Norwegian University of Life Sciences

Master's Thesis 2017 30 ECTS
Faculty of Environment Science and Natural Resource Management (MINA)

Separation and quantification of Microplastics from Beach and Sediment samples using the Bauta microplastic-sediment separator

SABNAM MAHAT
Master's in Environmental Science: Specialization in Sustainable Water and Sanitation, Health and Development

Separation and quantification of Microplastics from Beach and Sediment samples using the Bauta microplastic-sediment separator

Submitted by:
Sabnam Mahat
Student no: 994155
IMV-MINA

Main Supervisor: Research Prof. Dr. Åsgeir R. Almås (NMBU)
Co-supervisor: Dr. Hans Peter Arp (Norwegian Geotechnical Institute, NGI)

Preface

This thesis is a part of a 30-credit master's thesis at the Department of Environmental Science (MINA), NMBU within the Master's program in Environmental Sciences: Specialization in Sustainable Water and Sanitation, Health and Development.

It is with immense gratitude that I acknowledge my supervisor Research Prof. Dr. Åsgeir R. Almås. This thesis wouldn't have been possible without your help; from arranging all the formal procedures to always having an open door to all my queries and encouraging me all along.

I would like to thank JPI Oceans and Weather-Mic for funding the project, to Norwegian Geotechnical Institute, Oslo (NGI) for hosting me and most importantly to my co-supervisor Dr. Hans Peter Arp for your tireless support and supervision throughout my thesis. Thank you for always creating a comfortable and cheerful ambience during my intensive laboratory months. I consider it my honor to be a part of the NGI family, especially the Environment department. To Linn Merethe Brekke Olsen, with whom I would like to share the credit of my work, thanks for taking care of me and having my back all along; Dorothea Gilbert for your ideas and suggestions; Emma Jane Wade for your selfless help anytime and/or anywhere; Naiara Berrojalbiz for helping me with any technicalities I came across; many other people at NGI who uplifted me morally, thank you all for your best wishes. I also owe my genuine gratitude to Rajesh Joshi and Carl Emil Øyri for your generous guidance on my writing.

This thesis is dedicated to my parents Ratna Bahadur Mahat and Sajana Karmacharya Mahat who have given me the opportunity of education from the best institutions and have supported me throughout; my sister Sandhya Mahat, brother Samir Karmacharya and sister-in-law Monica Bajracharya, for always believing in me and loving me no matter what. I hope I make you all proud.

To my relatives for your support and encouragement, and to all my friends for saving me from monotony, thank you!

It would take another thesis just to put into words how grateful I am towards you all.

With love,

Sabnam

List of abbreviations

BMSS	Bauta Miroplastic-Sediment Separator
FT-IR	Fourier transform- Infrared spectroscopy
GF/C	Glass microfiber filter / grade C
GF/D	Glass microfiber filter / grade D
GF/F	Glass microfiber filter / grade F
HCL	Hydrogen Chloride
HDPE	High Density Polyethylene
HWM	High-water mark
LDPE	Low Density Polyethylene
MP	Microplastics
MPSS	Munich Plastic-Sediment Separator
NaCl	Sodium Chloride
NaI	Sodium Iodide
NGI	Norwegian Geotechnical Institute
PE	Polyethylene
PET	Polyethylene terephthalate
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
WWTP	Wastewater Treatment Plant

Units

µm	micrometer
C	Celsius
d	density
g	gram
h	hour
kg	kilogram
kPa	kilopascal
L	liter
M	molar
mg	milligram
min	minute
mL	milliliter
mm	millimeter
rpm	rotations per minute

Abstract

Microplastic pollution is a topic of scientific interest globally. The environmental impacts caused by microplastics have spurred research focusing on separation and quantification of microplastics from soil and sediments in both fresh and marine waters. Different approaches are used for studies and continuous improvements are made, thus developing a reliable, standardized separation and quantification method has been a challenge.

The Bauta microplastic-sediment separator, based on the concept of Munich plastic-sediment separator, was constructed at NGI, Oslo. An optimized separation protocol was developed using different dense solutions, and a range of laboratory microplastics (lab MP): LDPE pellets, PE fibers, HDPE pellets and PET powder.

Optimized method includes the use of Zinc chloride and Calcium chloride solution (d ~1.6 g/mL) as separation solution and a steel mesh (45µm) as filter. Laboratory sand (0.2-0.7 mm, d =2.6 g/mL) and beach sand (> 200 µm, d > 1.6 g/mL) were spiked with the lab MP to obtain recovery rates. Organic matter separated with the MP were dissolved following a digestion protocol which uses sodium hydroxide: urea: thiourea solution for dissolution, followed by 30% hydrogen peroxide and 10M sodium hydroxide for oxidization of organic matter. Microplastics (< 8mm and > 45µm) from environmental samples: beach plastic debris from Bygdøy sjøbad and effluent sediment from Bekkelaget WWTP were also separated.

Spiking resulted in lower recovery rates of PE fibers from lab sand and beach sand (77 ± 0.05 (s.d) % and 82 ± 0.10% respectively). The beach sand from Bygdøy sjøbad had a significant difference ($p < 0.05$) for the concentration of MP and hard-to-digest organic matter; the difference was found between the high-water mark (HWM) region and HWM -6m region, also between HWM and HWM +6m region. Bekkelaget WWTP samples had an average MP concentration of 27.92 ± 37.37 mg/kg.

This novel technique is a reliable approach to separate microplastics from soil and sediments. Microplastics were present in Bekkelaget WWTP sediment and Bygdøy sjøbad debris samples. The concentration of MP was highest at the high-water zone.

Advanced quantification of the separated microplastics based on their polymers should be performed using available identification techniques. This study will aid as an information tool for further optimization and development of more accurate separation, filtration, quantification and identification methods for microplastics from soil and sediments.

Abstrakt

Bauta mikroplastisk sediment separator, basert på konseptet av plast-sediment separator i München, ble konstruert ved Norges Geoteknisk Institutt, Oslo. En optimalisert separasjonsprotokoll ble utviklet basert på tester ved bruk av løsninger med forsjellig tetthet og laboratoriemikroplaster: LDPE-pellets, PE-fibre, HDPE-pellets og PET-pulver. Separasjonsprotokollen inkluderte bruken av sinkklorid og kalsiumkloridoppløsning (d ~ 1,6 g / mL) som separasjonsløsning og en stålmaske (45 μm) som filter. Mikroplastikk fra laboratoriet ble tilsatt laboratorie sand (0,2-0,7mm, d=2,6 g/mL) og strand sand (vasket Bygdøy sjøbad strand samlet sand, > 200 μm, d> 1,6 g / mL) for å få utvinningsrate. Fordøyelsesprotokollen inneholdt bruk av en løsning av natriumhydroksid, urea og tiourea for oppløsning, etterfulgt av 30% hydrogenperoksid og 10M natriumhydroksyd for oksidasjon av organisk materiale. Videre ble de optimaliserte metodene brukt til å skille mikroplastikk (<8 mm og >45 μm) fra miljøprøver: strand sand fra Bygdøy sjøbad og avløpssement fra Bekkelaget avløpsrensingsanlegg.

Tilsetting av mikroplastikk resulterte i forholdsvis lavere utvinningshastigheter av PE fibre fra laboratoriesand og strandsand (77 ± 0,05% s.d og 82 ± 0,10% s.d). Prøvene fra Bygdøy sjøbad hadde en signifikant forskjell ($p < 0,05$) for konsentrasjonen av mikroplastik og vanskelig å fordøye organisk materiale; forsjellen ble funnet mellom HWM-regionen og HWM-6m-regionen, og mellom HWM og HWM + 6m-regionen. Tilstedeværelsen av mikroplastikk ble verifisert i Bekkelaget WWTP-prøver med en gjennomsnittskonsentrasjon på 27,92 ± 37,37 mg / kg.

Denne nye teknikken er en pålitelig tilnærming til å separere mikroplastikk fra jord og sedimenter. Hypotesene viste seg å stemme, mikroplastikk er tilstede i strender der det ikke finnes tilsynelatende lokale kilder, og i avløpet fra Bekkelaget avløpsvannbehandling. Avansert kvantifisering av de adskilte mikroplastikene basert på deres polymerer bør utføres ved hjelp av utviklede identifikasjonsteknikker. Videre vil denne studien være et informasjonsverktøy for videre optimalisering og utvikling av mer nøyaktige separasjons-, filtrerings-, kvantifiserings- og identifiseringsmetoder for mikroplastikk fra jord og sedimenter.

Translation: Google translate

Edited: Carl Emil Øyri

Contents

1. Introduction

Plastics are synthetic polymers that are typically produced by polymerization of monomers derived from extraction of oil or gas (Thompson, 2009; Cole, 2011). The global production of plastics increased from 1.5 million tons / year in the 1950's to 250 million tons / year in 2011, and the production increases by 10 % annually (Claessens et al., 2011). The post-consumer plastic waste has been recorded as "plastic debris" in habitats from poles to the equator over the last 40 years (Thompson et al., 2004). The buoyant nature of plastic debris favors their dispersion around the globe, allowing them to accumulate in pelagic habitats whereas non-buoyant plastics accumulate in the seafloor and beach sediments (Thompson et al., 2004), where they persist for centuries (Derraik, 2002). Plastic debris undergo environmental degradation under land and marine exposure conditions e.g. high humidity, sunlight-induced heat buildup, fouling, mechanical abrasion by wave actions (Andrady, 1989). Degradation of plastic debris infers to increase in smaller plastic particles, referred here as "microplastics" (Browne et al., 2011).

National Oceanic and Atmospheric Administration (NOAA) defines microplastics as plastic particles in the form of fragments, fibers or beads that are less than 5 mm in their longest dimension and are either primary or secondary type (Cole et al., 2014; Arthur et al., 2008; Barboza and Gimenez, 2015). Primary microplastics are manufactured to be small plastic materials and have specific use e.g. virgin resin pellets used in plastics manufacturing process, microbeads used in cosmetics and personal care products. Secondary microplastics are those derived from degradation of larger plastic items that break down either through their natural life-cycle or due to weathering by ultraviolet radiations, wave actions and other mechanical abrasions (Duis and Coors, 2016).

There are various sources of microplastics; secondary microplastics resulting from weathering of plastic debris being a prominent source at present and in future as well (Barnes et al., 2009). It has also been proven that microbeads used in the personal care and cosmetic products, and microfibers released during washing of garments made up of polyester and nylon, end up in the grey water system (Browne et al., 2011). The microfibers and microbeads are inefficiently removed during the preliminary water treatment process because the municipal waste water treatment systems use filters with larger porosity, and thus microplastics end up in the effluent (Carr et al., 2016).

1.1. Environmental Impacts

Plastics are considered biochemically inert (Teuten et al., 2009) and include organic compounds like ethylene, propylene, vinyl chloride. A range of additives e.g. plasticizers and stabilizers, are added to enhance the properties and performance of plastics (Thompson et al., 2009), and leach out into the environment during degradation (Teuten et al., 2009). Hydrophobic chemicals e.g. oil and alkanes, persistent, bio-accumulative and toxic substances (PBTs) e.g. heavy metals and pesticides adhere to the surface of plastic debris from the environment. Some plastic additives and chemicals like Bisphenol A (BPA), are proven mutagens and carcinogens (Weber et al., 2011, Xu et al., 2013). On land, the chemicals leach out from landfills, where the plastic wastes are disposed (Kim et al., 2006) and the rivers and runoffs transport the leachate into larger water bodies. In seas and oceans, microplastics enhance the transport of these chemicals because of their larger surface area to volume ratio than their parent debris material and their smaller size make them bioavailable to a wide range of organisms in the trophic level (Epa, 2016, Barnes et al., 2009).

Entanglement of marine animals (including birds, turtles, marine mammals and fish) in plastic debris and ingestion of both macroplastics (> 5mm) (e.g. discarded fishing nets, disposable plastic bags) and microplastics (< 5mm) (e.g. microbeads, microfibers) have reported deaths of marine life (Andrady, 2011). NOAA estimates death of 100,000 marine mammals annually, as well as millions of birds and fishes. In addition to the plastic debris and microplastics having potential effects regarding ecosystem changes and on human health, the aesthetics of beaches, shorelines, coasts, sea floors and life of coral reefs have been jeopardized (Lytle, 2016).

1.2. Problem and Current state

Microplastics have gained much attention since their existence for more than four decades (Stolte, 2014) because of their pollution-related problems. Research examining the occurrence and fate of microplastics in the environment have considerably increased (Do Sul and Costa, 2014). Studies are focused on presence of microplastics in marine environment because of their unknown toxicity and potential to transport pollutants that are bioavailable across the trophic level (Andrady, 2011). The problem lies in the uncertainty about the degree to which the chemicals that are leached out in the environment pose threat to human and ecosystem health (Kershaw et al., 2011). Uncertainties are yet to be addressed regarding fate of microplastics after weathering in terms of time scales for fragmentation and degradation, the evolution of particle morphology and properties, and the hazards of the chemical mixture released by weathering. Scientists are also interested in studying the vertical transport i.e. sinking behavior of microplastics, and quantifying the microplastics sunken below the water surface and those buried in the sediments (Jahnke et al., 2017). Besides effluent sediments being a major source of microplastics in fresh and marine water bodies, sewage sludge is also used for land application as fertilizer (Singh and Agrawal, 2008) and assessing the organic and plant nutrient rich sludge for presence of other contaminants including microplastics is another dimension of understanding the fate of microplastics in the environment (Sujathan et al., 2017).

Many isolated reports on microplastics contamination of sandy, estuaries and subtidal habitats are present but they fail to quantify the global extent of contamination (Browne et al., 2011). Experimental methods are developed to separate microplastics using density separation and digestion of organic matter from beach and sediment soil samples. But the studies (e.g. Thompson, 2004; Claessens et al., 2011; Imhof et al., 2012; Nuelle et al., 2014) vary in their approach, for example: sample size, sampling locations, separator solution, polymers of microplastics, digestion methods etc. Thus, development of a standardized quantification method of plastics and microplastics in the marine sediment, beach sand, sewage sludge and soil has been a major challenge (Nuelle et al., 2014).

1.3. Objectives / Hypothesis

There were two main focuses of this study:

i. To develop an improved technique of separating microplastics (>75µm) using the Bauta MSS.
ii. To use the developed optimized methods to separate microplastics (< 45 µm and > 8mm) from real world samples: Beach sand and WWTP effluent sediment.

Two hypotheses were defined to validate the study aims:

i. Higher concentration of microplastics is expected along the high-water mark than other areas on Bygdøy sjøbad beach area, Oslo.
ii. Microplastics can be found in sediments from Bekkelaget WWTP effluent, Oslo

2. Materials and methods

2.1. Chemicals

All the chemicals used during solution preparation and digestion have been listed in the table below (Table 1) with their molecular formulas, manufacturers and the purities.

