of blue mussels (*Mytilus edulis*).

There was a significant correlation between the length and the weight of mussel with (Spearman's rho 0.702, $p<0.05$). Kruskal-Wallis test showed the distribution of weight is significantly different across the size groups. Dunn's test showed the significant difference in the distribution of weight in between the groups A and C.

3.2.1.2 MPs in sampled blue mussels:

MP was found present in each of the individual at this site. Total 124 MPs was identified in 34 samples through the visual identification, with the highest number of MPs was 10 in a single individual and lowest was 1 which were found in Size class C and size class A respectively. With the least number of samples in size class C, has the highest number of MPs were 5.91 (±2.66) per individual and it was also found that the total number of MPs is increasing with the size of the blue mussels.

The total number of MPs across the size groups was 124 with a mean number of MPs per individual was 3.64 (±2.59). During the identification, MPs were grouped into three-fibres, pellets and other MPs. In total, 17 fibres were found which was 14% of the total number of

MPs, and 43 black colored pellets were present with 35% of the total number of MPs (Fig. 3.15).

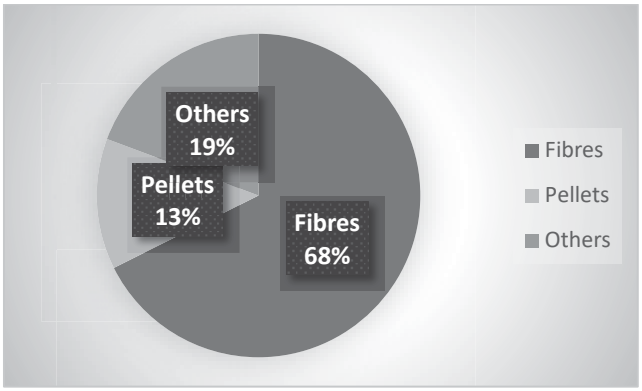


Fig. 3.15 Distribution of different types of MPs in the samples.

3.2.1.3 Length and MPs of the blue mussels:

The mean lengths of the groups A, B and C is 54.74 mm (± 2.54), 64.83 (± 1.85) mm and 72.73 mm (±3.11) and the mean number of MPs is 1.66 (±0.77), 3.54 (±2.01) and 5.91 (±2.66) per individuals in size groups A, B and C respectively (Fig. 3.16).

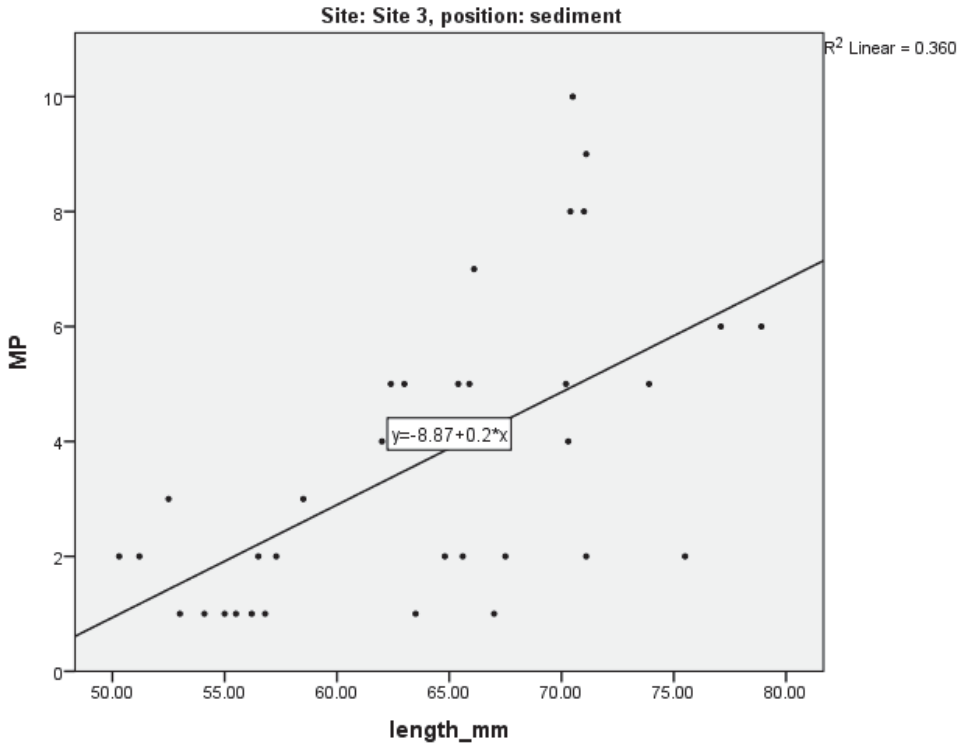


Fig.3.16 Relation between the length and number of MPs in blue mussels (*Mytilus edulis*).

There was a significant positive correlation between the length and the number of MPs found in each mussel (Spearman's rho 0.617, $p<0.05$). Kruskal-Wallis test showed the distribution of MPs is significantly different across the size groups. Dunn's test showed the significant difference between size groups A and C.

3.2.1.4 Weight and the number of MPs in the blue mussels:

The mean weight was 4.30 g (±0.69), 5.01 g (±1.02) and 6.51 g (±1.76) in size class of A, B and C respectively with the no of MPs 1.66 (±0.77), 3.54 (±2.01) and 5.91 (±2.66) per individuals in size groups A, B and C (Fig. 3.17).

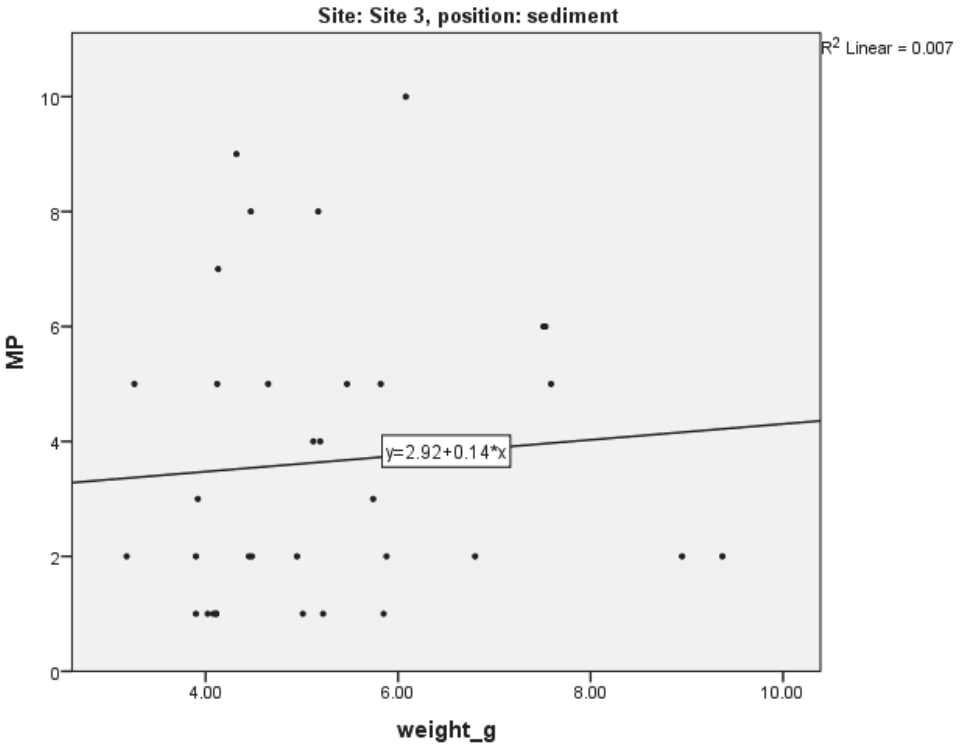


Fig. 3.17 Relation between the weight and number of MPs in blue mussels (*Mytilus edulis*).

