Sampling Microplastics in Beach Sediments and Analysis Using FTIR Spectroscopy

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Abstract:

The focus of the thesis is to find suitable methods to collect and separate microplastics from beach sediments, analyse them with microscope and identify the polymer types with FTIR spectroscopy. Microplastics are plastic particles that are less than 5 mm diameters and they originate from either fragmentation of macroplastics or production of plastics in micro meter size. This results in steadily increasing amount of microplastics litter in the environment. Microplastic pollution in aquatic environment is increasing daily and there are not enough resources to prevent it. Microplastic sampling in marine environment occurs on beach sediments, sea bottom and sea surface. This thesis focuses on sampling microplastics on sandy beaches with bulk and selective sampling methods. Samples were collected from different locations around the world. Micro particles were separated from sand samples by density separation and the floating micro particles were used in two different separation methods that are wet peroxide oxidation and vacuum filtration. Wet peroxide oxidation is a method to dissolve organic matters in the samples which failed during the experiment. Vacuum filtration method was used with filter paper that is effective for removing samples to analyse in FTIR spectroscopy. Many red and blue coloured fibers were found in each sample with microscopic analysis and they were failed to identify with transmission method in FTIR. Most of the microplastics found in the samples were HDPE and a few of them were identified as PP. Unexpectedly there were no PET found in the samples, however one sample was found as PVC with the possibility of 48% during the analysis of FTIR spectroscopy.

Keywords:	Microplastics, microplastic, FTIR, microplastics sampling, FTIR spectroscopy, microscope analysis, plastics, vacuum filtration, wet peroxide oxidation, WPO
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LIST OF ABBREVIATIONS

ATR	attenuated total reflectance
BF	bright field
DF	dark field
DIC	differential interference contrast
DSC	Differential scanning calorimetry
Fe (II)	iron (II) oxide
FTIR	Fourier transform infrared
HDPE	high density polyethylene
H_2O_2	hydrogen peroxide
KBr	potassium bromide
LDPE	low density polyethylene
LMT	lithium metatungstate
MPs	microplastics
NaCl	sodium chloride
NOAA	National Oceanic and Atmospheric Administration
PE	polyethylene
PET	polyethylene terephthalate
POM	particulate organic matter
POP	persistent organic pollutants
PP	polypropylene
PS	polystyrene
PVC	polyvinyl chloride
SEM	Scanning electron microscopy
SPT	sodium polytungstate
WPO	wet peroxide oxidation
WW	wet weight

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FOREWORD

I would like to thank the teachers and researchers in the department of Energy and Materials Technology at Arcada for their supportive attitude during my study time, my friends who brought samples from different countries and helped me during my project, and Sami-Pekka Hirvonen in Helsinki University for teaching me how to use the FTIR spectroscopy and giving me the opportunity to use it for my project.

Lastly, I would like to express my deepest gratitude and appreciation to my supervisor Stewart Makkonen-Craig for his encouraging and inspiring approach towards me and other students. His limitless support and trust towards me had a great help with my project.

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Derya Dilara Atas

1 INTRODUCTION

1.1 Background

Plastic particles were found in the sea for the first time in 1972 and since then the plastic pollution in the water increased rapidly. This is the result of the increasing plastic production all over the world (Mai, 2018). Plastic is a material that replace many different materials in different markets because of its high durability, high resistance, and lighter weight. Plastic production increased rapidly after 1950s since it became competitive in many fields (Qualman, 2017). The increase of plastic production brought the plastic litter along. Plastics have been released into the environment with poor waste management and deficiency of cities' infrastructures (H.S. Auta, 2017). This created a big problem in the environment; plastic pollution. Plastic pollution affects the environment with their appearance in the environment and its biological effect.

Macro size plastics that are left in the environment break in to smaller pieces in time and create micro size plastics. Besides that, there are micro size plastics manufactured for different industries that ends in the environment. Plastics less than 5 mm diameters are defined as microplastics. Microplastics pollution is mostly because of anthropogenic activities that is carried by rivers to the sea. Most of the microplastics are durable and floatable, that way they can be carried to the open sea by the rivers (Mai, 2018). Different sources show that there are huge variety of microplastics with multiple shapes, origins, and sizes. The characteristics of microplastics shows that their impact and distribution in the environment differs (Valeria Hidalgo-Ruz, 2012). Microplastics (MPs) can be found on beach sediments, sea beds, and on the water surface. MPs were found in fish long time ago and it is discovered that they are digested by fish in the aquatic environment. Microplastics have toxic chemicals that affects the marine life. Physical and chemical damage of the MPs to the marine life are highly rated. Because of their durability and negative effects, microplastics need to be removed from water.

1.2 Objectives

Microplastics are found in beach sediments, on sea beds, and on the sea surface. This thesis is focused on beach sediment samples because of limitations and lack of sources and equipment needed for sampling on sea beds and sea surface.

The aim of the thesis is to find out what method is efficient to do sampling of microplastics in beach sediments and which steps can be followed to do FTIR analysis for identification of microplastics.

Objectives of the thesis are:

- Following different sampling methods and understanding which method works during the experiment.
- Improving the methods to collect samples from beach sediments.
- Analysing microplastic types with FTIR spectroscopy that are found in the samples and observing what challenges occur during the experiment.

To have a better understanding of the effects of MPs, there need to be more investigation and analysis of MPs (NOAA, 2015). There are limited methodologies for analysing MPs. The most common methodologies of sampling, analysing and identifying of microplastics are implemented in the chemistry laboratory in Arcada University of Applied Sciences and polymer laboratory in Helsinki University to be documented in this report.

Literatures are reviewed for finding the most suitable methods that can be applied in this project. Samples are collected from various beaches in different countries and the same samples are processed in different steps in the chemistry laboratory of Arcada UAS. Two different methods are applied to collect microplastics from the sand samples and microplastics that are found are visually inspected under microscope. During the lab experiment, filtration method is improved for analysing samples with FTIR and regular FTIR was used after microscopic analysis and observed how small particles can be analysed.

2 LITERATURE REVIEW

This chapter explains briefly about the history of plastics, microplastics and their existence in marine environment, tells more about suitable methods to collect and analyse microplastics from beach sediments.

2.1 Microplastics in freshwater

Developing sustainable approach for production area increased the plastic amount all around the world by the middle of 20th century (Valeria Hidalgo-Ruz, 2012). Plastic has been leading many sectors as a preferred material because of its sustainability and durability. Increasing plastic production brought along a large amount of plastic waste which is released into the environment. This created considerable amount of plastic waste in the ecosystem. Landfills and freshwater are filled with plastics due to mismanaged waste and poor infrastructure. The awareness of the plastic pollution has increased significantly in the last decades all around the world. While many studies appeared for macroplastic litter in freshwater and beach sediments in last decade, microplastics observation in water and beach sediments started recently. Observation of microplastics contamination in marine environment increased rapidly last 10 years. Microplastics were found in marine animals, from small organisms to whales, in various size of sea animals. This shows that the microplastics are not able to be removed from marine environment easily, and it doesn't only affect the health of marine animals, it can also go into food chain and affect human health (Stolte, 2014).

Plastic pollution in marine environment has become a problem globally which affects ocean animals as well as humans. Increasing studies about microplastics shows the contamination of microplastics, the distribution in the marine environment, and its hazardous effects on marine species as well as methods to remove microplastics from freshwater. Up to 85 % of marine litter contains plastic which is released as municipal waste. Plastic production increased to 335 million tonnes by 2016 and 60 million tonnes of plastic is produced in Europe (PlasticsEurope, 2018). Plastic production is focused in different segments, the most demand for plastic is in food packaging. On the other hand, less than 5 % of plastics are reusable after first time used. Plastic waste in marine environment varies in different sizes. Macroplastics which are mostly thrown away end up in freshwater and they break into smaller pieces. This creates microplastics which are less visible compare to macroplastics. The size of the microplastic waste brings more problems. It's visibility in the environment is harder compare to macroplastics, and there are limited ways to collect microplastic litter. It has more effect in animal and human health because it can be digested easily by small and big animals in freshwater.

Microplastics get into freshwater in many different ways. Human activity on land or sea is the biggest factor which increase the amount of microplastics in the marine environment. Microplastic beads (see in Fig.1) that are used in cosmetics, synthetic fibres that are washed into waste water treatment plants, mismanaged waste on the beaches or in water activities, etc. Another way for microplastics to enter the marine environment is by climatic sources such as storm or current, as well as sewers (H.S. Auta, 2017). Rain washes the streets, substances that contain plastics and the particles that fall apart from tires are washed into drainage system. Microplastics also enter the marine environment through zooplankton fecal pellets. This means that the organisms, such as zooplankton, digest existed microplastics and eject them as fecal pellets. The ejected fecal pellets that include microplastics sank to the bottom of the sea and they are eaten by bigger species (H.S. Auta, 2017).

2.2 Definition of microplastics

Microplastics are plastic particles that are less than 5 mm size in diameter. Microplastics were observed for the first time in the beginning of 1970s, however they were not defined as microplastic until 2004. Plastics less than 5 mm size are defined as microplastics, although the lower size limit is not specified. Microplastics can be analysed down to 1 μm size, however there are a few documents doing identification on micro particles less than 50 μm (Alice A. Horton, 2017).

Figure 1. Microbeads (Dott, 2016).

There are two different types of microplastics; primary microplastics and secondary microplastics. Primary microplastics are manufactured in micrometre size. It is called microbead and it is aimed to be produced in small size range to be used in industrial area, and mostly cosmetic field. Microbeads are used in sandblasting which contains polyester or acrylic beads, cosmetic products such as pealing gel, shower gel, sunscreen and other cosmetic materials (see in Fig.1). Microbeads are also used in cleaning products. Microbeads used in personal care products contains polyethylene microbeads. Primary microplastics can easily go through the drainage systems and end up in waste water treatments plants. Some waste water treatment plants are able to remove up to 99.9 % of microplastics (Alice A. Horton, 2017).

Secondary microplastics are occurred with the result of macroplastic fragmentation. Macroplastics end up in landfills and marine environment and they are affected from UV radiation, temperature, water, oxygen and organisms. This changes the chemical and physical properties of plastics (Manca Kovač Viršek, 2016). Chemical changes make the plastic more brittle and it causes fragmentation (Alice A. Horton, 2017). Secondary microplastics originate from bigger plastic pieces which has been expanded in the landfills and marine environment, such as litter in landfills, synthetic fibres from clothes, peeled paintings from ships (Stolte, 2014). The amount of secondary microplastics in marine environment cannot be controlled and estimated. The amount of plastics goes into marine environment cannot be prevented because there are many different pathways go into the sea and unknown time period of the plastic litter that ended up in the sea. The size of the sea makes it impossible to estimate the amount of microplastics that is already in the marine environment (Matthew Cole, 2011).

Demand in plastic is growing over the years, and this results with increasing plastic production. Plastic production has increased from 322 million tonnes to 335 million tonnes in one year from 2015 to 2016. Most demand is in packaging by 39,9 %, building and construction sector follows with the demand of 19.7 % (PlasticsEurope, 2018) (see Fig.2). In 2016, 27.1 m tonnes of post-consumer plastic waste were collected. 31.1 % of the plastic waste was recycled and 27.3 % of the plastic waste ended in the landfills in the same year. It shows that for the first time recycled plastic waste was more than the plastic waste in landfills (PlasticsEurope, 2018).

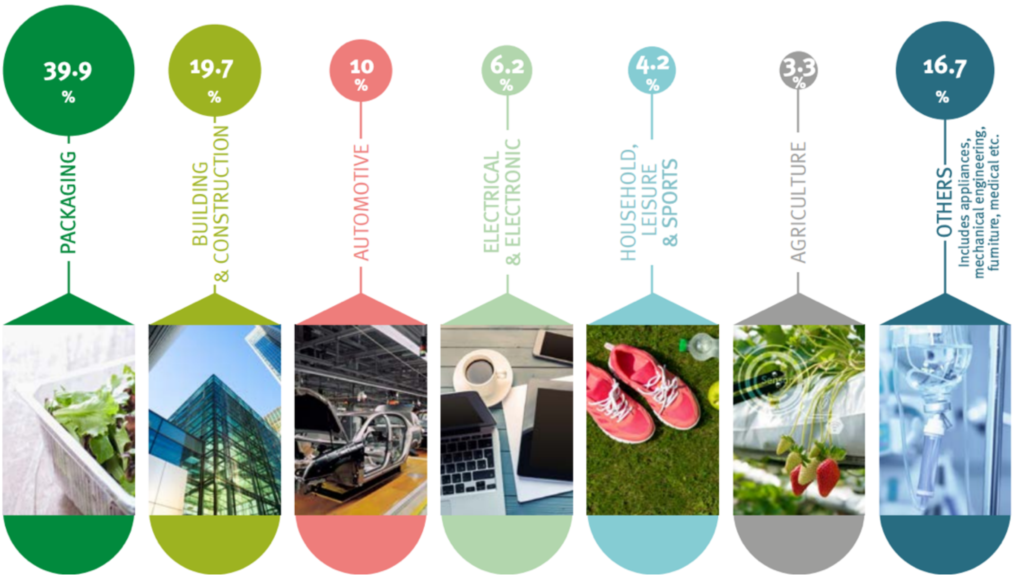


Figure 2. Plastic demand in market sectors in 2016 (PlasticsEurope, 2018).

Many countries have more than 50 % of their plastic waste in landfills, while Switzerland, Austria and Germany have almost no plastic waste in landfills. Except top ten countries including Finland and Norway, rest of the countries use landfills for their post-consumer plastic waste with the rate between 22 % to 81 % (PlasticsEurope, 2018). The number of plastic fragments originate from macroplastics which come from landfills and marine activities are not known. Secondary microplastics can be defined as the largest amount of microplastics in marine environment (Stolte, 2014).

2.3 Effects of Microplastics

Microplastics are toxic materials that are hazardous to the marine organisms. Ingestion of microplastics, additives dispersion of plastics, and contaminants that are caused by microplastics such as persistent organic pollutants (POPs) are the main effects of microplastics to the marine organisms (Julie C. Anderson, 2016).

Plastics have approximately 4 % of chemical additives, mainly plasticizers that are added to the plastics to increase their transparency, longevity, durability and flexibility. Main plasticizers are bisphenol A, phenols and phthalates which affect human health and marine species (Stolte, 2014). Additives involve persistent organic compounds which have a high toxicity level that enter the tissues of marine species that is in the food chain of marine animals and the existence of persistent organic compounds may affect the copulation among them (Stolte, 2014).

Organic pollutants can be added to plastics during production or it is absorbed when the plastic end up in the sea. Plastics contain organic contaminants and transport them to the marine environment. There are two types of adsorption occurs with microplastics; chemical and physical adsorption (H.S. Auta, 2017). Physical adsorption is related to big surface area of microplastic and chemical adsorption is related to hydrophobic surface of microplastic that pulls the organic pollutants towards the plastic (H.S. Auta, 2017). Plastics have hydrophobic surface with a charged biofilm or surface that can collect chemicals such as pesticides, persistent organic pollutants (POPs), and metals. These contaminants get attached to the plastic litters and separate from the litters over time depending on the environmental conditions (Albert A. Koelmans, 2017). POPs are organic compounds that are resistant to degradation in environment through chemical, photolytical and biological processes (L. Ritter, 2007). Absorption of microplastics are related with the type of plastic and its state (rubbery or glassy).

Microplastics can be ingested by a wide range of marine organisms from zooplankton and phytoplankton to marine mammals (Stolte, 2014). Digestion of microplastics by small organisms affect the whole food chain in the marine environment which leads to human health. One study shows that farmed mussels and oysters, that are sold in a fish market in

Germany, contain 0.36 microplastics/g wet weight (ww). The average consumption of mussels and oysters per person in European countries shows that a person can ingest between 1800 and 11,000 microplastic particles in a year (Julie C. Anderson, 2016).

Another study case was about trophic transfer and individual effect of polystyrene (PS) by Chae, Y. Algae were mixed with polystyrene (PS) nanoparticles and algae was given to Daphnia magna which is a zooplankton specie with a length between 1.5 mm and 5 mm, and later on the daphnia magna species were fed to Carrassius Carrassius (crucian carp) fish that has an average of 15 cm length. This was repeated for 61 days with the 3-day of feeding cycle. As a result, carp had less feeding sessions, there were changes in the behaviour, brain histology and in the muscle and liver. Ethanol level was increased in the liver and lysine was increased in muscle tissues (Julie C. Anderson, 2016). Daphnia magna, that was fed with PS nanoparticles, had swollen brains with more water in it (see Fig.3). Microplastics have a lipophilic environment and PS particles may gather in organs with more lipid, such as brain. This results in observation of considerable effects in fish (Julie C. Anderson, 2016).

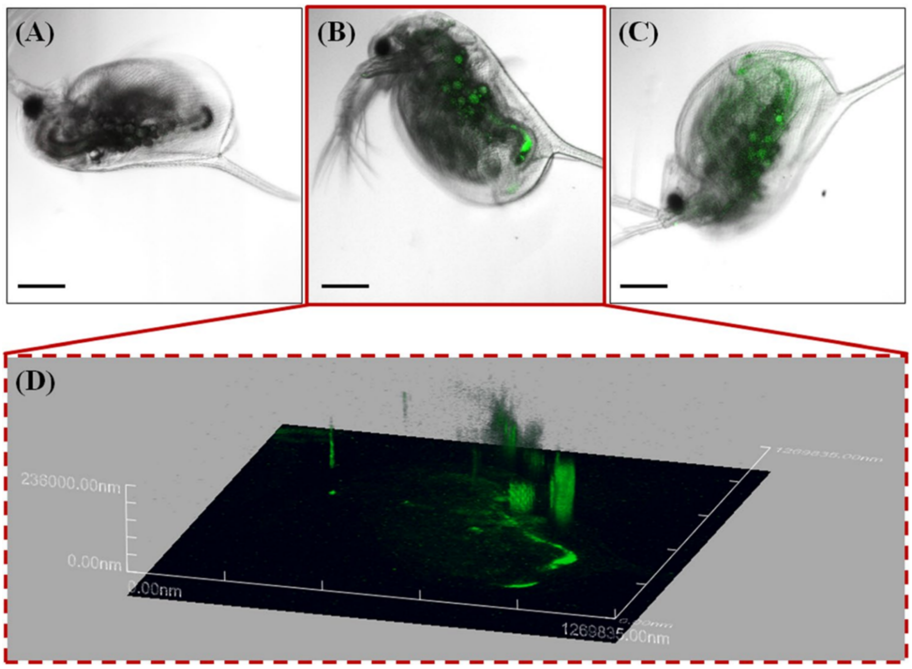
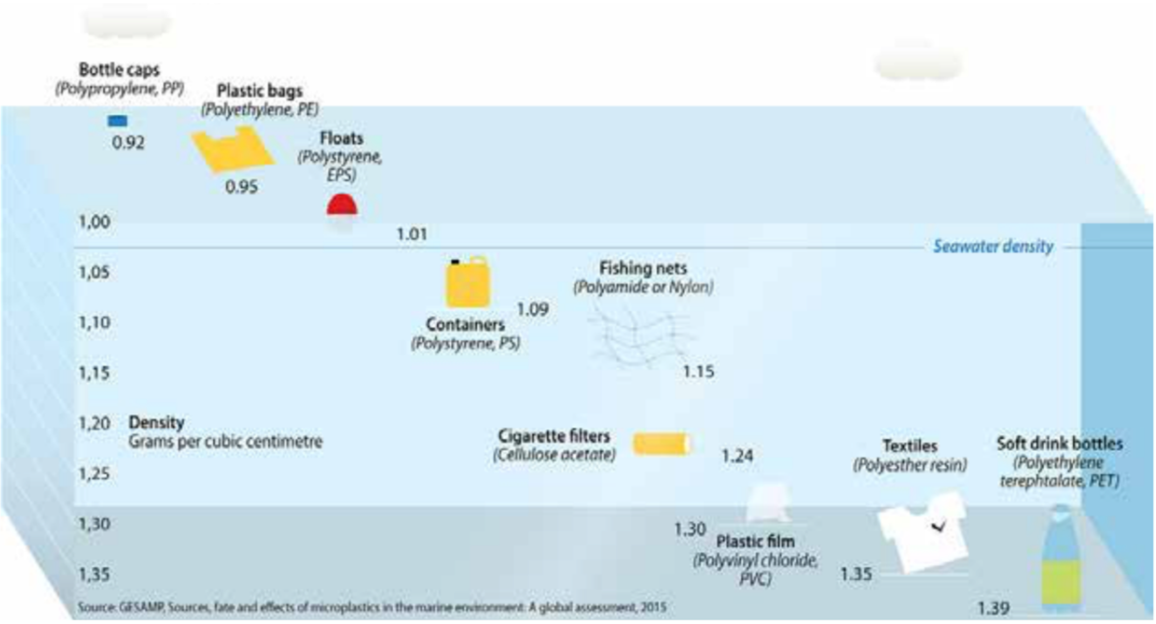


Figure 3. (a) Daphnia magna before feeding with nanoparticles of PS, (b) & (c) Daphnia magna after feeding with nanoparticles of PS, (d) 3D visualisation of Daphnia magna with PS nanoparticles. Scale is 200 µm (Yooeun Chae, 2018).

2.4 Sampling of microplastics in the marine environment

Microplastics are found in beach sediments, shorelines, sea bottom, and on the water surface. The distribution of microplastics are related to the ocean waves, density of the plastic particles, and the place where microplastics are released into freshwater. Microplastics are durable and floatable in the water. These features make them move around easily in the water with currents (H.S. Auta, 2017).



Source: GESAMP, Sources, fate and effects of microplastics in the marine environment: A global assessment, 2015

Figure 4. Systematic image that shows sinking and floating synthetic and semi-synthetic polymers (Kershaw, 2018).

Different types of plastics have different densities which distribute the plastics in water in different levels. Low density plastics float on the surface of the water, high density plastics sink to the sea bottom (see Fig.4). The most common polymer types that are found in the sea have different densities. This results with the change in their location in the sea whether they float or sink. The density differences in polymer types and their behaviour in the sea are shown on Table 1.

Table 1. Polymer types, their density and behaviours (Kershaw, 2018)

Polymer type	***Common areas used***	***Density (g/cm^{-3})***	***Behaviour***
Polyethylene	Storage containers, plastic bags	0.91 - 0.95	Float
Polypropylene	Bottle caps, rope, gear, strapping	0.90 - 0.92	Float
Water		1.00	
Polystyrene (expanded)	Cooling boxes, cups, floats	0.96 - 1.05	Float
Average seawater		1.025	
Polystyrene	Containers	1.04 - 1.09	Sink
Polyamide or Nylon	Ropes, fishing nets	1.13 - 1.15	Sink
Polyacrylonitrile (acrylic)	Textiles	1.18	Sink
Polyvinyl Chloride	Pipe, film, containers	1.16 - 1.30	Sink
Cellulose Acetate	Cigarette filters	1.22 - 1.24	Sink
Poly (ethylene terephthalate)	Bottles, strapping	1.34 - 1.39	Sink
Polyester resin + glass fiber	Textiles, boats	>1.35	Sink
Rayon	Sanitary products, textiles	1.50	Sink

2.4.1 Sampling on the sea surface

Floating microplastics can be found on the sea surface. The most common method to collect microplastic samples on the sea surface is manta net (Manca Kovač Viršek, 2016). Manta net is a cod-end that is attached to a fixed frame which stays on the surface of the water (see Fig.5). Manta net can be placed 3-4 m away from a boat. Sample collection occurs with 30 minutes of driving the boat in one direction. After 30 minutes of sample collection, microplastic samples can be removed from the cod-end with the help of a sieve which can have a 300 μm mesh size, and large objects can be extracted (Manca Kovač Viršek, 2016).