Table 1: List of chemicals used, name of the manufacturer and their purity

Chemicals Used	**Molecular / Linear formula**	**Manufacturer / Distributor**	**Purity (%)**
Zinc chloride	$ZnCl_2$	VWR International	97
Calcium chloride	$CaCl_2$	VWR International	90-98
Hydrogen peroxide	30% H_2O_2	VWR International	Analytical grade
Urea	$CO(NH_2)_2$	Sigma Aldrich	≥98
Thiourea	CH_4N_2S	Merck KGaA	≥ 98.0
Sodium Hydroxide	NaOH	Merck KGaA	Acidimetric, NaOH 99-100 Total Alkalinity calculated as NaOH 99-100
Sodium dodecyl sulfate	CH3(CH2)11OSO3Na	Sigma Aldrich	≥99.0 (Gas Chromatography)

2.2. Solution Preparation

Calcium chloride solution ($CaCl_2$)

10 L of $CaCl_2$ solution was prepared by mixing 350 g of analytical $CaCl_2$ (VWR International, Germany) saturated salt in 10 L of distilled water. Since the reaction is exothermic, the solution was prepared inside a fume hood and stored in a plastic carbouy. The carbouy was placed in a water trough to avoid overheating. A density of 1.3 g/mL was obtained and the solution was not filtered before using for separation.

Zinc chloride: Calcium chloride solution ($ZnCl_2$:$CaCl_2$)

30 L of $ZnCl_2$:$CaCl_2$ solution was prepared in 3 carbouys. For each 10 L of the solution, analytical $ZnCl_2$ saturated salt (VWR International, Germany), analytical $CaCl_2$ saturated salt (VWR International, Germany), and distilled water were used in ratio by weight 2: 1.4: 4.4 $ZnCl_2$: $CaCl_2$: H_2O respectively (Hudgins, C.M., 1964) (Appendix 1). Instructions for solution preparation revealed by Imhof et al. (2012) was followed. The instructions included use of appropriate personal protective equipment; the carbouy was placed in an ice trough inside a fume hood.

To check the density of fresh solution, 100mL of the solution was transferred using a 10mL plastic pipette into a pre-weighed Falcon-tube, and weighed again to calculate the density of the solution. The final densities of the solution batches prepared are listed in table 2.

Table 2: Final densities of $ZnCl_2$:$CaCl_2$ solution prepared in different batches

Carbouy	**Solution Temp ° C**	**Solution density g/mL**
1	12	1.57
2	12	1.58
3	12	1.58
Average solution density		1.57

A density of approximately 1.6 g/mL was obtained and the solution was filtered through Whatman glass fiber filter GF/D grade (pore size 2.7 µm) to remove precipitate. One run of 250 mL fresh $ZnCl_2$:$CaCl_2$ solution took 5-8 min to filter using a vacuum pressure filtration system. A cake of precipitate as shown in the figure 1 was retained over the filter paper.
A pressure of 80-100 kPa was required to filter 250 mL solution in around 8-10 minutes. After filtering 250 mL of fresh $ZnCl_2$:$CaCl_2$ solution, the filter paper was changed.

Figure 1: Precipitate filtered out from fresh $ZnCl_2$:$CaCl_2$ solution on a GF/D filter paper (2.7 µm)

For chemical digestion, a solution of NaOH: $CO(NH_2)_2$: CH_4N_2S was prepared in ratio by weight 8: 8: 6.5 respectively. 30% Hydrogen Peroxide (H_2O_2) diluted from 50% H_2O_2 in ratio 2:3 50% H_2O_2: H_2O (deionized) and 10M concentration of NaOH were also prepared using standard titration method.

2.3. Microplastic samples

4 types of laboratory microplastic (lab MP) were used for spiking, namely: HDPE pellets, PET powder >75µm, LDPE pellets and PE fibers (Good fellow, United Kingdom) The specific density of PE ranges from 0.92 to 0.97 g/mL and that of PET ranges from 1.37-1.45 g/mL (Hidalgo-Ruz et al., 2012)

10 g of PET powder was sieved through 75 µm filter and only the powder particles >75µm were used for spiking. 1 m long PE fiber was shredded into smaller fragments (Fig 2) and used for spiking.

Figure 2: PE fibers shredded into small fragments and 0.1 g weighed on a sensitive scale (0.1 g) for spiking

2.4. Design and setup

The concept was to facilitate density separation by using a high-density solution that allows light weight matter to float, hence separating them from the heavier matter in any soil and sediment sample. Inspired by the Munich Plastic-sediment separator (MPSS) developed by Imhof et al. (2012), Bauta Microplastic-sediment separator (BMSS) (Fig 3) was developed by Norwegian Geotechnical Institute (NGI), Oslo. The design was modified based on the functionality of BMSS. Currently, NGI owns three systems with different motor speeds (5:1, 10:1, 50:1) (Fig 3) so that the stirring of sediment, soil and sand samples can be adjusted based on the volume and type of sample.

Figure 3: Three Bautas in running condition at NGI. The left-most Bauta (5:1) contains Bygdøy sjøbad beach samples; Bekkelaget sediment introduction in under process in the middle Bauta (50:1); the right Bauta (10:1) is being used for spiking of lab sand

Figure 3 and Figure 4 (left) show a running setup of BMSS with aqueous Zinc Chloride: Calcium chloride solution inside the separator. Figure 5 shows a simplified and labeled design of BMSS designed by NGI.

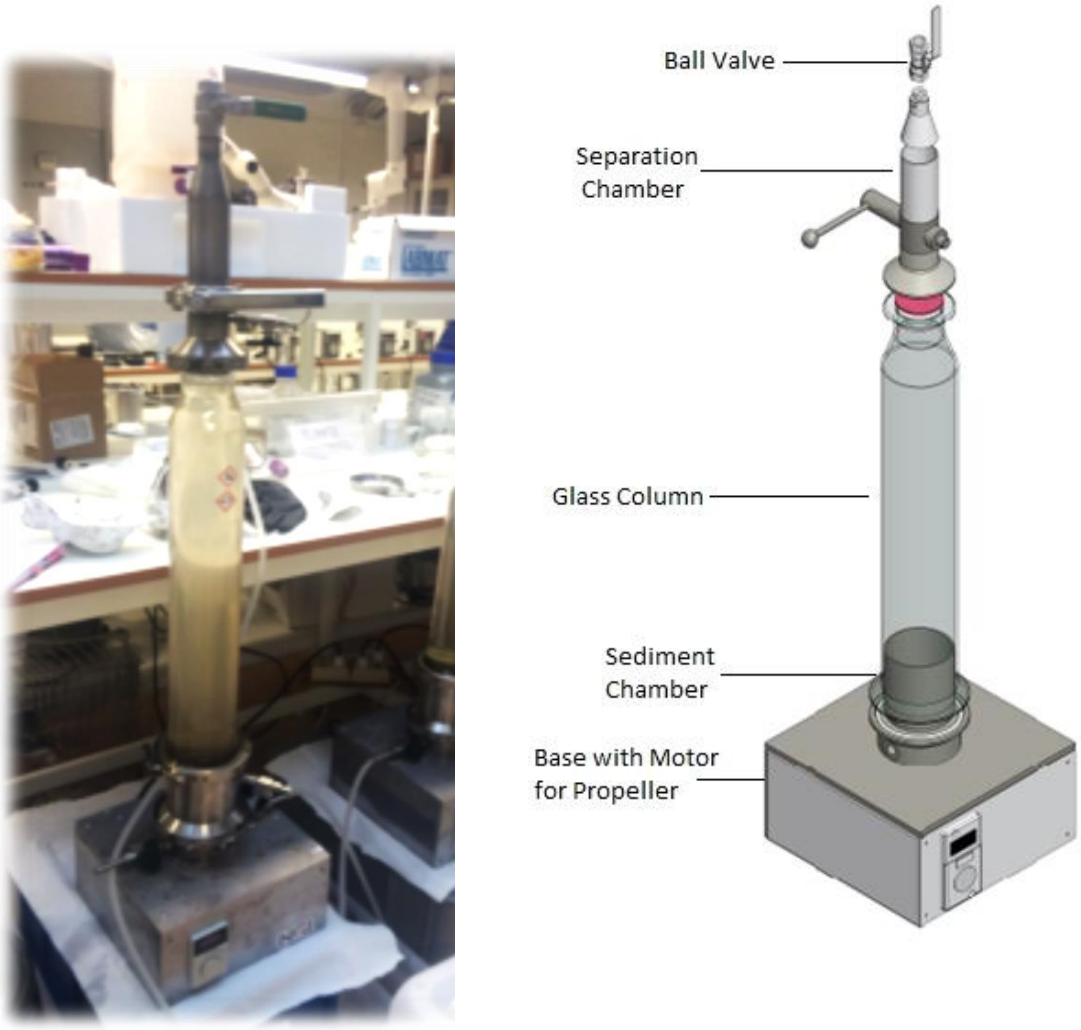


Figure 4: (Left) Bauta Microplastic-sediment separator assembled together and filled with $ZnCl_2$:$CaCl_2$ solution

(Right) Labelled design sketch of Bauta Microplastic-sediment separator

Source: Hans Peter Arp; Dorothea Gilbert; Philip B. Hayes, NGI

The BMSS is divided into three major components: the sediment chamber, the glass column and the separation chamber (Fig 4 right) and are described well along. These three components are equipped with Viton O-rings and connected using metal ring clamps.

Sediment chamber

The sediment chamber is a 126cm high cylinder that can hold 950 cm^3 volume of settled sediment. A propeller is connected to an electric motor which is mounted on a stainless-steel base (Fig 4 right) and serves as the bottom of the Bauta. The propeller speed can be adjusted up to 4000 rpm depending on the volume of sediment being analyzed on a single run. The sediment chamber is also mounted over this base (Fig 4 right). The propeller base is integrated with 2 valves: main outlet and inlet. In addition, the sediment chamber has a secondary outlet valve to drain out the separation solution.

Glass Column

The glass column is 650 cm tall transparent cylinder with a constricted neck at the top (Fig 4 right). The diameter at the top is reduced, while the body of column has an inner diameter of 90 mm. The height of the column allows better separation distance between the dense material and lighter microplastic particles and other organic matter.

Separation Chamber

The sample separation chamber (Fig 4 right) has a ½" ball valve and a shut-off valve. Both valves can be closed and the chamber containing light density sample can be disconnected from the BMSS. The chamber can hold a sample volume of 220mL. When inverted, the chamber allows extraction of the light density sample for filtration.

2.5. Method Optimization

Four methods were optimized for Separation, Filtration and Chemical Digestion.

Method 1: $CaCl_2$ solution (d=1.3 g/mL) was used as separation solution. The lab MP (PE powder and PE fibers) were introduced at the bottom in sediment chamber (section 2.4, Fig 4). The propeller speed was set to 4000 rpm. The PE powder and PE fibers stuck to the walls of glass column and the separation chamber (section 2.4, Fig 3). Lower recovery rates were obtained. Round glass microfiber filters GF/C grade (pore size 1.2 µm, diameter 47mm) and GF/F grade (pore size 0.7 µm, diameter 47mm) were used as filter papers.

Method 2: $CaCl_2$ solution (d=1.3 g/mL) was used as separation solution. The lab MP (LDPE pellets, HDPE pellets, PE fibers and PE powder) were introduced from the top of the glass column (section 2.4, Fig 4). The propeller was set to 180 rpm. The PE powder and PE fibers stuck to the walls of glass column and the sediment chamber (section 2.4, Fig 4). Vertical transport of PE fibers followed by homogeneous distribution along the solution column was observed resulting in longer density separation time and lower recovery rates. Round glass microfiber filters GF/C grade (pore size 1.2 µm, diameter 47mm) and GF/F grade (pore size 0.7 µm, diameter 47mm) were used as filter papers.

Method 3: $ZnCl_2$:$CaCl_2$ solution (d= 1.57 g/mL) was used as separation solution. Lab MP (PE fibers, LDPE pellets, PET powder, HDPE pellets) were introduced from the top of glass column (section 2.4, Fig 4). The propeller speed was set to 180 rpm. Solution precipitate was retained on the round glass microfiber filters GF/D grade (pore size 2.7 µm, diameter 47mm) and GF/C grade (pore size 1.2 µm, diameter 47mm) filtered using a bottom-top filter and vacuum pump.

Method 4: Fourth optimization step included finalization of $ZnCl_2$:$CaCl_2$ solution as separation solution. Lab MP (PE fibers, LDPE pellets, PET powder > 75, HDPE

pellets) were introduced from the top of the glass column (section 2.4, Fig 4). The propeller speed was set to 4000 rpm and reduced to 180 rpm after 30 minutes. The filter papers were replaced by steel mesh with porosity 45 µm. It was possible to rinse off $ZnCl_2$:$CaCl_2$ solution precipitate from the steel mesh using distilled water but impurities like Zinc chloride crystals and contamination from lab air were present. Thus, the dry weights of recovered MP were blank corrected (section 3.1, Fig 24A).

The separation protocols with the results are summarized in Table 2.

Table 2: Protocols used during optimization of the separation method, listing the separation solutions used, their densities, types of microplastics used for tests, direction of the sample introduction in the Bauta, the type of filters used during filtration of light weight particles and the type of filter used for solution purification

ID	Separation solution	Density g/mL	Microplastics	Sample introduction	Sample Filtration	Remarks
1.	$CaCl_2$	1.3	PE powder PE fibers	Bottom	GF/C (1.2 µm)	Stuck to the Bauta glass column walls and the separator chamber walls; lower recovery rates
2.	$CaCl_2$	1.3	LDPE HDPE PE fibers PE powder	Top	GF/C (1.2 µm) GF/F (0,7 µm)	Low density of $CaCl_2$ resulting lower recovery rates of PE fibers; Contaminated PE powder
3.	$ZnCl_2$: $CaCl_2$	1.57	PE fibers LDPE pellets PET powder HDPE pellets	Top	GF/D (2.7 µm) GF/C (1.2 µm)	Higher recovery rates of microplastics but precipitate of $ZnCl_2$:$CaCl_2$ solution on the filter paper

4.	$ZnCl_2$: $CaCl_2$	1.57	PET powder (> 75 µm) PE fibers LDPE pellets HDPE pellets	Top	Steel mesh (45 µm)	Higher recovery rates of microplastics, no precipitate of $ZnCl_2$:$CaCl_2$ solution on steel mesh

A protocol was already developed by Linn MB Olsen and Hans Peter Arp (Norwegian Geotechnical Institute, NGI) based on chemical digestions of organic matter ~1g. The method needed further testing for larger samples. A progressive protocol was hence developed (Table 3).