There was no significant correlation between the weight and the number of MPs found in each mussel individuals (Spearman's rho 0.239, $p=0.173$). Kruskal-Wallis test showed the distribution of MPs is not significantly different across the size groups.

3.2.3. Comparing number of MPs per individuals collected from water column and sediments.

The mean number of MPs per individual is 4.85 (±3.12) and 3.64 (±2.59) in the samples collected from water column and those from sediment respectively (Fig. 3.18a) and Fig. 3.18 b shows the mean number of MPs from the site.

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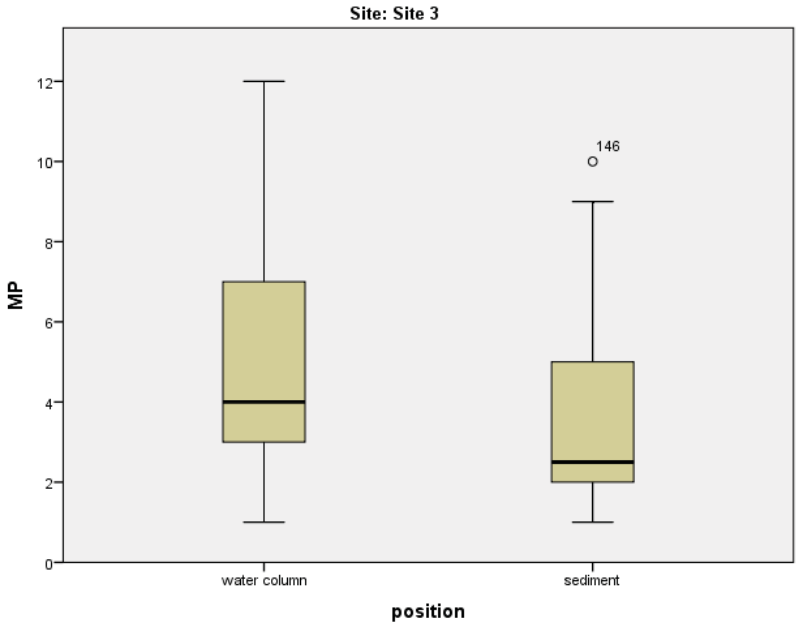
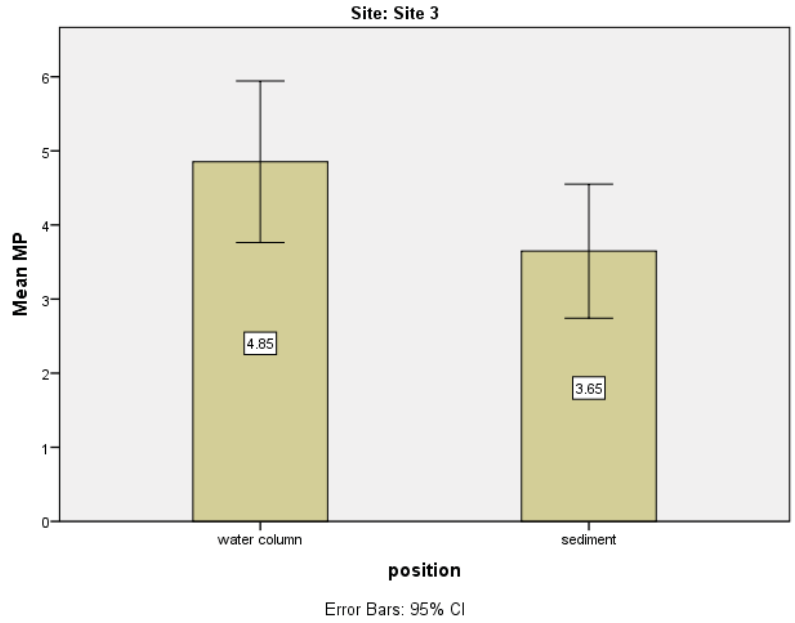


(a)



(b)

Fig.3.18 (a) Distribution of MPs in samples, (b) mean number of the MPs.

Mussels collected from the water column contained significantly more MP particles than in the mussels collected from the sediment. The distribution of the MPs is the same across the position (Mann-Whitney-U test, $p<0.05$).

3.3 Site 5:

3.3.1 Samples from water column.

3.3.1.1 Length and the weight of the blue mussels:

The mean lengths of the groups A, B and C is 55.76 mm (± 3.07), 65.78 (± 3.15) mm and 73.93 mm (±2.02) and mean weight 3.61 g (±0.88), 5.47 g (±1.63) and 7.61 g (±0.64) respectively. Fig. 3.19 describes the correlation between the length of the samples from water column of the site-5.

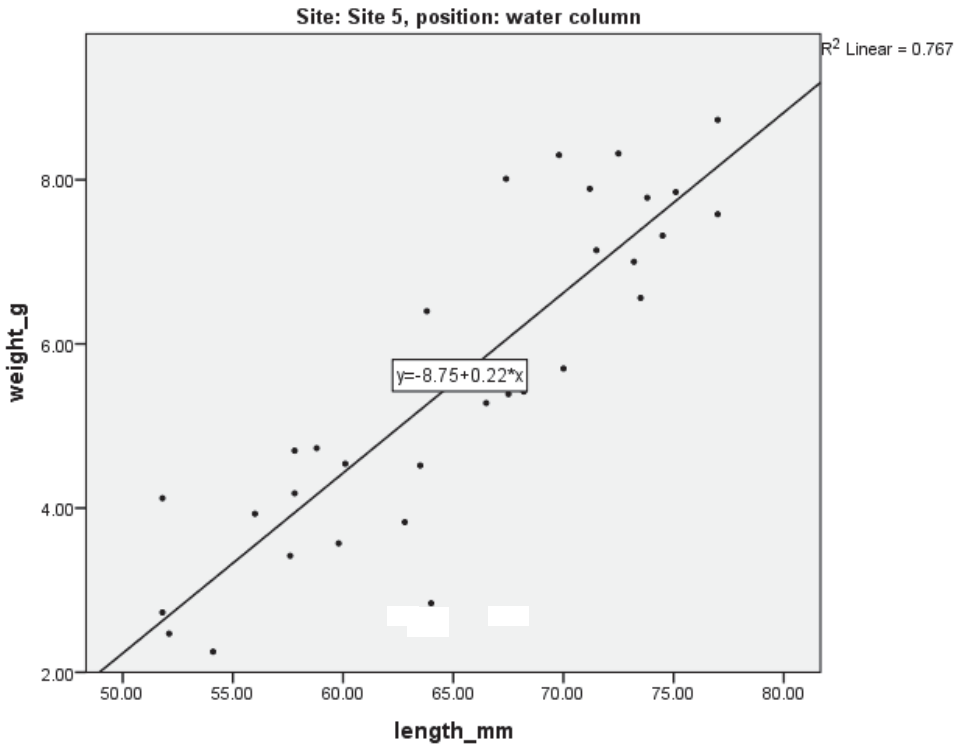


Fig. 3.19 Relation between the length and weight of blue mussels (*Mytilus edulis*).

There was a significant correlation between the length and the weight of mussel (Spearman's rho 0.849, $p<0.05$). Kruskal-Wallis test showed the distribution of weight is significantly different across the size groups. Dunn's test showed the significant difference in the distribution of the weight between the groups A and C.

3.3.1.2 MPs in sampled blue mussels:

MP was found present in each of the individual at this site. Total 224 MPs was identified in 31 samples through the visual identification, with the highest number of MPs was 14 in a single individual and lowest was 2 which were found in Size class A and size class B respectively. With the least number of samples in size class A, has the highest number of MPs were 8.4 (± 3.15) per individual and it was also found that the smallest size group A ha the highest number and size group has the least no of MPs.

The total number of MPs across the size groups was 224 with a mean number of MPs per individual was 7.22 (± 3.43). During the identification, MPs were grouped into three-fibres, pellets and other MPs. In total,111 fibres were found which was 14% of the total number of MPs, and 27 black colored pellets were present with 35% of the total number of MPs (Fig. 3.20).