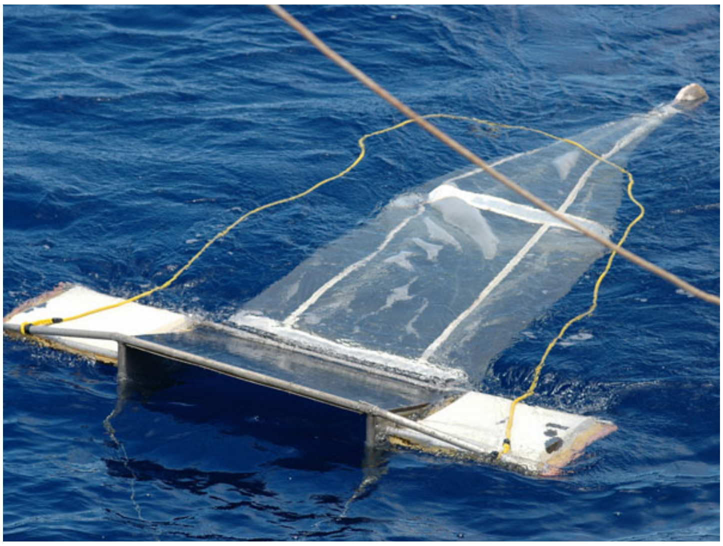


Figure 5. Manta net for microplastics sampling on the sea surface
(SchmidtOceanInstitute, 2019).

2.4.2 Sampling on the sea bottom

Microplastics which has more density than the seawater sink to the bottom of the sea and pile up there. Not only the microplastics that have more density than the sea water sink down, other microplastics which are floating on the sea surface end up on the sea bottom after the result of biofouling by organisms (H.S. Auta, 2017). Biofouling happens when organisms, algae, animals and plants start living on any wet surfaces. Buoyancy occurs on the surface of floating microplastics and it makes them sink. Floating microplastics sink faster than macroplastics, because they lose buoyancy faster than the bigger particles (Francesca M.C.Fazey, 2016).

There is a report focused on a work about collecting microplastics from the sea bed. Sampling location was chosen as the deepest part of the ocean (hadal zone, 6000-11000 m) (X. Peng, 2018). Water and sand samples from sea bed were collected from 2500-11000 m and 5500-11000 m deep in hadal zone where it is called Challenger Deep. The samples were identified with microscope and Raman spectroscopy, and found that many of the samples collected were microplastics (X. Peng, 2018).

2.4.3 Sampling on beach sediments

Beaches have microplastic litter as well as macroplastic litter. Macroplastic litters break down to smaller pieces on the beaches, besides the microplastics that already exists. Microplastics and fibers can change places overtime with water and wind factors. The existence of microplastics cannot be only related to macroplastics because of these factors (Stolte, 2014).

Microplastics can stay for a long period of time under the beach sediment surface in different depths during the aggradation due to the seasonal erosion of the beach sediments (Valeria Hidalgo-Ruz, 2012). This results with that particulate organic matters (POMs) accumulate in the sediments within different lengths, and microplastics which are in the depth of 0-5 cm can be found with POMs (Valeria Hidalgo-Ruz, 2012).

In one research case, the amount of microplastics found in beach sediments are calculated on south and north shores of Lake Garda in Italy by Imhof, H.K. (Hannes K. Imhof, 2013). The amount of microplastics collected from both shores was considerably different. Wind direction is from the south to north on Lake Garda, and the wind carries microplastics to the northern shore of the lake. More microplastic particles are found on the northern shore of the lake because of the wind direction. Wind also affected the water movement of the lake surface that carries the microplastics from surface of the water to the shore (Alice A. Horton, 2017). A significant detail of this case shows that the distribution of microplastics is influenced by many factors such as wind and water movement, as well as urbanisation, lack of waste management, fishing or touristic activities in the area (Alice A. Horton, 2017). Microplastic concentrations of different locations may differ a lot because of these reasons and sampling methods.

Figure 6. (a) Bulk sampling method (b) Volume-reduced sampling method (A Rocha International, 2018)

Many research papers have applied different sampling methods for beach sampling, these are bulk, selective and volume-reduced sampling (Valeria Hidalgo-Ruz, 2012). Bulk sampling is applied where the volume of the sample is not reduced and the whole sample is taken for the next steps (Valeria Hidalgo-Ruz, 2012) (see Figure 6. a). Different tide lines and different spots that are parallel to each other on the same tide line are chosen to have bulk samples. Samples are usually taken from different depths that varies between 3-15 cm deep (Odysseas Piperagkas, 2019). The most common depth for collecting samples from beach sediments is 5 cm, although some studies apply 0-2 cm depth for collecting beach samples, and a few studies apply up to 25 cm depth. (Valeria Hidalgo-Ruz, 2012) Bulk sampling is effective for sampling microplastics that are well mixed with the sand and not easy to observe with naked eye (Valeria Hidalgo-Ruz, 2012). Selective sampling occurs on the surface of the beach sediments where the microplastics can be easily observed with naked eye. This method works with collecting the microplastics one by one on the beach surface. Microplastics that are between 1 mm and 6 mm can be easily seen and picked in this method. However, irregular shaped microplastics and microplastics that are mixed with sand might not be observed well with this method. Volume-reduced sampling is a method when the volume of the sample is reduced immediately after when the samples are taken (see Figure 6. b). This method can be applied with different mesh size filters (Valeria Hidalgo-Ruz, 2012).

2.5 Separation methods of microplastics

Beach sediment samples involve sands, different type of organic matters (from plants to sea shells), and microplastics. It is important to use the right methods to separate microplastics from sand samples. Microplastics can be extracted from sand samples with different methods. The main method that is essential to separate microplastics is density separation, and complementary methods are WPO and vacuum filtration.

2.5.1 Density separation

Density of the plastics changes with polymer types and their production process (Valeria Hidalgo-Ruz, 2012). Density of plastics differs from 0.90 to 1.50 g/cm^{-3} (see Table 1). Sand and similar particles have a density of 2.65 g/cm^{-3}. Having a difference in densities of polymers and sand particles helps to separate microplastics from beach sediments with a saturated solution (Valeria Hidalgo-Ruz, 2012). Mixing saturated solution with sand sample and stirring it for few minutes are enough to make microplastics float on the surface. After stirring and leaving the mixture for a certain time, sand particles settle down and other substances (organic matters and microplastics) with lighter densities float on the surface.

Solutions that are used for density separation process are NaCl (sodium chloride, d=1.15 g/mL), LMT (lithium metatungstate, d=1.62 g/mL) (NOAA, 2015), SPT (sodium polytungstate, d=1.40 g/cm^{-3}) (Käppler, 2016), $CaCl_2$ (calcium chloride, d=1.47 g/cm^{-3}), and $ZnCl_2$ (zinc chloride, d=2.14 g/cm^{-3}) (Stolte, 2014).

2.5.2 Wet Peroxide Oxidation

National Oceanic and Atmospheric Administration-Marine Debris Program (NOAA, 2015) suggests a method that involves a step removing organic matters which is called wet peroxide oxidation. This method is decided to be used in the project because it is a suitable method to be applied in the chemistry laboratory in Arcada University of Applied Sciences and it is planned to be tested as if the WPO step removes all the organic matters as in NOAA literature.

WPO is a common method to remove organic matters from samples and from microplastics where the organic matters are attached to. This method involves a solution of 20 mL of 30% H_2O_2 and 20 mL of 0.05 M Fe (II) (NOAA, 2015). The solution helps removing the organic matters after heating the mixture up to 75 ℃ and repeating this step by adding many times 20 mL of 30% H_2O_2 until the organic matters dissolve (NOAA, 2015). Therefore, the following step becomes easier to identify microplastics from the samples.

2.5.3 Vacuum Filtration

Vacuum filtration is another method that helps separating floating particles from sand samples after density separation. Particles, that floats on the surface which occurs after the density separation process, are taken with the help of a glass pipet and poured on to the filter paper that is placed in a Buchner funnel which is attached to a vacuum pump (How to sample micro plastic, 2015). Most of the organic matters that floats on the surface can be avoided during this process and this way a clear visual inspection can be completed.

2.6 Identification methods of microplastics

Methods to collect and separate microplastics and identification methods are limited with similar steps used in different studies. Common methods to collect microplastics and fibres from sediments are selective sampling, bulk sampling, and volume-reduced sampling (Wenfeng Wang, 2018). After applying two of these methods to collect samples, density separation, filtration, and visual inspection via microscope is followed. To be able to identify the plastic types, analysing microplastics with micro FTIR or RAMAN spectroscopy can be followed. In this thesis, samples are collected with bulk sampling method, after samples are collected, density separation is done to follow wet peroxide oxidation and vacuum filtration as separation methods. After filtration occurred, visual inspection is done with microscopy analysis. Possible microplastics are collected after visual inspection and they are analysed by FTIR spectroscopy to identify the polymer types.

2.6.1 Microscope

Visual inspection is needed for separating the organic matters such as sea plants, remaining from sea animals, shells, salt as well as metal pieces and glasses from plastic particles. Depending on the size of the samples, it can be completed by visualizing the samples with naked eye or microscope (Valeria Hidalgo-Ruz, 2012).

Defining a particle as microplastic, there are important factors to be considered. A particle that can be observed need to have a homogenous and clear color. If the particle's color is transparent or white, it should be observed with high magnification. Fiber looking particles must have the same thickness along their length, and there shouldn't be any organic or cellular structure observed on the surface (Valeria Hidalgo-Ruz, 2012).

2.6.2 FTIR

Microplastic identification is occurred mostly by visual inspection. Visual inspection is very limited to identify microplastics precisely, therefore other identification methods are necessary for different type of polymers. Most common spectroscopic methods applied to identify microplastics are micro FTIR, regular FTIR, Raman and DSC.

Raman spectroscopy is a method that identifies microplastics precisely. Samples are scanned with a laser which is monochromatic. Sample molecules and atoms interact with laser lights by rotations, vibrations and low-frequency and it results with different frequency of the scattered light that is reflected from the sample. Laser wave lengths vary between 500 and 800 μm according to the system used (Martin G.J. Löder, 2015). Raman spectroscopy analyses the surface of microplastic and compare the results with a reference spectrum. This way the identification can be completed in one minute (Martin G.J. Löder, 2015).

DSC is a thermos-analytical method to identify synthetic polymers like thermogravimetry. TGA-DSC is a combination of both, and it observes the heat changes of polymers between solid and liquid phase transitions (Sven Huppertsberg, 2018). To have a clear identification of polymers, sample that is added in DSC should weigh between 5 and 15 mg (Humboldt Universitat zu Berlin, ei pvm).

Fourier transform infrared spectroscopy (FTIR) is one of the most common spectroscopic methods that identifies microplastics (Käppler, 2016). Characteristic spectroscopy occurs by vibrational measurement in FTIR. Molecule vibrations are detected by FTIR which determines the characteristic features of the material. The chemical structure of polymers is measured and compared with a reference spectrum which is recorded by the software in FTIR; thus, the result becomes accurate (Käppler, 2016). Fig.7. (a) shows a model of FTIR by Perkin Elmer. Sample can be placed in the middle of a metal plate as it is seen in number 5 in Fig.7. (b). There is a small crystal (ATR diamond) located in the middle of the metal plate where a certain force applied to analyze samples. This way, reflection occurs through the crystal. Beam splitter (number 2 in Fig.7. (b)) transmits half of the radiation and the other half of the radiation is reflected. Radiation hits the beam splitter and it divides into two beams. One of them goes through the beam splitter to the stationary mirror (number 3 in Fig.7. (b)), and the other one is reflected to the moving mirror (number 4 in Fig.7. (b)). The radiation is reflected back from the stationary and moving mirrors to the beam splitter. Half of the reflected radiation is transmitted, and the other half is reflected at the beam splitter. This results in one of the reflected radiations passes through, the other one goes back to the detector (Anon., 2019).

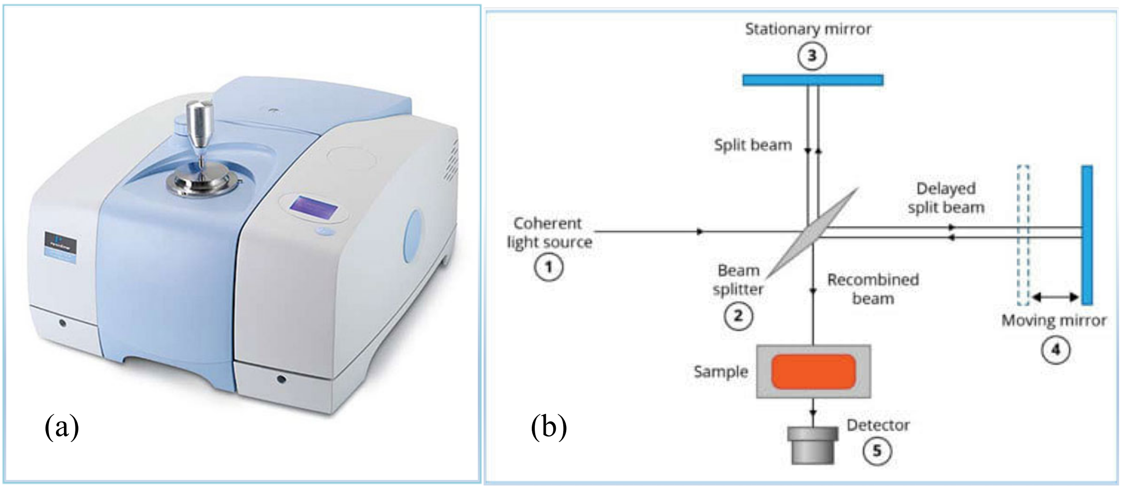


Figure 7. (a) Perkin Elmer Spectrum 100 FTIR Spectrometer (Anon., 2015)
(b) Light path and design of FTIR (Anon., 2015).

FTIR can analyze irregular shaped particles, however it is limited to identify particles that are only bigger than 500 μm (Mai, 2018). Micro-FTIR can analyse particles bigger than

20 μm size, that's why it is one of the most promising method to analyse the polymer types of microplastics (Mai, 2018). Regular FTIR has three different sampling methods which are ATR, transmission and diffuse reflectance (DRIFT) (Alberto Naranjo, 2008). ATR (attenuated total reflectance) is the common method that is used to identify materials with their surface structure. This method works with applying certain force on a material which is placed on a crystal. Transmission method is used for thinner materials ($< 25\ \mu m$) and tablets that are made by powder of materials ($< 2\ \mu m$) and potasium bromide (KBr) mixture which is pressed by hydraulic press (Alberto Naranjo, 2008).

2.7 Summary

Different methods are observed during the literature review, and wet peroxide oxidation method was chosen as the main separation method for this project. Vacuum filtration method was chosen as an alternative method in case of any complications. These two methods are particularly chosen, because they are suitable methods to perform in the chemistry laboratory of Arcada. WPO and vacuum filtration methods are planned to be applied during the experiment. Thus, the result of these two methods are compared with which one is the most suitable method to separate microplastic samples to analyse with FTIR.

During the literature review, it is observed that Raman and micro-FTIR are rare instruments to have access in Helsinki region for this experiment. DSC has an easy access, however DSC is not possible to use in the experiment because each microplastic sample weighs less than 5 mg. This is why regular FTIR is prefferred to be used during the experiment by the author.

3 MATERIALS AND METHODS

The purpose of the experiment was to use the chosen methods correctly to collect microplastics for analysing them with FTIR spectroscopy. Firstly, density separation method was used to collect particles with lower densities which includes fibers, organic matters and microplastics. WPO method and, as an alternative, vacuum filtration method was applied as separation methods, and each method had different filtration tools applied during the experiment such as metal sieve and filter paper. WPO method failed to have the estimated results, therefore the other method (vacuum filtration) was focused during the experiment. Thus, the result of these two methods were compared with which one is the most suitable method to separate microplastic samples to analyse with FTIR.

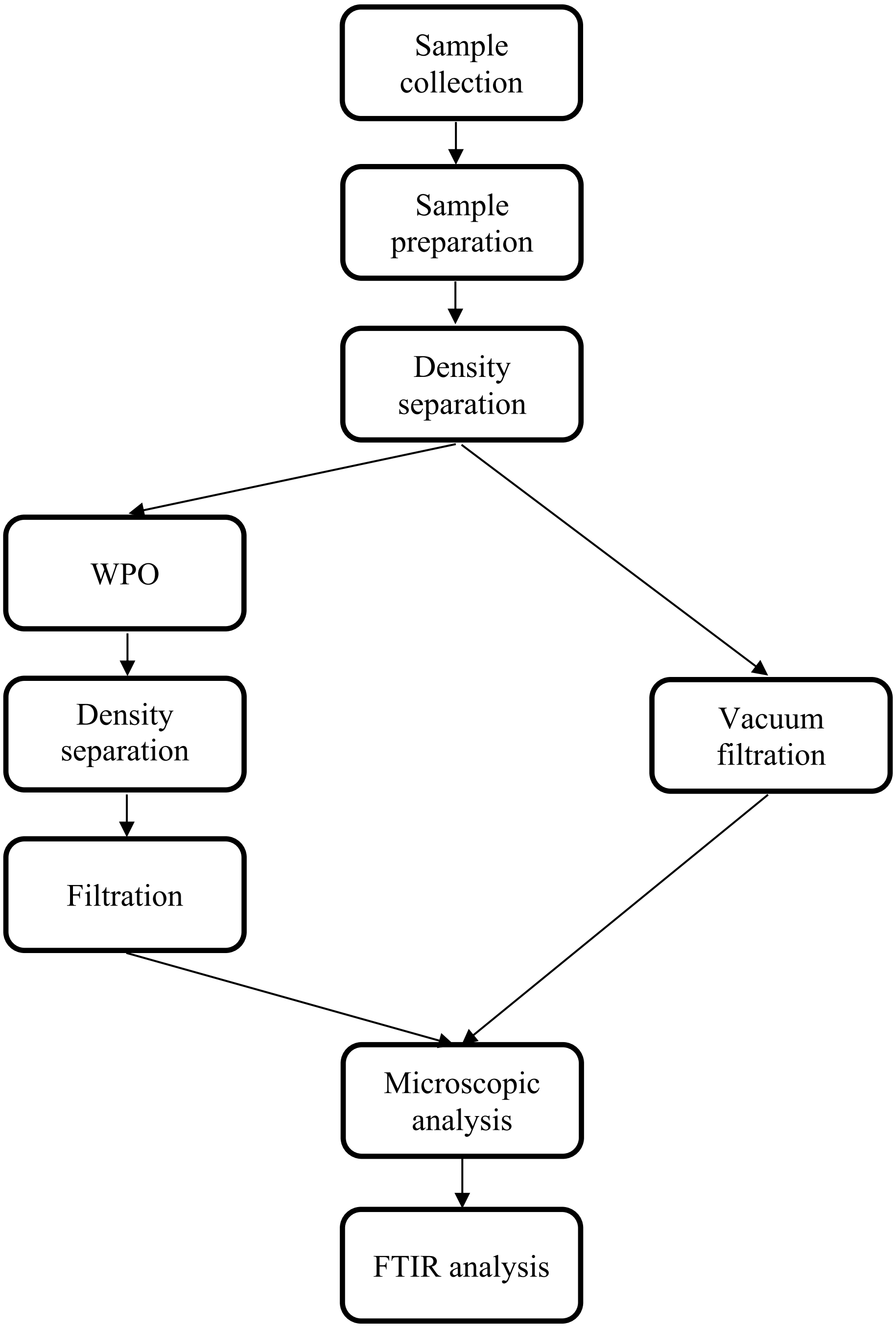


Figure 8. Flow diagram of methods applied during the experiment.

3.1 Materials and equipment used in laboratory

Materials used during the experiment were preferred to be metal, glass and ceramic. Plastic materials or tools were avoided during the experiment to reduce the contamination.

However, some of them were plastic because of limited options. During the experiment, plastic bottles were used to collect samples from different beaches, a rubber tube was used during vacuum filtration, and blue rubber gloves were used by the author.

The main materials and equipment used during the experiment were different size of glass beakers, metal forceps, oven, vacuum pump, filter paper, Buchner funnel, glass pipet, microscope, FTIR spectroscopy, sodium chloride, and other equipment that are attached to the report (see Appendix 1). Some equipment used during the experiment are shown on Fig. 9 a, b.

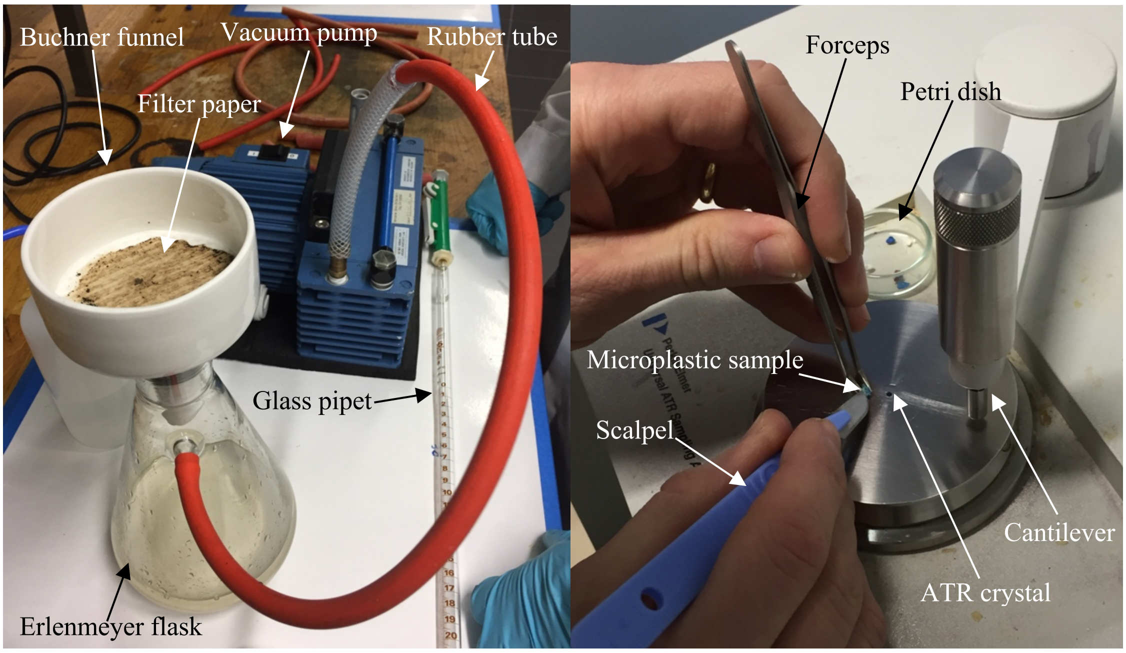


Figure 9. (a) Equipment used for vacuum filtration process (b) Equipment used during FTIR analysis.

3.2 Locations of samples

Samples were collected from several beaches in different countries. Local beaches were suspected too clean and had the risk of not finding any microplastics when the analytical methods were applied. Nevertheless, one local beach was selected for the project. Sample locations in different countries were selected from possible polluted beaches visited by friends during the sampling period of the project. There were samples collected from three different locations in Antalya, Turkey including a river beach, however they were not used in the analytical methods of this project, instead they were used in another thesis project by Ritesh Sharma. Another location that was planned to collect samples was the Seine river in Paris. It was observed that the riversides were covered with barriers all the way in and just outside of Paris. Therefore, the attempt was unsuccessful.