Table 3: Protocols for chemical digestion using Sodium hydroxide: Urea: Thiourea solution and Hydrogen Peroxide for dissolving organic matter

Id	Weight of sample (g)	Volume of 30% H_2O_2 (mL)	Shaking method	Soaking time (min)	Number of digestions
1	4 - 10	80	Magnetic stir bars Mechanical shaker	45	3
2	< 2	60	Magnetic stir bars	30	Max. 2

2.6. Spiking

40g of laboratory sand (grain size 0.2-0.7mm, d = 2.6 g/mL) was spiked with known weights of lab microplastics: PET powder, PE fibers, LDPE pellets and HDPE pellets. Approximately 0.1 g of PET powder (>75 µm) and ~ 0.1 g of shredded PE fibers were weighed. Similarly, 6 pellets of LDPE and 12 pellets of HDPE were used for spiking.

The beach plastic debris samples were collected from the sediment chamber (Section 2.4. Fig 4) after density separation in the Bauta (section 2.8). They were rinsed thoroughly with distilled water over a filter of 300 µm, oven dried at 110 °C for 72 hours and stored in glass jars. These samples are apparently free of organic matter and microplastics, referred here as "clean environmental samples (CES)".

The known weights of selected lab MP were added to 40 grams of CES. The sample introduction protocol (section 2.8) was followed for introduction of these spiked samples in the Bauta.

2.7. Sampling and Sample preparation

Beach plastic debris samples (including sand and organic matter) were collected from Bygdøy sjøbad in Oslo municipality and effluent sediment samples were collected from Bekkelaget WWTP, Oslo municipality to test the hypotheses.

2.7.1. Beach plastic debris samples

Sampling

The beach sampling location was chosen based on noticeable amount of marine and anthropogenic debris. 7 beach samples containing plastics debris with sand were collected from Bygdøy sjøbad, Oslo (59°54'39.4" N 10°39'58.8" E) (Fig 5) on 17th of March 2011, between 11:00 - 13:00 h.

Figure 5: Google satellite image of beach sampling site Bygdøy sjøbad (59°54'39.4" N 10°39'58.8" E), Oslo; the red area marks the area of sampling

The sampling points were the high-water mark (HWM), 6 meters above (HWM + 6m) and 6 m below (HWM - 6m) the high-water mark (Fig 6). A wooden scale was used to measure

an area of 40 x 40 cm^2 (Fig 6) and material up to 2 cm deep was scooped out with a stainless-steel spoon and/ or a soil scoop, into polypropylene (PP) plastic buckets (Fig 7). The distance between sampling points along the water marks was a random selection. Noticeable amount of marine and anthropogenic debris was present on site.

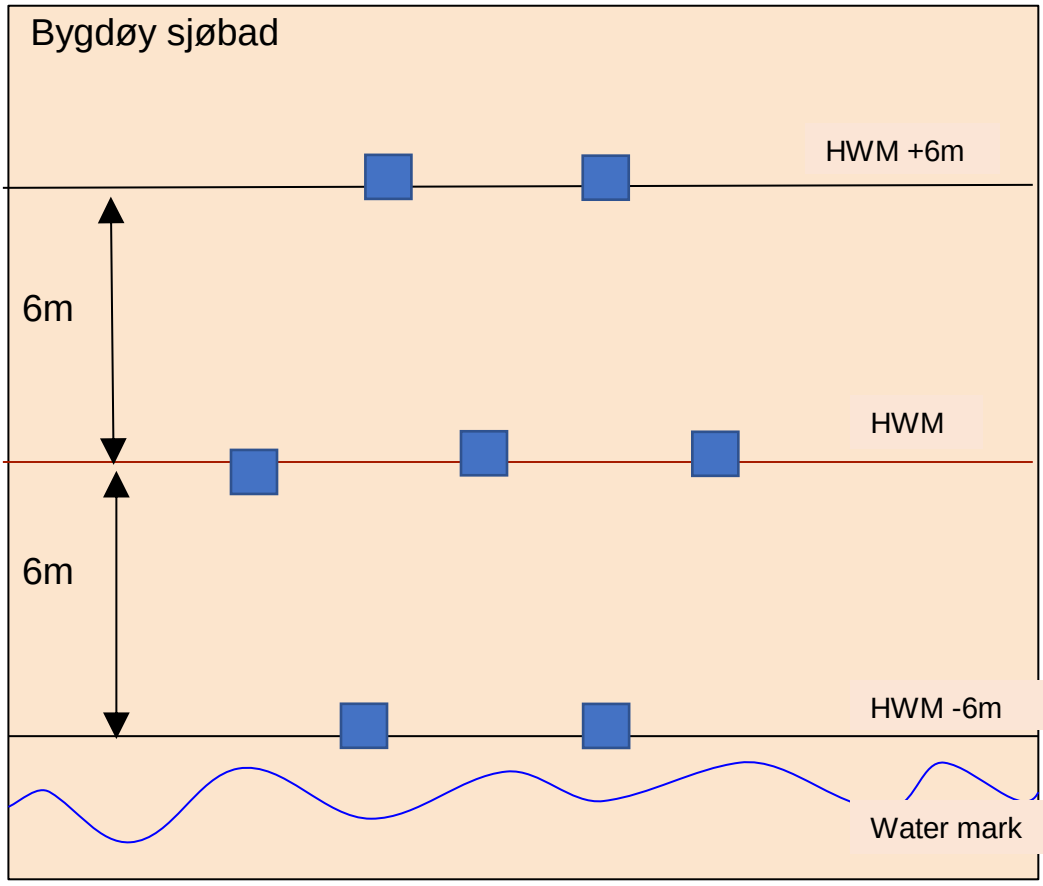


*Figure 6: Sampling design for Bygdøy sjøbad; the squares in each line represent the number of samples taken with a metal spoon and soil scoop, and has an area of 40*40cm^2, 2cm*

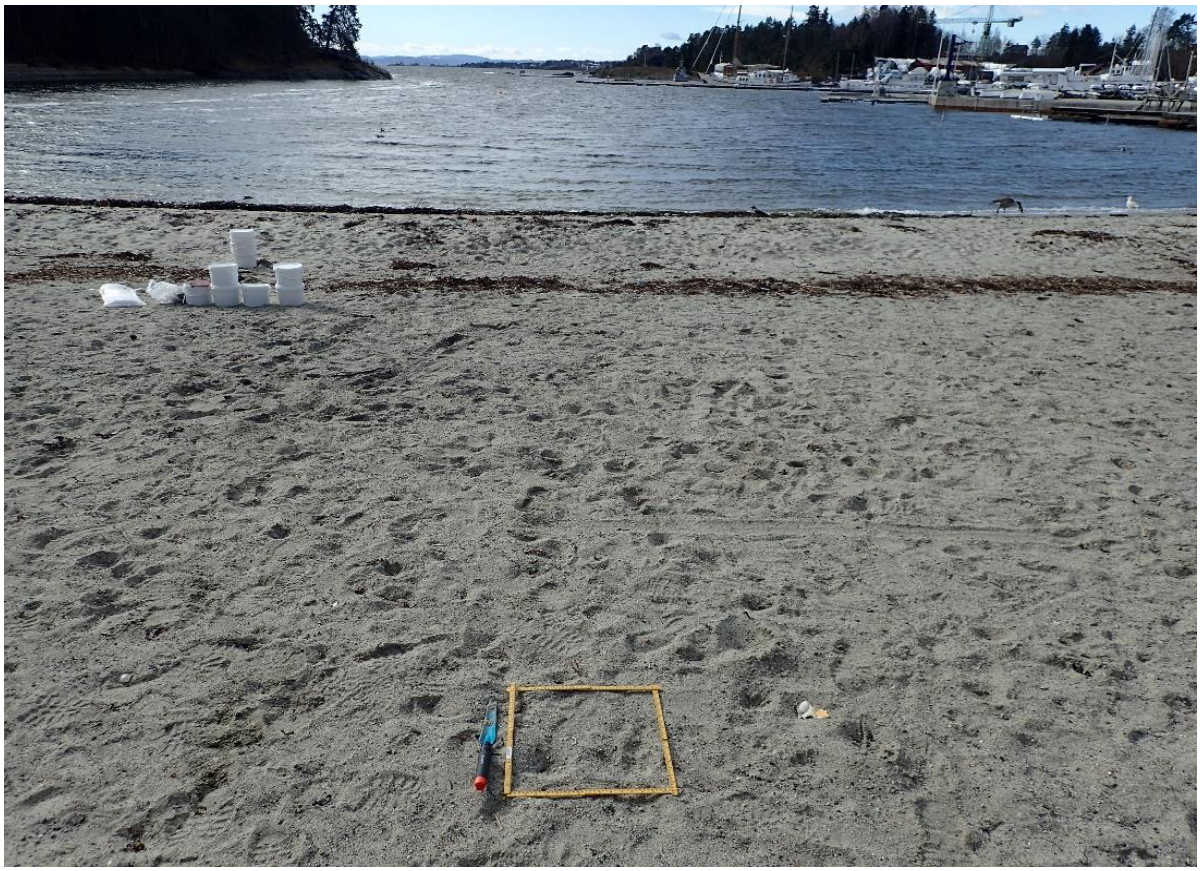


*Figure 7: Sampling site in Bygdøy sjøbad. A wooden ruler is placed marking an area of 40*40 cm^2 on one of the sampling points on HWM+6m. Further towards the sea, the HWM can distinguished by the presence of debris accumulated. The HWM-6m, nearest to the sea is noticeable by the accumulation of debris.*

*Figure 8: (Left) Demarcating 40 * 40 cm2 on the HWM before sample collection*

(Right) Beach debris up to 2cm deep being collected into a polypropylene (PP) bucket using a soil scoop

Photos source: Hans Peter Arp

Sample preparation

The collected beach debris samples were labelled and stored in pre-weighed PP buckets in laboratory at NGI. The buckets were weighed to obtain wet weights of the sample and covered with aluminum foil (Fig 9). The samples were air-dried at room temperature for 72 h, and their air-dried weights were recorded. Sieving of these samples through 8mm sieves (Fig 10) separated large debris and macroplastics > 8mm. The macroplastics were archived (Fig 11), and the debris (> 8mm) were discarded. The sieved samples (<8mm) were then sorted for large debris and organic matter using hand-picking method. A pair of metal tweezers were used to pick large debris. The samples were weighed again and distributed

Figure 9: Samples stored in PP buckets and air-dried at room temperature for 72 hours

Figure 10: Sieving of samples through 8mm sieve and discarding debris

Figure 11: Macroplastics hand-picked and stored in LDPE zip-lock bags

Figure 12: Sample distributed in pre-weighed aluminum trays and weighed before oven-drying at 60 °C, 24h

into pre-weighed aluminum trays. The aluminum trays were put into oven for drying at 60 °C for 24 h (Fig 12). The weight loss due to evaporation was noted.

2.7.2. Sediment samples

Sampling

Sediment samples from Bekkelaget WWTP (Fig 13) were collected by the NGI team on 9th March 2016, with assistance of the research vessel Trygve Braarud. 6 sediment samples were collected using a crane-mounted Van Veen sampler, mounted on a crane. Once collected, the latch on top of the Van Veen sample was opened, and either the top 5 cm or to 10 cm was collected using a spoon into a large polypropylene sample bucket.

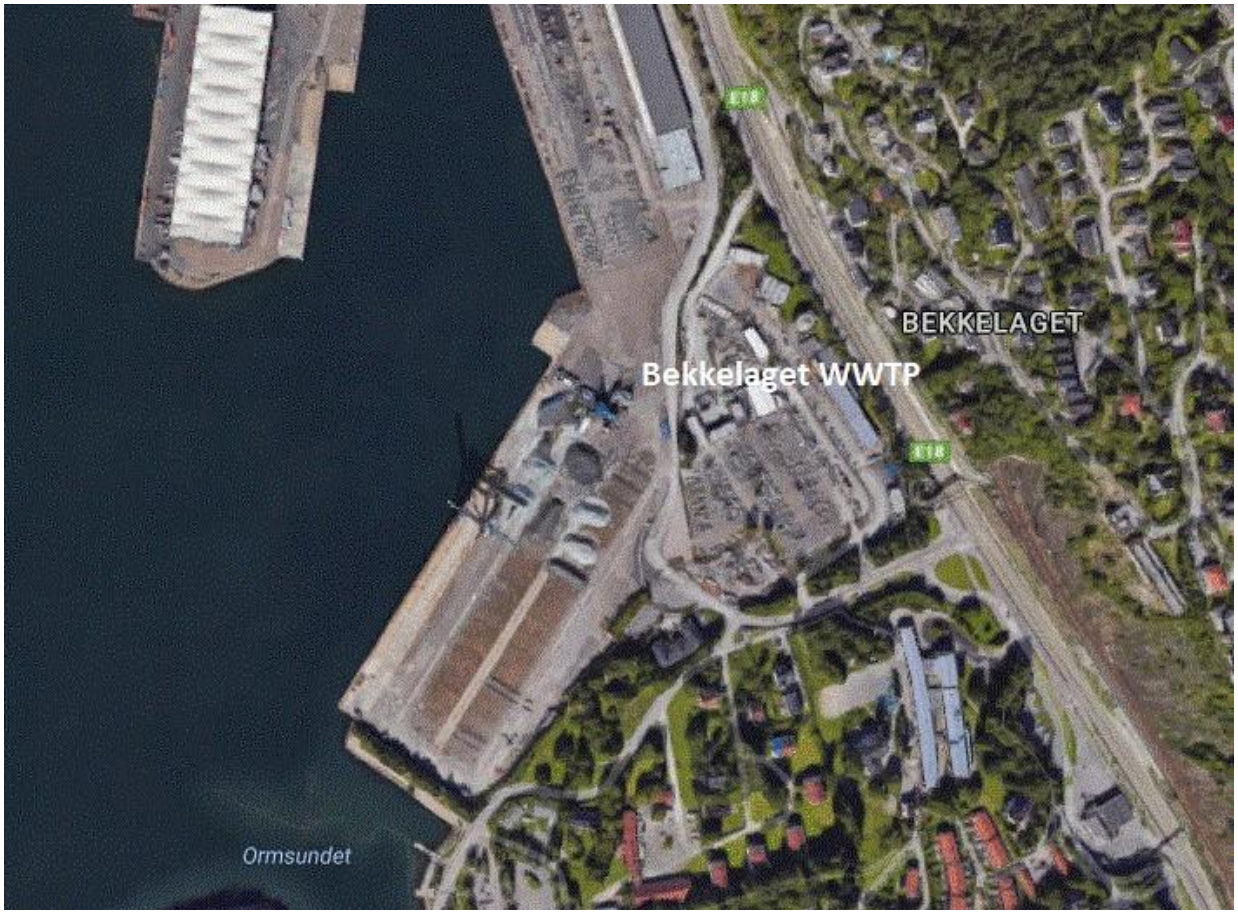


Figure 13: Satellite image of sediment sample site Bekkelaget waste water treatment plant in Oslo

Before analyzing the sediment samples for microplastics, their carbon and nitrogen content were analyzed. Carbon 13C and Nitrogen 15N were analyzed by taking approximately 0.1 g of dried, homogenized sediment, crushed in a mortar and pestle, and placed in small tin capsules (N.C. Technologies srl), and weighed. Subsequently, 1 M hydrogen chloride (HCl) was added drop wise to remove carbonates, with one drop about every 30 minutes. The carbonates were considered removed when no more bubbles appeared upon addition of 1 M HCl. The tin capsules were then placed in a 96-well plate, and sent to the Stable Isotope Facility of UC Davis for analysis. A description of the instruments and handling protocols can be found at: http:/stableisotopefacility.ucdavis.edu/13cand15n.htmL

Bekkelaget sediments varied in organic matter content in the form of biota, where F3 was rich in biota (Fig 14) and F1 was very black indicating absence of biota. Moreover, plastic was observed in the F3 sample.