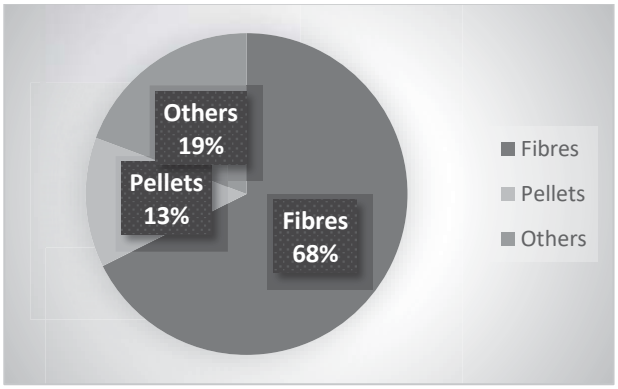


Fig. 3.20 Distribution of different types of MPs in the samples.

3.3.1.3 Length and MPs of the blue mussels:

The mean lengths of the groups A, B and C is 55.76 mm (± 3.07), 65.78 (± 3.15) mm and 73.93 mm (±2.02) and the mean no of MPs is 8.40 (±2.75), 6 (±3.68) and 7.4 (±3.62) per individuals in size groups A, B and C (Fig. 3.21).

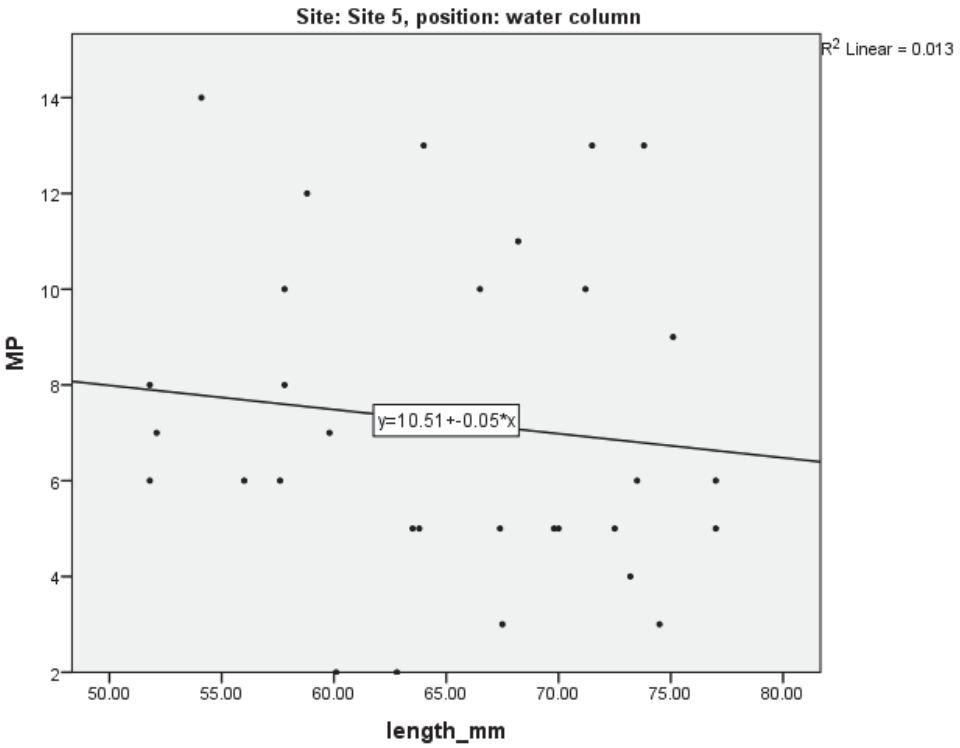


Fig.3.21 Relation between the length and number of MPs in blue mussels (*Mytilus edulis*).

There was a no significant correlation between the length and the number of MPs found in each mussel individuals (Spearman's rho -0.173, p=0.353). Kruskal-Wallis test showed the distribution of MPs is not significantly different across the size groups.

3.3.1.4 Weight and the number of MPs in the blue mussels:

The mean weight was mean weight 3.61 g (±0.88), 5.47 g (±1.63) and 7.61 g (±0.64) in size class of A, B and C respectively with the no of MPs is 8.40 (±2.75), 6 (±3.68) and 7.4 (±3.62) per individuals in size groups A, B and C (Fig. 3.22).

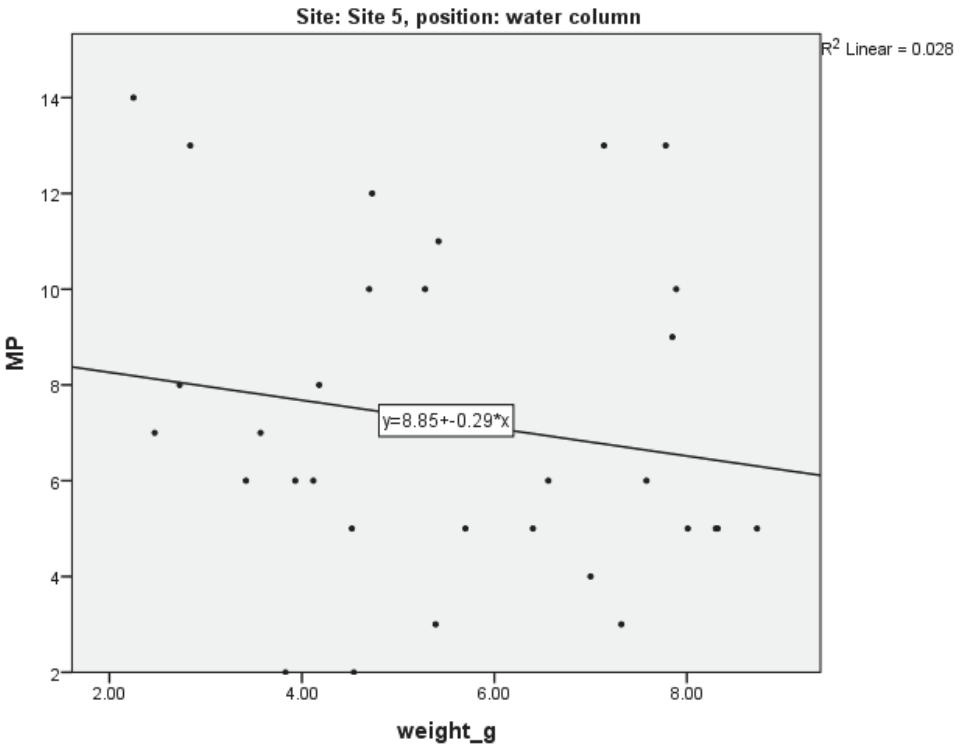


Fig.3.22 Relation between the weight and number of MPs in blue mussels (*Mytilus edulis*).

There was no significant correlation between the weight and the number of MPs found in each mussel individuals (Spearman's rho -0.222, p=0.230). Kruskal-Wallis test showed the distribution of MPs is not significantly different across the size groups.

3.3.2 Samples from sediment.

3.3.2.1 Length and the weight of the blue mussels:

The mean lengths of the groups A, B and C is 57.37 mm (± 2.84), 64.56 (± 2.09) mm and 72.18 mm (±2.44) and mean weight 3.83 g (±0.64), 4.80 g (±0.89) and 7.45 g (±0.645) respectively. Fig. 3.23 describes the correlation between the length of the samples from sediment of the site-5.