Samples were collected from different beaches listed below and their locations are shown on the Fig. 10.:

- Aberdeen beach, Aberdeen, United Kingdom
- Tigaki beach, Kos, Greece
- Kardamaina beach, Kos, Greece
- Lauttasaari beach (Kasinonranta), Helsinki, Finland
- Miramar beach, Mohammedia, Morocco
- Southsea beach, Portsmouth, United Kingdom
- Escondida beach, Maldonado, Uruguay
- Pocitos beach, Montevideo, Uruguay

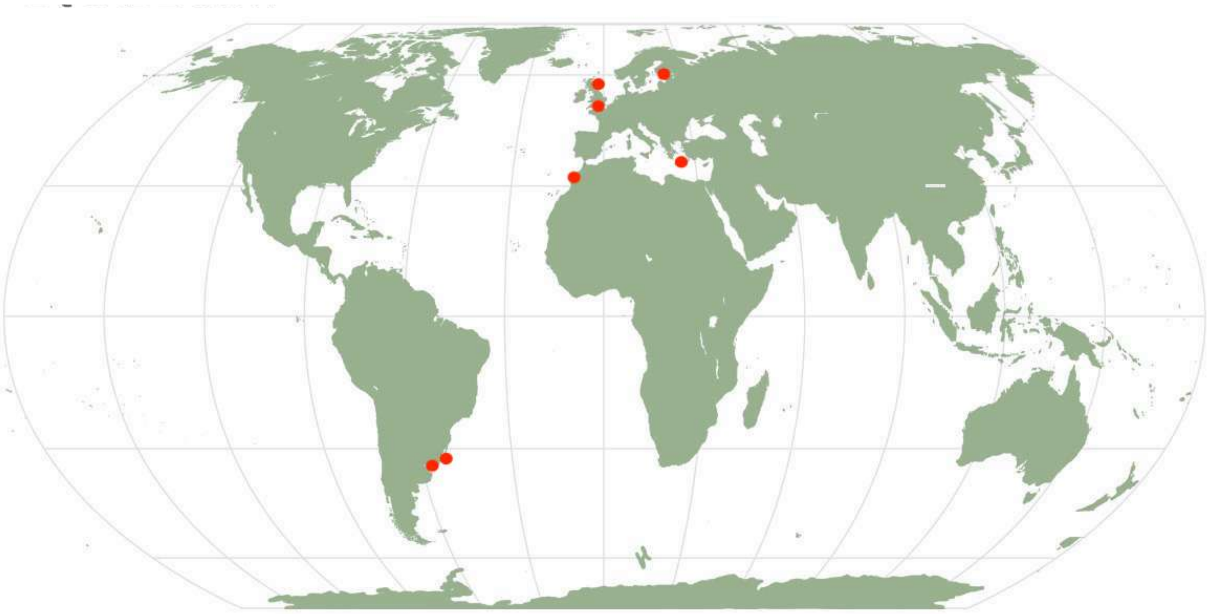


Figure 10. Beach samples locations on Earth map (Page, 2018).

3.2.1 Aberdeen beach

Aberdeen city is the commercial capital of Scotland in northeast. The main industry of the city has been ship building, fishing, paper and following with oil industry. (Britannica, 2019) Aberdeen beach is next to the Aberdeen harbour and located between two rivers; River Don and River Dee where these two rivers flow into the sea. The beach is known as a popular tourist location where many sport activities occurs. (Green, 2010)

Figure 11. High-tide zone on Aberdeen beach, UK.

Aberdeen beach samples were collected from the high-tide zone with the depth of 0-2 cm as it is seen on Fig.11.

3.2.2 Tigaki beach, Kardamaina beach

Kos is one of the most famous touristic location in summer season. The estimated number of people who visited Kos island during 2018 is calculated as more than 1.6 million. (Aris Ikkos, 2018) Tigaki and Kardamaina beaches are on Kos island in Greece which is in the Aegean Sea. Tigaki beach is located on the north coast of the island and Kardamaina is located on the south coast of the island. Both locations have sandy beaches where there are touristic activities occurs.

Tigaki beach samples were collected from the depth of 0-2 cm at storm line and Kardamaina beach samples were collected from the depth of 0-2 cm at high-tide zone. Both beach sediments where the samples collection occurred is seen on the Fig.12.

Figure 12. (a) Tigaki beach, Kos (b) Kardamaina beach, Kos.

3.2.3 Lauttasaari beach

Lauttasaari beach is called Kasinonranta which is on Lauttasaari island in Helsinki. It is a small sandy beach which is on the south coast of the island in Baltic Sea. Lauttasaari has visitors from local community, and there are no touristic and industrial activities in the surrounding.

Samples were collected from the depth of 5 cm at low-tide zone. Storm line and low-tide zone were observed, and some more samples were picked one by one from the surface of the sand. Fig.13. (a) and Fig.13. (b) were taken by the author in Lauttasaari beach where the sample collection occurred.

Figure 13. (a & b) Lauttasaari beach, Finland.

3.2.4 Miramar beach

Miramar beach is along the Atlantic Ocean in the city of Mohammedia in Morocco. There are many hotels located all along the beach, and harbor of the city is next to it. Beach has considerable amount of macroplastic litter as it is seen on the Fig.14.

Samples were collected from the depth of 5 cm at high-tide zone. Samples were also collected from the surface one by one after observation.

Figure 14. Macroplastic litter in Miramar beach.

3.2.5 Southsea beach

Southsea beach is in Portsmouth in UK. There are sport activities occur along the beach during the year, such as miniature golf, beach volleyball, tennis. There are facilities for habitants and tourists along the beach. (Anon., 2019) Samples were collected from the low-tide zone with the depth of 5 cm. It was observed that there were very few amount of marine litter on the beach as it is seen on the Fig.15.

Figure 15. Low tide line on Southsea beach.

3.2.6 Escondida and Pocitos beach

Escondida beach is in Maldonado, Uruguay on the Atlantic Ocean. The beach is between the cities Maldonado and Rocha with about 2 km length. (Anon., 2019) There are touristic and sport activities along the beach where a couple of hotels and playground are located.

Pocitos beach is along the city of Montevideo, Uruguay on the Atlantic Ocean. It is close to the city center and there are many sport activities take place on the beach. The beach is about 1.2 km length and it is convenient for the habitants of the city for many different activities. (Anon., 2019) Pocitos beach is at the end of the river La Plata. La Plata river is 290 km long and it flows in to the Atlantic Ocean. The river is a border between Uruguay and Argentina, and many big cities are located along the river such as Montevideo and Buenos Aires. (Wikipedia, 2019) The river carries litters from many different locations to the Atlantic Ocean which ends next to the Pocitos beach. Escondida and Pocitos beaches are sandy and there were not visible plastic litter seen. Samples were collected from the low-tide zone with the depth of 5 cm.

3.3 Sample collection

Tools for sampling of beach sediments were chosen to be metal, such as metal spoon and tweezers. Synthetic clothing was refused as much as possible during the sampling. Samples were taken from 5 cm depth and/or 0-2 cm depth with the help of a metal spoon. Some microplastic and macroplastic samples were observed while doing visual inspection and taken by tweezers or hand during the sample collection in Lauttasaari, Aberdeen, Tigaki and Kardamaina beaches. Low tide zone and high tide zone were focused for sampling areas. Samples were collected in a plastic bottle with an air-tight lid, and information of the sample (date, time, latitude and longitude) were marked on the bottles after each sampling occurred. About 250 g of dry or wet samples were collected from each location.

3.4 Sample preparation

Each sand samples were put in 800 mL and 500 mL glass beakers. Macro size particles were removed from the beakers and discarded from the experiment as it is seen on Fig.16. Samples were weighed on a scale before drying. They were put in to the oven for 90 ℃ to dry overnight. After drying in the oven, each sample was weighed again to record the actual weight of dried samples.

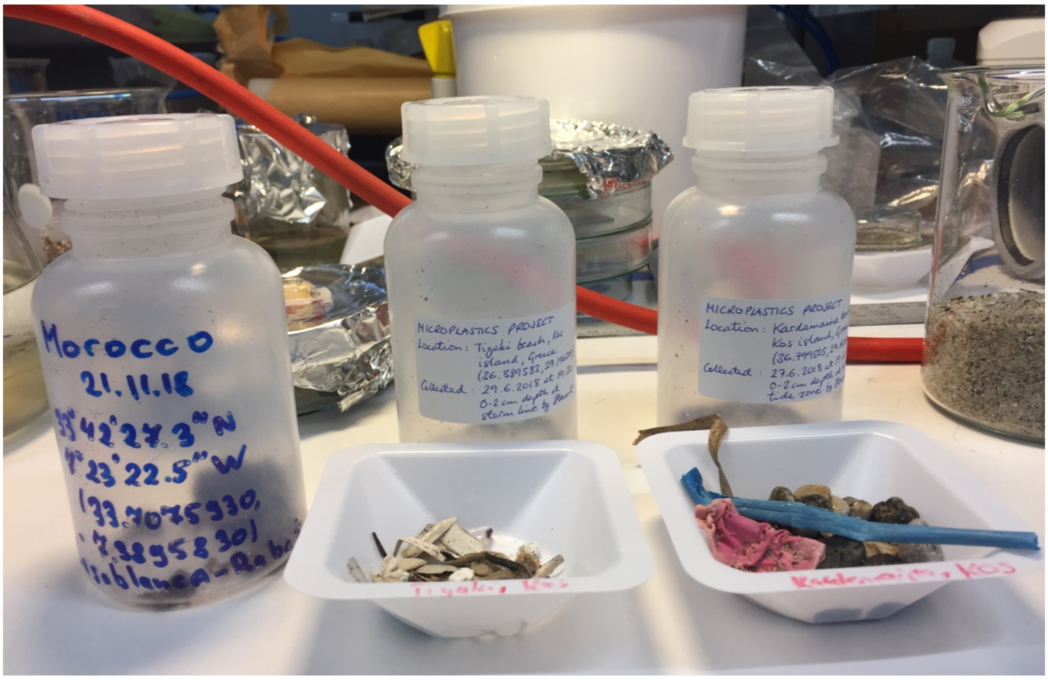


Figure 16. Discarded macro size particles.

Duration of the samples in the oven was not considered as a significant factor. Samples were left to dry in the oven between 20 to 24 hours as it is seen on Table 2.

Table 2. Beach samples duration in the oven and their mass before and after drying

	Duration in oven (t/h)	*Weight before drying (m/g)*	*Weight after drying (m/g)*
Aberdeen beach	20	147,41	146,03
Tigaki beach	24	441,53	438,18
Kardamaina beach	24	423,46	420,8
Lauttasaari beach-1 (Kasinonranta)	21	443,46	408,15
Miramar beach	24	398,41	382,88
Southsea beach	22,5	452,99	452,02
Escondida beach	22,5	436,97	421,46
Pocitos beach	22,5	441,39	406,78

The weights shown on the Table 2 includes weight of the samples and beakers. Aberdeen beach sample was only 50 g and the beaker (250 mL beaker) used for this sample weighted 97,41 g. Sample from Miramar beach, Morocco weighted 200,2 g and the rest of the samples weighted 250 g each. 500 mL glass beakers were used for all the samples except Aberdeen beach sample.

3.5 Density separation

This process is to separate particles with low density and high density. Sand has higher density than most of the plastic particles, and plastics such as polyethylene (PE) and polypropylene (PP) particles have lower density than the average sea water ($\sim 1.10\ g\ cm^{-3}$) (Mai, 2018).

5 M of NaCl (d=1.15 g/mL) or ~5.4 M LMT (lithium metatungstate, d=1.62 g/mL) can be used in density separation process (NOAA, 2015). There are also other solutions such as $ZnCl_2$ (d=2.14 g/cm^{-3}), $CaCl_2$ (d=1.47 g/cm^{-3}) (Stolte, 2014) and SPT (sodium polytungstate, d=1.40 g/cm^{-3}) which can be used in density separation process for floatation (Käppler, 2016). 5 M of NaCl was supposed to be used in the experiment because it is more environmentally friendly solution than the other solutions (Wenfeng Wang, 2018).

5 M NaCl was supposed to be mixed with 0.5 L distilled water for density separation process, however 2.5 M of NaCl was used because of unintended calculation error. Formula of molarity in 1 L is shown below (Anon., 2019).

$$Molarity = \frac{Moles\ of\ Solute}{Volume\ of\ solution\ (mL)} \times 1000\ mL$$

$$Molarity = \frac{{}^{Given\ mass\ of\ Solute}/_{Molar\ mass\ of\ solute} \times 1000\ mL}{Volume\ of\ solution\ (ml)}$$

Calculation of 2.5 M NaCl in 0.5 L distilled water:

$$2.5\ M = \frac{{}^{mass}/_{58.44\ g} \times 1000\ mL}{500\ mL}$$

$$mass = \frac{5 \times 58.44\ g \times 500\ mL}{1000\ mL}$$

$$Mass\ of\ Solute = 73.05\ g \text{ of NaCl}$$

73.05 g NaCl was weighed and added in 0.5 L distilled water. 73.05 g of NaCl and water was stirred for few minutes with the help of a metal spoon. After NaCl was dissolved in the water, it was poured gradually into the sample beaker while mixing the sample with a metal spatula for 5 to 10 minutes. After mixing, the beaker was left for 15 to 20 minutes to settle down. After waiting, visible floating particles were picked by metal forceps and macro size particles ($> 5\ mm$) were discarded. Fig.17 (a, b, c) shows the density separation process and floating particles.

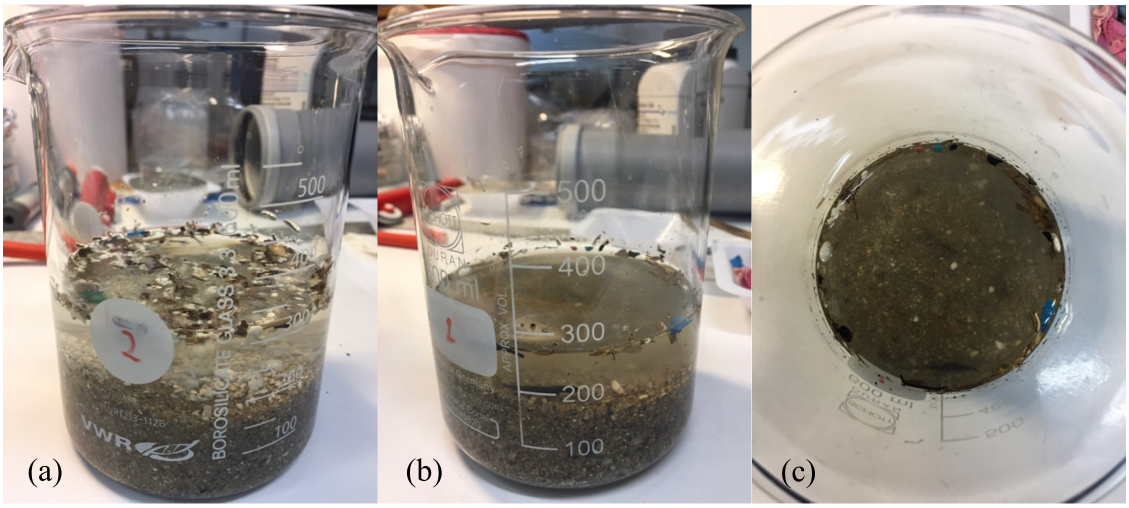


Figure 17. Floating particles of different sand samples during the density separation process.

3.6 Wet Peroxide Oxidation (WPO)

Wet peroxide oxidation was applied on 50 g of the beach samples listed below:

- Aberdeen beach, UK
- Tiyaki beach, Greece

Wet peroxide oxidation is a reactive mixture of chemicals. Before starting this analysis, laboratory safety rules need to be followed carefully.

WPO is a process that is used for removing organic materials from samples. Floating samples were collected in the previous step and dried in an oven with 90 ℃ for 24 hours. The floating samples contained organic matters. Most of the organic matters were planned to be removed during this process, therefore microscopic analysis of the samples would have less complications to observe microplastics. This process in the experiment was followed from a document of NOAA Marine Debris Program (NOAA, 2015).

Ingredients of the WPO mixture:
20 mL 0.05 M Fe (II) in 500 mL H_2O
20 mL 30% H_2O_2

The following ingredients were needed for 20 mL of 0.05 M Fe (II):
$7.5\ g\ FeSO_4 \times 7H_2O$
$500\ mL\ H_2O$
$3\ mL\ conc. H_2SO_4$

Fe (II) compound: $(NH_4)Fe(SO_4)_2 6H_2O$
$(NH_4)Fe(SO_4)_2 6H_2O = 392.13\ \ g/mol$ (Wikipedia, 2019)

$0.025\ mol\ Fe(II)$ for 500 mL mixture:

$$\begin{aligned} Moles\ Fe(II) &= V \times concentration \text{ (Ausetute, 2018)} \\ &= 0.5\ L\ \times\ 0.05\ \ mol/L \\ &= 0.025\ mol \end{aligned}$$

$Mass\ of\ Fe(II) = moles\ Fe(II) \times\ molar\ mass\ of\ Fe(II)$
$m = 0.025\ mol \times 392.13\ \ g/mol$
$m = 9.80\ g$

0.05 Fe (II) was made with the mix of 500 mL distilled water, 3 mL of concentrated sulfuric acid and 7.5 g of $FeSO_4 7H_2O$. 20 mL of 0.05 Fe (II) and 20 mL 30 % H_2O_2 were added in a 200 mL beaker with the dried samples. The mixture was left for 5 minutes in the room temperature before heating process started. Hot plate stirrer was set to 75 °C in a fume cabinet. After 5 minutes, a magnetic stir bar was added in the beaker and the mixture was put on the hotplate with a watch glass cover. The temperature of the mixture needs to be below 75 °C, therefore a thermometer was added in the beaker to control the temperature rise. To be able to reach 75 °C, the hotplate temperature was increased up to 100 °C, and when it was observed that the mixture's temperature got close to 75 °C, hotplate's temperature was decreased.

The mixture started boiling, therefore it was removed from the hotplate and let to cool down. It was observed that there were still organic matters in the sample beaker. Second time, 20 mL of 30 % hydrogen peroxide (H_2O_2) was added into the beaker and the same process was repeated. After adding the second 20 mL of 30 % hydrogen peroxide, reaction occurred faster than the previous step. When the mixture boiled, it was removed from the hotplate and observed that there were still organic matters in the beaker. This step was repeated 4 times and at the end there were still organic matters.

This experiment was supposed to be repeated until there were no organic matters in the mixture by adding 20 mL of 30 % hydrogen peroxide. After this step, 6 g of NaCl (salt) for 20 mL of the mixture should have been added to increase the density of the solution. And it supposed to be heated up to 75 °C to dissolve the salt in the solution. However, the process was stopped after observing the organic matters in the solution. (NOAA, 2015)

3.6.1 Density separation for WPO

Second density separation was involved only in the wet peroxide oxidation process. The second density separation needs to be done after the WPO analysis.

After repeating 4 times adding 20 mL of 30 % hydrogen peroxide to the mixture and observed that there were still organic matters in the mixture, macro sized organic materials were taken out manually. The mixture was moved to a new beaker, and distilled water was added to wash all the micro particles to the new beaker. The mixture in new beaker was covered loose with aluminum foil and left in the fume closet for overnight.

After the mixture was left to dry for overnight in the fume closet, the particles that sink at the bottom were discarded.

3.6.2 Filtration with sieves

Floating particles were separated from the mixture by a metal sieve with a mesh size of 30 μm. After this step, 25 μm metal sieve was used to collect smaller particles. Metal sieves with different mesh sizes were created by Ritesh Sharma who previously worked on his thesis project about experimental analysis of microplastics.

The beaker was rinsed with distilled water to move all the particles to the metal sieve with a mesh size of 30 μm. Particles that were bigger than 5 mm diameter were removed manually from the sieve and samples on the metal sieve were left to dry for couple of hours. After drying, double-sided tape was used to remove the particles that were stuck in the sieve (Fig. 18). And some of the particles were manually collected.

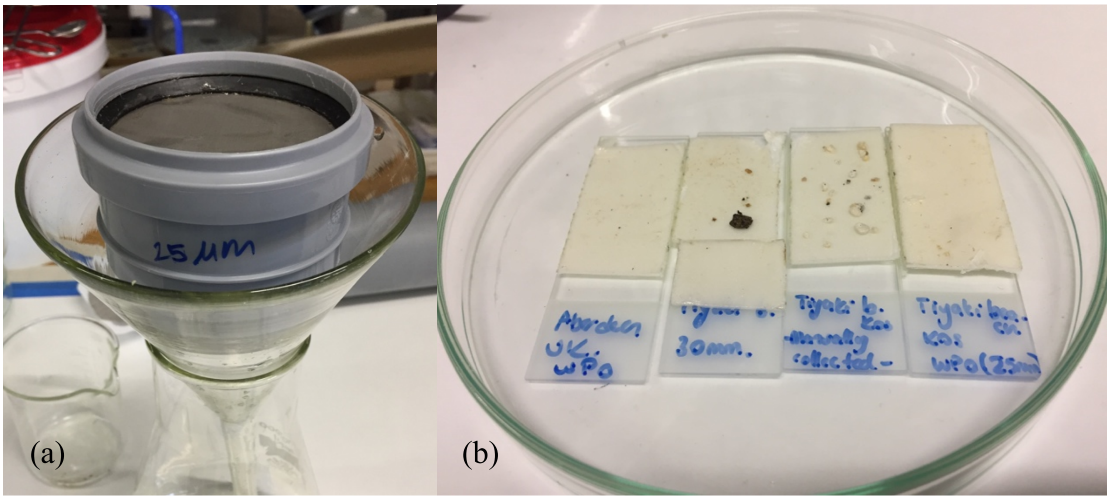


Figure 18. (a) Filtration with 25 μm mesh size sieve after WPO process. (b) Samples taken with double-sided tape after filtration with metal sieves.

3.7 Vacuum filtration

Vacuum filtration was involved as the second separation method that is used for the experiment as it is seen on the flow diagram in Fig.7. This process in the experiment was followed from an electronic source prepared by Damien Beri (How to sample micro plastic, 2015).

Vacuum filtration was applied on the following beach samples:

- Kardamania (Greece)
- Lauttasaari-1 (Finland)
- Lauttasaari-2 (Finland)
- Miramar (Morocco)
- Southsea (UK)
- Escondida (Uruguay)
- Pocitos (Uruguay)

Vacuum filtration is another separation method followed in the experiment after the WPO separation method was stopped (see in Fig.19).

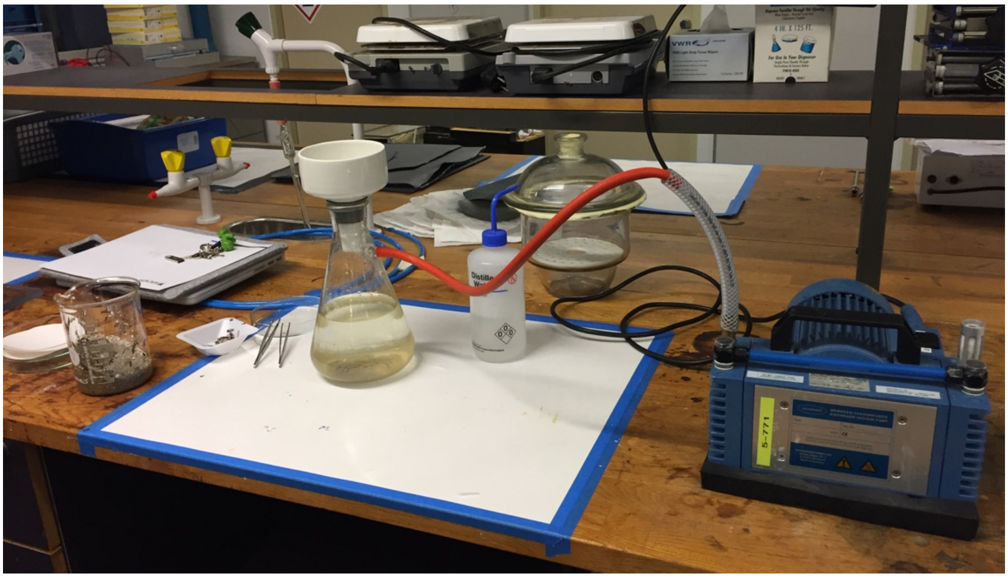


Figure 19. Vacuum filtration process.