Figure 14: Bekkelaget WWTP sampling site F3, rich in biota

Photo source: Hans Peter Arp

Sample preparation

Dry weights were obtained by placing approximately 100 g of wet, homogenized sediment in a pre-weighed aluminum tray. The aluminum tray was then placed in a drying oven at 110 ° C for a minimum of 16 hours. The weight loss due to evaporation was noted.
Among the 6 sediment samples taken from Bekkelaget, only 3 were tested for microplastic presence: F1 (59°83’63” E 66°39’57.9” N), F3 (59°82’74” E 66°39’57.6” N), F4 (59°82’59” E 66°39’69.4” °N).

The samples were stored at 4°C. The weights of the polypropylene sample buckets with the sample were taken without their lids after the samples reached room temperature. A long glass rod was used to homogenize the samples (Fig 15) and 500 g of the samples were weighed in pre-weighed aluminum trays for introduction into the Bauta (Fig 16).

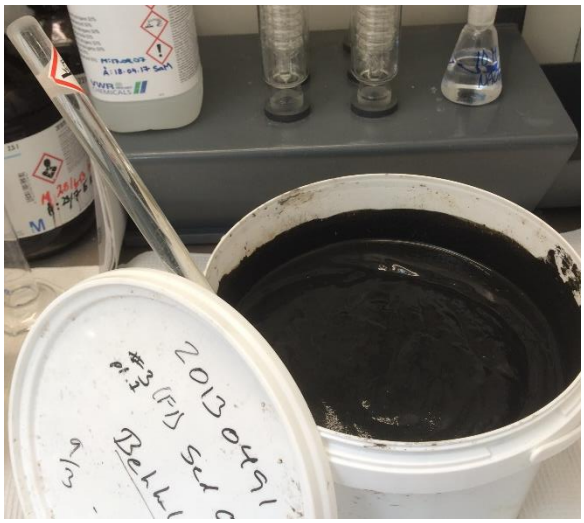


Figure 15: Homogenization of sediment sample (F1) from Bekkelaget WWTP. A long glass rod is used to mix the sediment well.

Figure 16: 500 g Homogenized sediment sample (F4) in a pre-weighed aluminum tray

2.8. Separation

The separation method follows a density separation approach like the "Munich Plastic Sediment Separator (MPSS)" by Imhof et al. (2012). The MPSS was an important motivation for the construction of the Bauta Microplastic-Sediment Separator (BMSS). Laboratory manual for microplastics analysis developed by NOAA (Masura et al., 2015) was also used as a guideline.

Assembly of Bauta

The sediment chamber and glass column were mounted on the Bauta base (section 2.4) and checked for leak-proof mounting using ring clamps. The top of glass column was covered with an aluminum foil to avoid contamination from air. A silicon tube was used to connect the inlet valve of Bauta to the carbouy containing filtered $ZnCl_2$:$CaCl_2$ solution. The column was filled with the solution up to 7cm below the top: a level just below the constriction. The propeller was turned on at maximum speed (4000 rpm) to stir up any impurities at the bottom and the walls, and to maintain homogeneity of the solution with regards to the density.

Sample Introduction

A slurry was prepared by adding filtered $ZnCl_2$:$CaCl_2$ solution to dry beach plastic debris sample in aluminum tray (section 2.7.1). Making a slurry reduced surface tension between the sand particles and $ZnCl_2$: $CaCl_2$: H_2O molecules, allowing the sand particles to sink without much particles sticking to the glass column wall. The slurry was scooped out carefully using a metal spoon into the Bauta. A large glass funnel was used on the column for safety from splashing. The top of the solution was stirred with the spoon. Then, the aluminum tray, spoon and funnel were washed with additional $ZnCl_2$:$CaCl_2$ solution into the column. It was made sure that the solution level did not exceed 1 cm below the top of column for safety from spilling.

Unlike beach debris sample, the sediment samples were already a slurry with about 60% moisture content. 500 g of homogenized sample (section 2.7.2) was introduced into the

Bauta using a metal spoon and glass funnel. The top of the solution was stirred with the spoon. Then, the aluminum tray, spoon and funnel were washed with additional $ZnCl_2$:$CaCl_2$, as mentioned before.

Separation in Separation chamber

The separation chamber (section 2.4) was rinsed with $ZnCl_2$:$CaCl_2$ solution (d = 1.6 g/mL) using a glass pipette over a Schott bottle of 250 mL before mounting it on the Bauta. The propeller speed was reduced to 180 rpm and solution was filled-in through the inlet. The propeller was stopped after 20 minutes. The level of solution along with the light density floating matter was monitored through the open end of ball-valve (Fig 4) of the separation chamber. The carbouy's tap, inlet valve and the ball valve was closed after the chamber was filled. The shut-off valve and inlet valve were opened and closed a minimum of 5 times to remove any air trapped in the inlet pipe. However, the shut-off valve was left open to allow more floating materials to rise inside the separation chamber.

To dismount the separation chamber, 300 mL of solution needed to be drained out through the secondary outlet valve. The pressure valve was opened and the used $ZnCl_2$:$CaCl_2$ solution was collected in a Schott bottle for filtration. A safe level of the solution is at least 3 cm below the top of glass column. The separation chamber was then dismounted and was followed by filtration.

2.9. Filtration

The sediment chamber was inverted and supported on an iron stand over a glass funnel with long stem, and a Schott bottle (250 mL) underneath (Fig 17).

Figure 17: Setup for filtration; sediment chamber is inverted and supported on an iron stand. The glass funnel has a steel mesh (45 µm) inside. A Schott bottle is kept underneath the funnel, with the stem of the funnel inside the Schott bottle

A pre-weighed steel mesh (45 µm, size 11 * 11 cm^2) was adjusted inside the funnel and wetted with Alfa-Q water. The shut-off valve was opened first to release the pressure, and the ball valve was opened carefully to prevent overflowing and spilling of $ZnCl_2$:$CaCl_2$ solution with separated sample. The separated sample was filtered and the $ZnCl_2$:$CaCl_2$ (filtrate) was collected in Schott bottle for reuse. The sediment chamber was rinsed with

distilled water, using a wash bottle. A glass pipette was used to pipette in any samples on the outer rim of the chamber. The filtrate was collected in a polypropylene bucket. Rinsing the steel mesh is important to wash off any Zinc chloride salt on the steel mesh.

The steel mesh was then folded and sample was sealed (Fig 18 and 19) carefully into an envelope. The sealed steel mesh with filtered samples were labelled in a pre-weighed glass jars and oven-dried at 60 °C for a minimum of 17 hours. The dry weights of filtered samples were obtained.

The samples in the steel mesh were secured for chemical digestion by using pre-weighed steel wires

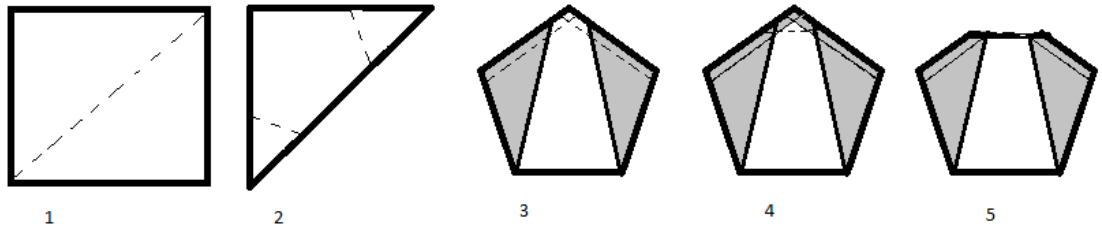


Figure 18: Folding technique of a square steel mesh to seal sample after filtration

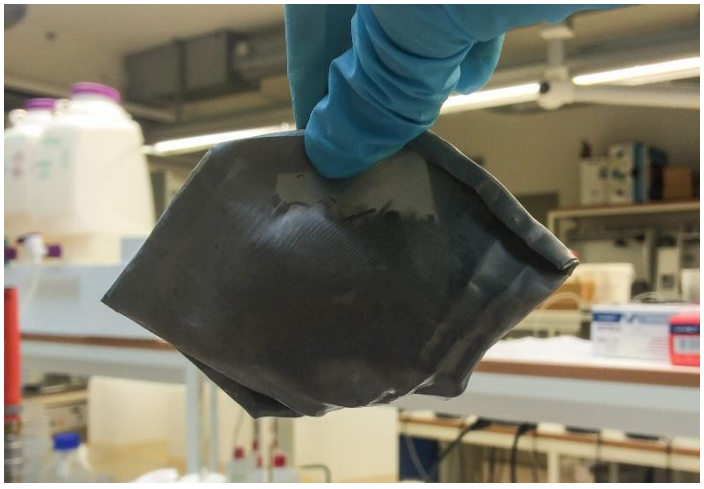


Figure 19: Folded Steel mesh (45µm) into an envelope containing sample

2.10. Chemical Digestion

The dissolution of cellulose and chitin in the organic matter was performed using a solution of Sodium hydroxide: Urea: Thiourea [NaOH: $CO(NH_2)_2$: CH_4N_2S] (protocol developed by Linn Merethe Brekke Olsen, NGI, in 2016) and adjusted to better fit the needs of the samples.

80 mL of NaOH: $CO(NH_2)_2$: CH_4N_2S solution per 2 grams dry weight of filtered sample was used. The samples were soaked in the NaOH: $CO(NH_2)_2$: CH_4N_2S solution (Fig 20) for approximately 45 minutes at -20 °C. Samples were monitored after 30 minutes inside the freezer to avoid crystallization of the solution (Fig 21). The vials were taken out from freezer and placed inside a fume hood on a stir plate. A magnetic stir bar was included in the vial. The samples were stirred for 30 minutes or until the solution reached room temperature. Some samples with larger volume of solution took 1.5 h to reach the room temperature. Samples were rinsed a minimum of 15 times with Milli-Q water; soaking for 15 minutes every 5^{th} wash to get rid of the residual solution and any dissolved organic matter.

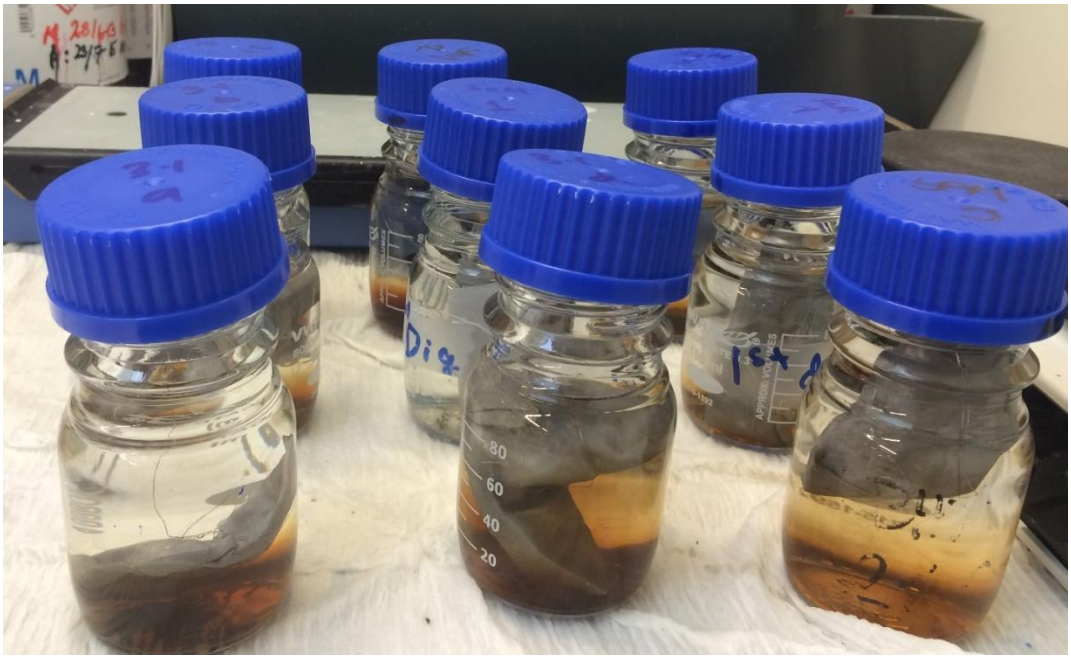


Figure 20: Soaking samples enclosed in steel mesh in NaOH: $CO(NH_2)_2$: CH_4N_2S solution. The coloration of solution was observed resulting from dissolution of cellulose

Figure 21: Crystallization of H_2O_2 solution at -20 °C when left in freezer for longer than 45 min

60 mL of 30% Hydrogen peroxide (H_2O_2) per 2 grams of sample was added into the vial for oxidation. 1.5 mL of 10M NaOH was added as a catalyst. The vials were enclosed with an open cap containing a steel mesh to let out the mist produced (Fig 22).

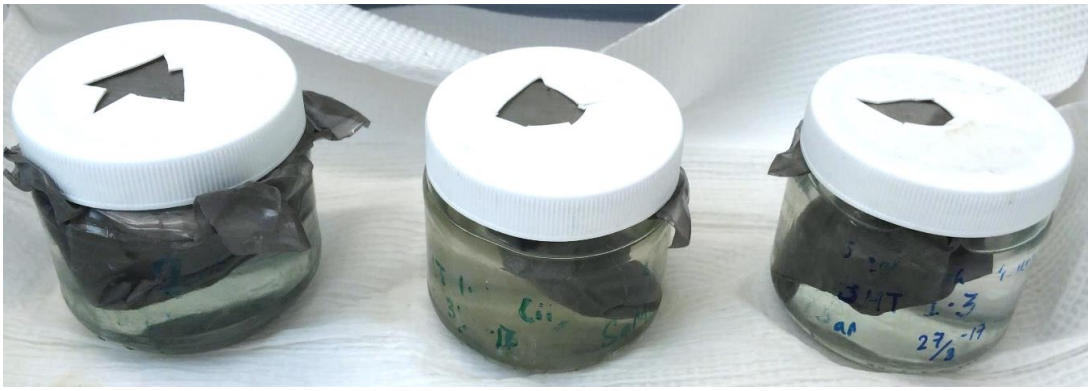


Figure 22: Vials containing samples for chemical digestion enclosed with an open cap to let out the mist produced during reaction.