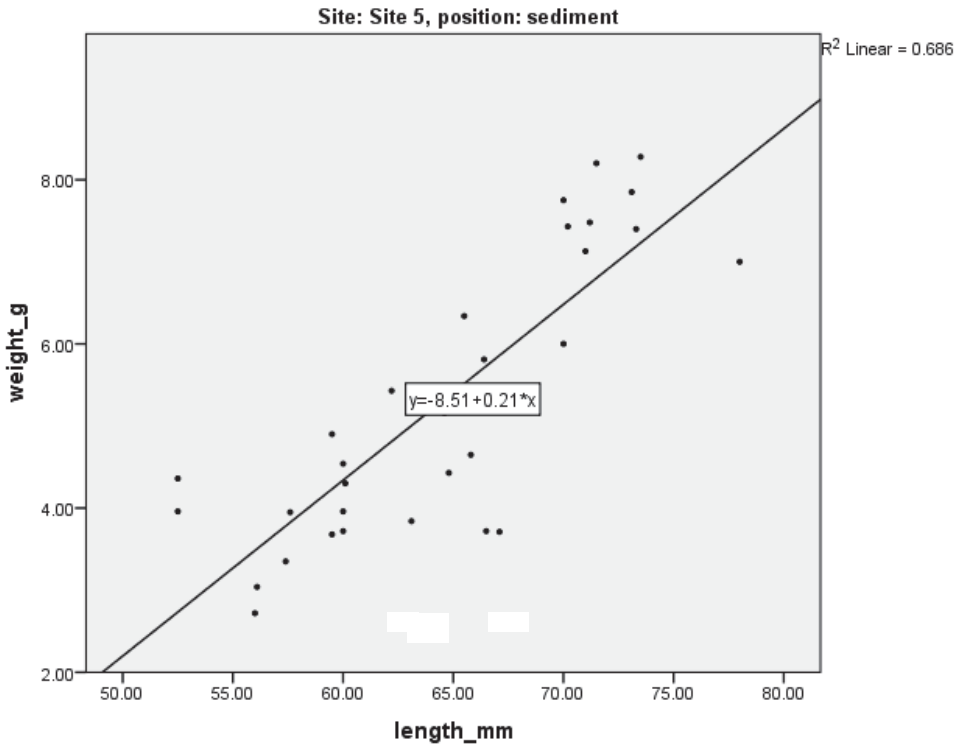


Fig. 3.23 Relation between the length and weight of blue mussels (*Mytilus edulis*).

There was a significant correlation between the length and the weight of the mussel individuals (Spearman's rho 0.789, $p<0.05$). Kruskal-Wallis test showed the distribution of weight is significantly different across the size groups. Dunn's test showed the significant difference in the distribution of the weight between the groups A and C and in between the groups B and C.

3.3.1.2 MPs in sampled blue mussels:

MP was found present in each of the individual at this site. Total 176 MPs was identified in 32 samples through the visual identification, with the highest number of MPs was 19 in a single individual and lowest was 1 which were found in Size class C and size class B respectively. With the least number of samples in size class C, has the highest number of MPs were 6.9 (±4.55) per individual and it was also found that the smallest size group C has the highest number of MPs.

The total number of MPs across the size groups was 176 with a mean number of MPs per individual was 5.18 (±3.58). During the identification, MPs were grouped into three-fibres, pellets and other MPs. In total,119 fibres were found which was 14% of the total number of

MPs, and 23 black colored pellets were present with 35% of the total number of MPs (Fig. 3.24).

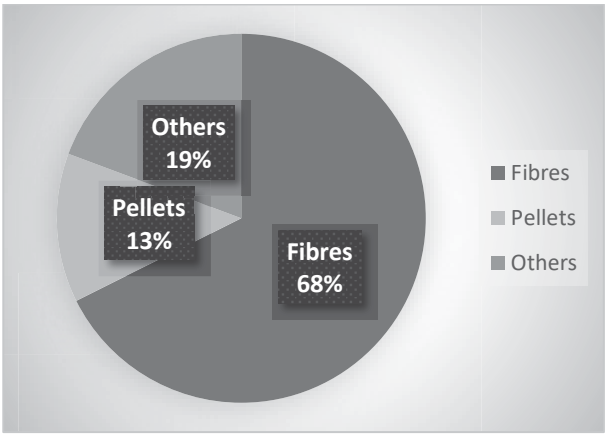


Fig. 3.24 Distribution of different types of MPs in the samples.

3.3.1.3 Length and MPs of the blue mussels:

The mean lengths of the groups A, B and C is 57.37 mm (± 2.84), 64.56 (± 2.09) mm and 72.18 mm (±2.44) and the mean number of MPs is 6.54 (±3.04), 3.18 (±1.06) and 6.9 (±4.55) per individuals in size groups A, B and C (Fig. 3.25).

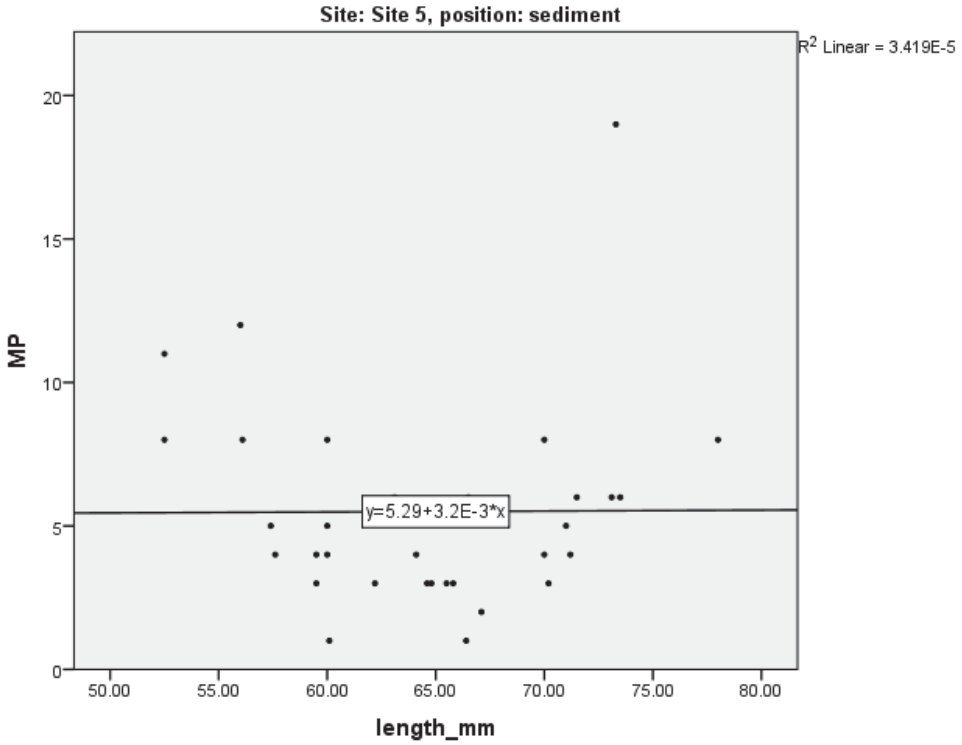


Fig.3.25 Relation between the length and number of MPs in blue mussels (*Mytilus edulis*).

There was no significant correlation between the length and the number of MPs found in each mussel (Spearman's rho -0.048, p=0.795). Kruskal-Wallis test showed the distribution of MPs is not significantly different across the size groups. Dunn's test showed the significant difference between the groups A and B and between B and C.

3.3.1.4 Weight and the number of MPs in the blue mussels:

The mean weight was mean weight mean weight 3.83 g (±0.64), 4.80 g (±0.89) and 7.45 g (±0.645) in size class of A, B and C respectively with the no of MPs is 6.54 (±3.04), 3.18 (±1.06) and 6.9 (±4.55) per individuals in size groups A, B and C (Fig. 3.26).