Floating samples were taken out after the density separation process with different size glass tubes and they were poured on a filter paper which was placed in a porcelain buchner funnel while the vacuum pump was on. This step was repeated until there were no floating particles in the beaker. While pouring the floating particles in the porcelain funnel, a vacuum pump was used to absorb the water in Erlenmeyer flask which was placed under the porcelain funnel as it is seen in the Fig. 20 (a, b).

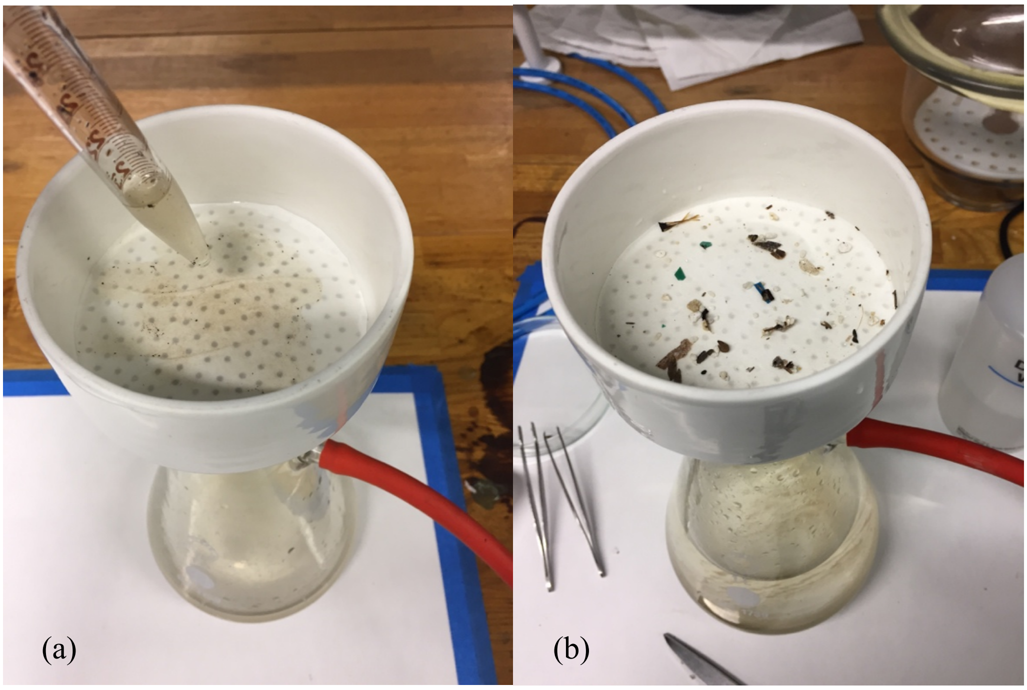


Figure 20. (a) Pouring floating samples from density separation process via glass pipet. (b) Samples on a filter paper after vacuum filtration process.

The filter paper that was used in this method is Qualitative filter paper with slow filtration rate and particle retention is 2 μm. This filter paper was used because it was the only suitable option as a filter paper in the chemistry laboratory of Arcada University of Applied Sciences.

3.8 Microscope analysis

Microscopic analysis of the experiment occurred in the chemistry laboratory of Arcada University of Applied Sciences with Zeiss Axio Scope.A1 microscope.

Visual inspection of the samples that were collected after density separation and filtration was an essential process. Visual inspection was done by microscope when the filtered samples were dry. (Valeria Hidalgo-Ruz, 2012) To reduce the contamination, synthetic clothing should be refused while doing microscopic analysis.

Samples were observed with 5 X, 10 X, 20 X, and 50 X magnification. During the microscopic analysis; dark field (DF), bright field (BF), and differential interference contrast (DIC) filters were used.

After the filtration was applied with metal sieves, double-sided tape was used to take the samples. The samples were placed on a tape which is placed on a microscope glass slide and left to dry for couple of hours.

After vacuum filtration was applied, samples were left to dry overnight in a covered plate to reduce contamination. To observe microparticles on a round filter paper, following method was applied. Observation started at one corner of the filter paper, and a straight path down was followed. When reached the edge of the paper, took a u turn and straight path up was followed until the other edge was reached. This way, the round filter paper was observed without missing any spot on the paper. Microscopic analysis of samples on a filter paper is seen in Fig. 21 (b).

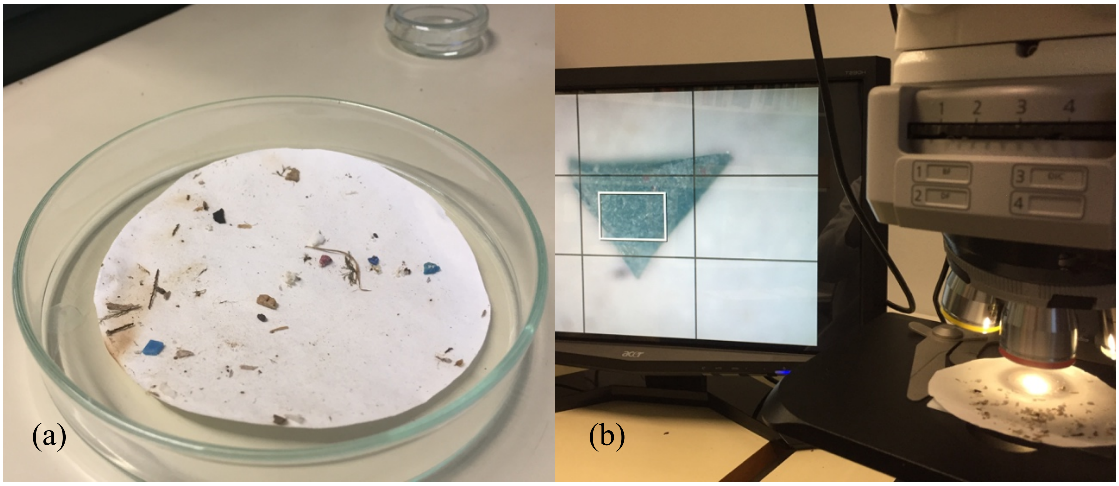


Figure 21. (a) Samples on a filter paper after drying overnight. (b) Microscope analysis of samples on a filter paper.

3.9 FTIR analysis

FTIR analysis was occurred in a polymer laboratory in Chemistry Faculty of Helsinki University. The FTIR used for this experiment was Perkin Elmer Universal ATR Sampling Accessory (Perkin Elmer Spectrum 100 FTIR Spectrometer) and the software which run the spectrums is Spectrum v5.0.1.

After doing microscopic analysis and collecting possible microplastic particles, they were observed with FTIR spectroscopy to identify what material they consisted of. Firstly, size of a minimum particle that could be analysed by FTIR was observed, and possible microplastic particles that had big enough size were picked from the filter papers after microscopic analysis, and each of them were analysed by ATR-FTIR spectroscopy. Each micro particle was put on the spot where ATR diamond is, and certain force was applied. The force applied on the sample was observed on the spectrum software and force gauge was chosen to be between 100 kg and 115 kg to have a good measurement. After the force was applied, software ran the spectrum and it showed the most possible options of what the material consisted of on a spectrum list with a graph of the spectrum. Placement of microplastics on the ATR diamond and calibrated force applicator is shown on Fig. 22.

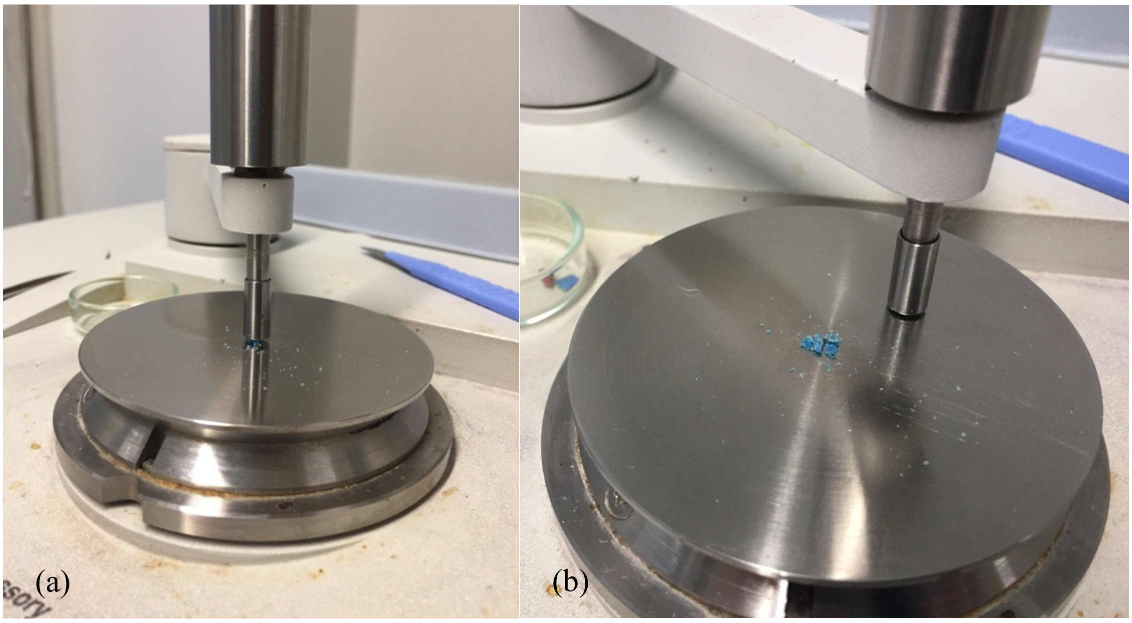


Figure 22. (a) & (b) Microplastics analysis with ATR-FTIR.

Fibers that were found during the microscopic analysis were collected for FTIR analysis and transmission method was used to analyze the samples. This method identifies difficult solid materials with powders. (PerkinElmer, 2005) A tablet was made of potassium bromide (KBr) powder and it was used in FTIR to identify particles. $\sim 20\ mg$ of potassium bromide powder was added in a small mortar (4 cm diameter size bowl) where a fiber was placed. They were grinded together with a pestle for up to 10 minutes and the fiber became small pieces in the mixture. The mixture was added in a KBr pellet press which was placed in a hydraulic press later. A KBr tablet was made of this mixture by the hydraulic press which has a force gauge up to 15 tons. ~ 10 tons was applied to make each tablet. These tablets which were grinded with different fibers that were found in beach samples were placed in a plastic pellet holder in FTIR sample chamber. Chamber door was closed, and spectrum ran to show results on the graph.[1] (Jordan)

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[1] For more information of the steps: Michael R. Jordan, *Using the IR,* (Oklahoma Baptist University).

4 RESULTS AND DISCUSSION

4.1 Results of WPO process

WPO process was repeated by adding 20 mL of 30 % hydrogen peroxide four times to the mixture while heating the mixture up to 75 °C. This process was stopped after adding 30 % hydrogen peroxide for the fourth time, because it was observed that many of the organic matters were still present in the beaker as it is seen in Fig. 23. (a & b).

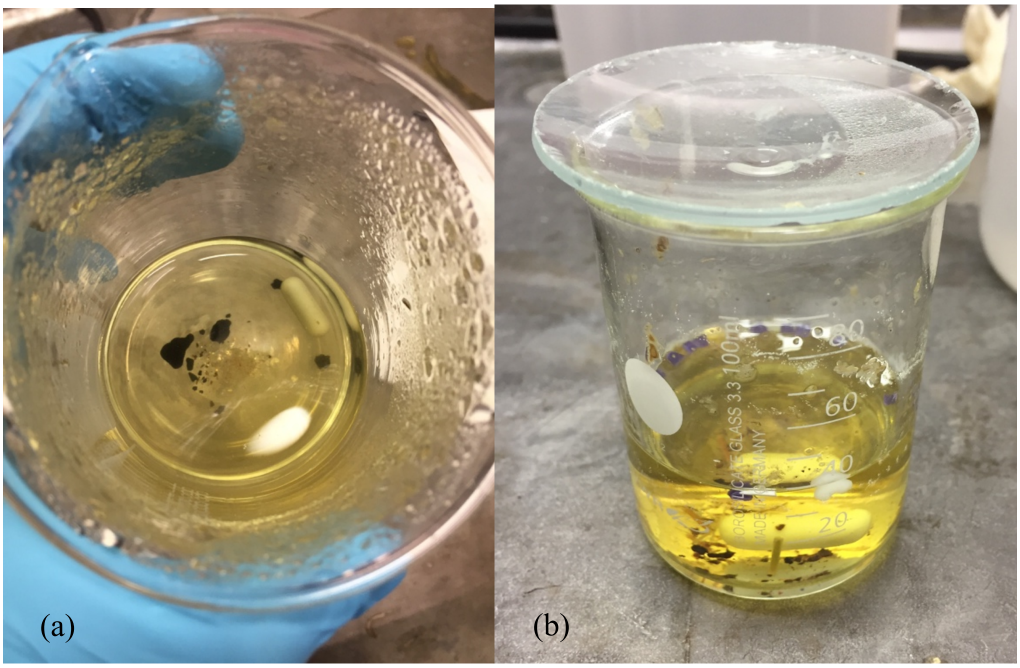

Figure 23. (a & b) Remaining organic matters after WPO process.

This method was used for two different beach samples. The process was stopped after observing organic matters in both samples.

4.2 Results of vacuum filtration process

Vacuum filtration process was applied for seven different samples. Many organic matters were observed after density separation; however, they were not discarded during this method. The method was successful for separating the floating samples from NaCl solution with a glass pipet and a vacuum pump. Using a filter paper during this method was helpful to observe the particles under microscope and manually collect them for FTIR analysis.

4.3 Results of microscope analysis

Microscope analysis was completed for all the micro particles that were collected through the experiment. Besides microplastics, a lot of fibers, organic matters and sea shells were observed during the microscopy analysis. Considerable amount of fibers with different colors were observed in each beach samples even though some beach samples didn't contain any micro plastics. These fibers consisted of one color; blue, red, black, and white. Two different fiber samples are shown in Fig. 24. a & b.

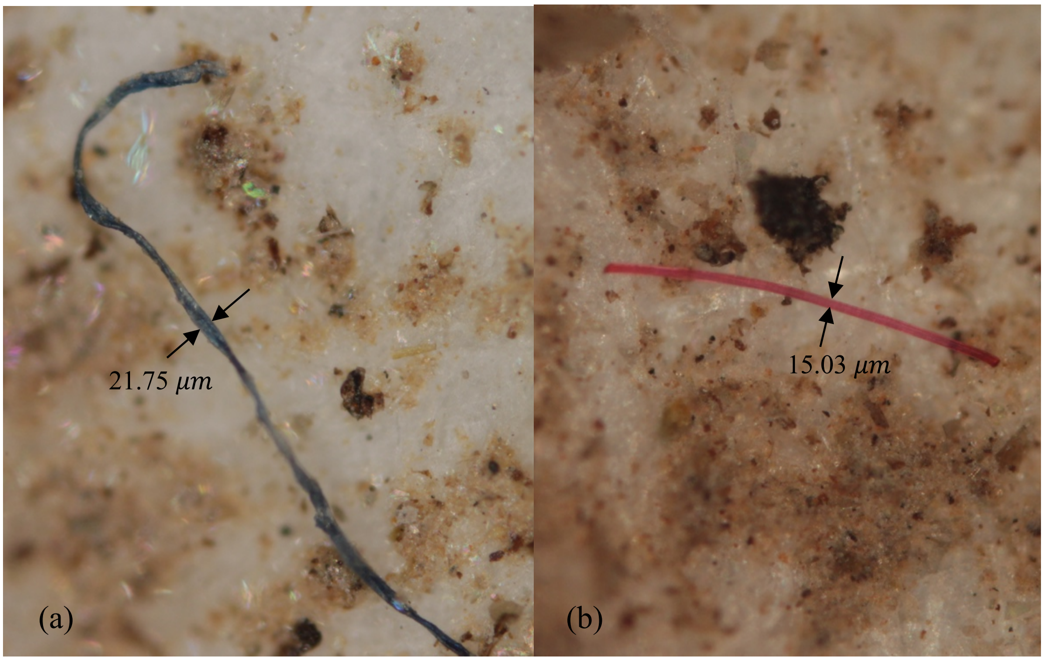

Figure 24. (a) 10 X magnification, DF. Blue fiber. (b) 10 X magnification, DIC. Red fiber.

Many of the microplastics that were found during the microscopy analysis had flat surfaces and uneven edges. Some MPs had round shapes and wavy surfaces, however there were not many microplastics found with irregular shapes. Each MPs that were found during the experiment consisted of a single color. During the experiment; pink, blue, dark blue, white, green colored MPs were found. Examples of two different microplastic samples are shown in Fig. 25. a, b, c, d.

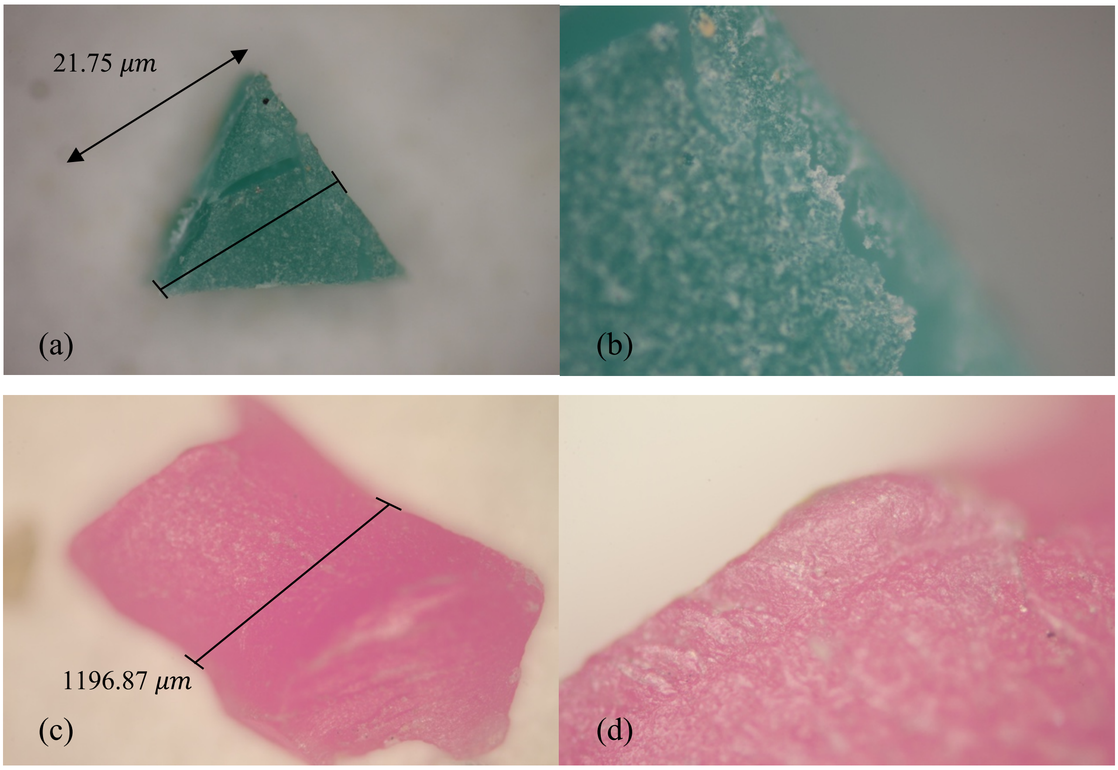

Figure 25. (a) Green microplastic sample with 5 X magnification, DF. (b) Green microplastic sample surface with 20 X magnification, DF. (c) Pink microplastic sample with 5 X magnification, BF. (d) Pink microplastic sample surface with 20 X magnification, BF.

Many micro size organic matters were found in the samples during the microscope analysis. A few of them had hard and flat surfaces and broken edges like microplastics, this is why they were mixed with microplastics. Examples of the organic matters are shown on Fig. 26. a, b.

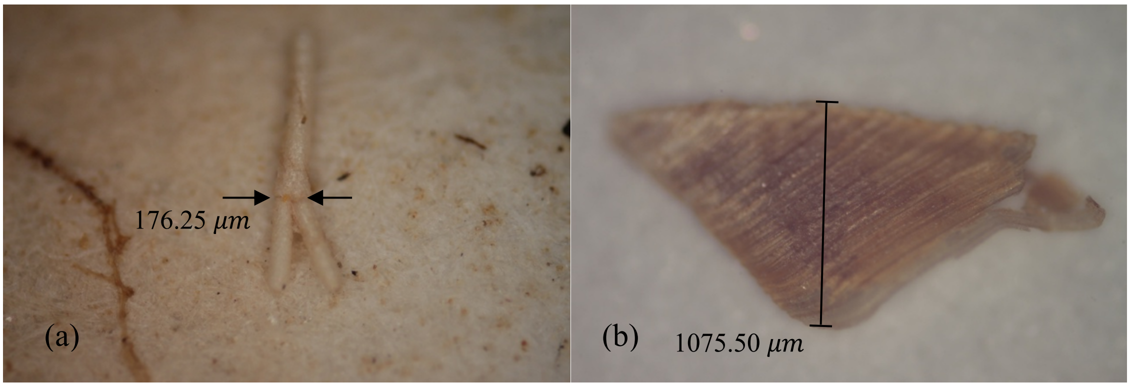

Figure 26. (a) Organic sample with 5 X magnification, DF. (b) Organic sample with 5 X magnification, BF.

4.4 Results of FTIR analysis

FTIR analysis was done after visual inspection of each micro particles. During microscope analysis, possible microplastic particles were manually collected from the filter papers and saved for FTIR analysis. There were many micro particles identified as organics during the FTIR spectroscopy that were previously observed as possible microplastics during the microscope analysis. Most of these particles were plant based, and the rest was made of animal shells. The particles that were identified as microplastics were mainly high-density polyethylene (HDPE), and there were a few polypropylene (PP) found during the identification process. There was one microplastic identified as polyvinyl chloride (PVC) with the possibility of 48%. There was no polyethylene terephthalate (PET) found during this experiment. Spectra of each polymer type and organic matters found in beach sediments, and the comparison of the samples' spectra are shown on Fig. 27, 28, 29, 30, 31, 32.