The solution with the samples were stirred over magnetic stir plates at room temperature for at least 3 hours. The reaction was violent as mentioned in NOAA manual and the solution boiled up producing Sodium peroxide (Na_2O_2) and an exothermic mist. To avoid any accidents from overflowing solution and burns from exothermic mist during the reaction, the sample vials were placed inside tall plastic containers (Figure 23).

Figure 23: Sample vials kept inside a tall plastic container for safety from spilling of boiling solution and the exothermic mist produced

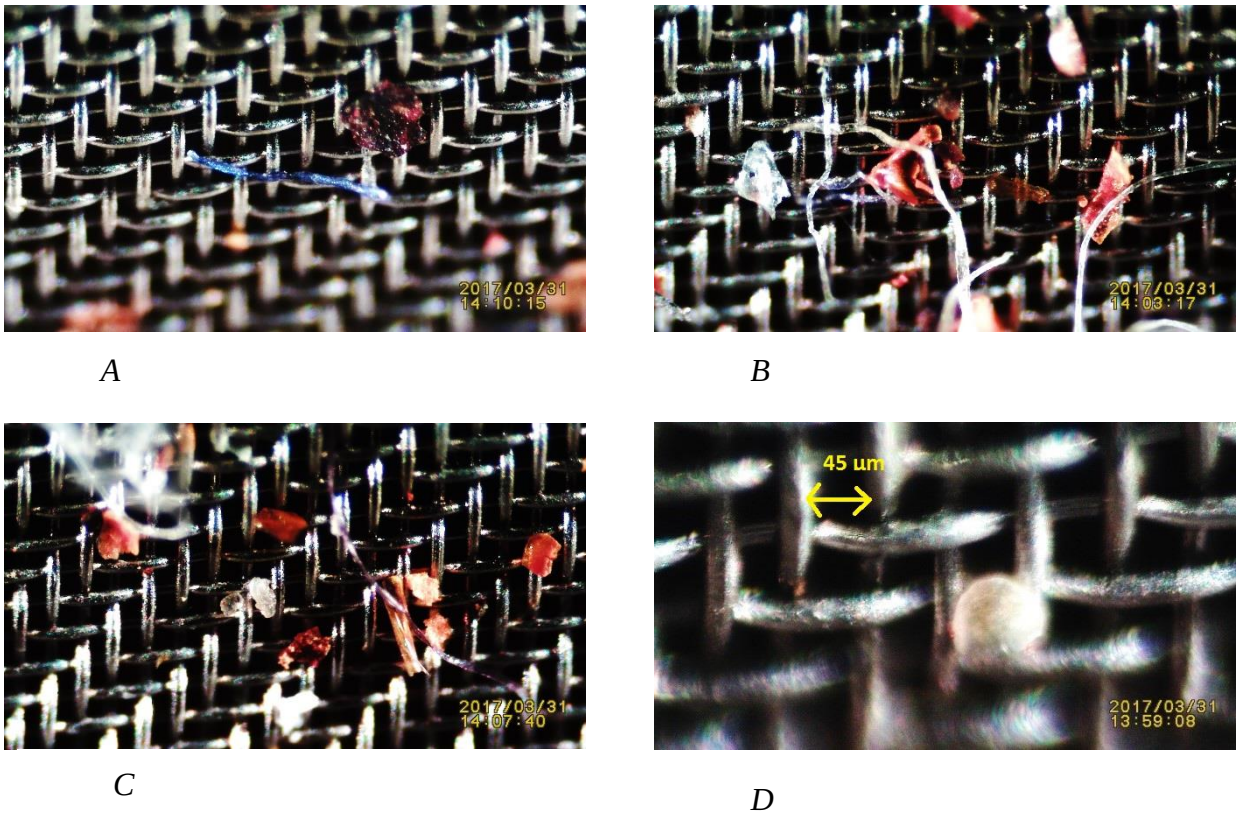
The samples were rinsed a minimum of 10 times: soaking for 15 minutes every 5th wash) and oven dried at 60 °C overnight.

The dry weights of the steel mesh with digested sample were noted and later calculated using gravimetric analysis for the weight of microplastics and hard-to-digest organic matter (section 3.5).

3. Results and Discussions

3.1. Blanks

The steel mesh (45µm) used for filtration of separated light weight samples had a mass recovery rate of more than 100 %. When observed under a microscope, zinc crystals were seen stuck in between the pores of the steel mesh along with other impurities like cloth fibers (Fig 24A). Thus, seven blanks were taken, each before introducing spiked samples. The impurities contributed to 0.01g average additional weight. Four of the seven unopened blanks were tested for chemical digestion following the chemical digestion protocol (section 2.10). After first step digestion, small reduction of weight of blanks was observed, yet $ZnCl_2$ crystals retained on the steel mesh. (Appendix 2)



A *B*

C *D*

Figure 24: Impurities on blanks taken on steel mesh (45 µm) and observed under a microscope before chemical digestion

The impurities on blanks might have resulted from laboratory air contamination, clothes worn in the laboratory, poorly cleaned instruments and improper storage of samples (Hidalgo-Ruz et al., 2012). The rinsing of steel mesh was also not able to remove $ZnCl_2$ crystals. However, ultrasonic bath for 3min was effective in $ZnCl_2$ crystals removal (Imhof et al., 2012).

3.2. Optimization

Design: The present design was obtained after some adjustments during the method optimization. An important addition was a pressure valve on the separation chamber. Lowering of the solution was easier with release of pressure through the sediment chamber without any spillage.

Second modification was the addition of a secondary outlet valve to drain out cleaner solution after the samples have sufficiently settled.

Third and the most important modification was inverting the handle of the ball -valve. As mentioned previously, the sediment chamber is placed in an inverted position (section 2.9) during sample extraction. A glass funnel is kept below the separation chamber. Previously, the ball-valve handles used to open downwards i.e. towards the funnel. This could be a source of microplastics. Therefore, the handle with the ball valve was dismounted, rotated and fixed in such that it opened upwards and had less chances of contaminating the steel mesh.

Method 1: PE powder and PE fibers were stuck to the walls of glass column (Fig 25) and the separation chamber. Lower recovery rates were expected. Microplastics tend to stick more when the inner wall of the Bauta column was dry.

Method 2: Vertical transport of PE fibers resulted in homogenous distribution of the fibers along the column (Fig 26). The density separation took about 4-5 hours even when the propeller was turned off. Many fibers were evidenced to be suspended along the vertical length of the column.

Figure 25: (Left) Introduction of PE powder in the separation chamber. $CaCl_2$ solution is filled from the bottom.

(Right) PE powder stuck to the Bauta glass column wall.

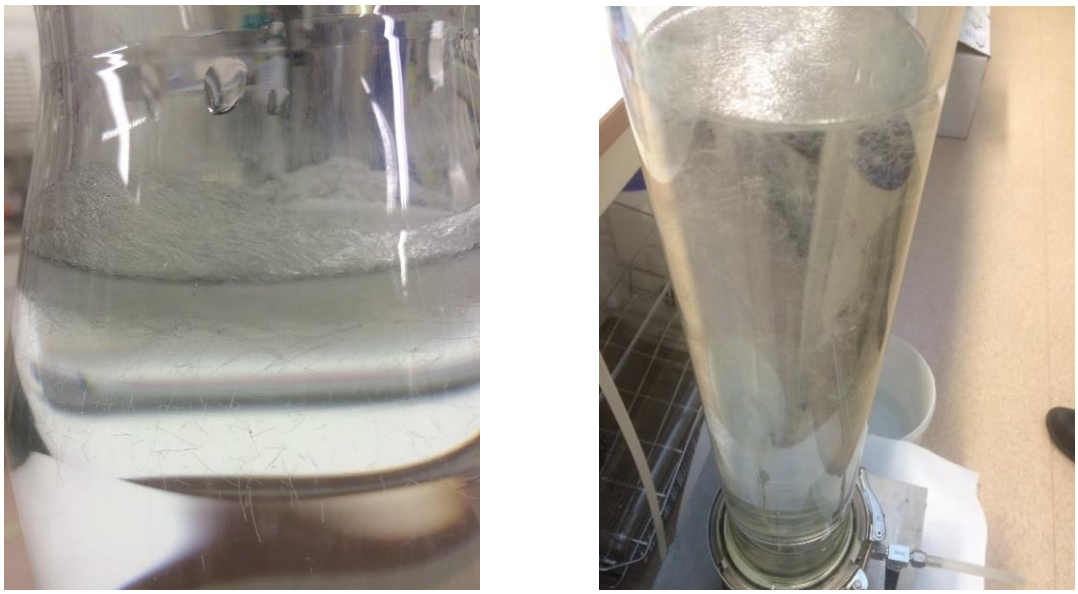


Figure 26: (Left) PE fibers stuck to the Bauta glass column wall at the constriction when introduced in the sediment chamber. (Right) Distribution of PE fibers in the solution column.

PE fibers had an average recovery rate of 77 ± 0.05 (s.d) % and the graph (Fig 27) shows the recovery rates of three PE fiber replicates with their standard deviations (s.d).

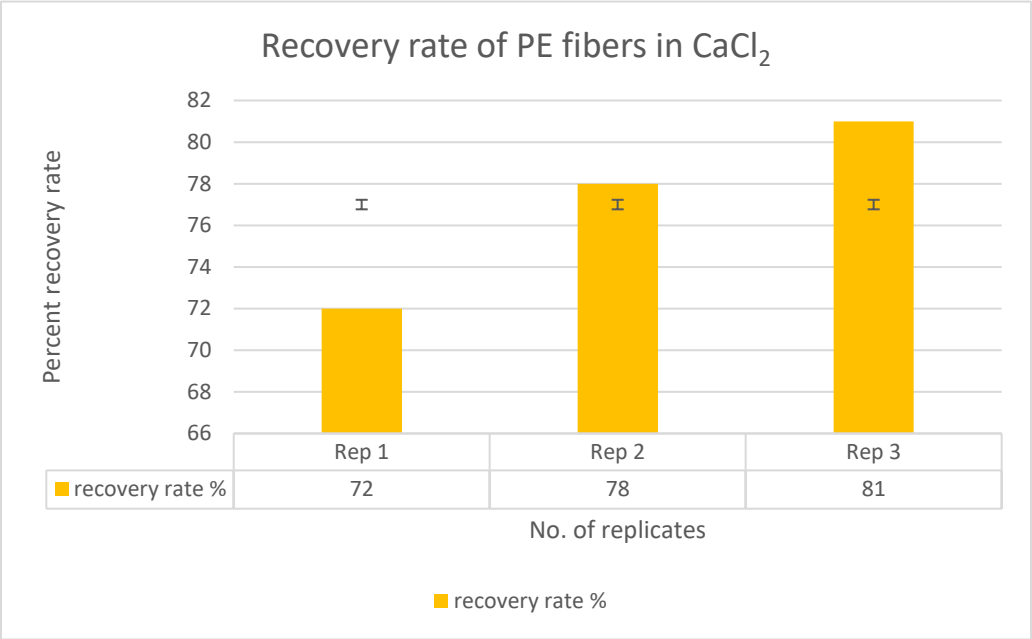


Figure 27: Recovery rates of PE fibers replicates from $CaCl_2$ solution with standard deviations. The microplastics were filtered out on GF/D and GF/F filter papers.

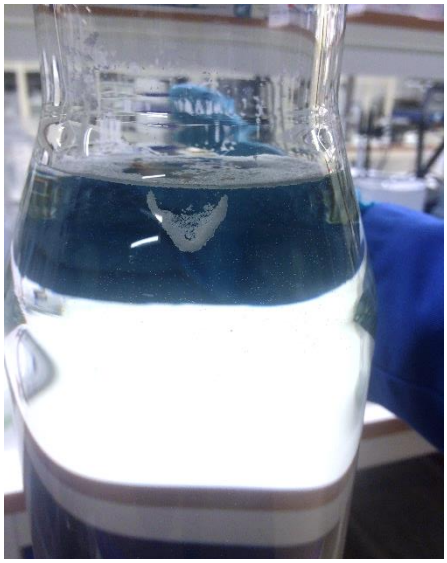


Figure 28: PET powder stuck to the constriction of the glass column when $CaCl_2$ was used as separation solution and sample was introduced from top.

PET powder was stuck to the walls of the constriction of the glass column (Fig 28). Thus, replicates were not taken.

The constriction was one of the major drawbacks of the Bauta design. Design of MPSS (Imhof et al., 2012) reveals a tall conical standpipe (referred as glass column in BMSS) to provide better separation distance and is shaped without sharp edges to prevent attachment of ascending particles on the inner wall.

Additionally, literatures e.g. (Browne et al., 2010, Von Moos et al., 2012) support that turbulence keeps the microplastics suspended temporarily in the water-column, especially in sea waters (d=1.03 g/mL).

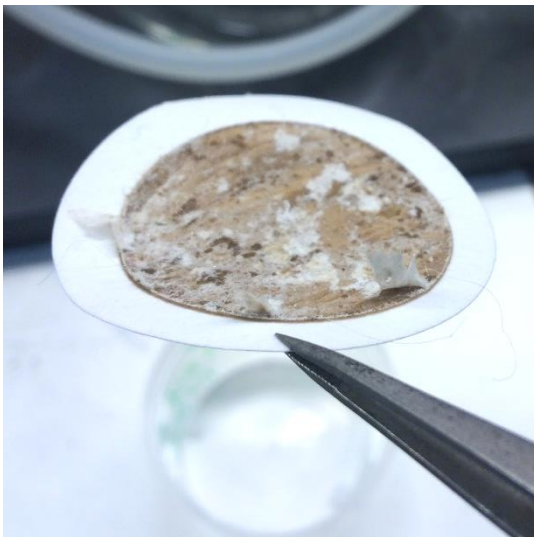


Figure 29: Precipitate of $ZnCl_2$: $CaCl_2$ on GF/C glass fiber filter paper during method optimization.

Method 3:

Although a solution of $ZnCl_2$:$CaCl_2$ was a better alternative to imitate saline conditions in large volumes in laboratory and its density (1.6 g/mL) proved sufficient for separation of the selected lab MP, noticeable amount of precipitate (Fig 29) was retained on the filter paper GF/C and GF/D grades.

Use of aqueous Zinc chloride alone is toxic as the chemical itself is highly oxidizing and corrosive in nature, whereas calcium chloride is comparatively non-toxic and can also be used in food as a coagulation agent (Stolte, 2014). The use of other salts like Sodium Iodide

(NaI) as separation solution with same density was possible but NaI is expensive (Claessens et al., 2013).

3.3. Mass recovery of microplastics from Spiking

The spiked laboratory sand (grain size 0.2-0.7mm, d = 2.6 g/mL) had a mass recovery of 100 ± 0.00 (s.d) % for PET pellets. Nevertheless, average recovery rate of PET powder was 77 ± 0.08 % (Fig 30) (Appendix 3.1).

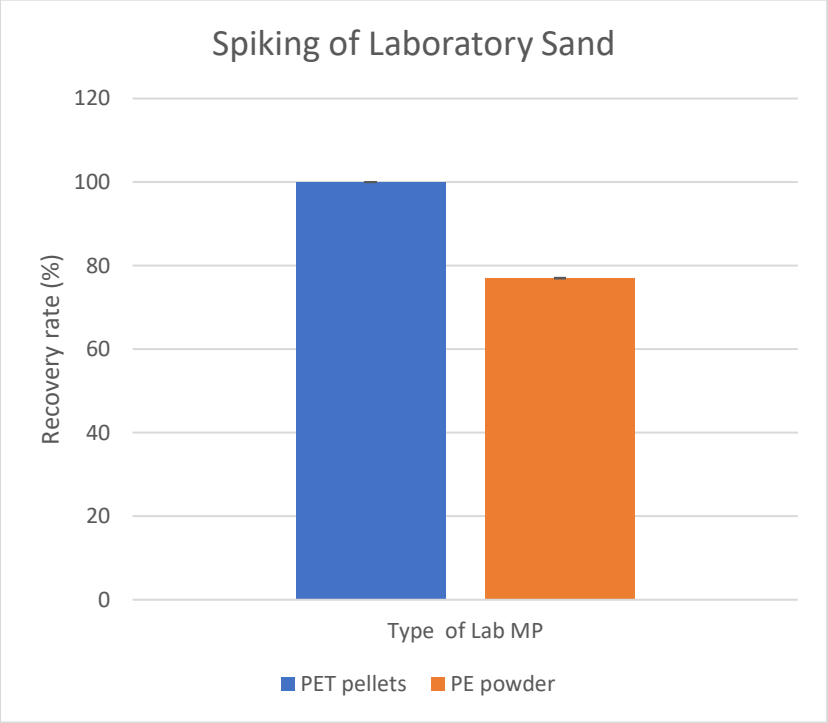


Figure 30: Recovery rates (%) with standard deviation of PET pellets and PE powder from spiked laboratory sand (grain size 0.2-0.7mm, d = 2.6 g/mL). $ZnCl_2$:$CaCl_2$ was used as separation solution

Similarly, when clean environmental samples (CES) were spiked, 100 ± 0.00 (s.d) % recovery rates were obtained for the LDPE and PET pellets. The PET powder (>75 µm) resulted in 93 ± 0.02 % and PE fibers resulted in 82 ± 0.1 % average recovery rate (Fig 31).

These samples did not undergo chemical digestion but they were blank corrected (Appendix 3.2).

The recovery efficiency is used to predict the recovery rates of microplastics from the environmental samples.

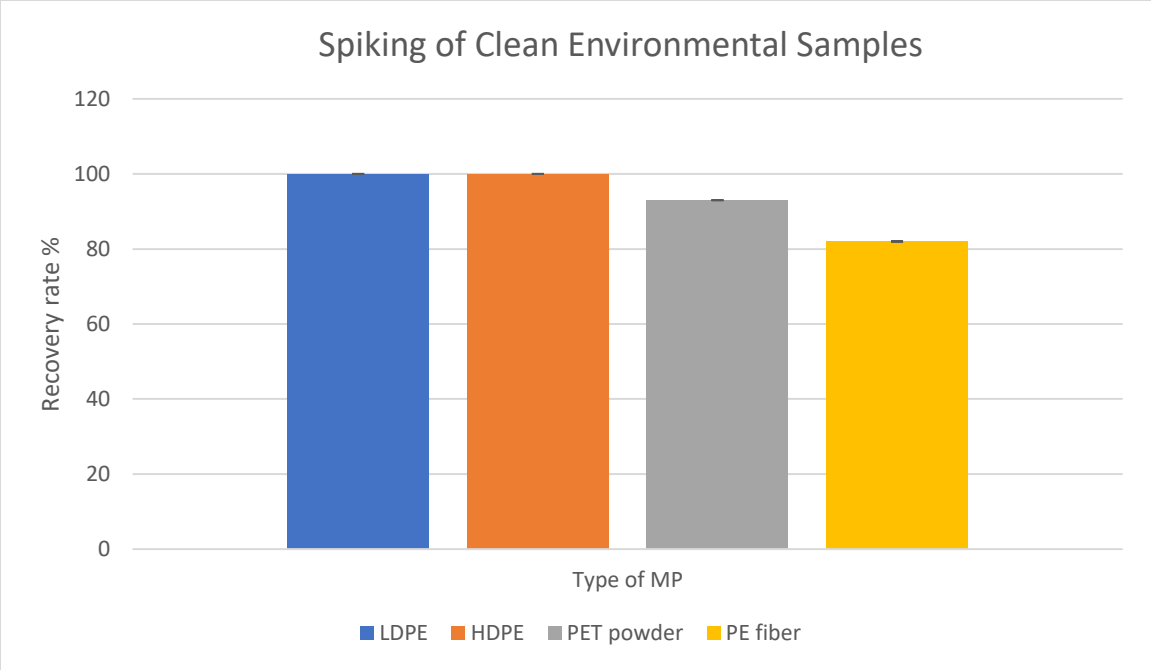


Figure 31: Recovery rates (%) with standard deviation of LDPE pellets, HDPE pellets PET powder and PE fibers from clean environmental samples (grain size > 300 µm, d >1.6 g/mL). $ZnCl_2$:$CaCl_2$ was used as separation solution. PE fibers has the lowest recovery rates.

PE fibers had the lowest recovery rates among four types of MP used for spiking. PE fibers remained suspended in the solution column because of turbulence, like the results revealed by Hidalgo-Ruz et al., (2012). PE fibers and similar microplastic particles cannot be removed easily because seas and oceans are impossible without any turbulence; therefore, PE fibers and powder remain suspended in the water column below the surface for very long, thus posing greater threat to marine life.

PET powder and PE fiber were stuck to the wall of the Bauta column, especially at the constriction. The solution level was lowered down a few times and filled in again in the

Bauta to obtain higher recovery rates. Moreover, these microplastics were stuck on the outer rim of the junction between the glass column and the separation chamber. Thus, some PET powder and PE fiber were lost, resulting in lower recovery rates than the LDPE and HDPE pellets.

3.4. Beach and Sediment Sample Characterization

The sediment samples from the Bekkelaget WWTP revealed higher water contents (58.14 ± 3.07 (s.d) %) than the beach samples (average water content 5.24 ± 0.02 %).

Even after oven drying at 110 °C for 16 hours, the sediment samples were a slurry. Furthermore, a finer grain size was exhibited in sediment samples with black colored sediment and a greater clay proportion than the beach samples.

The beach plastic debris samples, however, contained higher organic matter, in both wet and dry sieved samples (Table 4).

Sediment samples from Bekkelaget WWTP contained varying amount of organic matter. Table 5 shows the average total organic carbon and nitrogen content in the analyzed samples in this study.

The determined water contents and the resulting dry weights of the sediments and beach samples were used for the analysis.

Table 4: Sample characterization of the beach plastic debris sample; the wet and dry weights of samples and the determined water contents are summarized.

S. N	wet wt. (kg)	dry wt. (kg)	Water content %)	Characterization
HT.1	1.1	1.1	4.34	Wet, presence of organic matter like needles, twigs and bird feathers and fragments of shells, few small pebbles ~2mm, grayish sand, macroplastics and MP pellets observed
HT.2	1.2	1.1	7.56	Wet, presence of organic matter, few pebbles (~6mm), grayish sand, microplastics pellets observed
HT.3	1.3	1.2	6.34	Wet, presence of significant organic matter, shells, few pebbles ~2mm, grayish sand, macro and microplastics observed
HT-6.1	8.2	7.7	6.30	Wet, presence of organic matter and fragments of shells, few pebbles ~2mm, darker sand than High tide samples
HT-6.2	1.2	1.1	7.72	Wet, substantial organic matter and fragments of shells, few pebbles ~2mm, darker sand than High tide samples
HT+6.1	1.3	1.2	1.97	dry, less organic matter, more gravel, brownish and grayish coarse sand, MP pellets observed
HT+6.2	2.2	2.2	2.36	Dry, less organic matter, lots of gravels, brownish and grayish coarse sand, piece of cloth fabric in sample

Table 5: Sediment samples from Bekkelaget WWTP with their average total organic carbon and nitrogen contents

S. N	Avg. Total Organic Carbon	Avg. Total Organic Nitrogen
F1	4.4 %	0.441 %
F3	3.4 %	0.313 %
F4	3.8 %	0.343 %

36.22 g of macroplastics (> 8mm) were sieved out from the beach plastic debris samples. These macroplastics were continuously exposed to wave actions and photodegradation. Thus, they could be the possible sources of secondary microplastics in the beach samples that were analyzed. There are no local sources of primary microplastics around Bygdøy sjøbad, however, several plastic resin pellets were observed during sampling. The adjacent sea water is likely to be the source of microplastics off the shore, and thus the phenomenon supports that water transport microplastics to remote coastal area, where there are no local sources of primary microplastics (Browne et al., 2011).

3.5. Separation of microplastics from Beach plastic debris samples

Beach plastic debris samples contained mostly sand particles. Consequently, the density separation was speedy. Since the propeller was rotating at the speed of 180 rmp to stir up

the settling debris and avoid burying of microplastics, some sand and silt particles remained suspended.

Based on the behavior of microplastics in the spiked samples (section 3.3), microplastics present in fibers and powder form was expected to float up me beach samples used for chemical digestion protocol testing were digested twice or thrice depending on the amount of organic matter present after previous digestion step. Based on the amount of undigested sample present after first digestion, the chemical digestion was repeated. The chemical digestion protocol is under development, thus results from one digestion step have been used. The high average concentration of microplastics and hard-to-digest debris material (9.75 ± 3.92 mg/kg) was obtained in the high-water mark zone, as defined by the hypothesis. The beach zones higher and lower to the high-water mark had average MP concentration of 0.65 ± 0.2 mg/kg and 0.38 ± 0.51 mg/kg respectively.

The average concentration of MP separated from the beach plastic debris using the developed methods is shown in Figure 32.

Figure 32: Microplastics and hard-to-digest debris material from Bygdøy sjøbad beach plastic debris samples after chemical digestion

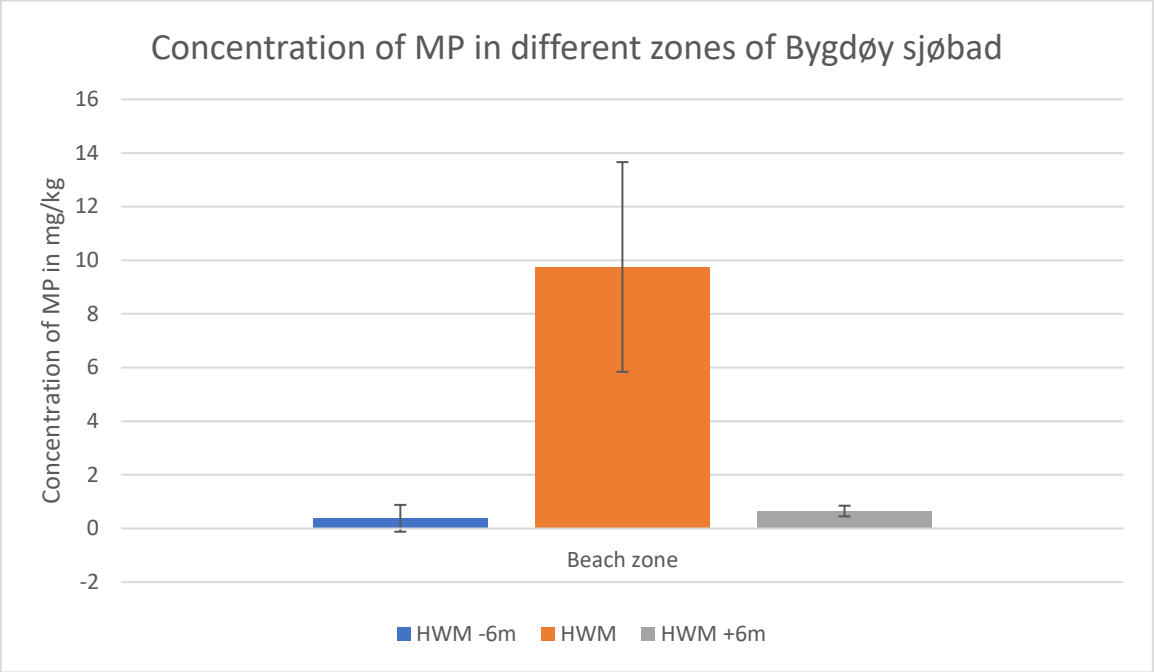


Figure 33: Concentrations of microplastics (mg/kg) present in different beach zones of Bygdøy sjøbad and their standard deviations. The concentration is the highest in high water mark region on the beach where most of the marine debris accumulate.

Average concentration of microplastics in different zones of Bygdøy sjøbad beach zone (Fig 33). ANOVA test (using R) showed that samples from Bygdøy sjøbad had a significant difference ($p < 0.05$) for the concentrations of MP and hard-to-digest organic matter; the difference was found between the high-water mark (HWM) zone and HWM -6m zone, also between HWM and HWM +6m zone (Appendix 3).

Further, Tukey test (Appendix 4) showed that HWM and HWM -6m; HWM +6m and HWM were significantly ($p <0.1$) different whereas no significant difference was seen between HWT+6m and HWT-6m zones in Bygdøy sjøbad.

Hidalgo-Ruz et al., (2012) also revealed similar results from 44 studies conducted on beach samples (referred here as beach plastic debris sample). The highest number of microplastics were found in the high tide line (referred here as high-water mark). This concurs to the results obtained in this study.