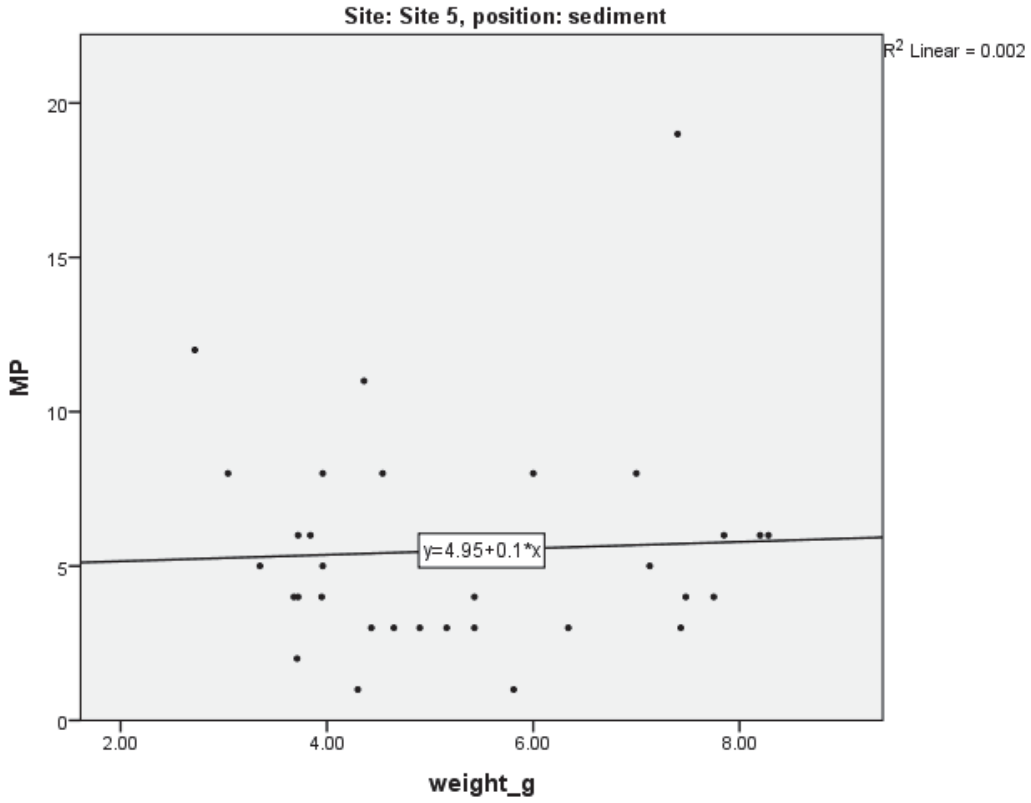
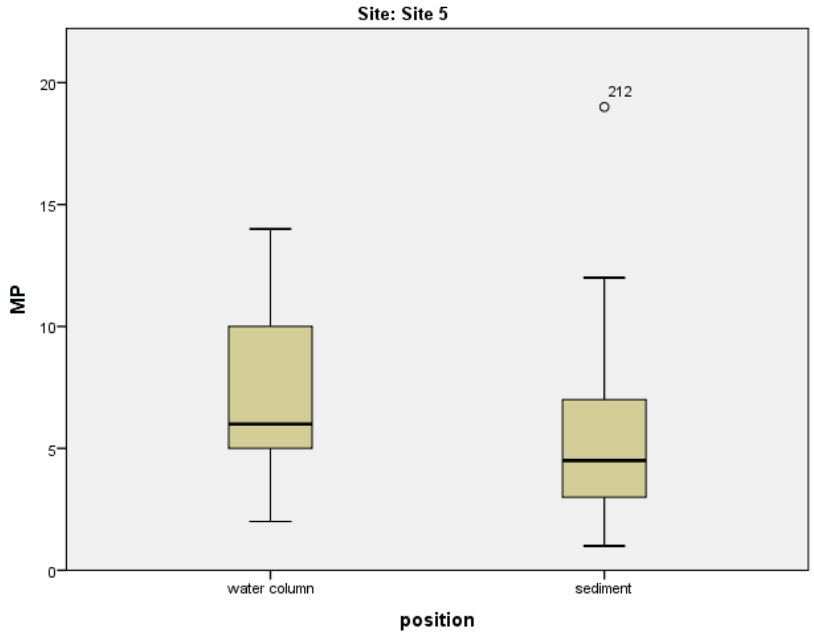


Fig.3.26 Relation between the weight and number of MPs in blue mussels (*Mytilus edulis*).

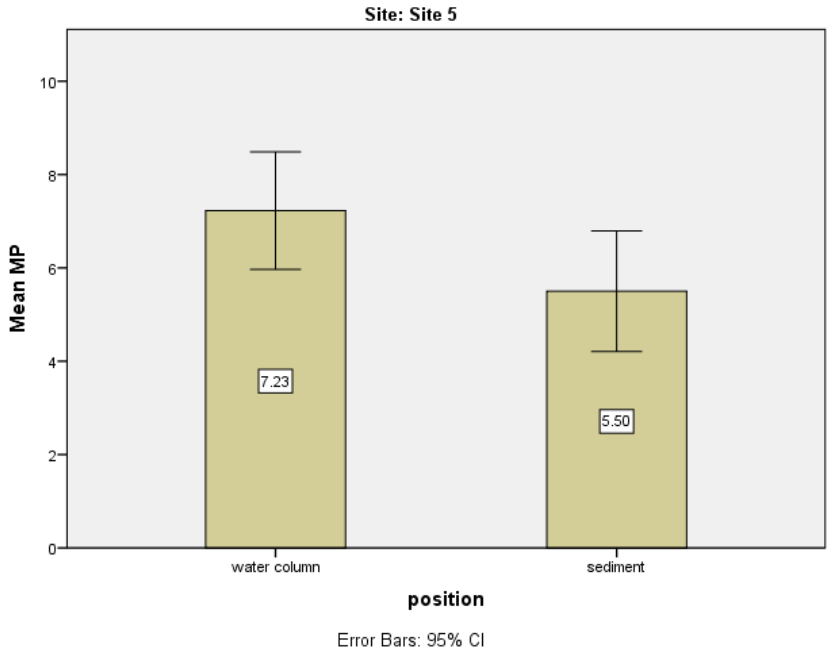
There was no significant correlation between the weight and the number of MPs found in each mussel (Spearman's rho -0.43, p=0.817). Kruskal-Wallis test showed the distribution of MPs is not significantly different across the size groups.

3.3.3. Comparing number of MPs per individuals collected from water column and sediments.

The mean number of MPs per individual is 7.22 (±3.43) and 5.5 (±3.58) in the samples collected from water column and those from sediment respectively. It shows that there is significant difference in the number of MPs per individuals (Fig. 3.27a) and Fig. 3.27 b shows the mean number of MPs from the site.



(a)



(b)

Fig.3.27 (a) Distribution of MPs in samples, (b) mean number of MPs.

Mussels collected from the water column contained significantly more MP particles than in the mussels collected from the sediment. The distribution of the MPs is not the same across the position (Mann-Whitney-U test, $p<0.05$).

4. Discussion:

4.1 Assessment of the method:

4.1.1 sampling and soft tissue collection-

During the sampling, there was no issues to be concerned on. The names of the sites were used by the NIVA, Oslo- Site-ORM, Site-3 and Site-5. After collection from these sites, mussels were rinsed with fjord water thoroughly to remove any fouling and then put into plastic bags. And there might be a question arises, whether any MPs got into the sample through their gut during rinsing or not. As, only the closed mussels were collected and mussel's lids were tightly closed during rinsing and transportation, so it can be said that no contamination has occurred. Lusher *et al.* 2017a, suggested that the depth of the collection site may affect the result as there is a chance of water-borne and air-borne MPs (Lusher *et al.* 2017a). In this study, samples are collected from different depths and this is another concern. Putting samples in the plastic bags is another important issue to be considered for opportunistic contamination. During the procedure, no MPs were found which might be originated from the plastic bags. Using plastic bags is also supported by Phuong *et al.* 2018.