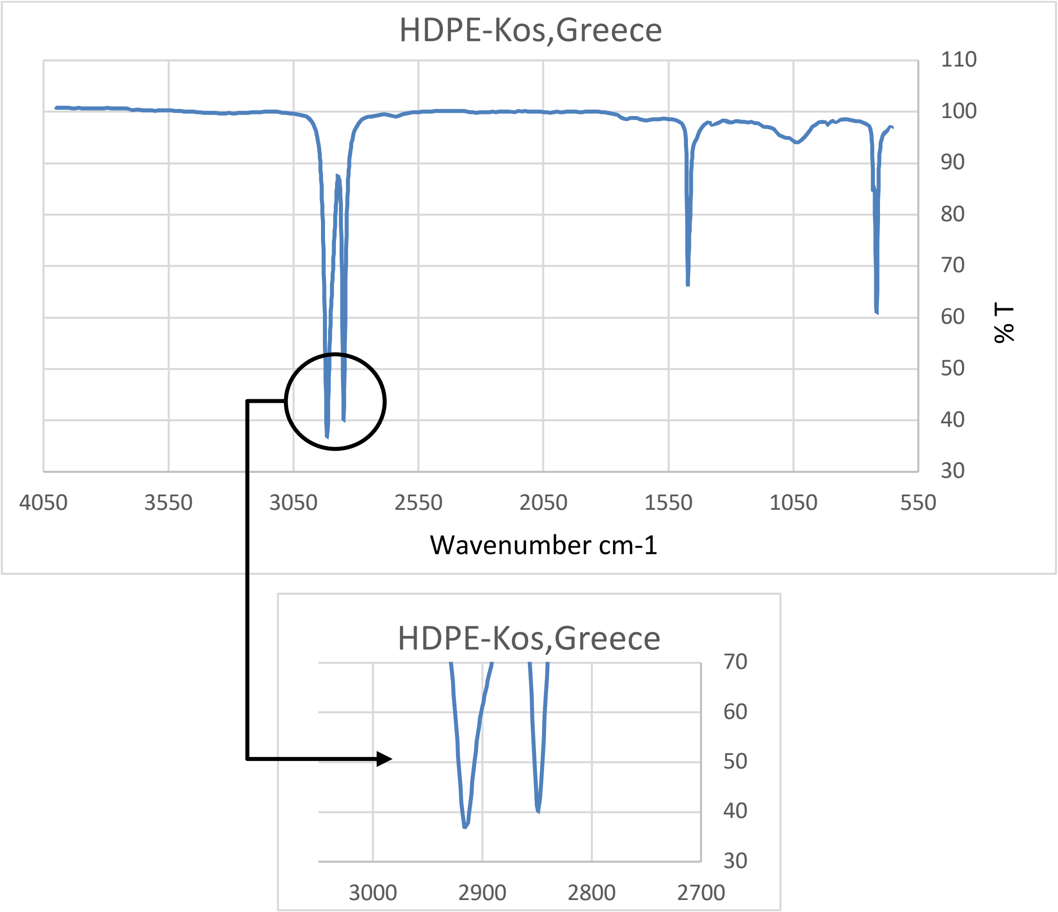

Figure 27. HDPE microplastic sample spectra

Figure 27 shows the spectra of HDPE microplastic sample which was found in Tigaki beach in Kos, Greece. The sample contained only blue colour. Result of the FTIR spectroscopy showed that the sample was HDPE with a 98% possibility. This sample was first put on the ATR diamond plate and analysed. The first result was unclear because of a worn surface. The sample was cut into small pieces with a help of a scalpel and the small pieces were analysed. Result showed a clear peak for HDPE. Two sharp and long peaks were observed between 3000 cm^{-1} and 2800 cm^{-1} and they are aliphatic C-H bond of methylene and methyl groups of polyethylene. The peaks below 3000 cm^{-1} were defined as sp^3 C-H stretch (Alberto Naranjo, 2008). Another strong peak was observed between 1500 cm^{-1} and 1450 cm^{-1}. The shape of the peak was defined as C-H in methylene groups of polyethylene (Alberto Naranjo, 2008). The peak between 750 cm^{-1} and 650 cm^{-1} doesn't need to be defined because it is in the finger print region. However, it could be defined as Ortho substitution pattern for an aromatic compound. Finger print region is between 1500 cm^{-1} and 500 cm^{-1}. This region can have different absorptions that can be unique to each compound. (Score, 2019)

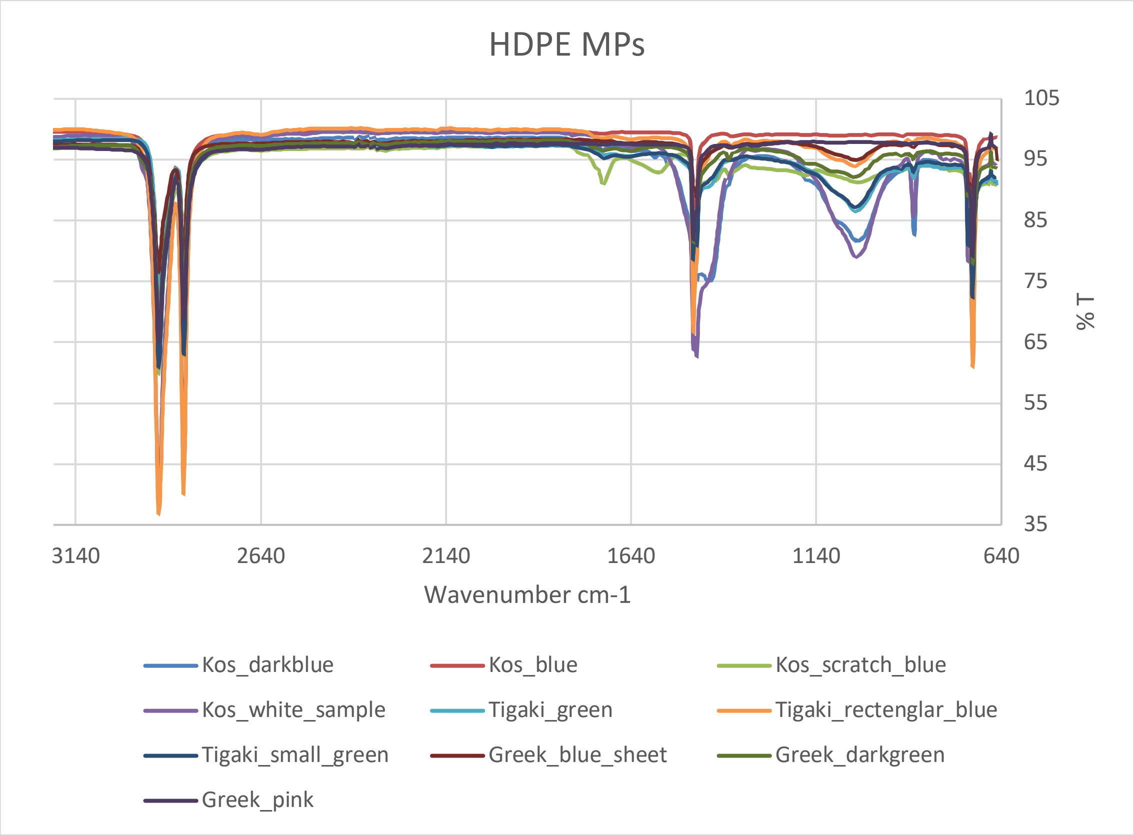

Figure 28. Comparison of HDPE microplastic samples

Figure 28 shows the spectra of all the HDPE microplastic samples. Long peaks of the samples were observed between 3000 cm^{-1} and 2800 cm^{-1} are aliphatic C-H bonds. Intensity of the long peaks shifts for each HDPE microplastics however the energy absorbed by aliphatic C-H bonds shows the same result for each sample.

Kos_scratch_blue sample (green line on Fig. 28) has a slightly different result from the other samples. The clear peak on 1714 cm^{-1} is ketones carbonyl extension (Alberto Naranjo, 2008). It was observed that there was no C-H bond of methylene groups of polyethylene while it was observed in the rest of the samples. The slight differences between the samples could be explained as that samples would have had different production processes and they had different environmental conditions since they were released in the environment.

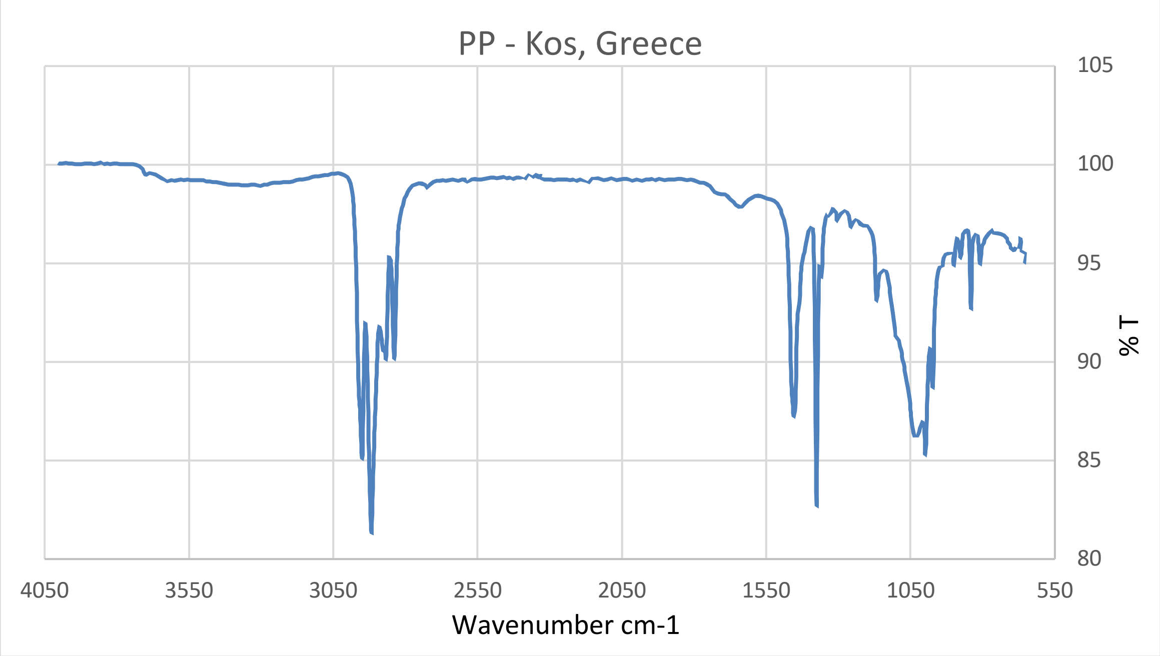

Figure 29. PP microplastic sample spectra

Figure 29 shows the spectra of PP microplastic sample which was found in Tigaki beach in Kos, Greece. Colour of the sample was pink, and it didn't contain any other colour. Result of the FTIR spectroscopy showed that the sample was PP with an 85.7% possibility. The peaks between 2990 cm^{-1} and 2820 cm^{-1} are C-H stretches. First two strong peaks are sp^3 C-H stretches, and last two are aldehyde C-H. The first peak below 1500 cm^{-1} showed a CH_2 deformation. Second peak which was the longer in this region showed a symmetric CH_3 deformation. The rest below 1400 cm^{-1} was observed as isotactic polypropylene bands (Jordi, 2011).

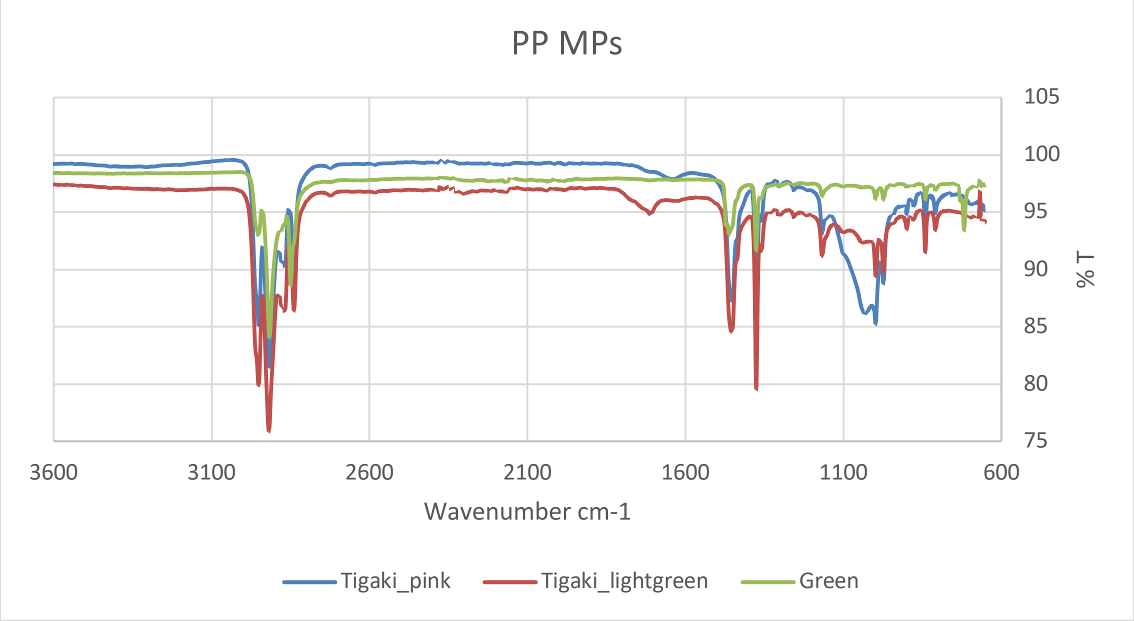

Figure 30. Comparison of *PP microplastic samples*

Figure 30 shows the spectra of all the possible PP microplastic samples. Long peaks of the samples were observed between 2970 cm^{-1} and 2830 cm^{-1} are C-H stretches. Intensity of the peaks between 2970 cm^{-1} and 2830 cm^{-1} differs for each PP microplastics however the energy absorbed by C-H bonds shows the similar result for each sample. The third peak of the green sample at 2850 cm^{-1} is polyethylene oxide which differs this sample from the other two (Alberto Naranjo, 2008). Tigaki_pink (blue line) and Tigaki_lightgreen (red line) have similar intensity in the fingerprint region, however green sample from Kos (green line) has slightly different peaks.

The FTIR spectra result of the green sample from Kos didn't show PP in the possible options of what sample contains, however it showed Poly (4-Methyl-1-Pentene) with an 81% possibility. Despite this, the sample was compared with PP samples in the same graph because of the similarities in peaks. The chemical changes of the samples via UV radiation, water, oxygen, temperature and organisms (POMs) that are stuck on microplastics may show different results on spectra. Also, different environmental conditions of each samples affect the results.

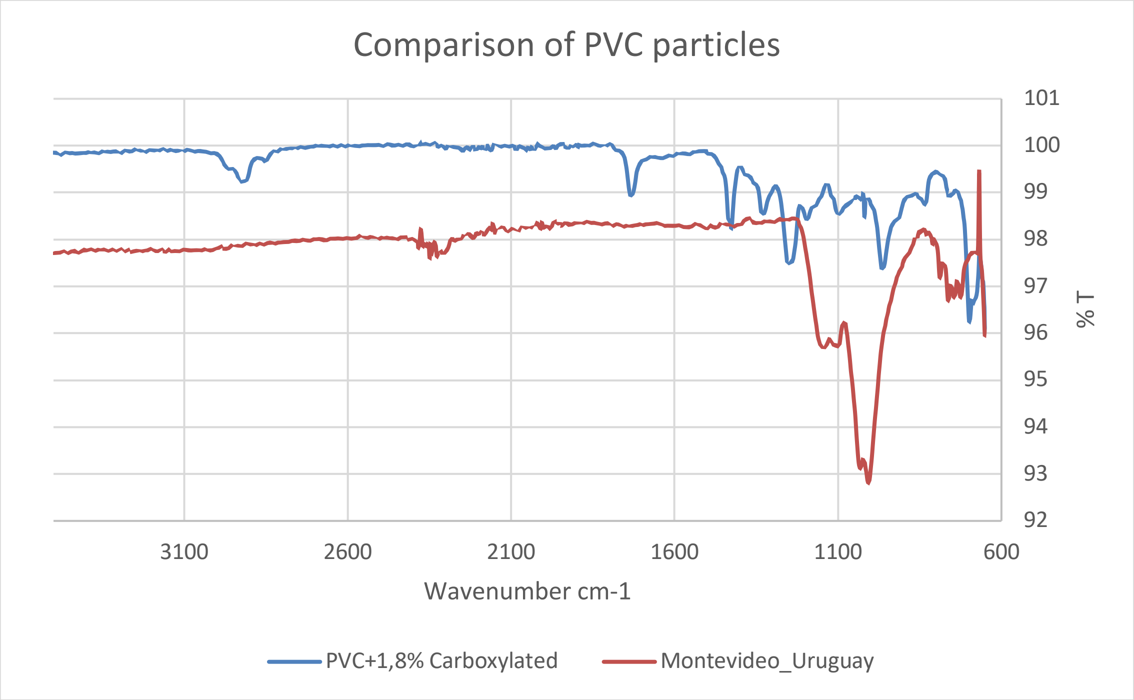

Figure 31. Comparison of pristine PVC material and PVC microplastic

Figure 31 shows the spectra of PVC microplastic sample which was found in Montevideo, Uruguay (red line) and a pristine PVC material that was used as a reference (blue line). Colour of the PVC microplastic sample from Uruguay was white. The sample had a weak spectra analysis, and the highest possible material obtained was PVC with a 48% possibility. The reference material was identified as PVC+1,8% Carboxylate in the spectra. The peaks of the sample from Uruguay between 1180 cm^{-1} and 1080 cm^{-1} are C-C stretches. The peaks between 1050 cm^{-1} and 950 cm^{-1} are CH_2 rocks. The last line which is half completed peak would be a C-Cl stretch as it is in the region between 675 cm^{-1} and 650 cm^{-1}. However, the spectra was completed at 650 cm^{-1}.

The peak at 1730 cm^{-1} of pristine PVC is a carbonyl extension. The peaks at 1420 cm^{-1}, 1322 cm^{-1} and 698 cm^{-1} shows the main peaks for PVC (Alberto Naranjo, 2008). However, first two peaks were not observed on the spectra of PVC sample from Uruguay. The sample from Uruguay had only 48% possibility that it is PVC, and the spectra of these two materials show very clear differences.

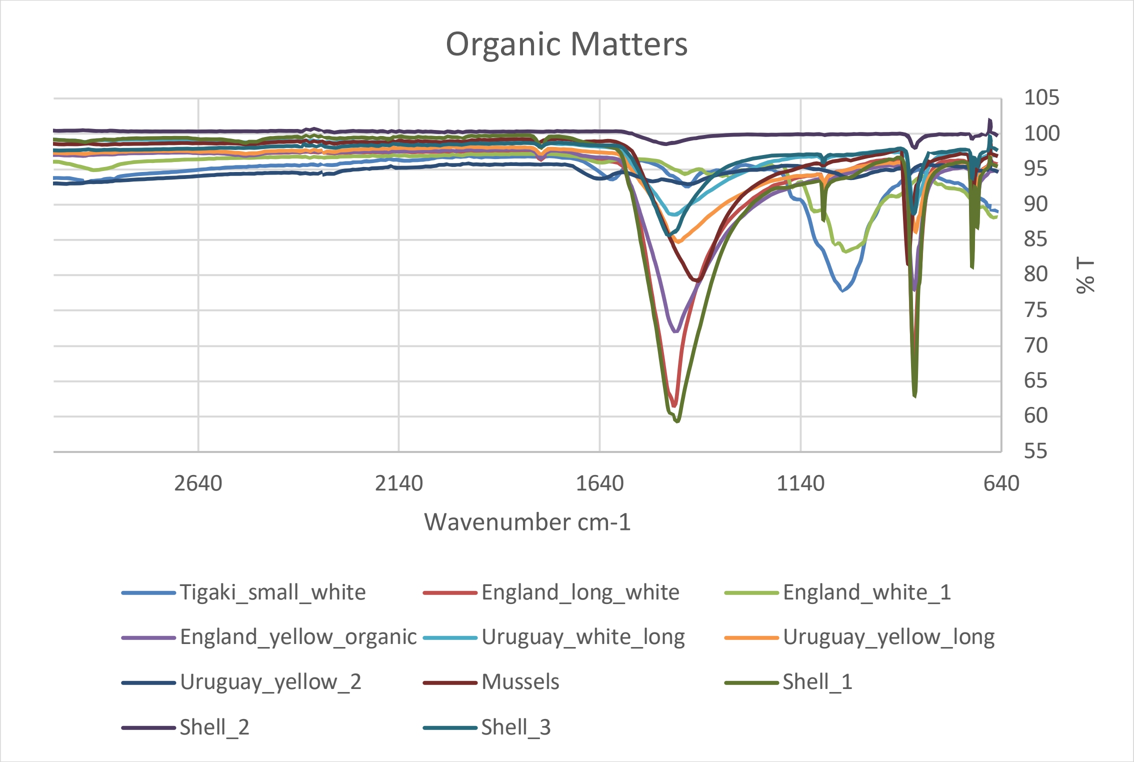

Figure 32. Comparison of organic matters

Organic matters that were found during the experiment were analysed and some of them were identified by FTIR spectroscopy. Figure 32 shows the spectra of the organic matters. The broad peaks around 1450 cm^{-1} shows an aliphatic C-H flexion (Alberto Naranjo, 2008). Intensity of the peaks shifts for each organic matter. The long peaks and a broad peak below 1140 cm^{-1} show variety between each organic particle.

Each of the samples' spectra are attached to the report (see Appendix 2).

4.5 Analysis of WPO

This method was followed from NOAA Marine Debris Program (NOAA, 2015) to observe if the method would be successful with following all the steps in the report. This method was applied in NOAA guidelines with a successful result. However, it was not clearly mentioned how many times it was repeated to achieve. During the experiment, this method was applied by adding H_2O_2 four times. However, it failed to dissolve organic matters.

WPO was applied to two different samples and 30 % hydrogen peroxide was added four times to dissolve organic matters. There were no considerable changes with the amount of organic matters in samples after adding 30 % H_2O_2 the fourth time and then the process was stopped by the author. All the chemicals were added with right amount and the steps were followed carefully as it is mentioned in NOAA guidelines. The reason of the failure could be because the step with adding 30 % H_2O_2 was not repeated enough.

The author removed organic matters manually from the sample beaker after the failure of WPO method. This method was not used in the experiment for other samples after it failed. Floating samples in WPO process, which were separated by metal sieves, were taken by double-sided tapes, and the samples on tapes were not used in FTIR analysis.

4.6 Analysis of vacuum filtration

Vacuum filtration was a simple method to apply for separating and filtering floating particles. It was a suitable process to work with filter paper. This method is not useful when using metal sieves with different mesh sizes.

When floating particles were taken with a glass pipet, it was simple to avoid the organic matters going into the pipet. This resulted with having less amount of organic matters on the filter paper with microplastics, therefore it was easier to do visual inspection.

4.7 Analysis of FTIR spectroscopy

Perkin Elmer Universal ATR Sampling Accessory (Perkin Elmer Spectrum 100 FTIR Spectrometer) in Helsinki University was used for identifying microplastic samples. Different size samples were ran with FTIR to define the minimum size that can be identified with the instrument. It was observed that minimum 0.5 mm diameter size samples can be analysed and identified with this instrument.

Many of the microplastic samples that were ran with ATR-FTIR spectroscopy had clear spectrums and they were identified as polymers with high possibility. Some of the microplastic particles were observed with less possibility of polymer types, because they, including organic particles, had poor surface quality. POMs stuck on the surface of microplastics and organic matters, therefore this resulted with weak spectra.

Some microplastic samples had rough surface and therefore the spectra had weak results. Some of these samples were cut into small pieces and some others were grinded to be able to have more clear results in spectrum. There were samples that the surface was peeled to find a clear result as it is seen on Fig. 33.

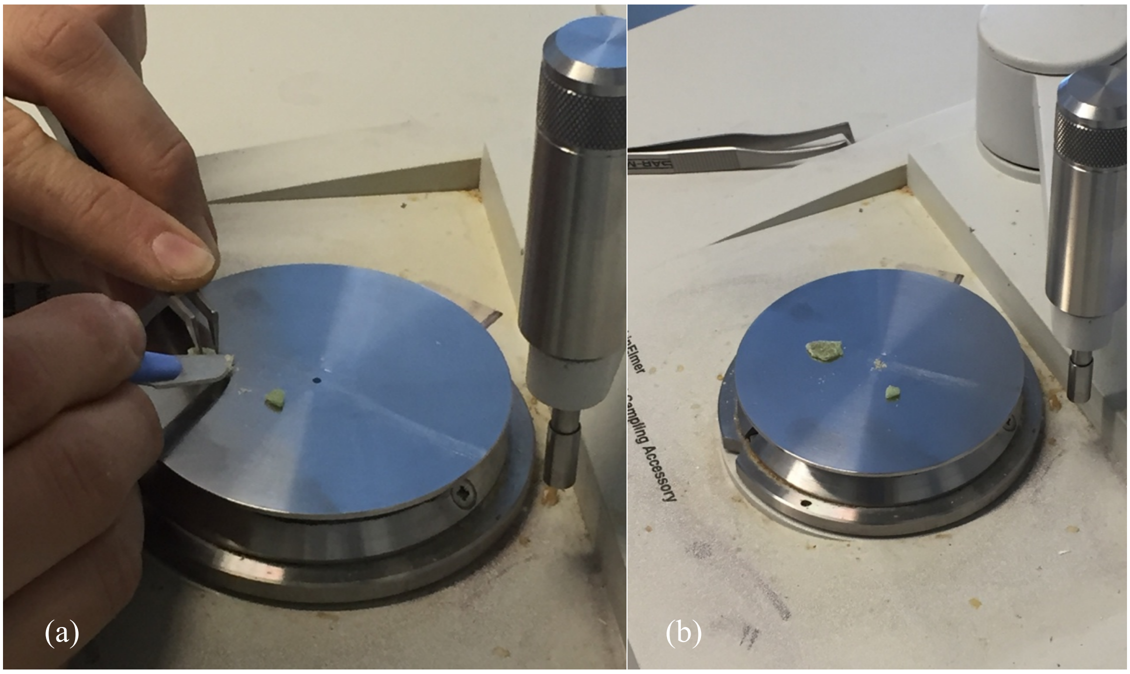

Figure 33. (a & b) Steps of peeled microplastic samples to be analysed in FTIR.

Fibers that were found during the microscopic analysis were analyzed with transmission method in FTIR spectroscopy. This method was applied to three different fiber samples. Fibers were grinded in potassium bromide (KBr) powder and tablets that were made by grinded fibers and KBr powder was added in the FTIR sample chamber. Spectrums of the fiber samples had no clear result because of poor analysis of the KBr tablets. Fibers were not grinded well because of their mechanical structure (see Fig.34 a). Tablets showed tiny pieces of synthetic or semi-synthetic fibers and they couldn't be transformed into powder (see Fig. 34). Therefore, spectra had weak wave fluctuations as it is seen in Fig.35.

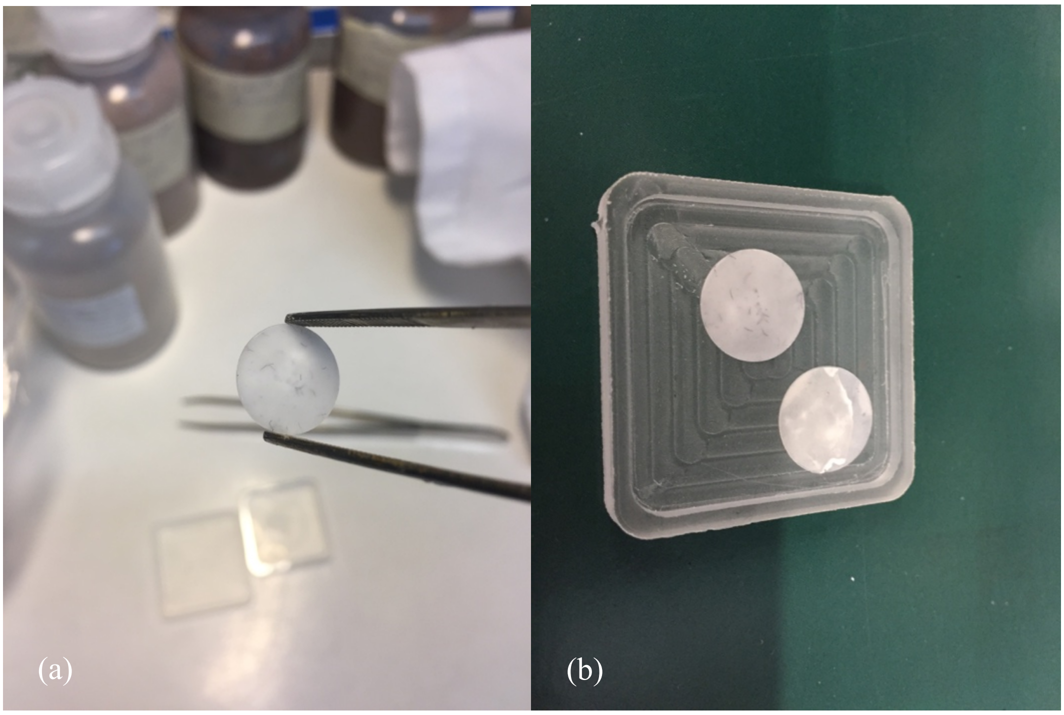

Figure 34. (a) Visible fiber particles in KBr tablet, (b) KBr tablets with uneven mixture.

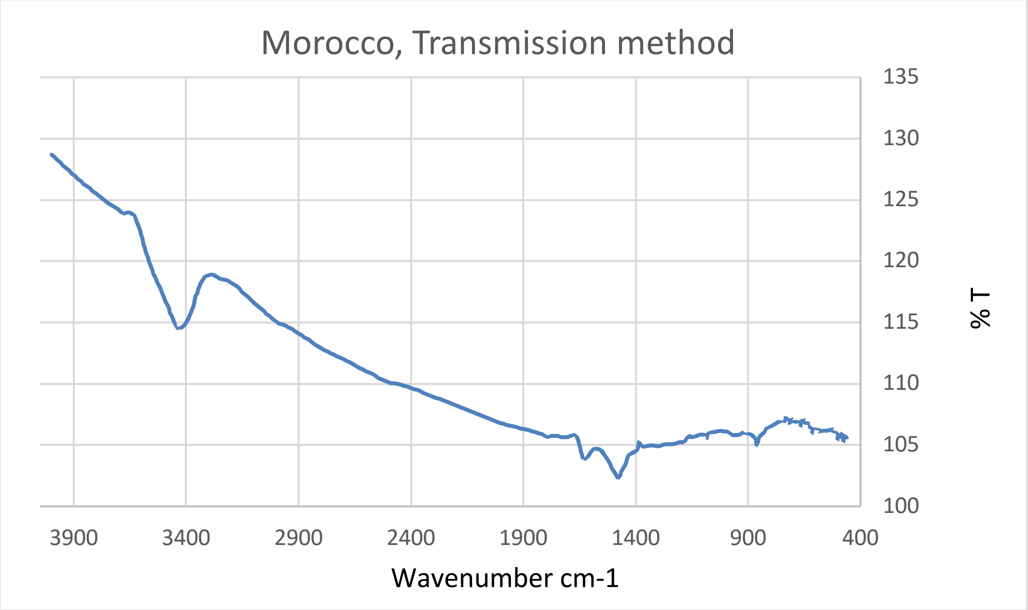

Figure 35. Fiber sample from Morocco with transmission method.

Many organic matters were found during the identification process. Organic matter broke apart while the force was applied with calibrated force applicator in FTIR. It was not possible to identify them because of their weak mechanical properties. Sea shells and mussels that broke during the ATR-FTIR analysis are shown on Fig. 36.

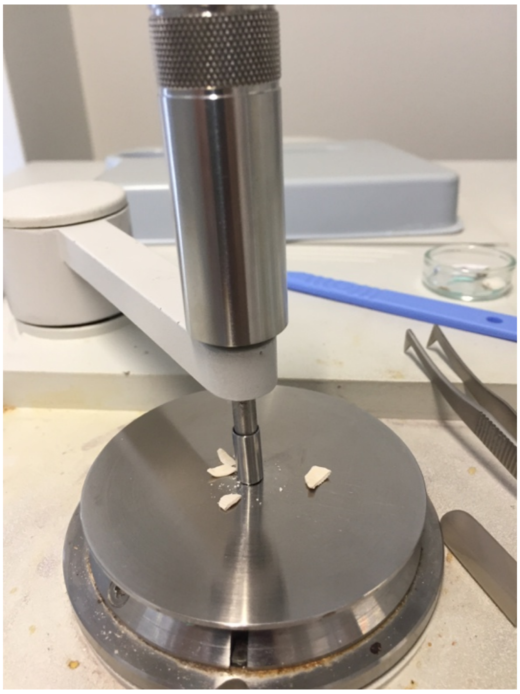

Figure 36. Broken sea shell in ATR-FTIR analysis.

5 CONCLUSION

In this project, scientific resources about microplastics were reviewed, sand samples were collected from different beaches, sampling methods were tested, visual inspection was done with microscope and one of the microplastic identification methods (FTIR) was used to identify the microplastics that were found in collected sand samples.

Limited amount of sand samples was collected (200-250 gr) from different beaches. The method used to collect samples was correct, however the amount of each sample was not enough to go through the separation and identification processes. Small amount of sand samples decreases the possibility of finding more MPs. An alternative to this could be doing filtration (via metal sieve with different mesh sizes) of sand samples at the sample locations. Collecting up to 1 kg of sample at different points on low tide and/or high tide zone of sample locations and sieving the samples with different mesh size sieves can show a better result in future experiments.

PET is one of the most common plastic types that is released into the marine environment, however there was no PET found during the experiment. NaCl was used in density separation process for flotation of microplastics. This decreased the chances to find microplastics in the samples that have higher density than NaCl. It is better to use other solutions such as $ZnCl_2$, $CaCl_2$, LMT and SPT to separate polymers with high densities (PET, PVC, PS, etc.). 5 M of NaCl was supposed to be used during the density separation process, instead 2.5 M of NaCl was used because of a calculation error. This error decreased the possibilities to find more MPs with lower densities in the samples.

Wet peroxide oxidation is a successful process in NOAA guidelines (NOAA, 2015), however it is not clearly mentioned how many times it was repeated to have a successful result. During the experiment, this method was failed after adding H_2O_2 four times. There were not enough resources about different methods to dissolve organic matters in samples, that’s why this method should be applied many times in the future experiments until organic matters dissolve.

Metal sieves with different mesh sizes were used in the filtration process and double-sided tape removed the samples that are stuck on sieves. This method was suitable only for visual inspection, because samples were impossible to collect from the metal sieves and double-sided tape. To be able to identify micro particles with FTIR, filter papers should be used for filtration and visual inspection in future experiments.

Previously it was suggested that identifying microplastics with micro-FTIR was more suitable method than regular FTIR as it was reviewed from scientific resources (Mai, 2018). During this project, regular FTIR was used to identify microplastics and it was confirmed that this method is suitable to analyse MPs down to 0.5 mm diameter. Synthetic and semi-synthetic fibres were grinded with KBr to be analysed with transmission method in FTIR. KBr tablets showed weak results in spectra. There was no heating applied in this process while grinding KBr and fibers together, however it could help grinding fibers if they were heated first at a certain temperature. This should be considered in future experiments.

The objectives of the thesis were achieved except improving methods to collect samples from beaches. The same collecting methods (bulk and selective sampling) were applied for each sample, however effective sample collection methods were observed and suggested previously. Overall, the location of the samples and collecting methods are very significant to have a successful result. The methods to dissolve organic matters in the samples should be improved in future experiments, and solutions with high density should be considered for flotation of microplastics. Microscopic analysis is not enough to observe microplastics, because organic matters which have surface similar to plastics can be mixed with microplastics and polymer type would not be known by visual inspection. It is necessary to have an identification method such as FTIR, micro-FTIR or RAMAN.

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APPENDIX 1

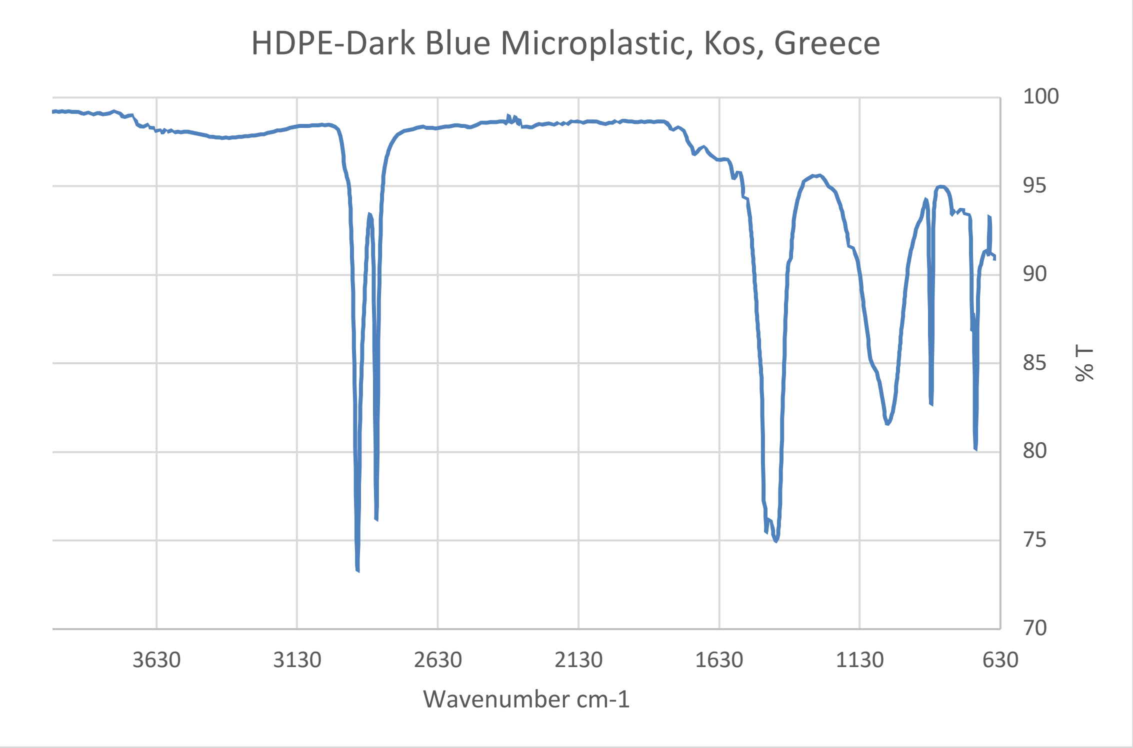
List of materials and equipment used during the experiment. Arcada, 2019
The list of the materials was explained in chapter 3.

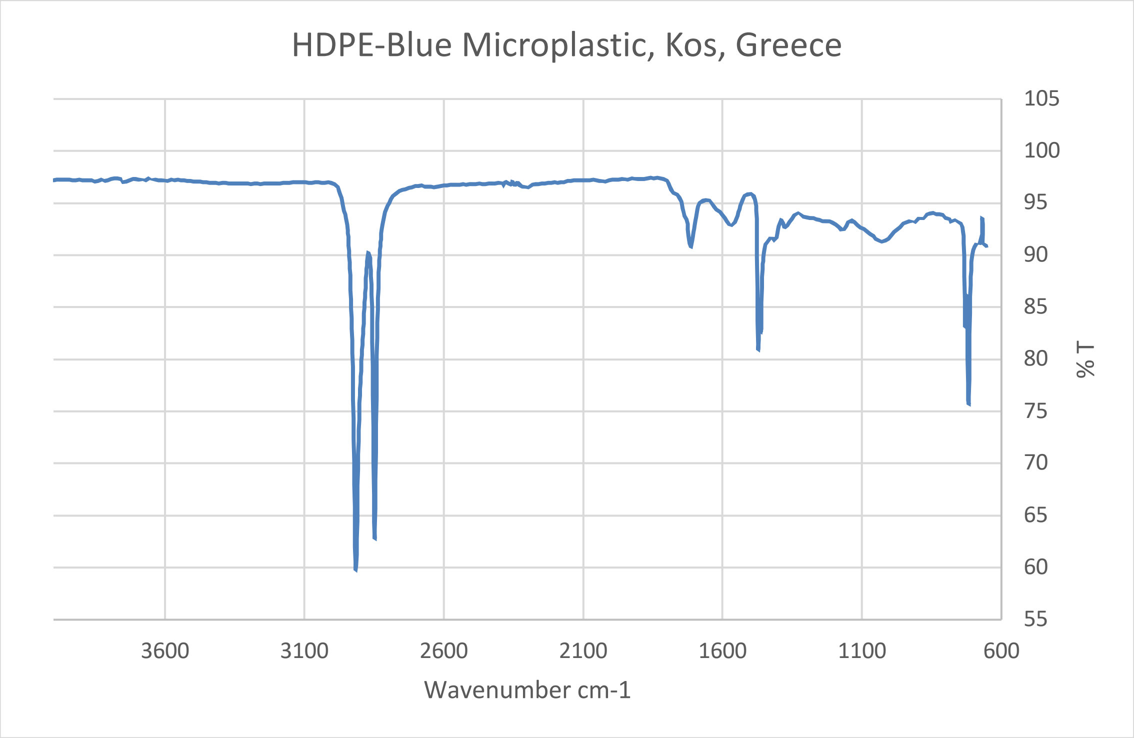
- 800 mL, 500 mL, and 250 mL glass beakers
- Petri dishes
- Different sizes of metal spoons and spatulas
- Forceps
- Scalpel
- Watch glass
- Erlenmeyer flask
- Florence flask
- Funnel
- Graduated pipet, glass pipet
- Ring stand
- Glass dropper
- Rubber tube
- Filter paper
- Aluminium foil
- Microscope glass slide
- Double-sided tape
- Porcelain Buchner funnel
- Small porcelain bowl and stick for grinding
- Oven
- Vacuum pump
- Thermometer
- Magnetic stir bar
- Laboratory weighing scale (precise to 0.1 mg)
- Hotplate magnetic stirrer
- Hydraulic press
- Microscope

- FTIR spectroscopy
- Distilled water
- 0.05 M Fe (II) solution, 30 % H_2O_2, NaCl, KBr

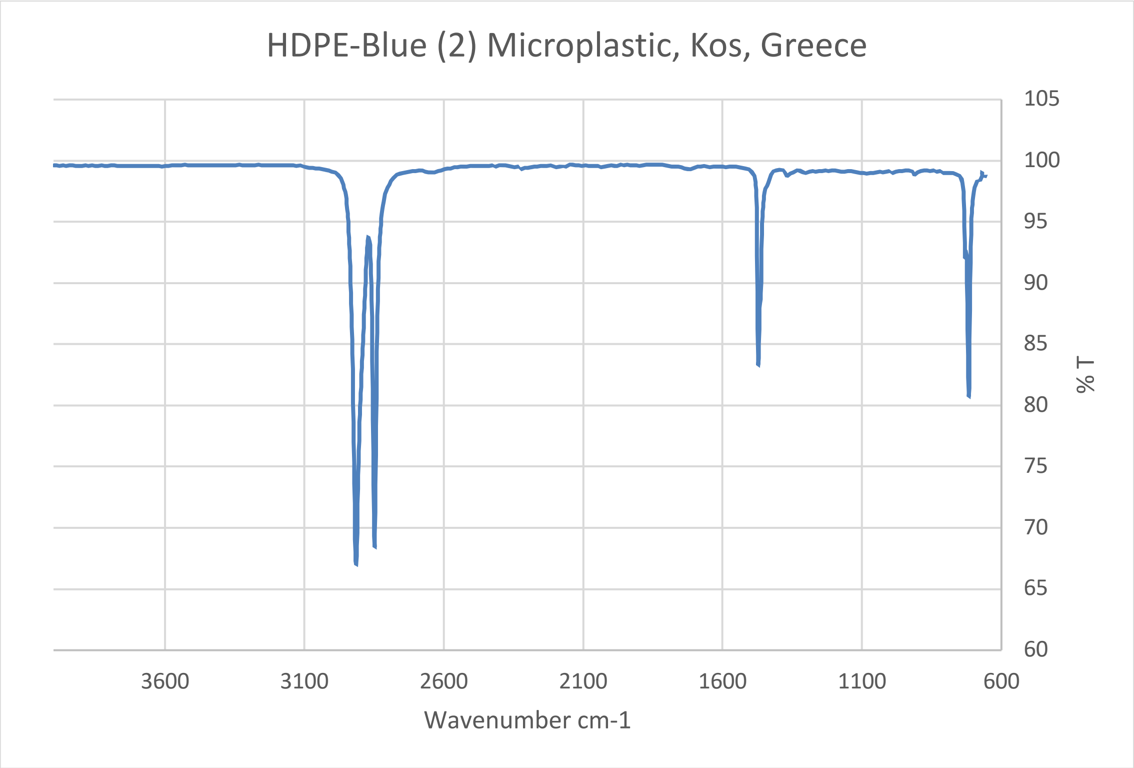
APPENDIX 2

ATR-FTIR and transmission method spectra of microplastic samples and organic matters that were collected and separated during the experiment. Helsinki University, 2019. These spectra were discussed in chapter 4.

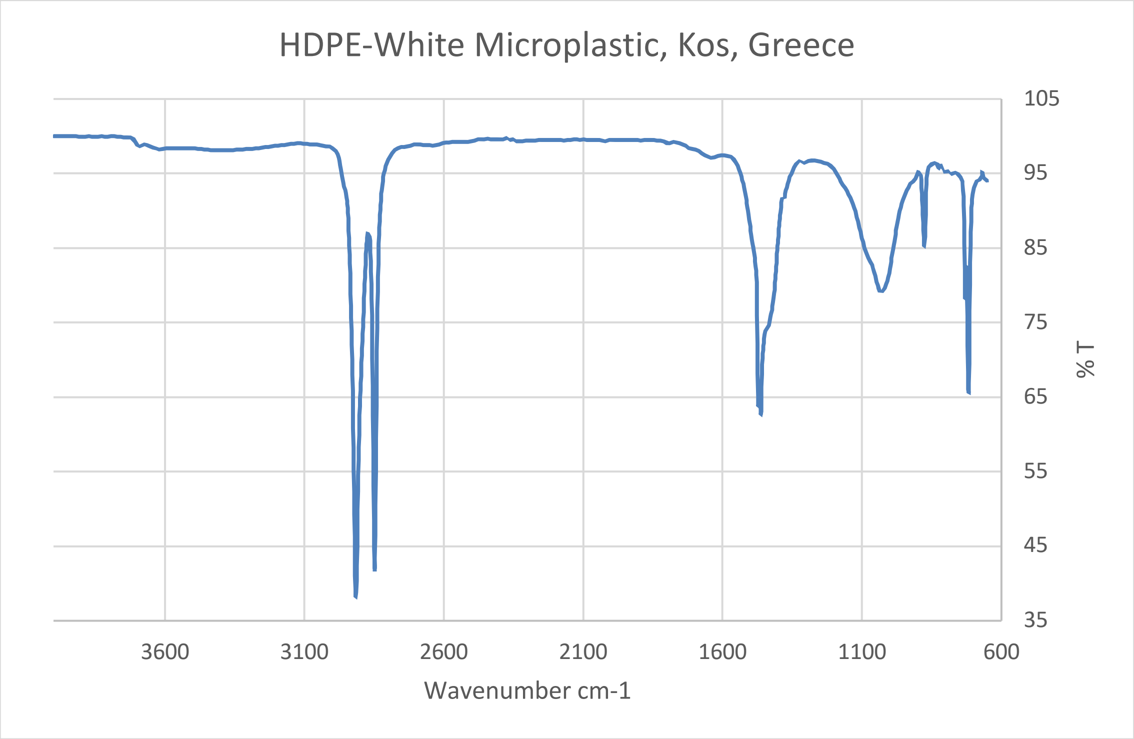


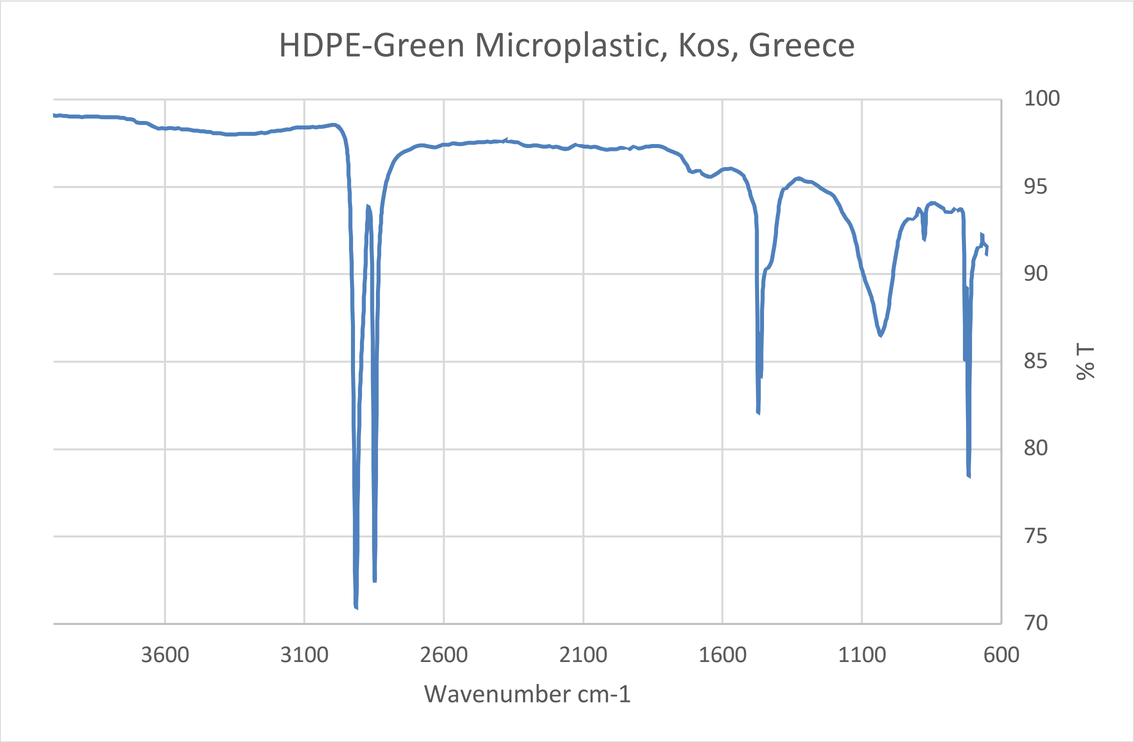


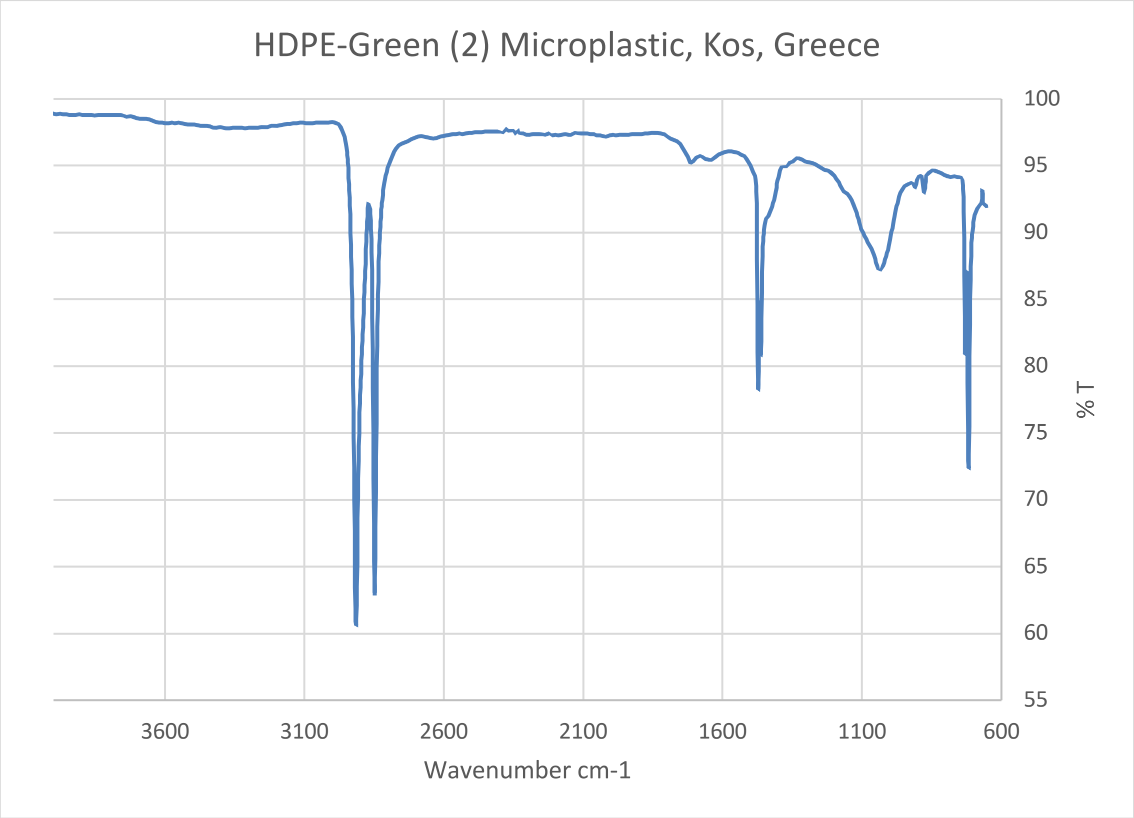
HDPE-Blue Microplastic, Kos, Greece

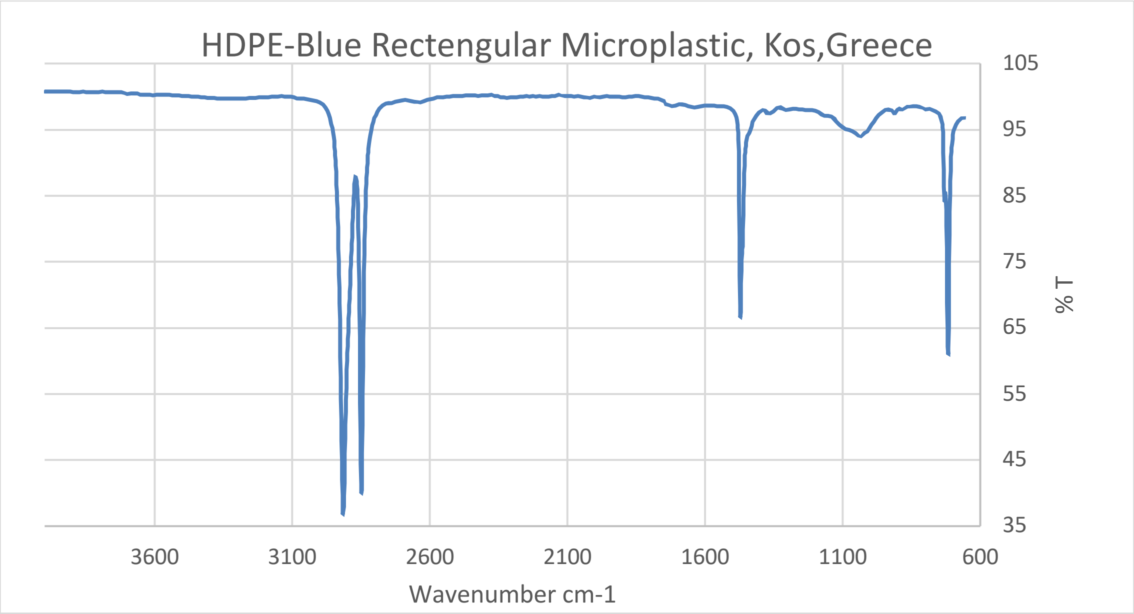


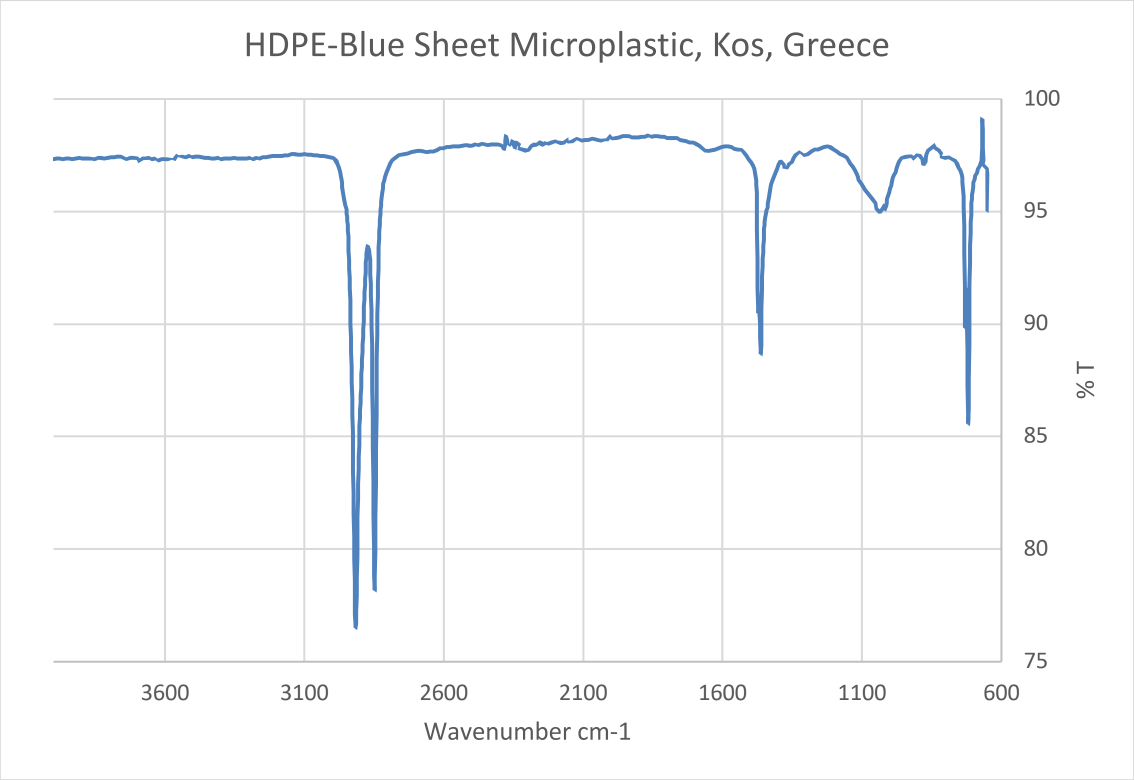
HDPE-Blue (2) Microplastic, Kos, Greece

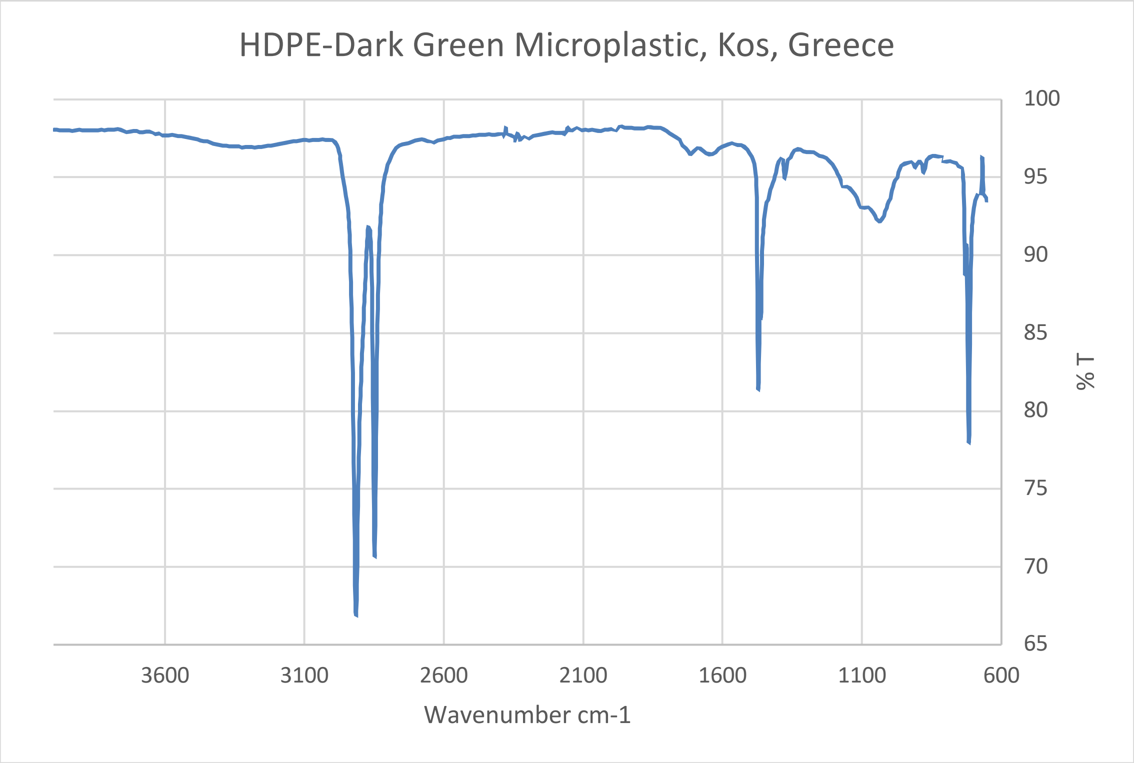

HDPE-White Microplastic, Kos, Greece
105
95
85
75
65
55
45
35
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1

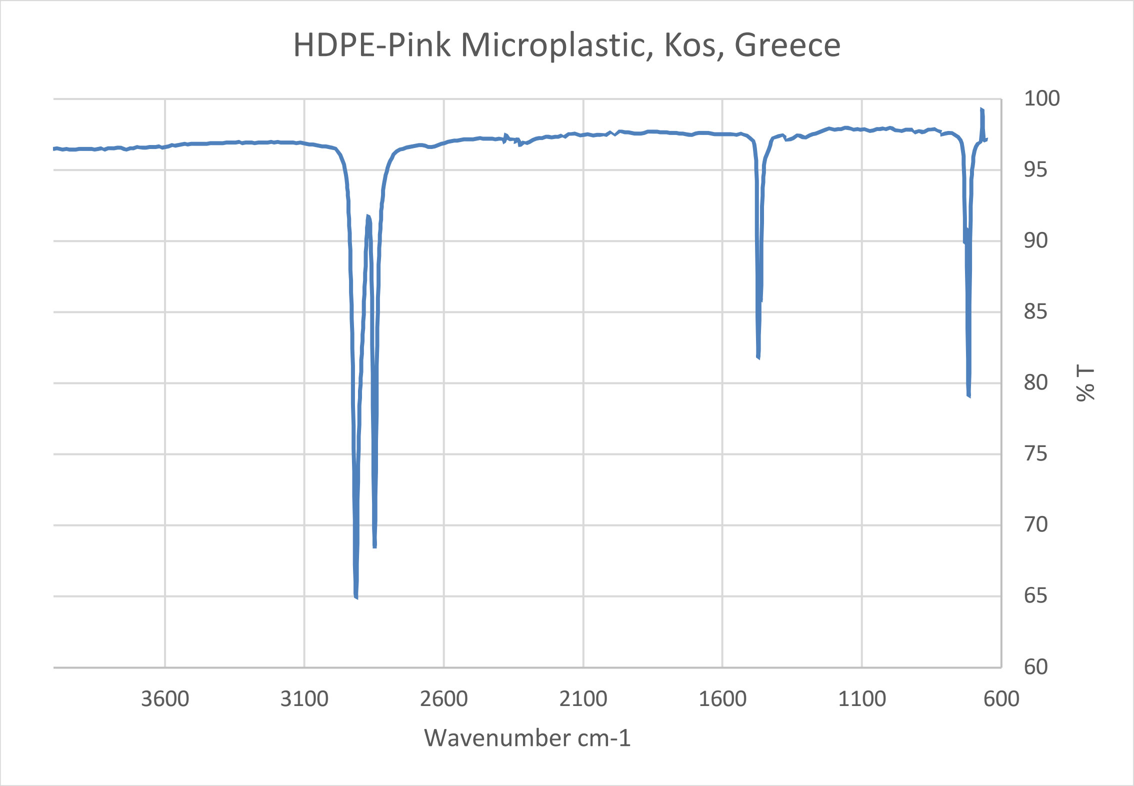

HDPE-Green Microplastic, Kos, Greece
100
95
90
85
80
75
70
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1

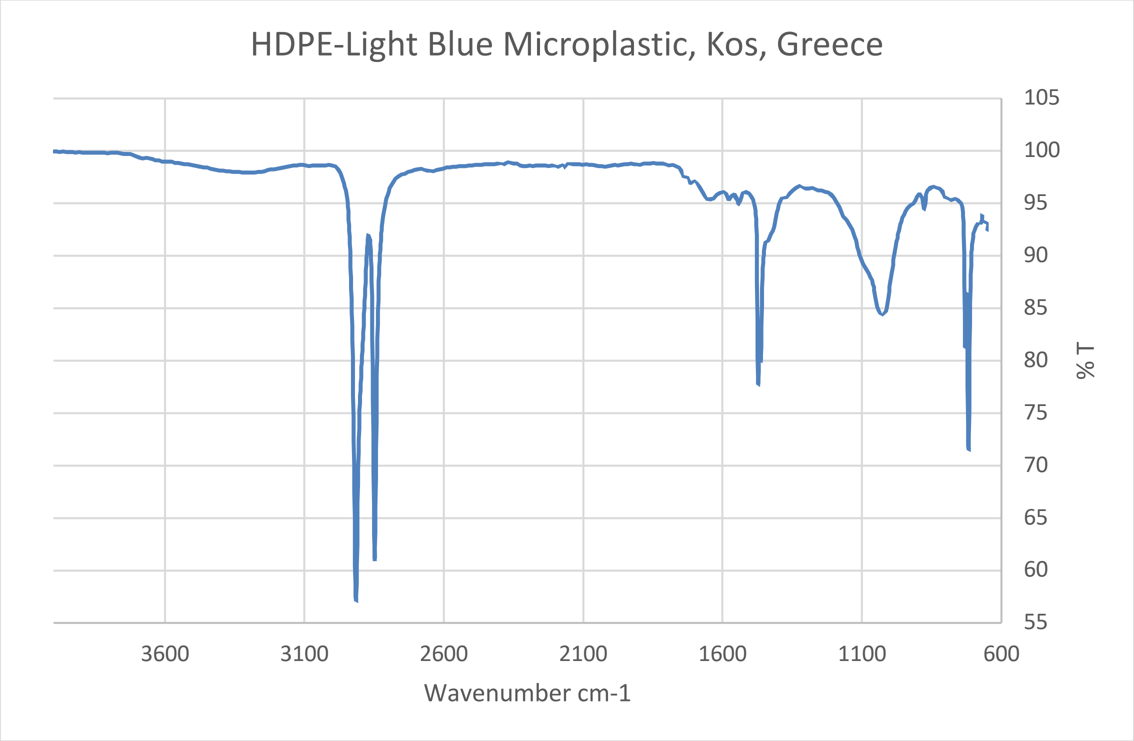

HDPE-Green (2) Microplastic, Kos, Greece
100
95
90
85
80
75
70
65
60
55
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1

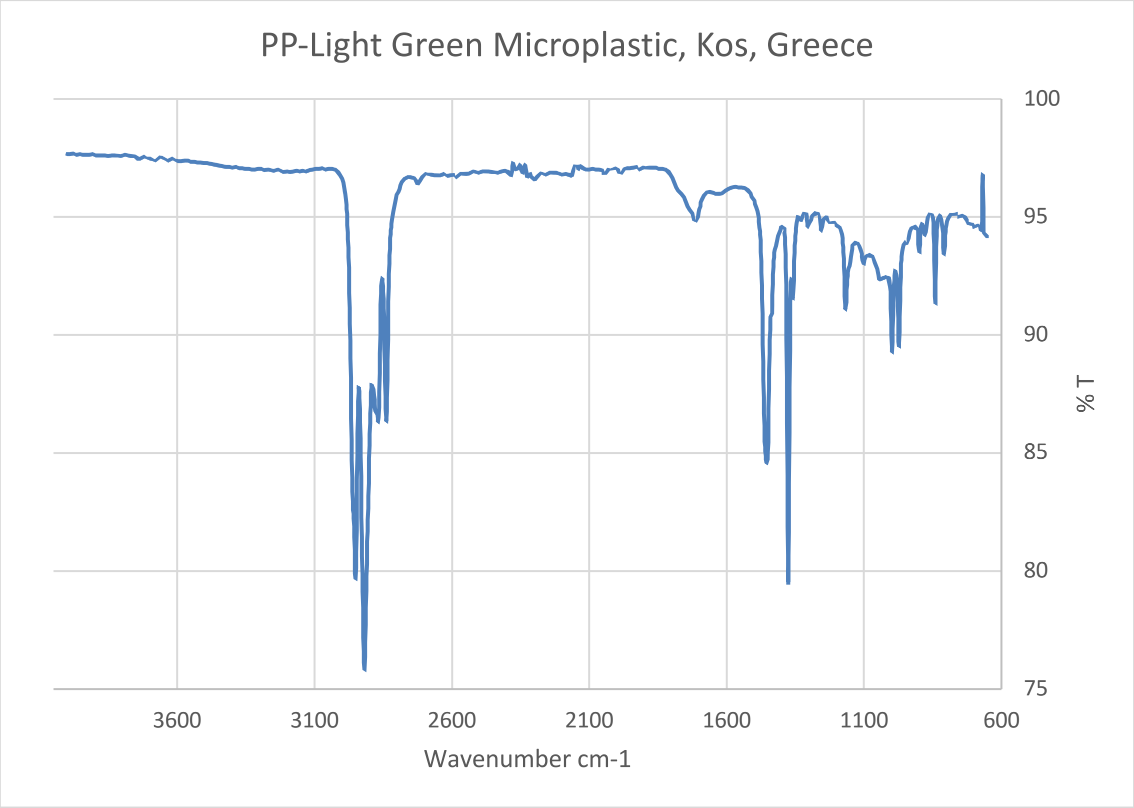

HDPE-Blue Rectengular Microplastic, Kos,Greece
105
95
85
75
65
55
45
35
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1

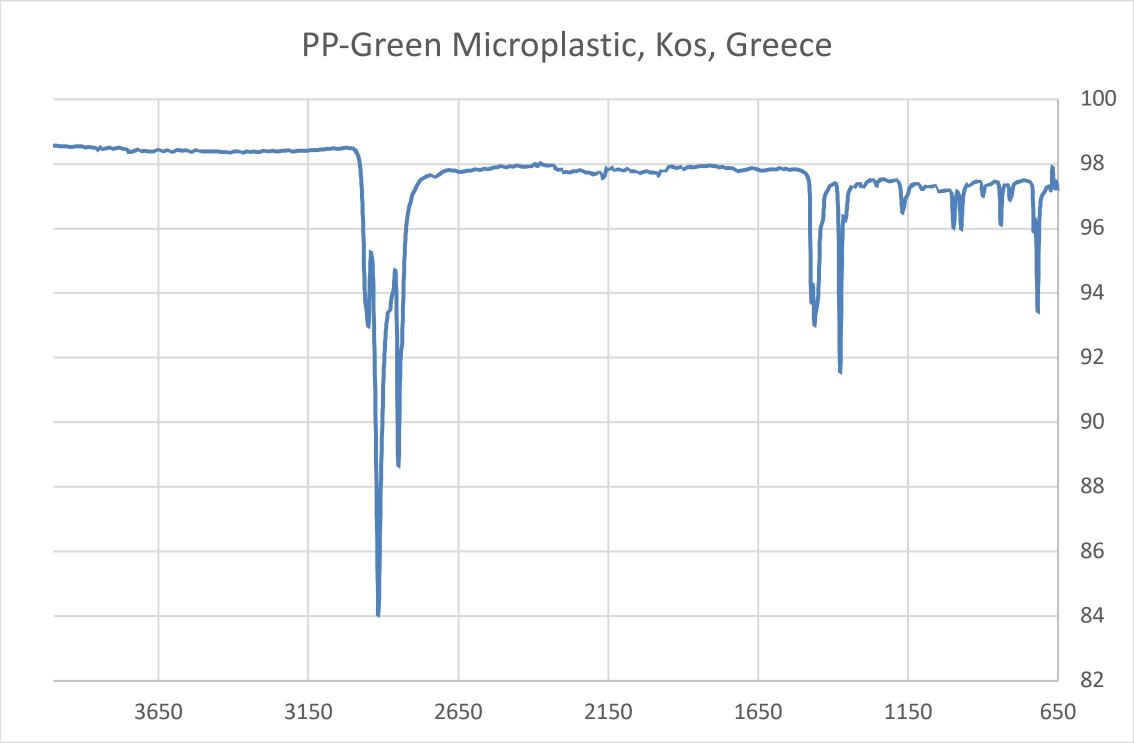

HDPE-Blue Sheet Microplastic, Kos, Greece
100
95
90
85
80
75
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1

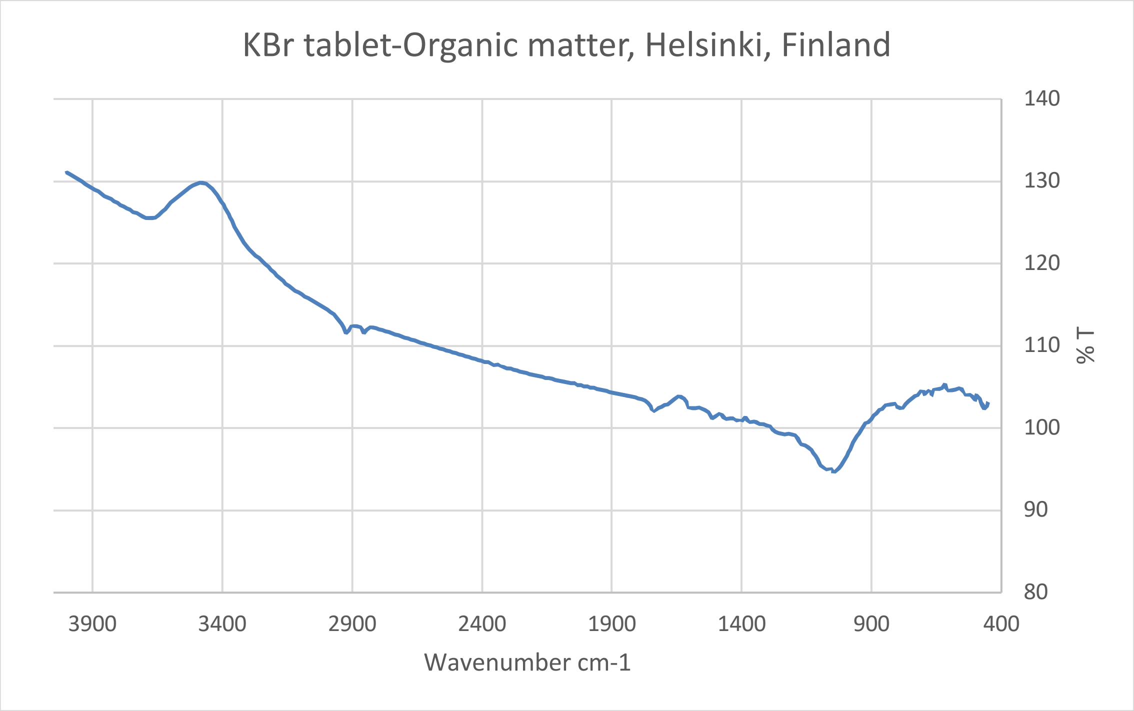

HDPE-Dark Green Microplastic, Kos, Greece
100
95
90
85
80
75
70
65
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1

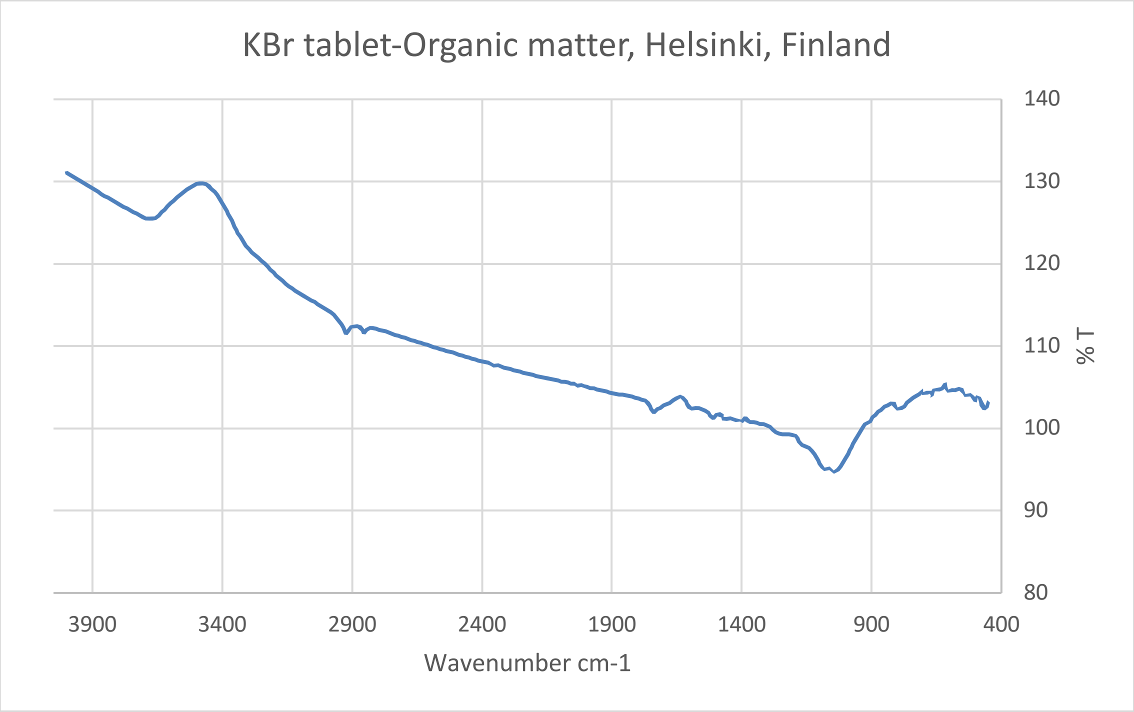

HDPE-Pink Microplastic, Kos, Greece
100
95
90
85
80
75
70
65
60
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1

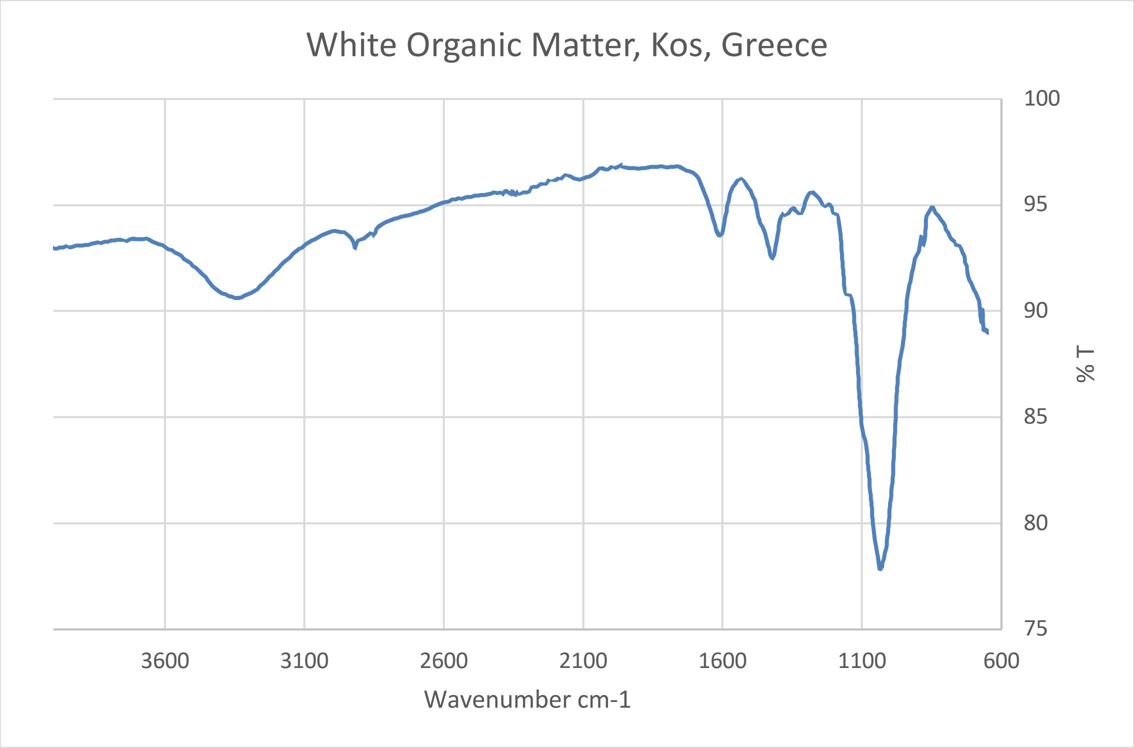

HDPE-Light Blue Microplastic, Kos, Greece
105
100
95
90
85
80
75
70
65
60
55
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1

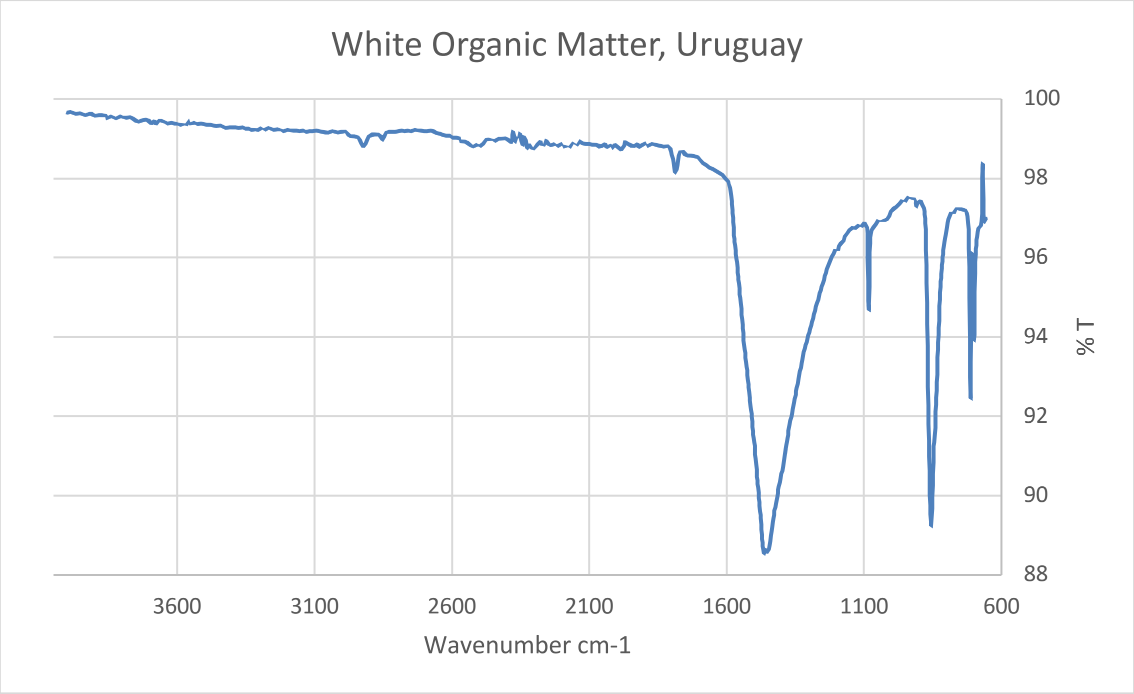

PP-Light Green Microplastic, Kos, Greece

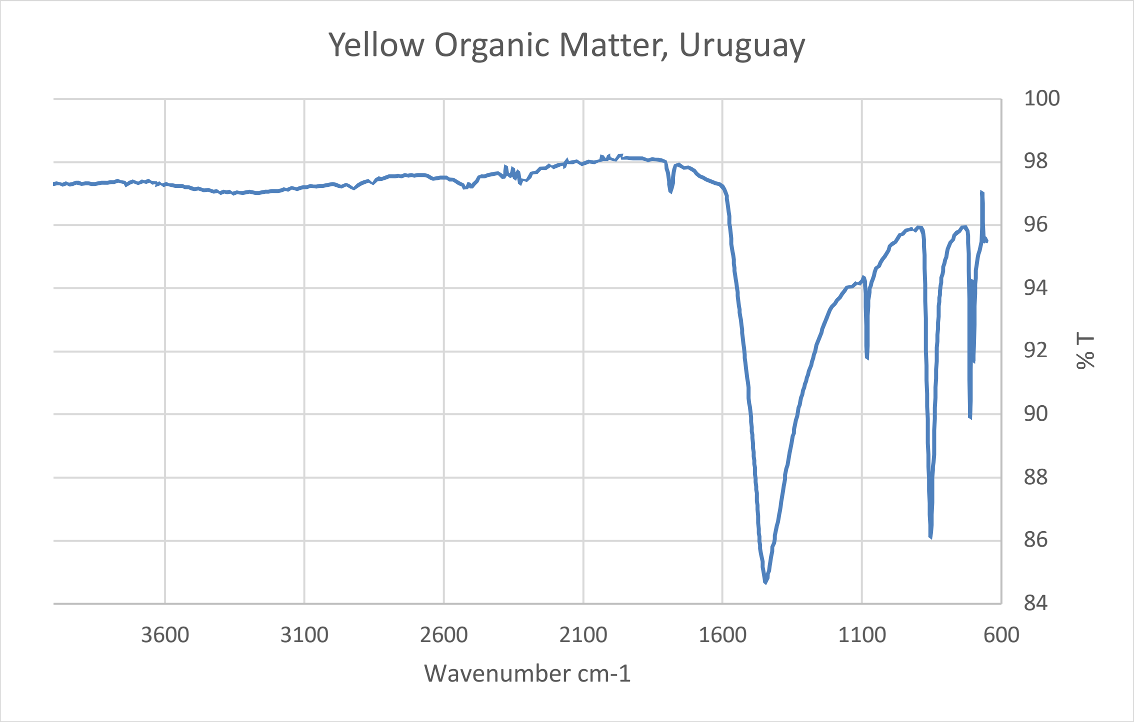

PP-Green Microplastic, Kos, Greece

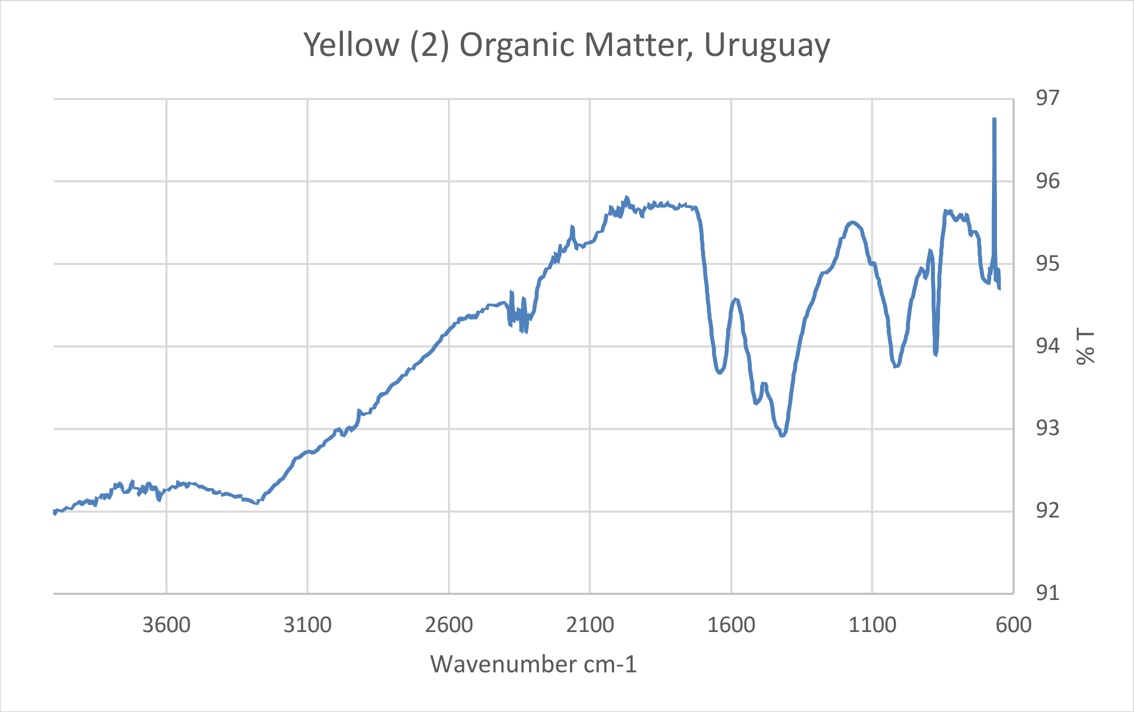

KBr tablet-Organic matter, Helsinki, Finland
140
130
120
110
100
90
80
% T
3900
3400
2900
2400
1900
1400
900
400
Wavenumber cm-1

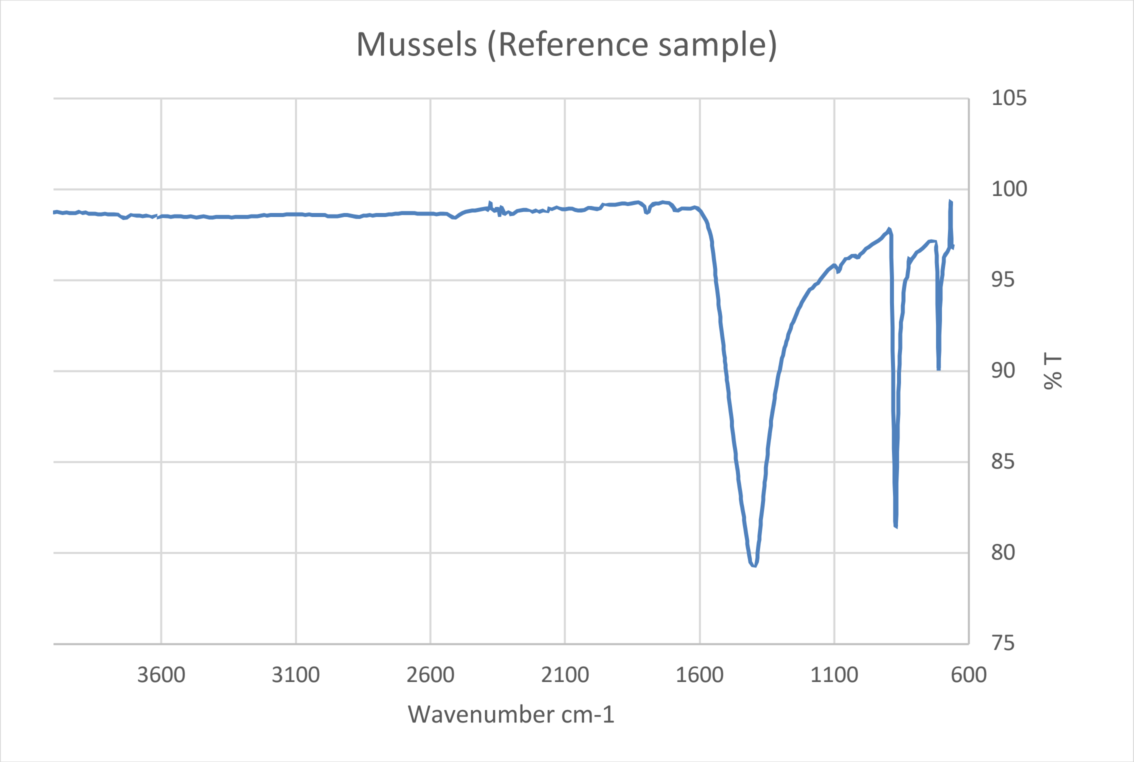

KBr tablet-Organic matter, Helsinki, Finland
140
130
120
110
100
90
80
% T
3900
3400
2900
2400
1900
1400
900
400
Wavenumber cm-1

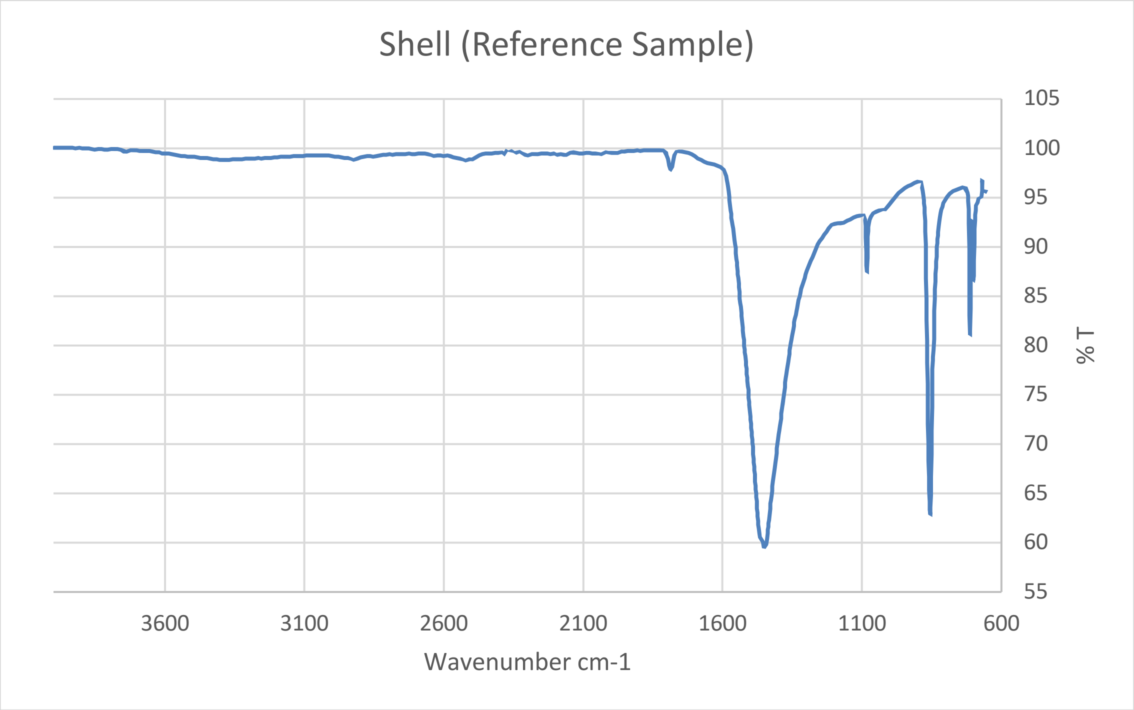

White Organic Matter, Kos, Greece
100
95
90
85
80
75
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1


White Organic Matter, Uruguay
100
98
96
94
92
90
88
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1


Yellow Organic Matter, Uruguay
100
98
96
94
92
90
88
86
84
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1


Yellow (2) Organic Matter, Uruguay
97
96
95
94
93
92
91
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1


Mussels (Reference sample)
105
100
95
90
85
80
75
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1


Shell (Reference Sample)
105
100
95
90
85
80
75
70
65
60
55
% T
3600
3100
2600
2100
1600
1100
600
Wavenumber cm-1