3.6. Separation of microplastics from Sediment samples

Sediment samples from Bekkelaget WWTP seemed to contain marine clay. As a result, the settling in the Bauta took comparatively longer than the Bygdoy beach debris samples. Only 20 cm of the total height of the glass column was apparently clear after 1.5 h of the sediment sample introduction in the Bauta. During the sediment sample preparation and density separation, microplastics were not visible in the sample. Moreover, the sediment samples contained very less organic matter compared to the Bygdøy beach debris samples. Therefore, filtration and extraction of light weight sediment sample from the Bauta on a steel mesh was comparatively easier.

Microplastics were observed on the steel mesh only after the samples were chemically digested. However, further identification is necessary.

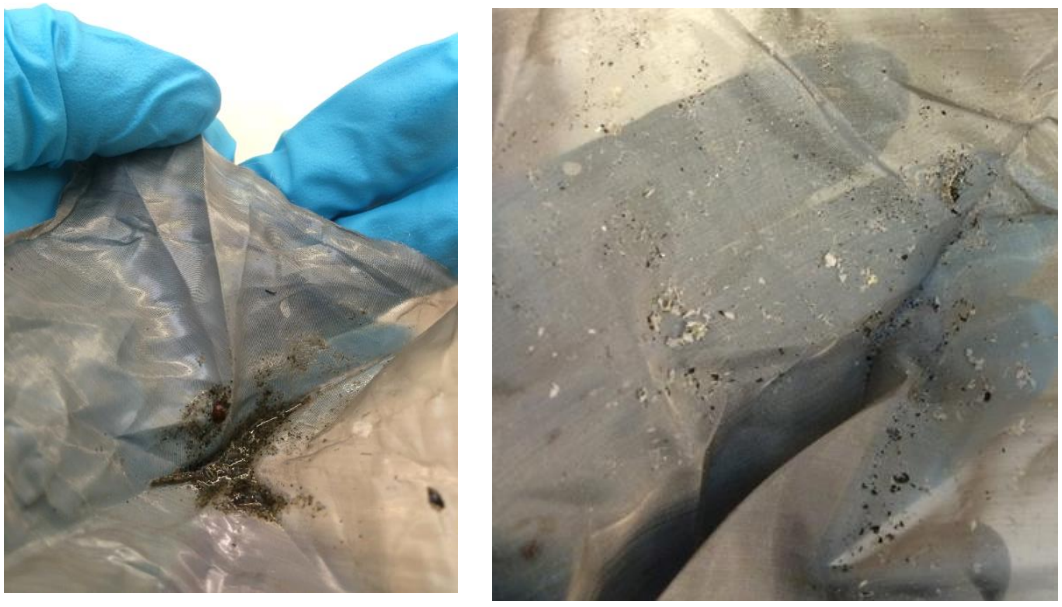


Figure 34: Before (left) and after (right) chemical digestion of sediment sample F1 on steel mesh (45 µm)

Presence of MP was observed in Bekkelaget WWTP samples with an average concentration of 27.92 ± 37.37 mg/kg (Fig 35).

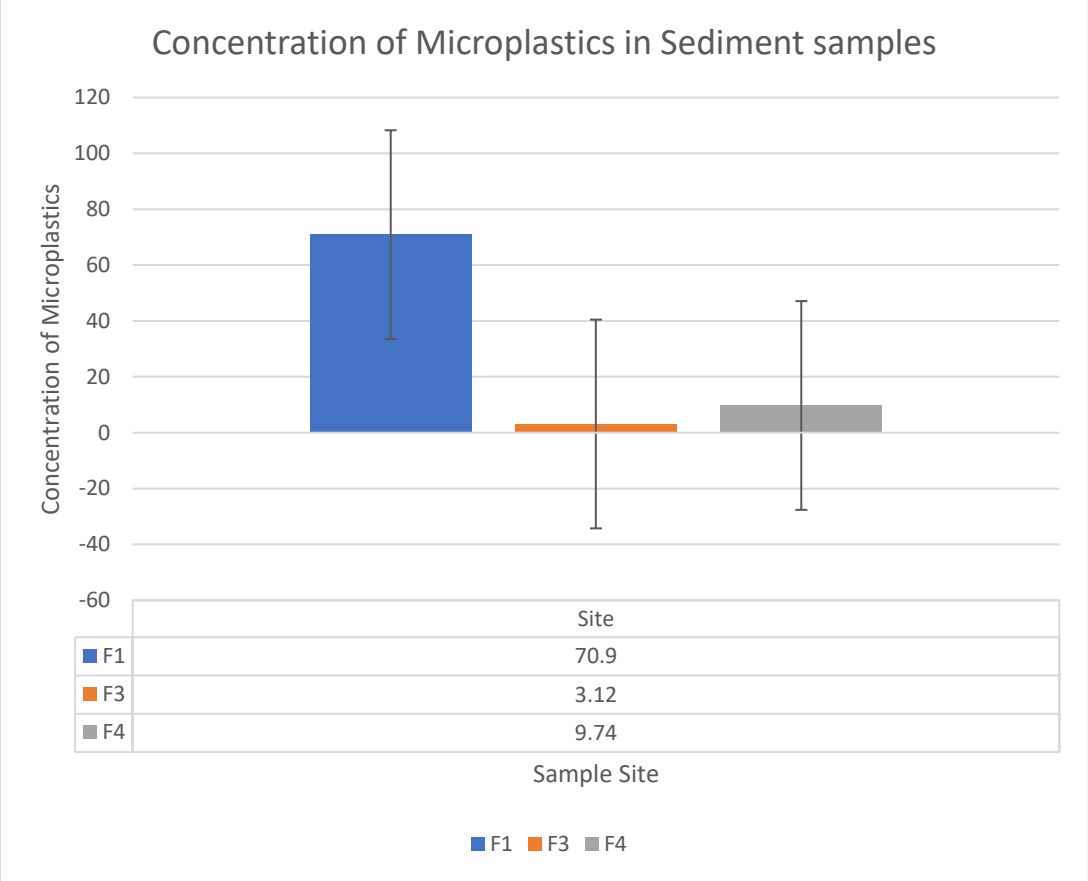


Figure 35: Concentration of microplastics (mg/kg) obtained in each sampling site of Bekkelaget WWTP effluent with the average standard deviation. Concentration is found the highest in F1 sediment sample. The sampling depth was 0-10cm for F1 while for F3 and F4, sampling depth was 0-5cm. Average concentration of the three samples is 27.92 ± 37.37 mg/kg.

High standard deviation was obtained; possible reason could be, F3 and F4 were sampled from depth 0 – 5cm while F1 was sampled from depth 0-10cm. Deviations might have occurred due to differing sampling places. The samples are not replicates of each other. In addition, only three samples were analyzed and their individual values of concentration of microplastics have large difference.

The $ZnCl_2$:$CaCl_2$ solution used for beach sand and sediment sample separation was filtered through 2 layers of steel mesh (45 µm) overlapped on each other. To further purification, GF/D filter was used.

4. Conclusions

From the obtained recovery rates of microplastics (100% of LDPE and HDPE, 93 % of PET powder (>75 microns) and 82 % of PE fibers), it is concluded that the Bauta MSS provides a reliable tool for the quantitative analysis of a wide range of microplastics regarding their size, shape and specific densities. Standard deviations between 0.00 - 0.1 % imply that the separation and extraction method using Bauta MSS are less susceptible to error compared to the density separation using classic method (3.98% - 16.6%) and MPSS (0.00 - 1.8%) according to a study by Imhof et al., (2012). NOAA methods analyzed microplastics of size 5mm – 0.3mm while Bauta MSS proved efficient for microplastics size < 8mm and > 45µm. From spiking results, it can also be concluded that when a matrix of unknown concentration of microplastic containing beach sand, soil and sewage sludge, is separated, pellets can be expected to have maximum recovery rates and fibers to have lowest recovery rate.

1 kg of beach debris sample took about 3 days to process that included air-drying for 72 hours, sieving through 8mm sieved, oven drying over – night at 60 °C, separation in Bauta and filtering on a steel mesh (45 µm).

The beach debris can be air dried in less than 72 hours by storing them in paper cardboard boxes instead of polypropylene buckets. Cardboard boxes allow larger surface area for drying of the samples. The sample processing time was mostly dependent on the amount of floating organic matter; as large volume of organic matter along with other floating plastic debris needed to be filtered out in smaller steel mesh. Also, presence of large debris was a limitation of filtration method and the microplastics are susceptible to loss during extraction method of light weight sample on a steel mesh.

On the other hand, the Bauta could process 700 g of beach sand and 500 g of sediment sample in one run, implying that the Bauta MSS advances the time efficiency of microplastics research.

A solution of Zinc chloride and Calcium chloride with density 1.6 g/mL was a better alternative regarding toxicity and cost-effectiveness, and for preparing dense saline solution in laboratories for density separations tests.

Results revealed that the high-water zone of a beach has higher average concentration of microplastics (9.75 ± 3.92 mg/kg) compared to other zones (0.38 ± 0.51 mg/kg and 0.65 ±

0.2 mg/kg). Although further identification is required for determining microplastics, traces of microplastics were visually observed after successful separation from sediments of waste water treatment system effluent (27.92 ± 37.37 mg/kg).

Hence, it can be concluded that both defined hypotheses (section 1.3) proved to be true. This study provides a robust and reproducible technique of separating microplastics (>45 µm) from beach debris and waste water treatment plant effluent sediment. Also, a reliable method for chemical digestion has been developed and is currently under optimization at NGI.

4.1. Further method optimization recommendations

The methods were developed to best fit the present design and were optimized, but further modifications are possible. One of the major challenges includes the height of Bauta. Though the current design provides an effective separation distance between the floaters and sinkers, some dense particles like marine clay takes longer to settle down. Moreover, shorter glass column will favor less volume of separation solution and shorter density separation time.

An important optimization could be modifying the glass column and removing sharp angles like the constriction at the top (section 2.4, Fig 4). The constriction is a place for the microplastics fibers and powder to stick on the Bauta glass column wall, and hence resulted in lower recovery rates of the fibers and powder.

Another modification in the design could be a solution meter or transparent body of the separation chamber for better visibility and monitoring of the solution level. This will prevent overflow and spilling of the separation solution from the top of separation chamber. Better filtration method with powerful vacuum pump is recommended to save time and increase efficiency. Furthermore, to increase efficiency of recovery rates, Zinc crystals on the steel mesh can be removed using 1g of Sodium dodecyl sulfate in an ultrasonic bath for 3 minutes.

In addition, for samples with large organic matter like needles and twigs that passed through sieves of 8mm, for example Bygdøy plastic debris sample (High-water mark), filtration was challenging. Large organic matter clogged the ball valve opening and a long thin glass rod

was used to push out the samples. The extraction of samples was also done through the shut-off valve opening by reverting the chamber over a larger glass funnel and steel mesh of size 13 x 13 cm^2 when necessary. There are potentials for development of filtration methods in future.

For further method verification, larger sample size for correct estimation of the microplastic concentration results in environmental samples is recommended.

4.2. Implications for future studies

This thesis can provide first-hand scientific information on the recovery rates of selected range of microplastics from the Bauta MSS. Large volumes of soil and sediments can be efficiently separated in less time using the developed separation method. Spiking tests are possible with more plastic polymers like polypropylene (PP) and polyvinyl chlorides (PVC). The verification of presence of microplastics in the sediment sample from Bekkelaget can direct attention towards analysis of more sediment samples from effluents and promote improvement of sewage treatment methods.

This thesis can aid as a reliable separation method to answer curiosities regarding presence of microplastics in marine and beach sediments, sewage sludge and soil. In addition, it can be used as a reference for further optimizations yet to be achieved.

5. Reference

Andrady, A. L. 1989. Environmental degradation of plastics under land and marine exposure conditions. Proceedings of the Second International Conference on Marine Debris..

Andrady, A. L. 2011. Microplastics in the marine environment. *Marine Pollution Bulletin,* 62**,** 1596-605.

Arthur, C., Baker, J. & Bamford, H. 2008. Proceedings of the International Research Workshop on the Occurrence, Effects, and Fate of Microplastic Marine Debris. NOAA Marine Debris Program, sep 9-11. National Oceanic and Atmospheric Administration.

Barboza, L. G. A. & Gimenez, B. C. G. 2015. Microplastics in the marine environment: current trends and future perspectives. *Marine Pollution Bulletin,* 97**,** 5-12.

Barnes, D. K., Galgani, F., Thompson, R. C. & Barlaz, M. 2009. Accumulation and fragmentation of plastic debris in global environments. *Philosophical Transactions of the Royal Society of London B: Biological Sciences,* 364**,** 1985-1998.

Browne, M. A., Crump, P., Niven, S. J., Teuten, E., Tonkin, A., Galloway, T. & Thompson, R. 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. *Environmental Science & Technology,* 45**,** 9175-9179.

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

Carr, S. A., Liu, J. & Tesoro, A. G. 2016. Transport and fate of microplastic particles in wastewater treatment plants. *Water research,* 91**,** 174-182.

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

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M. B. & Janssen, C. R. 2013. New techniques for the detection of microplastics in sediments and field collected organisms. *Marine Pollution Bulletin,* 70**,** 227-33.

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C. and Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. *Scientific reports*, *4*, p.4528..

Derraik, J. G. 2002. The pollution of the marine environment by plastic debris: a review. *Marine Pollution Bulletin,* 44**,** 842-852.

do Sul, J. A. I. & Costa, M. F. 2014. The present and future of microplastic pollution in the marine environment. *Environmental Pollution,* 185**,** 352-364.

Duis, K. and Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. *Environmental Sciences Europe*, *28*(1), p.2.EPA. 2016. *Persistent, Bioaccumulative and Toxic Chemicals (PBTs)* [Online]. [Accessed 1st June 2017].

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C. & Thiel, M. 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environmental Science & Technology,* 46**,** 3060-75.

Hudgins, C.M., 1964. Solubility and Density Studies of the CaCl2-ZnCl2-H2O System at 0 and 25 C. *Journal of Chemical & Engineering Data*, *9*(3), pp.434-436.

Imhof, H. K., Schmid, J., Niessner, R., Ivleva, N. P. & Laforsch, C. 2012. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. *Limnology and Oceanography: Methods,* 10**,** 524-537.

Jahnke, A., Arp, H. P. H., Escher, B. I., Gewert, B., Gorokhova, E., Kühnel, D., Ogonowski, M., Potthoff, A., Rummel, C. & Schmitt-Jansen, M. 2017. Reducing uncertainty and confronting ignorance about the possible impacts of weathering plastic in the marine environment. *Environmental Science & Technology Letters,* 4**,** 85-90.

Kershaw, P., Katsuhiko, S., Lee, S. & Woodring, D. 2011. Plastic debris in the ocean. United Nations Environment Programme.

Kim, Y.-J., Osako, M. & Sakai, S.-i. 2006. Leaching characteristics of polybrominated diphenyl ethers (PBDEs) from flame-retardant plastics. *Chemosphere,* 65**,** 506-513.

Lytle, C. L. G. 2016. When the mermaids cry: The Great Plastic Tide. *Coastal Issues/Coastal Care.*

Masura, J., Baker, J., Forster, G. & Arthur, C. 2015. Laboratory methods for the Analysis of Microplastics in the Marine Envirnment: Recommendations for quantifying synthetic particles in waters and sediments.

Nuelle, M. T., Dekiff, J. H., Remy, D. & Fries, E. 2014. A new analytical approach for monitoring microplastics in marine sediments. *Environmental Pollution,* 184**,** 161-9.

Singh, R. & Agrawal, M. 2008. Potential benefits and risks of land application of sewage sludge. *Waste management,* 28**,** 347-358.

Stolte, A. 2014. *The detection of microplastics in beach sediments : Extraction methods, biases and results from samples along the German Baltic coast.* Masters, Universitat Rostock.

Sujathan, S., Kniggendorf, A.-K., Kumar, A., Roth, B., Rosenwinkel, K.-H. & Nogueira, R. 2017. Heat and Bleach: A Cost-Efficient Method for Extracting Microplastics from Return Activated Sludge. *Archives of Environmental Contamination and Toxicology***,** 1-8.

Teuten, E. L., Saquing, J. M., Knappe, D. R., Barlaz, M. A., Jonsson, S., Björn, A., Rowland, S. J., Thompson, R. C., Galloway, T. S. & Yamashita, R. 2009. Transport and release of chemicals from plastics to the environment and to wildlife. *Philosophical Transactions of the Royal Society of London B: Biological Sciences,* 364**,** 2027-2045.

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

Thompson, R. C., Swan, S. H., Moore, C. J. & vom Saal, F. S. 2009. Our plastic age. *Philosophical Transactions: Biological Sciences,* 364**,** 1973-6.

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

Weber, R., Watson, A., Forter, M. & Oliaei, F. 2011. Review Article: Persistent organic pollutants and landfills-a review of past experiences and future challenges. *Waste Management & Research,* 29**,** 107-121.

Xu, W., Wang, X. & Cai, Z. 2013. Analytical chemistry of the persistent organic pollutants identified in the Stockholm Convention: A review. *Analytica Chimica Acta,* 790**,** 1-13.

Appendix

Appendix 1: Graphical representation of solubility data of $ZnCl_2$: $CaCl_2$: H_2O and the isodensity lines (Hudgins C.M., 1964)

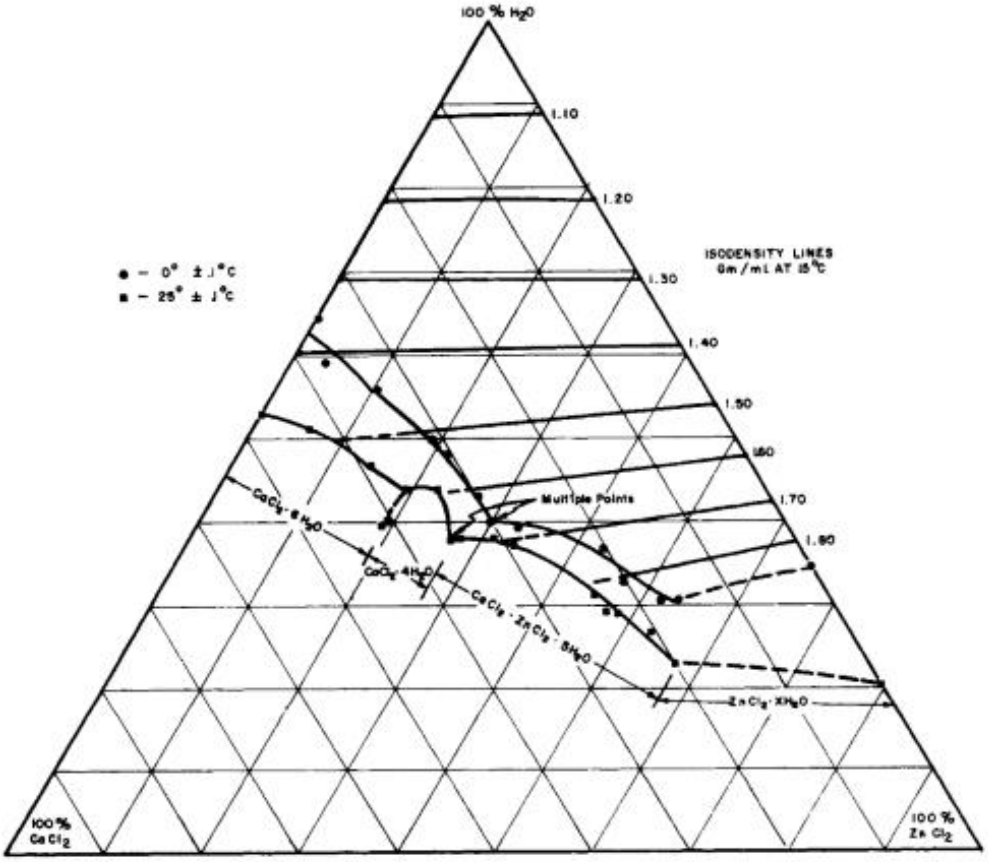


Figure 1. Solubility limits of the $CaCl_2$–$ZnCl_2$–H_2O system at 0° and 25° C. Density curves at 15° C.

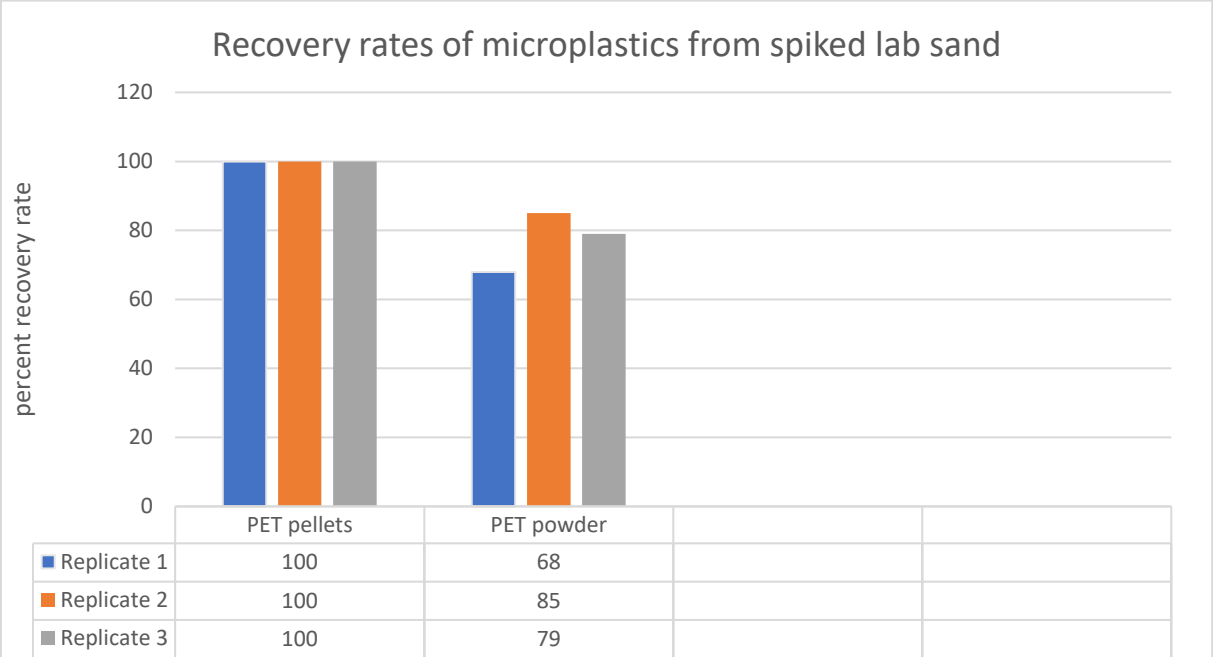
e. 3, JULY 1964

Appendix 2: Blanks taken on steel mesh (45 µm). The first four steel mesh were chemically digested to check whether the Zinc chloride crystal and impurities are reduced by the digestion process.

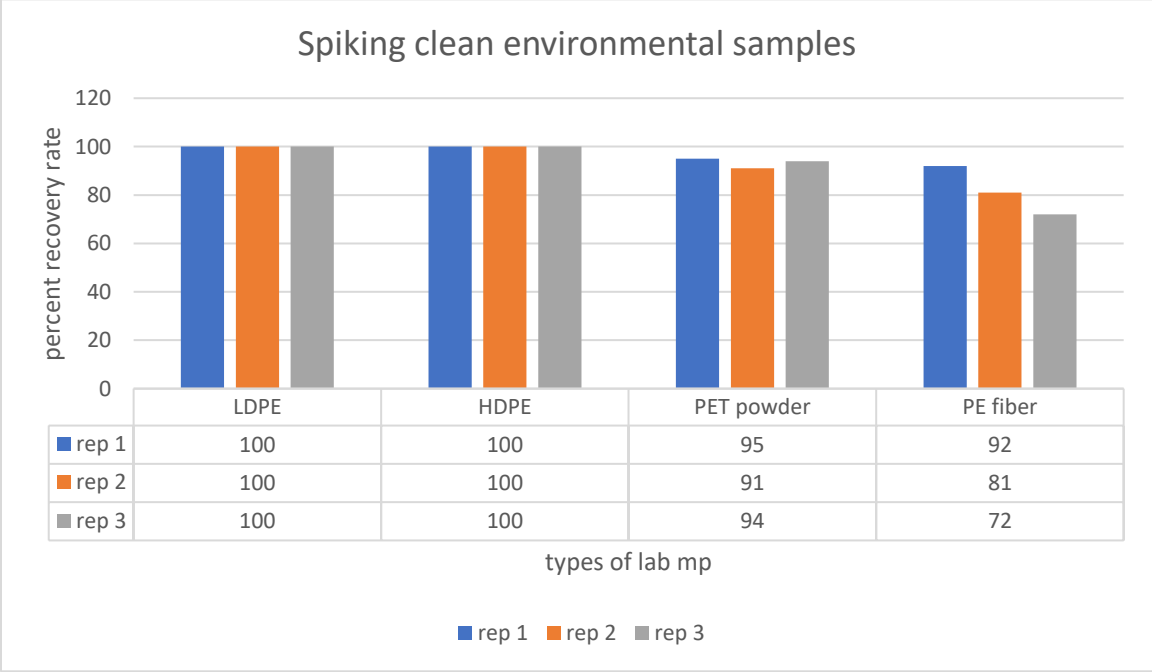
S. N	Initial wt. (g)	Final wt. (g)	Wt. diff. (g)	Wt. after digestion	Reduced wt.	Wt. diff	Impurities
1	2.3089	2.331	0.0221	2.3292	0.0018	0.0203	0.96%
2	4.3097	4.3135	0.0038	4.3099	0.0036	0.0002	0.09%
3	4.2126	4.2316	0.019	4.2127	0.0189	0.0001	0.45%
4	1.0788	1.0791	0.0003	1.0789	0.0002	0.0001	0.03%
5	0.6799	0.6911	0.0112				1.65%
6	2.3295	2.3338	0.0043				0.18%
7	1.6662	1.6796	0.0134				0.80%
						Average	**0.59%**
						Standard deviation	**0.01%**

Appendix 3

Appendix 3.1: Cluster graph of recovery rates of microplastic from spiked laboratory sand sample replicates



Appendix 3.2: Cluster graph of recovery rates of microplastic from spiked clean environmental sample replicates



Appendix 3.3: Chemical digestion of Beach samples and the average concentration of microplastics and hard-to-digest organic matter and their standard deviations

S. N	ID	Final wt. MP	blank corrected wt.
HT.1	BHT 1.1 (i)	0.64	0.63
	BHT 1.1 (ii)	3.34	3.33
	BHT 1.2 (i)	4.8	4.79
	BHT 1.2 (ii)		
	a	0.16	0.15
	b	0.21	0.20
	c	1.42	1.40
	d	1.18	1.17
	e	1.03	1.02
	f	1.55	1.54
	BHT 1.2 (iii)	0.06	0.05
	BHT 1.3	1.38	1.37
	BHT 1	15.76	15.64
HT.2	BHT 2.1		
	1	0.89	0.88
	2	0.81	0.80
	3	0.55	0.54
	4	0.34	0.33
	5	0.55	0.54
	BHT 2.2		
	1	0.88	0.87
	2	0.94	0.93
	3	0.87	0.86
	4	0.86	0.85
	BHT 2.3 (i)	1.00	0.99
	BHT 2.3 (ii)	1.28	1.27

	BHT 2	8.97	8.86
HT.3	BHT3.1		
	a	0.55	0.54
	b	1.15	1.14
	c	0.55	0.54
	d	1.43	1.42
	BHT 3.2		
	a	1.98	1.97
	i	0.75	0.74
	ii	1.42	1.41
	BHT 3.3	0.84	0.83
	BHT 3	8.66	8.58
Average concentration at High water mark			**9.75**
Standard deviation			**3.92**
HT-6.1	BHT -6.1	0.03	0.02
HT-6.2	BHT -6.2	0.83	0.82
Average concentration at High water mark – 6m			**0.38**
Standard deviation			**0.51**
HT+6.1	BHT +6.1.1	0.88	0.87
	BHT +6.1.2	0.14	0.13
	BHT +6.1	1.02	1.00
HT+6.2	BHT+6.2.1	0.23	0.22
	BHT+6.2.2 (i)	0.33	0.32
	BHT+6.2.2 (ii)	0.02	0.01
	BHT+6.2.3	0.58	0.57
	BHT +6.2	1.17	1.12
Average concentration at High water mark + 6m			**0.65**
Standard deviation			**0.20**

Appendix 4: Anova test and Tukey test for Beach plastic debris samples

Source: Rajesh Joshi

ANOVA test

ANOVA test was performed using R.

```
Model used: MpConc ~ Type

            Df Sum Sq Mean Sq F value Pr(>F)
Type         2 117.39   58.69   7.926 0.0406 *
Concentr    4  29.62    7.41
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

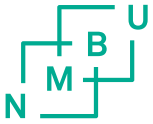
Further, Tukey’s test was performed to see the significant difference between the **type**.

```
Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = MpConc ~ Type, data = Book1)

$Type
                     diff        lwr       upr     p adj
HWM-6m -HWM         -8.3234489 -17.177025 0.5301275 0.0603842 .
HWM+6m -HWM          -8.2260098 -17.079586 0.6275666 0.0625574 .
HWM+6m - HWM-6m     0.0974391  -9.601168 9.7960462 0.9992935

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```



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

Postboks 5003
NO-1432 Ås
Norway