Using the dry or wet weight of the soft tissue is another area of concern. Few researches support the use of dry weight of soft tissue (for instances, Karlsson *et al.* 2017). They also suggested that dry weight is better for comparisons between studies Beyer *et al.* 2017 found using wet weight is advantageous, but they also mentioned it as less reliable. So, elaborate researches are still needed to find the appropriateness to use dry or wet weight of the soft tissue. During the sample processing, lesser number of steps should be followed so that especially airborne contamination can be minimized. The effect of drying on MPS is still unknown and drying might make the MPs more brittle. As, wet weight was successfully used in different studies like- Van Cauwenberghe *et al.* 2015; Li *et al.* 2016; Phuong *et al.* 2018), in this study wet weight was used.

Use of 10% KOH solution was used to digest soft tissues in the study which was tested at NIVA and was adapted from to Dehaut e*t al.* (2016). This method was tested efficiently and so was used in this study. Some organic materials were left on filters which made the MPs identification more challenging.

4.1.2 Visual analysis

After filtration, with the aid of visual identification, MPs were quantified as the first step. Visual analysis was done with the help of a microscope. Proper training is needed to identify the MPs and non-MPs. The process itself is subjective, time consuming and intensively laborious. This is used as a simple initial step identification of MPs (Hidalgo- Ruz *et al*. 2012; Song *et al.* 2015; Phuong *et al.* 2018). According to Hidalgo-Ruz *et al.* (2012), visually identified MPs are later not confirmed as plastics it is about in 70% cases.

4.2 MPs in blue mussels from the Oslo fjord

All blue mussel's samples were found contaminated by the MPs. The range of the number of MPs per individual is 1-20. The average number of MPs in per individuals of blue mussels in this study indicates that blue mussels at Oslo Fjord are more contaminated than other blue mussels in Norway (Table 1). A higher average number of MPs was found in the study compared to the average found in a study by Bråte et al. (2018) (Table 1). As a larger area was investigated with larger variations in environmental conditions in their study, it should be logical to assume that their results are influenced by more variability of factors.

This study revealed that blue mussels from Oslo fjord contained more MPs per individual than blue mussels in similar studies worldwide (Table 1). The following studies investigate larger areas. Van Cauwenberghe et al. (2015a) found microplastic abundances relatively similar to the findings in the Belgian coast and in the UK where the sample sites were located close to coastal harbours and there were higher shipping and industrial activity were present. The highest number of MPs which was 34 per individual were found in Canada by Mathalon and Hill (2014) which is almost seven times higher in compare to this study. The studied sites have similarity to the sampling sites in Canada which is surrounded by potential sources for contamination. More researches should be done to determine the reasons for these high differences.

Table-1. A selection of worldwide MPs studies performed on blue mussels.

Sample Area	**Average concentration**	**Comment**	**Reference**
Oslo fjord	5.18 MP/ind.	Range 1-20 MP/ind.	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)
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)
China	2.2 MP/g (range 0.9 – 4.6)	2/3 of the coastline of mainland in China investigated	(Li *et al.* 2016)
North Sea coast of France, Belgium and the Netherlands	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 (±0.22)		(De Witte *et al.* 2014)
Canada	34 MP/ind.		(Mathalon & Hill 2014)

The uptake of MPs by the organisms is dependent on a collection of parameters present in the organisms like- size, shape and density of the MPS that determine the position of MPs in the water column or in the sediment, and hence their bio-availability (Van Cauwenberghe *et al.* 2015a). Blue mussels are filter feeders and they have high capacity of MPs ingestion (Setälä et al. 2016) and they were reported to ingest more numbers of the smaller size MPs (<0.1 mm, 0.1 mm–0.5) (Digka *et al.* 2018). Larger blue mussels have higher body mass which means they have higher capability of their filtration (Navarro and Thompson 1996). The result indicates that the larger sized mussels contained more number of MPs per individual except

for the samples collected from the water column of Site-5 which can be an indicator that the floating type of MPs were not abundant in the sampled site or may be the absence of smaller MPs on the sampled site.
MPs are found in all the samples. Total 1104 number MPs were found in 213 samples across three different size classes which were collected from two different positions. MPs were grouped as fibres, pellets and 'Others' which includes- fiber bundle, fragment, sphere (or bead), film, and foam. Out of this total, 376 fibres which is the approximately 34% of the total MPs; 218 black pellets which was approximately 20% of the total MPs and MPs which were grouped as others were 46% of the total MPs.

Fibres like polyesters and acrylics were the most common MPs in sediments in former sewage disposal plants (Browne *et al.* 2011) and polyester was the most abundant polymer in mussels (Catarino *et al.* 2018) and was the second most abundant polymer (Li *et al.* 2016). In Norwegian coast, polyester was also the most common polymer found in Atlantic cod (Bråte et al 2016). Both polyester and acrylic are raw material in clothing industry and could be the possible source for the fibres. As there is fibres in the results, it can be suggested that, the domestic waste water (e.g. from washing machines) is an important source of the fibres. Along with WWTP, atmospheric fallout may be a significant source for fibres as these even can spread through air (Dris *et al.* 2015). Another study by Cai *et al.* (2017) showed high percentage (73%) of fibres in the atmospheric fallout. So, it could be predicted that the possible sources of fibres found in the samples could be from the clothing and other textiles, may also from any form of disposed cotton wool, or from paper and or even from cigarette butts. These fibres are expected to be degraded more quickly compared to the other types of MPs, those were still in the mussels and were not damaged by KOH solution.

In total, samples from site-5 where the highest percentage (71.68%) of fibres were found. Site-3 has the smallest percentage of the fibres (19%) may be from the natural sources which clearly cannot indicate their exact sources, but it is most likely due to human activities. This indicates the potential effect on the biota in the fjord. This could be an area of more researches to find the effects of anthropogenic particles like- cotton, viscose rayon, synthetic plastics etc. have on biota of Oslo fjord.

The black colored pellets found in the samples might be from run off form the road which includes – asphalt, wear and tear from tires and different road markings and this group demands high attention for further researches (Magnusson *et al.* 2016). And as because of their black

color, UV cannot degrade those quickly (Kole *et al.* 2017). This type of MPs had potentiality to have additives on them and in this way, these have effect on biota of Oslo fjord. These additives may be hazardous due to their toxicity to biota-from green algae to water frogs to rainbow trout (Stephensen *et al.* 2003 a& Kole *et al.* 2017).

Samples collected from the water column from the site-ORM, showed clear indication that the mussel size affects the numbers of microplastic particles. It was 2.75 (±1.94) MPs per individual, 4.27 (± 2.12) MPs per individual and 9.18 (±4.16) MPs per individuals in size class A, B and C respectively. In the samples from sediments this trend was not clearly observed as 4.5 (±2.44) MPs per individual 4 (±1.83) MPs per individual and 7.8 (±2.85) MPs per individual. In both cases the largest size class C has the highest number of the MPs per individual.

Samples collected from both water column and sediments from site-3 showed the clear indication that the mussel size affects the numbers of microplastic particles. Samples from water column had 2.08 (±0.99) MPs per individual, 5.72 (±2.05) MPs per individual and 7 (±3.43) MPs per individuals in size class A, B and C respectively. It was 1.66 (±0.77) MPs per individual, 3.54 (±2.01) MPs per individual and 5.99 (±2.66) MPs per individuals in size class A, B and C respectively in the samples collected from sediments.

Samples collected from both water column and sediments from site-5 did not show any indication that the mussel size affects the numbers of microplastic particles. Samples from water column had 8.4 (±2.75) MPs per individual, 6 (±3.68) MPs per individual and 7.4 (±3.62) MPs per individuals in size class A, B and C respectively. It was 6.54 (±3.04) MPs per individual, 3.18 (±1.66) MPs per individual and 6.9 (±4.55) MPs per individuals in size class A, B and C respectively in the samples collected from sediments.

So, the hypothesis that the mussel size affects the numbers of MPs can be rejected in two sites-site-ORM and site-5 and it can be retained in site-3 only. This can be explained by the sources, types and sources, route to the fjord, abundance and distribution (Thiel *et al.* 2003); interaction with biota of the MPs (bioavailability, transferring between the trophic levels) and how the MPs can interact with meteorological and hydrological factors of the environment ((Andrady 2011). Biological factors of blue mussel samples-like filtration rate, ability to uptake of MPs, hydrological issue (water temperature, pH, salinity, O_2 availability) and availability of foods

might be also considered and could be interesting area for researches which would enlighten with clearer conceptions in these regards.

Samples collected from site-ORM had 4.97 (±3.74) MPs per individual and is 5.15 (±2.78) MPs per individual collected from water column and sediments respectively. Samples from site-3 had 4.85 (±3.12) MPs per individual and 3.64 (±2.59) MPs per individual collected from water column and sediments respectively. Samples from site-5 had 7.22 (±3.43) MPs per individual and 5.5 (±3.58) MPs per individual collected from water column and sediments respectively. So, samples collected from the sediments had lower number of MPs compared to those from water column in Site-3 and Site-5. Site-ORM showed that the samples from sediments contained more number of MPs per individual.

As MPs were present in all samples collected both from water column and the sediments, the distribution of the MPs in the sampled sites were abundant. This is due to the factors like-types of polymer which is responsible for different densities, size and shape of the polymers (Thiel *et al.* 2003); the different parameters of the aquatic environment like- salinity, water temperature and biomass (Andrady 2011, Zhao *et al.* 2014); the meteorological conditions like-rain and wind, air temperature (Kukulka *et al.* 2012) and the turbulence in the water (Reisser *et al.* 2015). Movement of boats, wind and the surface currents may cause turbulence in the water column and can redistribute MPs within the water column (Lusher *et al.* 2015). Higher shipping activity can contribute to the higher abundance of the MPs (Lusher *et al.* 2015). The turbulence also affects the redistribution of MPs in the water column (Dai *et al.* 2018).

Due to higher density, PVC was found at the bottom layer in the water column (Dai *et al.* 2018). Different physical shapes of MPs are also responsible for their distribution in the water column (Kooi *et al.* 2016). Fibres were the most common type of MPs found in the 'in near-surface' and' near-bottom layers' of the water column (Bagaev *et al.* 2017).

Biofouling and the surface property of the MPs also could be the reason for the distribution of MPs in water column and in the sediment (Dai *et al.* 2018). Physical characteristics of MPs like-rough surfaces, irregular cracks and pores may increase the ability of attachment with foreign particles like- clay minerals or quartz grains (Corcoran 2015), and these may decrease the buoyancy of MPs (Kowalski *et al*. 2016). Weathering also may contribute to the attachment of MPs with microorganisms which helps in the formation of biofilm and causes to increase the densities of MPs which may affect the buoyancy (Corcoran 2015, Fazey and Ryan 2016, Harrison *et al.* 2011, Kaiser *et al.* 2017, Kowalski *et al.* 2016, Long *et al*. 2015). Floating MPs can be sunk from the surface which may be affected by the aggregations of algae water

(Bergmann *et al.* 2017). Smaller sized MPs are more likely to sink than large ones (Dai *et al.* 2018) and this was also observed by Fazey and Ryan (2016).

However, sinking of MPs may not always resulted in the attachment with the benthic sediments (Rummel *et al.* 2017). This study showed inconsistence between the abundance of MPs in the water column and in the sediments. MPs tend to settle with finest sediments in the deeper parts of the sea water and in area with lower depressions (Bagaev *et al.* 2017). Abundance of the MPs in the sediments might be affected by both the rate of sinking of the MPs and the physical properties of the benthic sediments (Dai *et al.* 2018).

MPs with greater densities sink to the bottom of the sea (Andrady 2011, *Reisser et al.* 2013, Jorissen 2014). Sediments are considered as the long-term sink for MPs as they have the potential to accumulate those (Nuelle *et al.* 2014 and Cózar *et al.* 2014). High abundance of MPs was reported within the sediments and it can be up to 3.3% of the weight of the sediment in heavily effected se beaches (Van Cauwenberghe *et al.* 2015a, Van Cauwenberghe *et al.* 2015b, Boucher *et al.* 2016). Coastal shallow sediments along with the deep-sea sediments areas are now established as the sinks for the MPs (Alomar *et al.* 2016, Pham *et al.* 2014).

4.3 Correlation

One potential correlation was investigated in this study- the correlation between the size (length in mm) of the blue mussels and the no of MPs. Another question was tried to answer-which samples had more number of the MPs per individuals- samples from water column or from sediments.

A positive correlation between the size and the number of MPs per individual was expected. Blue mussels collected from both the water column and the sediment at the same site should be impacted by some common variables. However, a positive significant correlation was found between the length and the number of MPs per individual except for the water column samples from the site-5. A reasonable assumption may be the larger sized mussels have higher body mass, higher filtration rate and higher ability to retain more number of MPs. According to Van Cauwenberghe et al. (2015a), a blue mussel (30-40 mm) has a filtration rate of 2 L/h, and no of MPS retained in the blue mussel is usually determined by intake and egestion (Qu *et al.* 2018). Smaller MPs are expected to be found in blue mussels (Bråte *et al.* 2018; *Li et al.* 2018; Qu *et al.* 2018) and sediments are proposed as the final destination of MPs in the environment (Lusher *et al.* 2017a).

Since the length of the mussel is correlated with its weight (which also are heavier mussels), the larger mussels contain more MPs per individual. Larger mussels filter more water, and this is why they also contain more MPs. But the distribution of the MPs was not same across the wet weight of all the individuals sampled. Factors such as degradation, weather, wind, waves also could have a great impact and distribute the plastics randomly. According to (Lusher et al. 2017a), rural locations had more number of MPs available in the environment than urban and industrial locations. This might be seemed unreasonable, but it indicates the influence the environmental variables which affect the long-distance distribution of MPs.

5 Conclusion

This study on MPs as an environmental pollution confirms that MPs were present in all (100%) the samples of blue mussels. MPs were found in all the samples across all size groups collected from all site and all positions. The Larger sized mussels contain more number of MPs per individuals. The number of MPs increased with the sizes across all the samples except for the samples collected from water column at site-5. It was also found that site-5 had a significant higher no of MPs in the samples collected from water column than site-3 and site-5. The reason for this difference is currently not understood, and further research on sources and pathways of MPs in the inner Oslo Fjord is needed. Results from this study show higher no of MPs in comparison to other studies investigated. The samples collected from sediment had less number of MPs compared to the samples collected from the water column in the site-3 and in site-5.

Three issues were investigated in this study. Firstly, to find out the occurrence and abundance of the MPs across the collected samples and it was recorded that all samples had MPs present in them. Secondly, the correlation between the length of the blue mussels and the number of MPs per individual, where significant correlation was found among the samples collected from water column of site-ORM and from site-3. MPs are assumed to accumulate faster in larger sized mussels and could be the reasoning for this finding. Thirdly, to find out which samples from two positions (water column and sediment) has the more number of MPs per individual and it has been found that samples from sediments have less number of MPs per individual among the samples collected from site-3 and site-5. Factors like- temperature of the water, airborne MPs, wind, waves and currents can have a large impact, and might contribute to random distribution of MPs. Extended researches are to be done for the better understanding of the distribution of MPs and the correlation between different matrixes.

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Appendix

Appendix A

Table1. Test for normal distribution of the data.

Tests of Normality

Site		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
SIite ORM	length_mm	.115	82	.009	.955	82	.006
	weight_g	.069	82	.200*	.987	82	.576
	MP	.166	82	.000	.878	82	.000
Site 3	length_mm	.118	68	.020	.956	68	.016
	weight_g	.125	68	.011	.947	68	.006
	MP	.166	68	.000	.897	68	.000
Site 5	length_mm	.109	63	.060	.964	63	.065
	weight_g	.125	63	.016	.937	63	.003
	MP	.190	63	.000	.914	63	.000

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Table 2. Spearman's correlation coefficients.

Correlations[a]

			length_mm	MP
Spearman's rho	length_mm	Correlation Coefficient	1.000	.732**
		Sig. (2-tailed)	.	.000
		N	42	42
	MP	Correlation Coefficient	.732**	1.000
		Sig. (2-tailed)	.000	.
		N	42	42

**. Correlation is significant at the 0.01 level (2-tailed).

a. Site = Site ORM, position = water column

Correlations[a]

			length_mm	MP
Spearman's rho	length_mm	Correlation Coefficient	1.000	.302
		Sig. (2-tailed)	.	.058
		N	40	40
	MP	Correlation Coefficient	.302	1.000
		Sig. (2-tailed)	.058	.
		N	40	40

a. Site = Site ORM, position = sediment

Correlations[a]

			length_mm	MP
Spearman's rho	length_mm	Correlation Coefficient	1.000	.664**
		Sig. (2-tailed)	.	.000
		N	34	34
	MP	Correlation Coefficient	.664**	1.000
		Sig. (2-tailed)	.000	.
		N	34	34

**. Correlation is significant at the 0.01 level (2-tailed).

a. Site = Site 3, position = water column

Correlations[a]

			length_mm	MP
Spearman's rho	length_mm	Correlation Coefficient	1.000	.617**
		Sig. (2-tailed)	.	.000
		N	34	34
	MP	Correlation Coefficient	.617**	1.000
		Sig. (2-tailed)	.000	.
		N	34	34

**. Correlation is significant at the 0.01 level (2-tailed).

a. Site = Site 3, position = sediment

Correlations[a]

			length_mm	MP
Spearman's rho	length_mm	Correlation Coefficient	1.000	-.173
		Sig. (2-tailed)	.	.353
		N	31	31
	MP	Correlation Coefficient	-.173	1.000
		Sig. (2-tailed)	.353	.
		N	31	31

a. Site = Site 5, position = water column

Correlations[a]

			length_mm	MP
Spearman's rho	length_mm	Correlation Coefficient	1.000	-.048
		Sig. (2-tailed)	.	.795
		N	32	32
	MP	Correlation Coefficient	-.048	1.000
		Sig. (2-tailed)	.795	.
		N	32	32

a. Site = Site 5, position = sediment

Correlations[a]

			length_mm	weight_g
Spearman's rho	length_mm	Correlation Coefficient	1.000	.618**
		Sig. (2-tailed)	.	.000
		N	42	42
	weight_g	Correlation Coefficient	.618**	1.000
		Sig. (2-tailed)	.000	.
		N	42	42

**. Correlation is significant at the 0.01 level (2-tailed).

a. Site = Site ORM, position = water column

Correlations[a]

			length_mm	weight_g
Spearman's rho	length_mm	Correlation Coefficient	1.000	.673**
		Sig. (2-tailed)	.	.000
		N	40	40
	weight_g	Correlation Coefficient	.673**	1.000
		Sig. (2-tailed)	.000	.
		N	40	40

**. Correlation is significant at the 0.01 level (2-tailed).

a. Site = Site ORM, position = sediment

Correlations[a]

			length_mm	weight_g
Spearman's rho	length_mm	Correlation Coefficient	1.000	.784**
		Sig. (2-tailed)	.	.000
		N	34	34
	weight_g	Correlation Coefficient	.784**	1.000
		Sig. (2-tailed)	.000	.
		N	34	34

**. Correlation is significant at the 0.01 level (2-tailed).

a. Site = Site 3, position = water column

Correlations[a]

			length_mm	weight_g
Spearman's rho	length_mm	Correlation Coefficient	1.000	.702**
		Sig. (2-tailed)	.	.000
		N	34	34
	weight_g	Correlation Coefficient	.702**	1.000
		Sig. (2-tailed)	.000	.
		N	34	34

**. Correlation is significant at the 0.01 level (2-tailed).

a. Site = Site 3, position = sediment

Correlations[a]

			length_mm	weight_g
Spearman's rho	length_mm	Correlation Coefficient	1.000	.849**
		Sig. (2-tailed)	.	.000
		N	31	31
	weight_g	Correlation Coefficient	.849**	1.000
		Sig. (2-tailed)	.000	.
		N	31	31

**. Correlation is significant at the 0.01 level (2-tailed).

a. Site = Site 5, position = water column

Correlations[a]

			length_mm	weight_g
Spearman's rho	length_mm	Correlation Coefficient	1.000	.789**
		Sig. (2-tailed)	.	.000
		N	32	32
	weight_g	Correlation Coefficient	.789**	1.000
		Sig. (2-tailed)	.000	.
		N	32	32

**. Correlation is significant at the 0.01 level (2-tailed).

a. Site = Site 5, position = sediment

Correlations[a]

			weight_g	MP
Spearman's rho	weight_g	Correlation Coefficient	1.000	.437**
		Sig. (2-tailed)	.	.004
		N	42	42
	MP	Correlation Coefficient	.437**	1.000
		Sig. (2-tailed)	.004	.
		N	42	42

**. Correlation is significant at the 0.01 level (2-tailed).

a. Site = Site ORM, position = water column

Correlations[a]

			weight_g	MP
Spearman's rho	weight_g	Correlation Coefficient	1.000	-.066
		Sig. (2-tailed)	.	.686
		N	40	40
	MP	Correlation Coefficient	-.066	1.000
		Sig. (2-tailed)	.686	.
		N	40	40

a. Site = Site ORM, position = sediment

Correlations[a]

			weight_g	MP
Spearman's rho	weight_g	Correlation Coefficient	1.000	.415*
		Sig. (2-tailed)	.	.015
		N	34	34
	MP	Correlation Coefficient	.415*	1.000
		Sig. (2-tailed)	.015	.
		N	34	34

*. Correlation is significant at the 0.05 level (2-tailed).

a. Site = Site 3, position = water column

Correlations[a]

			weight_g	MP
Spearman's rho	weight_g	Correlation Coefficient	1.000	.239
		Sig. (2-tailed)	.	.173
		N	34	34
	MP	Correlation Coefficient	.239	1.000
		Sig. (2-tailed)	.173	.
		N	34	34

a. Site = Site 3, position = sediment

Correlations[a]

			weight_g	MP
Spearman's rho	weight_g	Correlation Coefficient	1.000	-.222
		Sig. (2-tailed)	.	.230
		N	31	31
	MP	Correlation Coefficient	-.222	1.000
		Sig. (2-tailed)	.230	.
		N	31	31

a. Site = Site 5, position = water column

Correlations[a]

			weight_g	MP
Spearman's rho	weight_g	Correlation Coefficient	1.000	-.043
		Sig. (2-tailed)	.	.817
		N	32	32
	MP	Correlation Coefficient	-.043	1.000
		Sig. (2-tailed)	.817	.
		N	32	32

a. Site = Site 5, position = sediment

Appendix B.

Table 1. Number of different microplastic particles found in blue mussels.

Types of MPs	Site-ORM		Site-3		Site-5	
	Water column	sediment	Water column	sediment	Water column	sediment
Fibres	46	45	38	17	111	119
Black Pellets	46	45	34	43	27	23
Others (beads, fragments, film & foam)	117	116	93	64	86	34
Total	209	206	165	124	224	176

Appendix C.

Table 1. Sample distribution.

Size class & number of Samples	Site-ORM		Site-3		Site-5	
	Water column	sediment	Water column	sediment	Water column	sediment
A (51-60 mm)	16	16	12	12	10	11
B (61-70 mm)	15	14	11	11	11	11
C (71-80 mm)	11	10	11	11	10	10
Total	42	40	34	34	31	32

Total samples= 213

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway