



**Extraction of microplastics from beach  
sediments using principle of density flotation  
in SMI unit and microscopic analysis.**

Deepa Bhandari

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Author:	Deepa Bhandari
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Supervisor (Arcada):	Stewart Makkonen-Craig
Examinar	Mirja Andersson
Abstract:	
<p>The general objective of this research is to extract microplastics from marine sediment using principle of density floatation using apparatus sediment microplastic isolation (SMI) unit and analysing them in arcada's laboratory. Also, the thesis will explore more about the sources of microplastics, its adverse effects on aquatic animals and review of methods of analysing microplastics in marine environment.</p> <p>During experiment, seven different sand samples were taken for analysis and density separation was done using NaCl salt solution and extracted samples after density separation was vacuum filtered and further taken for microscopic analysis.</p> <p>Results show that various coloured microplastics and fibres were present in all the sand samples.</p>	
Keywords:	Microplastics, SMI, Microscopy, Density Separation, Vacuum Filtration, PVC, NOAA.
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## **Abbreviations**

SMI: Sediment Microplastic Isolation

NOAA: National Oceanic and Atmospheric Administration

MPs: Microplastics

PVC: Polyvinyl Chloride

PET: Polyethylene Terephthalate

HDPE: High Density Polyethylene

LDPE: Low Density polyethylene

PP: Polypropylene

PS: Polystyrene

NaCl: Sodium Chloride

FTIR: Fourier- Transform Infrared Spectroscopy

H<sub>2</sub>O: Water

ZnCl<sub>2</sub>: Zinc Chloride

## **FOREWORD**

I would like to express my deep sense of gratitude to my supervisor Mr. Stewart Makkonen-Craig for guiding and helping me throughout the thesis, without his supervision this thesis would not have gone this far. Along with him, I am very much thankful to all my friends who helped me in collecting numerous samples from different parts of the world.

I am thankful to the entire material processing technology department of Arcada for making me capable to do this research. Moreover, my family provided me the immense support during my study period so, I would like to dedicate this thesis to them.

Helsinki

April 2019.

# 1 INTRODUCTION

## 1.1 Background

Microplastics are those microscopic particles which are less than 1 mm also the plastics that are less than 5 mm are included in microplastics (Thompson, 2014). In total there are 45 different varieties of plastics and they have almost dozens of variations on them. Mainly, there are two different types of plastics and they are classified as thermoplastic and thermoset plastics in which thermoset plastics cannot regain its original shape once it has been cooled down to room temperature. Some examples of thermoset plastics are Epoxy resins and polyurethanes. Whereas, thermoplastics are those which can be changed into any shape once it is heated and they are useful for making fibers, films etc. (Biron, 2010) The production of plastic has increased rapidly where in 1945 it has reached to 1.5 million tones and the result of annual production reached to 80 million tons in 1989. Only in Europe the plastic production has a turnover of 300 million euros and 1.6 million people have been employed (L. Lundquist, 23rd January 2001).

Mostly the polymer resins are mixed with additives to make the plastic materials change in its appearance and those additives contains inorganic fillers and different chemicals which has an adverse effect in animals and human beings. In the natural environment and in landfills some quantities of plastic materials have been accumulated in which around 10 percent of the plastic waste if the municipal waste. For example, plastics from newspapers has a high accumulation of plastic waste and different glass materials and various larger plastic materials are broken down into small fragments through rainwater, sunlight, UV rays and they are carried out through rain water, floods into the seas, oceans etc. Afterall, the fragmented plastic particles which are carried out into the marine environment has an adverse effect on the marine life. Due to plastics being small they are distributed in the marine environment, and they are found in many marine organisms and many experimental studies has found that it affects marine animals physically and chemically and it may lead to death (R. C. Thompson, 27 July 2009) (Christopher M. Schalk, 2018).

The main aim of this thesis is to extract microplastics from various beaches around the world and analyzing them in arcada laboratory using apparatus sediment microplastic isolation unit.

## 1.2 Objectives

- **Literature review about sources of MPs and its various effects on aquatic animals.**

This part includes about various sources of MPs being produced in the human environment and how they enter the marine environment. Also, various problems in aquatic animals with examples are described briefly.

- **Review about various methods of analyzing microplastics in marine environment.**

Various laboratory methods of extracting microplastics from marine environment are discussed in this section including water, bed and beach samples.

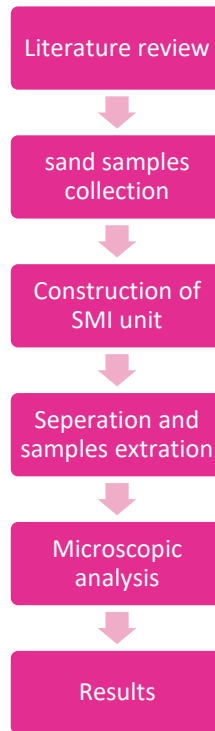
- **Using principle of density floatation for extracting microplastics.**

Sodium chloride (NaCl) solution is used as a density separator liquid to extract microplastics.

- **Sediment microplastic isolation (SMI) unit is used as an apparatus to extract microplastics.**

SMI unit is an easy and efficient apparatus to extract microplastics which is used in this research.

### 1.3 Thesis Structure



This thesis explained briefly about literature review of various sources of MPs, effects on aquatic animals and various methods of extracting MPs in water, bed and sand samples. SMI unit was constructed in arcada's laboratory and all the sand samples from six different countries were extracted using principle of density floatation on SMI unit. All the extracted samples were taken for microscopic analysis and various results obtained were analyzed and their size were measured using GIMP software.

## 2 LITERATURE REVIEW

The topic mainly focuses about the overview of plastics, sources of microplastics, its adverse effects on environment, human beings and marine animals and solutions for how it can be controlled in entering the marine environment.

### 2.1 Sources of microplastics

Mainly microplastics has two different sources called primary sources and secondary sources but through the current study and understanding it has some other sources also and all of them are described below: -



*Figure 2-1: Microplastics (NOAA, 20/03/18)*

- Primary sources

Primary microplastics are those microplastics which are produced with purpose or intentionally for example, microbeads, capsules, fibers or pellets in which microbeads are used in facial scrubber and other different personal use products and different microfibers which are used in making textiles and resin pellets are used for manufacturing of plastics. These primary microplastics are manufactured as smaller than the size of 5 mm so, they can easily pass through the sewage treatment as they cannot be filtered. For example it was found that a bottle of skin cleanser contains (100-200  $\mu\text{m}$ ) polystyrene with irregular fragments (R. C. Thompson, 27 July 2009) (Beaulieu, 2017).

- Secondary microplastics

Secondary microplastics are those plastics which are formed through breaking of larger plastics due to physical, chemical or environmental behavior. For example, the action of light (photolysis), heat and oxygen (thermal oxidation), water (hydrolysis) are the fragmentation pathways of plastics. For example, if the plastic particles used in the beaches and during fishing are not disposed properly, they make a direct way into the ocean (Beaulieu, 2017).

## **2.2 Effects of microplastics on marine animals and environment**

Plastic pollution has been the main problem in the marine environment. According to different research and studies it has been found that 4.8-12.7 million tons of plastic found to be passing through the marine environment and those plastic debris could be from land fill part and rest from different human activities. As plastics turns into microplastics due to different factors (wave, rain, sunlight, UV etc.), and whenever they enter marine environment they are ingested by marine animals. Microplastics are found to be ingested by different marine species like cetaceans, seabirds, molluscs, echinoderms, Zooplankton, Desforbes, etc. These ingested microplastics causes different kind of effects among marine animals such as physical and chemical where it has been reported that it cause reduction in food chain, problem in growth, reproduction and various chemicals present in the

plastics during production, mixed additives causes cryptic sub-lethal effects and it may be collected in top level of the foods web and may lead to toxicity (Eleonora Guzzetti A. S., 2018).

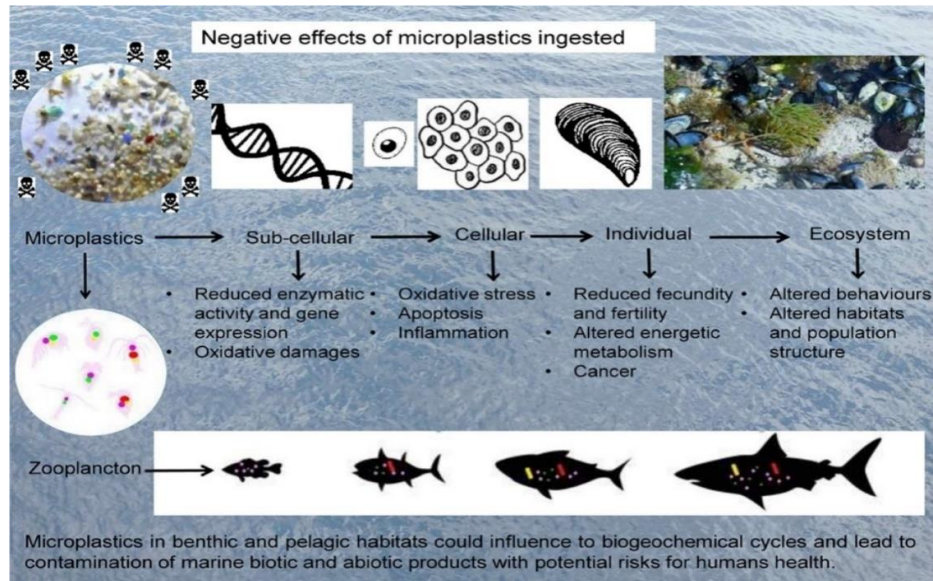


Figure 2-2: Effects of ingested microplastics. (Eleonora Guzzetti A. S., 2018)

### 2.2.1 Effects of polystyrene microplastics in collaboration with microbiome and metabolism in larval zebrafish

To find the real effects of polystyrene microplastics in composition of microbiome and metabolism in larval zebrafish, two sizes (5 and 50  $\mu\text{m}$  diameter) of fluorescent and virgin polystyrene microplastics were taken and effects on larval Zebrafish was analyzed after 7 days of experiment using 100 and 1000  $\mu\text{g/L}$  of couple of polystyrene microplastics. After the experiment, the result was, polystyrene microplastics cause changes, microbiome at phylum and genus levels in larval zebrafish. According to metabolomic analysis when larval Zebrafish are exposed to polystyrene, they get changes in metabolic feature and changing metabolites were engaged in energy metabolism, metabolism of glycolipid, inflammatory response, neurotoxic response, Nucleic acid metabolism, oxidative stress etc. In case of genes, exposure to polystyrene it causes variation in glycolysis type of gens and lipid metabolism genes and it reduces the processing activities of catalase and amount of glutathione (Zhiqin Wan, 2019).

### **2.2.2 Effects of ingested polystyrene microplastics in brine shrimp, *Artemia parthenogenetica***

Zooplankton being primary consumers in a marine environment in food web it plays an important role in transportation of energy and along with this pollutant are also transferred along the marine ecosystem. Various ingestion experiments have been made among zooplankton in order to identify their food behavior in marine life. The brine shrimp, (*Artemia*) was used as a practical test organism to identify the effect of microplastics in the marine environment. During the experiment, Microplastics ranging the size of (1-20  $\mu\text{m}$ ) being accumulated in *Artemia nauplii* were made ingested by fish as prey food. *Artemia nauplii* being small, and short life period are used in lab experiments to calculate the amount of toxicity. The main target of this study was to quantify the very less 10  $\mu\text{m}$  polystyrene particles in which ingestion could be seen easily in a parthenogenetic, in both exposure regimes called acute (24-h) and chronic (14-d) and the other aim included about the growth, development, survival and effects in intestinal cells.

During the study, couple of 10  $\mu\text{m}$  polystyrene samples were taken and two different tests were done, (toxicity test and ingestion test). During toxicity test, parthenogenetic were tested with untagged polystyrene microspheres and during ingestion test parthenogenetic were tested with green tagged combined with fluorescent which has an excitation of (468 nm/ emission and 508 emission) polystyrene microspheres. FTIR was used to calculate the chemical distribution among polystyrene microsphere. After the test being done in *A. parthenogenetic*, lowest polystyrene concentrations in which the ingestion was visualized in a test organism were  $12 \pm 0.57$  particles/mL ( $6.7 \pm 0.32$   $\mu\text{g/L}$ ) and  $1.1 \pm 0.16$  particles/mL ( $0.61 \pm 0.088$   $\mu\text{g/L}$ ), respectively. There seems to be no any abnormalities on survival, development or growth in a test organism, but abnormalities were observed in intestinal epithelial cells when exposed to polystyrene microspheres, in which decreased, and microvilli being disordered and increased in number of mitochondrion and autophagosome were appeared and these things could affect in nutrition and energy metabolism process. Finally, it was concluded that though no acute or chronic toxicity effects on *A. parthenogenetic* which was found over 24-h or 14-d exposures, but it could affect on intestinal epithelial cells which was seen through a microscope(Ying Wang, January 2019).

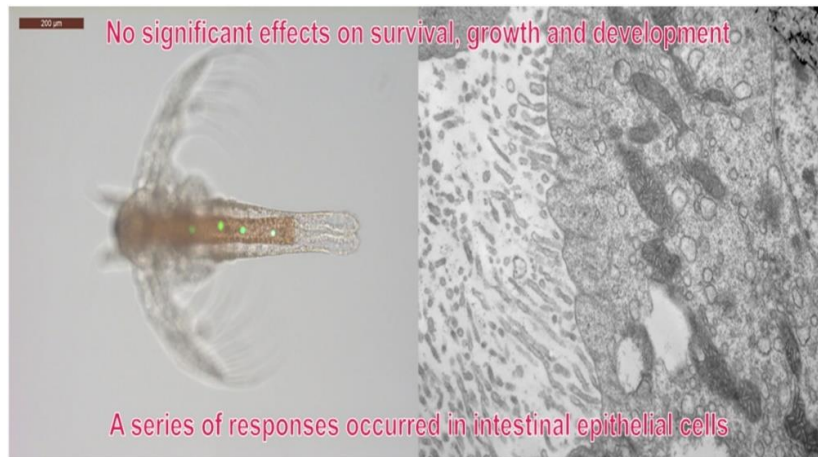


Figure 2-3: Effect of ingested polystyrene in brine shrimp. (Ying Wang, January 2019)

### 2.2.3 Toxic effects of microplastic on marine microalgae *Skeletonema costatum*; Interaction of microplastic and algae.

During the investigation of toxic effects of microplastics on marine microalgae (*Skeletonema costatum*), the algal growth inhibition test and shading effect test was done as shown in the figure above and in addition algal photosynthesis readings were also calculated. During the test, when microplastic and algae were interacting each other SEM images were used to observe them. Microplastic (mPVC) of average diameter 1 µm was used as a testing microplastic and from that test it was concluded that microplastic of 1 µm diameter had obstruction in growth of microalgae and obstruction growth rate at the highest level reached up to 39.7 percent after being exposed to 96 h. In the same way testing it with plastic (bPVC) with average diameter of 1 mm showed no any obstruction in growth of microalgae. Again, when test was done with mPVC (50 mg/L) with high concentration which did not gave any negative effects on the process of algal photosynthesis in which, under mPVC treatments chlorophyll content and efficiency of photosynthesis was slowed down. After all, the microplastic toxicity on algae was not due to shading effect, but comparing with non-contact shading effect, adsorption and aggregation due to interaction of microplastic and microalgae were concluded as a biggest problem for toxicity of microplastic on marine macroalgae.

Shading effect was not one reason for toxicity of microplastic on algae in this study. Compared with non-contact shading effect, interactions between microplastic and microalgae such as adsorption and aggregation were more reasonable explanations for toxic

effects of microplastic on marine microalgae. The SEM images provided a more direct and reasonable method to observe the behaviors of microplastic (Cai Zhang, January 2017).

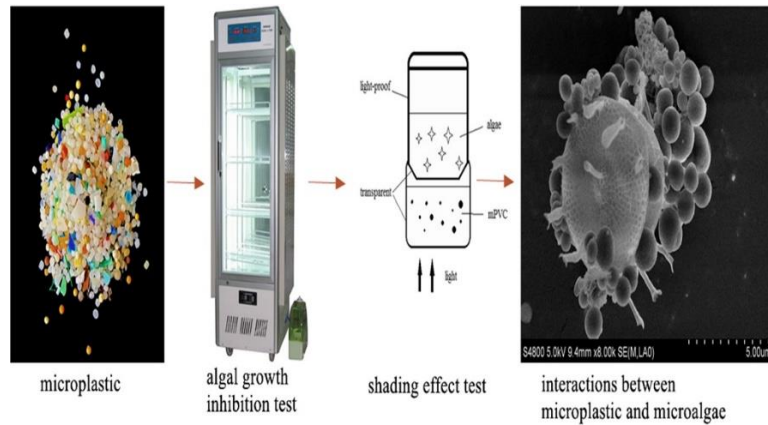


Figure 2-4: Interaction of microplastic and algae. (Cai Zhang, January 2017)

## 2.2.4 Investigation of transfer of microplastic from trophic level in marine top predators

Microplastics being small are ingested by marine animals directly or indirectly. Direct ingestion is likely to be accidental ingestion of particles or misidentification of selection of food for microplastics. Mainly, trophic transfer is caused from laboratory stuffs for low trophic level organisms like crabs but then transfer in wild animals and other higher trophic level is not identified yet. Different studies and research have concluded that microplastics present in gastrointestinal tract of different fish species is transferred to predators. It was found that 11 % of mesopelagic fish has microplastics accumulated and approximately 463 million microplastics could be ingested by one striped dolphin (*Stenella coeruleoalba*) during the cycle of consumption of prey that are already accumulated with microplastics. Analysis was done among scat samples from captive grey seals (*Halichoerus grypus*) and wild- caught Atlantic mackerel (*Scomber Scombrus*) from which analysis was done taking all digestive tract. To be aware of appeared organic materials an enzymatic digestion protocol was taken, and clear visual detection was done to watch synthetic particles without damaging them. All appeared plastics were confirmed their type using Fourier- Transform Infrared (FTIR) Spectroscopy. After test, Scat samples with (48%; n=15) and third fish (32%; n=10) appeared to be containing 1-4 microplastics. Those microplastics were found to be in different color like black, red and blue.

The most abundant microplastic appeared in both samples was ethylene propylene among which 12 various polymers were found and the size were detected to be 1.5 mm and 2 mm. Throughout this experiment it was concluded that trophic transfer could be the biggest problem and reason to transfer microplastics for example from fish to other bigger marine animals. (Sarah E. Nelms, 2018)

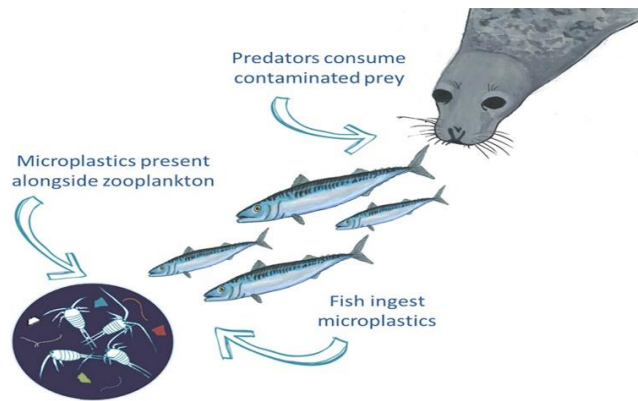


Figure 2-5: Cycle of microplastic ingestion among marine animals. (Sarah E. Nelms, 2018)

## 2.3 Types of plastics

Plastics are mainly categorized into 7 different types: -

1. Polyethylene Terephthalate (PET)  
PET was introduced in 1940 by J. Rex Whinfield and James T. Dickson. PET prevents letting oxygen to get inside so, mainly it is used for making drinking bottles, food packaging purposes. These PET materials are marked with the SPI code 1.
2. High-Density polyethylene (HDPE)  
High density polyethylene was introduced by Karl Ziegler and Erhard Holzcamp in 1953. This material has its high resistance to extreme heat and cold. HDPE is commonly used to make milk bottles, juice containers, medicine bottles, shampoo and conditioner bottles. HDPE is marked with SPI code 2.
3. Polyvinyl Chloride (PVC)  
PVC was produced twice by French physicist Henri Victor Regnault in 1838 and German chemist Eugen Baumann in 1872. PVC is known as a the least recycled

among plastic material where less than 1 % is recycled annually. It is also marked as a poisonous plastic which is toxic and has harmful effects on human beings and environment. PVC is used to make blister wrap, tubes in medical purpose, toys, blood bags. It is marked as SPI code 3.

4. Low-Density Polyethylene (LDPE)

The first polyethylene produced was LDPE, which is well known as a godfather of the material. These materials have structurally ordered form. LDPE is regarded as a safe material for human use. It is used to make food bags, drink cups, plastic wraps, bottles for honey, oils. It is marked as a SPI code 4.

5. Polypropylene (PP)

PP was introduced by J. Paul Hogan and Robert L. Banks of Philips Petroleum company in 1951. PP is mainly used as a hot food keeper. This is also safe for food and drink use as LDPE. It is marked as SPI code 5.

6. Polystyrene or Styrofoam (PS)

German apothecary Eduard Simon introduced PS. This was discovered accidentally when he isolated a substance from a natural resin. Later, this polymer was researched and studied by German chemist Hermann Staudinger. Since, it has a light weight and it is used more as a plastic material it is hazardous in the environment. Good example of PS is it is used to make egg cartoons. It has a SPI code of 6.

7. There are also other left plastic materials like: polycarbonate, polylactide, acrylic, acrylonitrile butadiene, styrene, fiberglass and nylon (Mertes, 2017).

## 2.4 EU plastic strategy

Today the European union has been working curiously on the matter of plastic recycling. They have promised that by 2025 at least 10 million tons of recycled plastics could be allocated if they could get the enough pledges, but according to the demand only 5 million tons are expected. More than 60 pledges were received recently by the end of October and most of them were provided by polystyrene industries, PET packaging owners, different plastic recycling companies.

This strategy of EU on plastics is a part of big transformation in economic stability. Different positive agendas have been made in perspective of recycling plastic where, by 2030 all plastic packaging on EU market will be recyclable, willful use of microplastics will be banned and plastics that has only single use will be highly reduced. Responsible person from sustainable development First Vice- president Frans Timmermans said: “ if we don’t change the way we produce and use plastics, there will be more plastics than fish in our oceans by 2050”. According to data collection Europeans produce 25 million tonnes of plastic debris annually, but only 30 % of the debris goes for recycling. The new EU strategy says it will tackle all the problems that are being faced due to plastic pollution and it will change the way plastic products are designed, produces, recycled and used. Plastic strategy is mainly composed of ten keys cycle, which counts progress in transforming to a circular economy of EU and national level.

The EU strategy covers the following factors:

- **Recycling will be made profitable for business:** To improve the recyclability of plastic debris new ways of packaging and designs of plastics will be developed. Collection and sorting of plastics waste will be done in a standard way across the EU, which saves around 100 euros amount per ton waste being collected. Also, it will help in producing more added value for competitive plastic industries.
  
- **Curb plastic waste:** In this section, EU legislation has already been able to reduce the number of plastic bags being used in most of the member states. Other plans include about use of single use plastics, fishing gear and launching various

awareness programs based on EU rules going to be purposed in 2018 with full evidence and stakeholder consultation.

- **Restriction of littering at sea:** This section aims about the wastes that are collected from ship or collected at sea are not left around or by side, but they are supposed to manage properly in land. Reducing the amount of waste production in ships and ports also include in the aim.
  
- **Drive investment and innovation:** Minimisation of plastic debris from source is the primary part so, commission will give guidance to all European business holders and national authorities. Recycling process will be made more productive and fruitful and all the recycled plastics will be traced to make them free of dangerous substances.
  
- **Boost change across the world:** As, European union is working on its own project, it also works with worlds matter regarding plastic waste problem collaborating with international countries globally to solve the problem and to keep the international standards. For example, EU has already worked out cleaning the ganga river in India (available, 16 January 2018).

### 3 REVIEW OF LABORATORY METHODS FOR ANALYSIS OF MICROPLASTICS FROM MARINE ENVIRONMENT

This section explains about the different laboratory methods being carried out for the analysis of microplastics from marine environment by careful study and laboratory work granted by NOAA Marine debris program to the University of Washington, Tacoma.

#### 3.1 Methods for the analysis of microplastics in water sample.

In this method surface net is used during collecting water sample for the analysis of plastic litter. The sample may contain plastics like, hard, soft, fibers so, the solids obtained are filtered in 0.335 mm surface net through 5.6-mm or 0.3-mm sieves and the appropriate size of solids are taken out. To calculate the exact mass of the solid the sieved solid material is dried in an oven and to get rid of organic matters present there the solid sample is taken to do experiment with wet peroxide oxidation (WPO) with Fe (II) which works as a catalyst. After this, density separation process is carried out in which WPO mixture is added to NaCl and the plastic litter is found to be floating on the top. To identify the concentration of microplastic, the floating plastic litter is accumulated in density separator using 0.3-mm filter and dried with air and plastic materials is separated and weighed and the sample is taken for microscopic examination (Masura, July 2015).

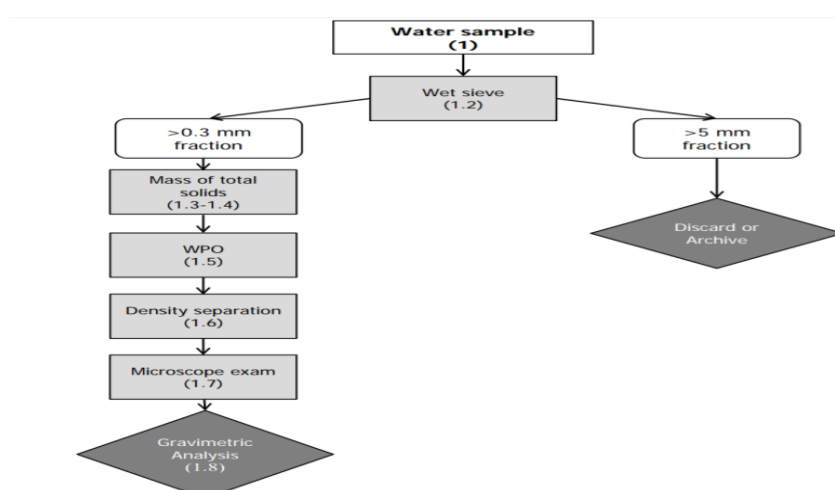


Figure 3-1: Flow diagram for analysis of microplastics in water samples. (Masura, July 2015)

### 3.2 Methods for the analysis of microplastics in beach samples.

In this method, sand samples are collected using shovel or spade and to get rid of large plastic litter sieving is done for dry sand sample up to 5 mm. Various plastic materials can be identified through this method like: polyethylene, polypropylene, polyvinyl chloride and polystyrene. Microplastics ranging from 5 mm to 0.3 mm are easily analyzed with this method. Dried Samples are taken to wet peroxide oxidation and the plastics that are resistant to WPO are considered as the microplastics and density separation and floatation process is done in 5 M NaCl( $d=1.15$  g/mL) or 5.4 M lithium metatungstate ( $d=1.62$  g/mL) solution, and the sample obtained is taken to microscopic analysis at 40X power (Masura, July 2015).

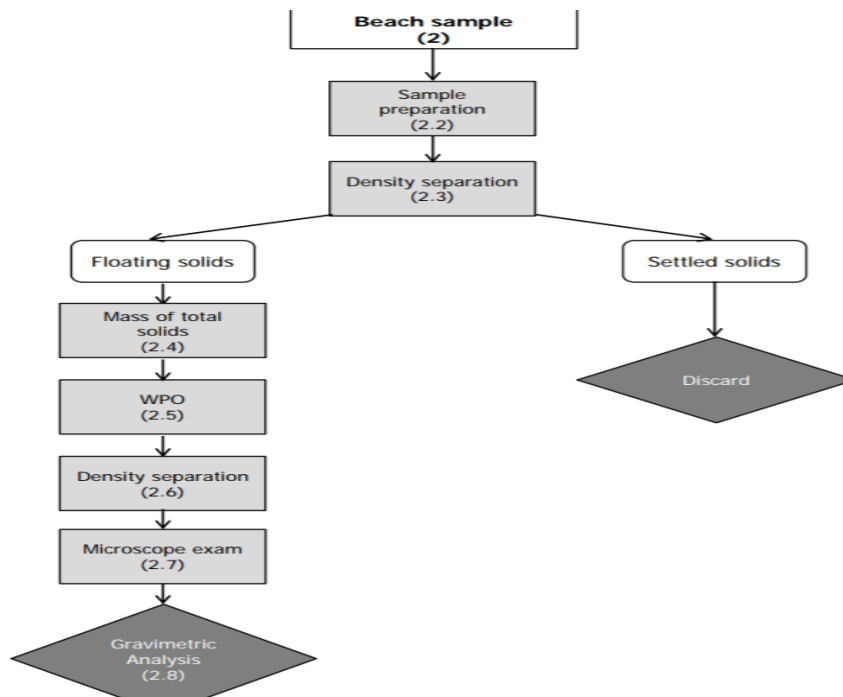


Figure 3-2: Flow diagram for analysis of microplastics in beach sample. (Masura, July 2015)

### 3.3 Methods for the analysis of microplastics in Bed Samples

Analysis of plastic litter in bed sediments collected with the help of corer or grab sampler is done by this method. In this method initially dried samples are disaggregated, and

disaggregated samples are sieved with the help of 5-mm and 0.3-mm sieves. Microplastics that are accumulated with sieve 0.3-mm are taken to wet peroxide oxidation experiment (WPO) where Fe (II) is present as a catalyst and this process helps the sample to get rid of organic matters. After this, WPO mixture is taken for density separation process and NaCl solution is used to float the plastic debris called floatation. Floated particles are filtered using 0.3-mm filter and dried with air and plastic material is removed and concentration of microplastic is determined by weighing it and the sample is taken for microscopic observation under 40X magnification (Masura, July 2015).

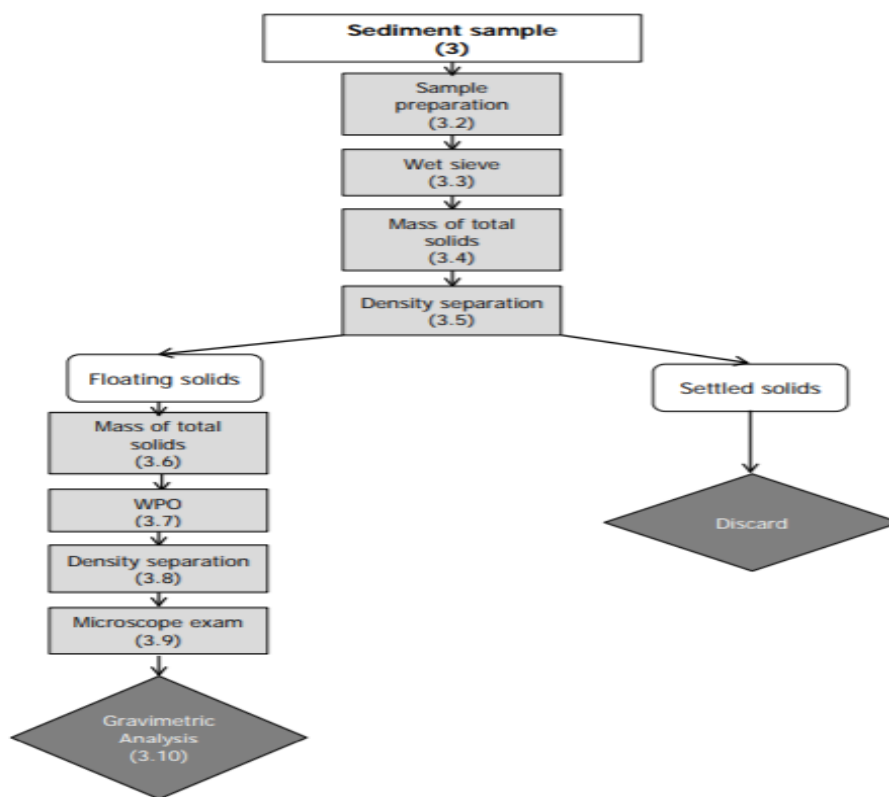


Figure 3-3: Flow diagram for analysis of microplastic in bed samples. (Masura, July 2015)

## 4 EXPERIMENT AND METHODOLOGY

This section describes about the whole process of microplastic extraction done from the beach sediments and details about Sediment microplastic isolation unit.

The experiment is done following the principle of density floatation using the apparatus sediment microplastic isolation (SMI) unit and microscopic analysis of the samples are done in Arcada's chemistry laboratory. SMI unit in this project was used being motivated by reading the available articles about using SMI unit and its functions from various web-sites and researchers in Finland. One of the research done in XAMK university of applied science showed that samples collected from Kotka containing full of organic materials were extracted using Sediment microplastic isolation method invented by SYKE lab personnel so that the pouring of samples would be easier during filtration process (Karjalainen, 2018 ).

Afterall, SMI unit was constructed in arcada's laboratory following the Coppock's DIY instructions (al, 2017). Sediment microplastic isolation (SMI) unit is a small-scale and portable apparatus designed by Coppock (Rachel L. Coppock a, 2017). This design has extracted microplastics from all the sediments samples in a single step and this has a mean efficiency of 95.8% ( $\pm$ SE 1.6%; min 70%, max 100%). During, the density separation process Zinc chloride, of density 1.5 g/cm<sup>3</sup> was recommended to use to allow fine sediments to settle down and enabling the denser plastics to float. This method is highly portable, cheap and can be carried to laboratory and in the field very easily. Using this method, the presence of microplastics in marine sediments could be achieved very easily in a single step (Rachel L. Coppock a, 2017).

### 4.1 Equipment and materials used

Various equipment's were used during the experimental process and they are as follows: -

- 117.5- and 118.5-mm size of transparent PVC pipe which has a diameter of 50 mm.
- PVC square plate.
- PVC ball valve of 50 mm diameter.
- 2×PVC valve union fittings.
- 2×PVC pipe couplers.
- PVC glue.
- Acetone (75%), Ethyl methyl ketone (15%), Cyclohexanone (10%).

- Sodium chloride (NaCl).
- Glass beakers: 800 ml and 500 ml.
- Weighing balance.
- Magnetic plate.
- Magnetic stirrers.
- Stir bar.
- Distilled water bottle.
- Petri dish.
- Parafilm.
- Vacuum filter.
- Filter paper.
- Metal forceps.
- Sieve.
- Microscope.

## 4.2 Preparation of PVC primer.

PVC primer was prepared in arcada laboratory to clean PVC materials before starting any operation to get them rid of any external contamination. PVC primer was prepared using, Acetone (75%), Ethyl methyl Ketone (15%) and Cyclohexanone (10%) and they were mixed together.

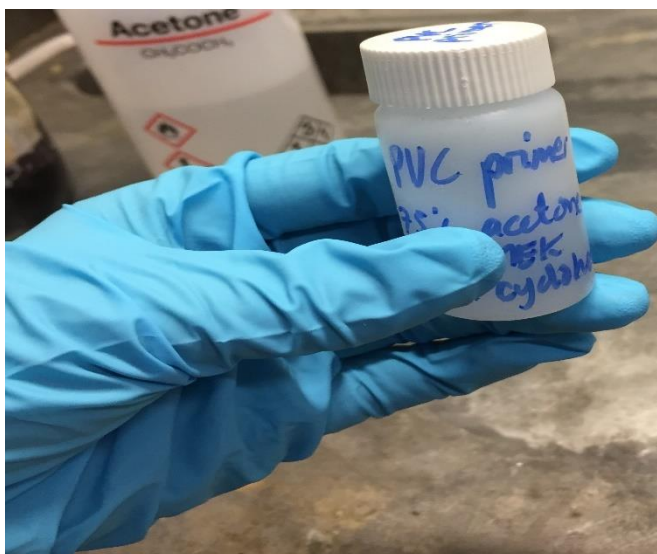


Figure 4-1: PVC primer.

### **4.3 Construction of Sediment Microplastic isolation (SMI) unit.**

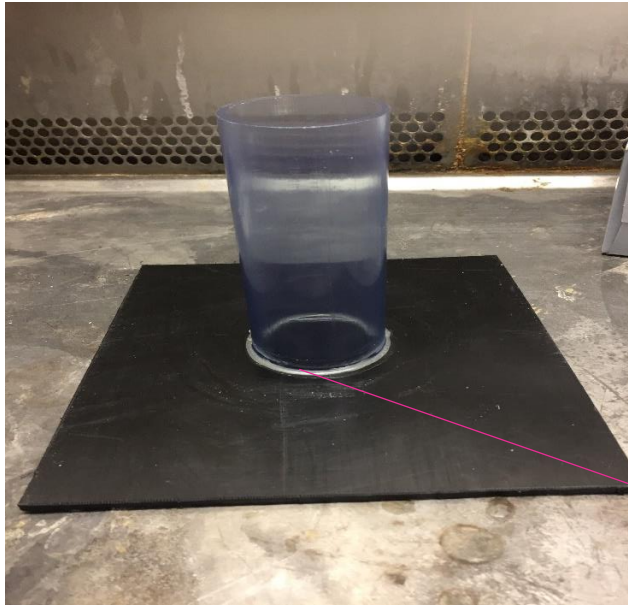
SMI unit was constructed assembling all the component parts together. The process of assembling parts during construction are described briefly below: -

List of kits used in SMI unit are: -

- 117.5- and 118.5-mm transparent PVC pipes which has a diameter of 50 mm.
- PVC ball valve of diameter 50 mm.
- PVC base plate.
- 2×PVC valve union fittings.
- 2×PVC pipe couplers.
- Solvent PVC glue.

#### **Step 1.**

PVC glue named as tangit PVC-U was used to fix transparent PVC to a base plate. Method used in fixing them was glue was applied all over the base of the pipe and into the area of the plate where the base of the pipe sets. Before placing pipe coupler at the top of the pipe, ball valve union fitting was inverted from the pipe and surface of base plate and pipe were cleaned using primer made at arcada to get rid of any external contamination. Again, parts were attached together, and heavy piece of iron was kept at the top of pipe so that a force applied could attach them together easily and quickly.

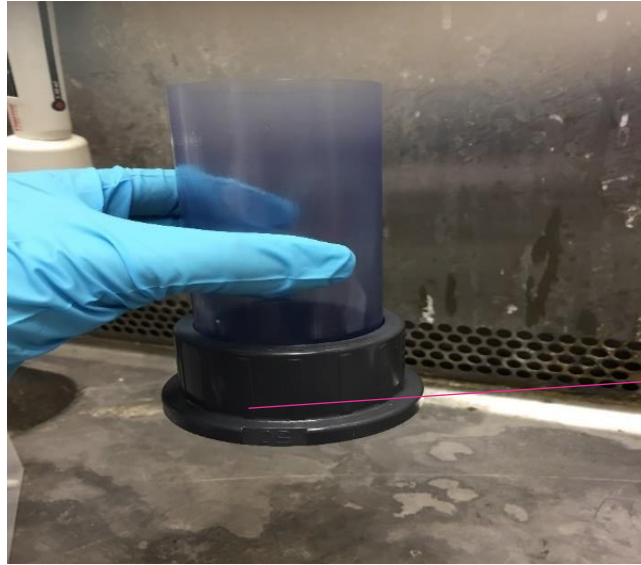


**PVC glue applied area**

*Figure 4-2: Glue applied to attach pvc pipe and base plate.*

## **Step 2.**

Pipe couplers and Pipe were attached together, and PVC glue was used to the end of the pipe and to the surface of the coupler where both parts were to be attached together and using the same procedure both pipe lengths were attached with pipe couplers. Surface of both parts were cleaned with PVC primer made at arcada.



**Pipe coupler**

*Figure 4-3: pipe coupler and pipe attached together.*

### **Step 3.**

In this step, base plate attached together with PVC pipe is assembled with one pipe coupler being faced upward with one ball valve union fitting at the end of pipe, PVC ball valve, other PVC pipe length attached with pipe coupler and other ball valve union fittings are assembled together and finally the SMI unit is constructed.



**Ball valve union fittings**

*Figure 4-4: Ball valve union fittings.*

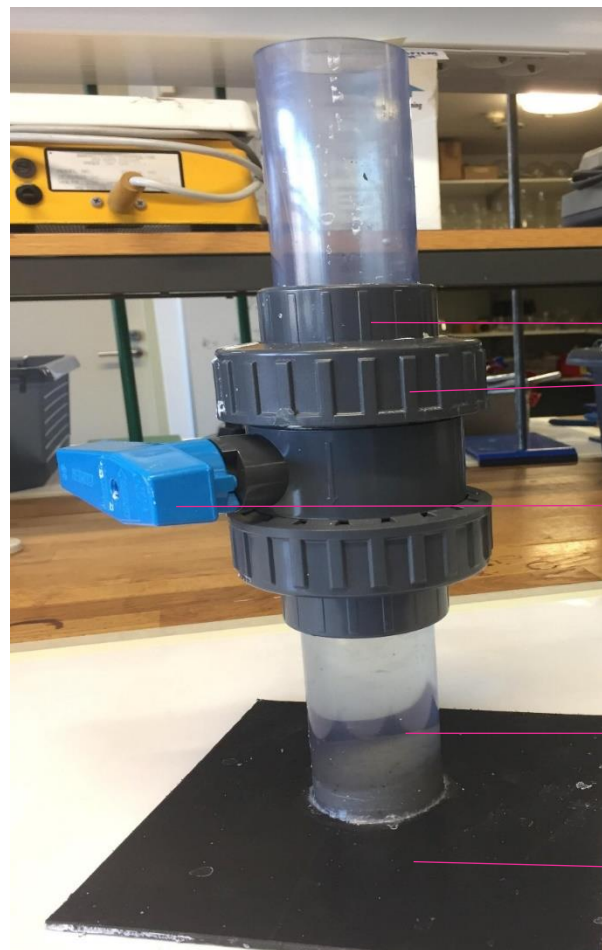


**PVC ball valve**

*Figure 4-5:PVC ball valve*

#### **Step 4.**

In this section, all the parts are assembled together and final sediment microplastic isolation (SMI) unit is made ready.



**Pipe coupler**

**PVC valve union fitting**

**PVC ball valve**

**PVC transparent pipe**

**Base plate**

*Figure 4-6:Final SMI unit*

#### 4.4 Detail about sand samples and sampling location.

Sand samples were collected from seven different beaches around the world which are listed below. The main reason of taking sand samples from these beach locations includes being highly crowded tourist area, pollution due to industries and factories and highly residential area near beach.

- ❖ Lilaste beach (Gulf of Riga), Latvia. (samples taken from tidal zone and far from tidal zone).
- ❖ Läänemeri beach, Estonia.
- ❖ Goa Morjim beach, India.
- ❖ Balangan beach, Bali Indonesia.
- ❖ Praia da Barra, Brazil
- ❖ Praia da Boa Viagem (Rio de Janeiro).



Figure 4-7: Sand samples from seven different beaches of various countries.

- Lilaste beach (Gulf of Riga), Latvia.

Lilaste is located 25 km far from Riga which is the part of Baltic sea has a beautiful forest lake with clean water and sandy beach. During, summer many peoples go there to enjoy vacation. From, this beach two different samples one near sea and other far from sea were taken (available, Civilization : Riga and Jurmala, 2016).



*Figure 4-8: Sand samples taken from lilaste beach, ( Provided by friend).*

- Läänemeri (Kuressaare) beach, Estonia.

Läänemeri (Baltic sea) is in Kassari bay and in Estonia the Läänemeri sea is in western part and this beach is the warmest beach. Being warm, in this beach many peoples are attracted during summer. From this beach sand sample was taken near the sea.



*Figure 4-9:- Kuressaare beach, Estonia. (Malv, 2018)*

- Goa Morjim beach, India.

This beach lies in the northern part of the state and many tourists are attracted in this beach and mainly this beach is famous in its sea turtles nesting in a ground and is very peaceful. From this beach sand sample was collected a bit far from the tidal zone.



*Figure 4-10:Morjim beach Goa, India (McClymont, 2019)*

- Balangan beach, Bali Indonesia.

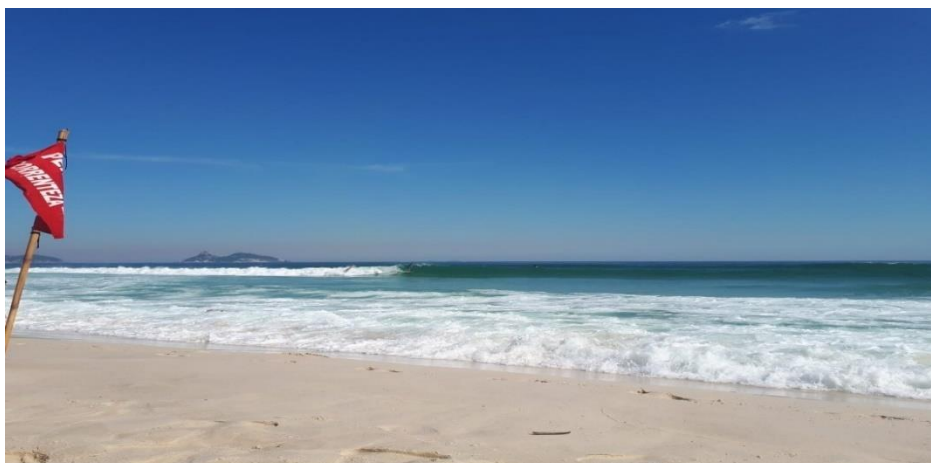
Balangan beach is in southern part of Bali and is very close to Bali International Airport. It takes only 60 minutes to reach there from Kuta Beach Bali. Many visitors are attracted to this beach so, this beach is the busiest beach in Bali. Sand sample was collected from tide line of the beach.



*Figure 4-11: Balangan beach, Bali (Dave, 2019)*

- Praia da Barra, Brazil.

Praia da Barra is located near the Barra da Tijuca and west of Rio de Janeiro. It has 15 kilometers of sand also boasts as the longest beach in the city (K, 2013) Sand sample was collected next to tidal zone from this beach.



*Figure 4-12: Praia da Barra, Brazil (provided by friend).*

- Praia da Boa Viagem (Rio de Janeiro).

This beach has seven miles of white sand, coconut palms. In front of the building Acaiaca, there is one of the busiest beaches which stretches between the streets Felix de Brito e Melo and Antonio Falcao. As this beach has sharks, dips are not allowed on that area. From this beach sand sample was collected next to tidal zone. ([www.tripadvisor.com](http://www.tripadvisor.com))



Figure 4-13: Praia da Boa Viagem, Rio de Janeiro (Provided by friend).

## 4.5 Density separation and Vacuum Filtration process.

Density separation is a process of separating solid particles with various densities with the help of separating liquid in an appropriate device to proceed the process. (Pagenkopf, Apr 14, 1998)

### 4.5.1 Saturated NaCl as microplastic separating liquid.

In this project NaCl solution was used as separating liquid to extract microplastics from all the samples.

**Calculation of amount of NaCl to be taken during the separation process: -**

Salt	Density (g/cm <sup>3</sup> )	Amount of NaCl added to 1 L water (g)
NaCl	1.2	337

From, the above table the appropriate amount of salt to be taken for distilled water being used is calculated,

$$1000 \text{ mL of } H_2O = 337 \text{ gram of NaCl}$$

$$1 \text{ mL of } H_2O = \frac{337}{1000} \text{ g of NaCl}$$

$$\text{Hence, } 1 \text{ mL of } H_2O = 0.337 \text{ g of NaCl}$$

After, calculating the appropriate amount of NaCl that could be used, in each procedure 134,8 g of NaCl was used for 400 mL of Distilled water to make a saturated salt solution. In each saturated salt solution sand sample was mixed and density separation process was done using sediment microplastic isolation unit and the mixture was mixed vigorously using magnetic pulses in magnetic bar and the separated MPs were vacuum filtered using filter paper and sample extracted was taken for microscopic analysis.

#### 4.5.2 Overall procedure applied during density separation and vacuum filtration.

Saturated NaCl solution was produced by mixing 400 mL of distilled water with 134.8 g of NaCl. Salt and water were mixed vigorously in a clean glass beaker with the help of metal stirrer until the salt was dissolved in water and the saturated NaCl solution was filtered through 50 µm mesh to prevent solution from external contamination. 50 g of sand was weighed in a weighing machine. Before starting density separation process, all SMI parts were rinsed with distilled water to avoid external contamination and during extraction, saturated NaCl solution and 50 g of sand were poured into a sediment microplastic isolation (SMI) unit and the unit was kept above magnetic stirring plate to mix the sediment. SMI unit was kept for 5 minutes above magnetic stirring plate and the sediment was mixed properly with the help of 3 magnetic pulses and the unit was left over until the supernatant part was clear of the sediments and the ball valve was closed properly

where, some meshes were found to be floating in the supernatant and the supernatant was taken to filter in a vacuum filter using the filter paper of size 125 mm.

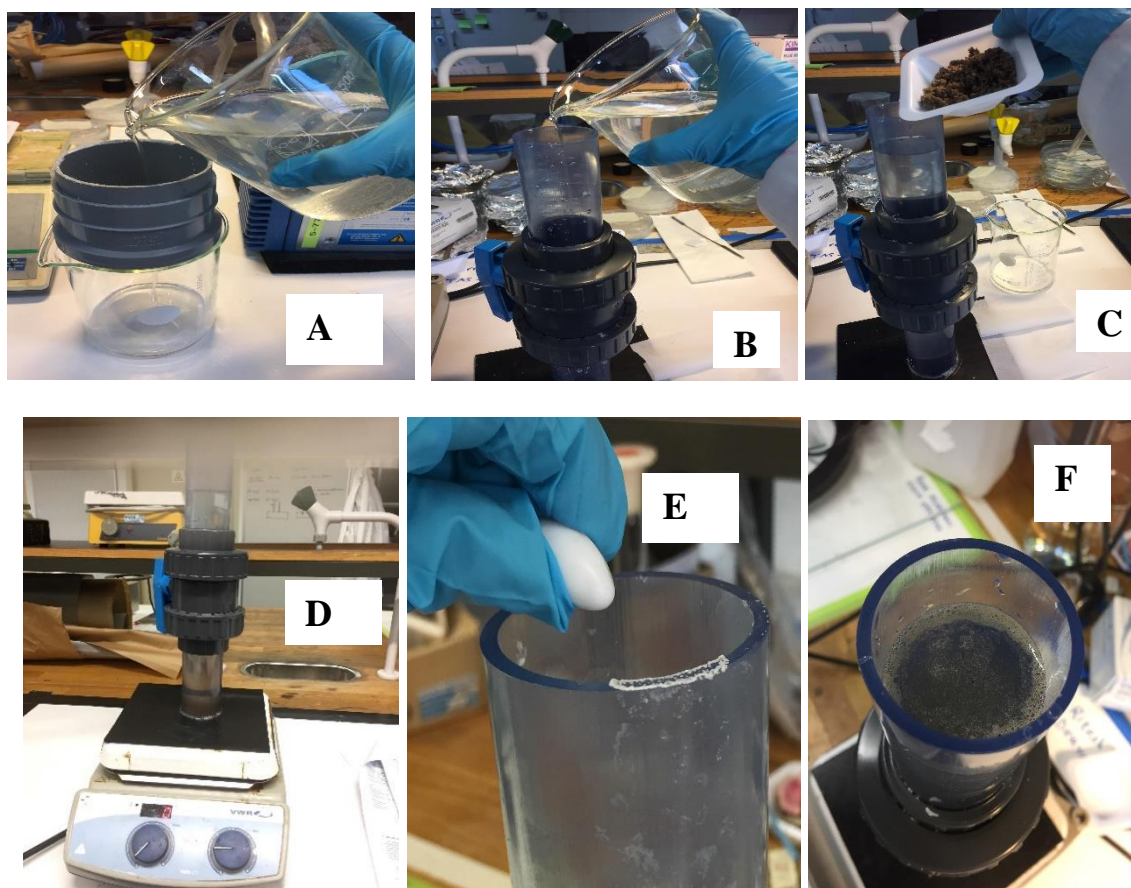


Figure 4-14:(A) Filtration of NaCl solution, (B) NaCl solution poured into SMI unit, (C) sand sample poured into SMI unit, (D) SMI unit with samples kept over magnetic bar, (E) Stirring pulse poured into SMI unit, (F) floated samples after density separation.

### **Vacuum filtration process**

Vacuum filtration process was done using Buchner funnel and a side arm flask where, side arm of the flask was connected to a vacuum source using a thick-walled pipe. Next, Buchner funnel was kept above the flask and the filter paper was cut into the size where it could be placed onto the funnel covering all the holes of the funnel so that the mesh couldn't pass through the holes into the flask. Filter paper was made wet using some distilled water to adhere the paper into the plate of the funnel and the vacuum source was turned on following the supernatant mixture poured onto the filtered paper. Liquid was

pulled through funnel and the mesh was settled on to the filter paper which then was transferred to a clean petri dish and sealed with parafilm until the sample was taken under microscopic examination. The same procedure was applied for all the remaining samples.

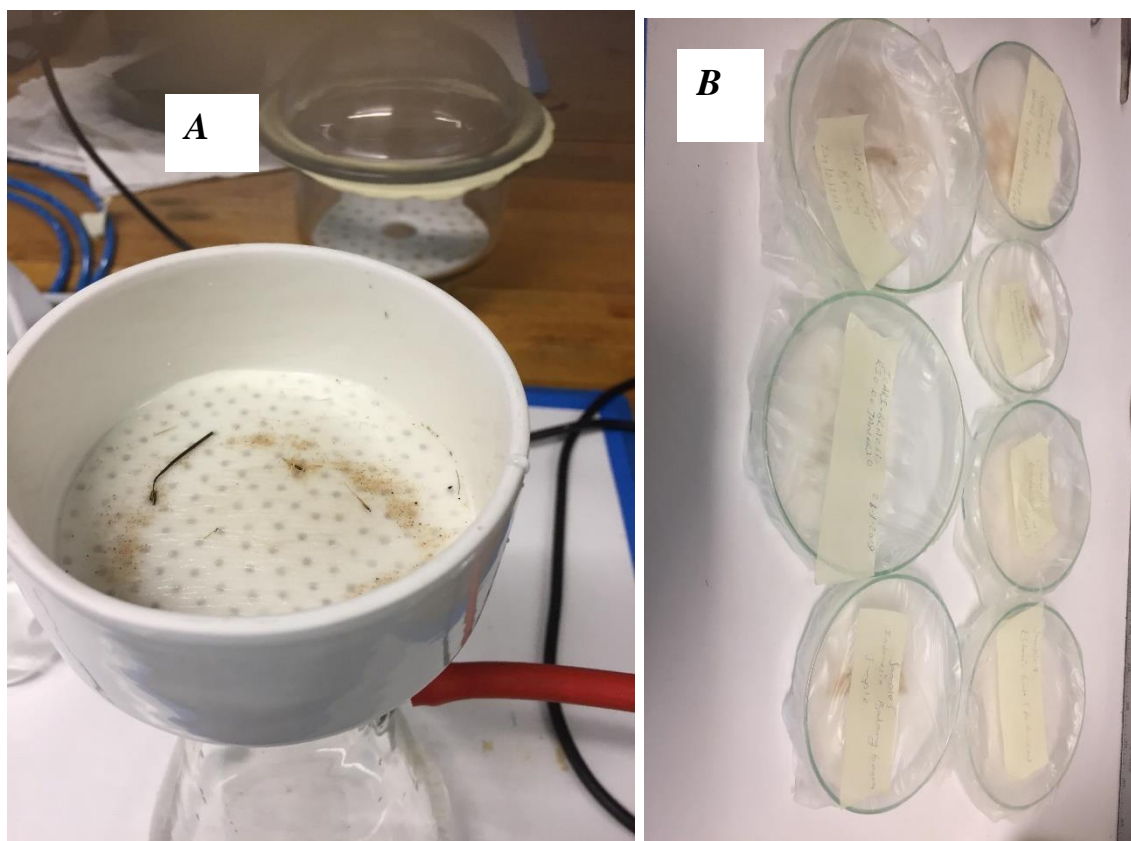


Figure 4-15: (A) Vacuum filtered MPs samples, (B) samples made ready for microscopic examination.

## 5 RESULT AND ANALYSIS

Following the process of density separation using SMI unit, NaCl was used as a salt to extract light weight particles. Seven different sand samples from various beaches around the world were tested to extract MPs.

### 5.1 Results obtained from all seven sand samples

- Sample 1: Lilaste beach (Gulf of Riga), Latvia- far from tidal zone.

From the sample of lilaste beach, results obtained after microscopic examination are shown below. From the result obtained, three various fibers of different colors were found and in comparison, to fibers MPs were found very few. From the figure below, figure (1) a blue colored fiber was obtained, which was inspected under 10× magnification with dark field. Figure (2) blue colored MPs sample was found under 10× magnification with bright field. In the same way in figure (3) and (4), three various fibers were found with distinctive colors both under 10× magnification with bright field respectively.

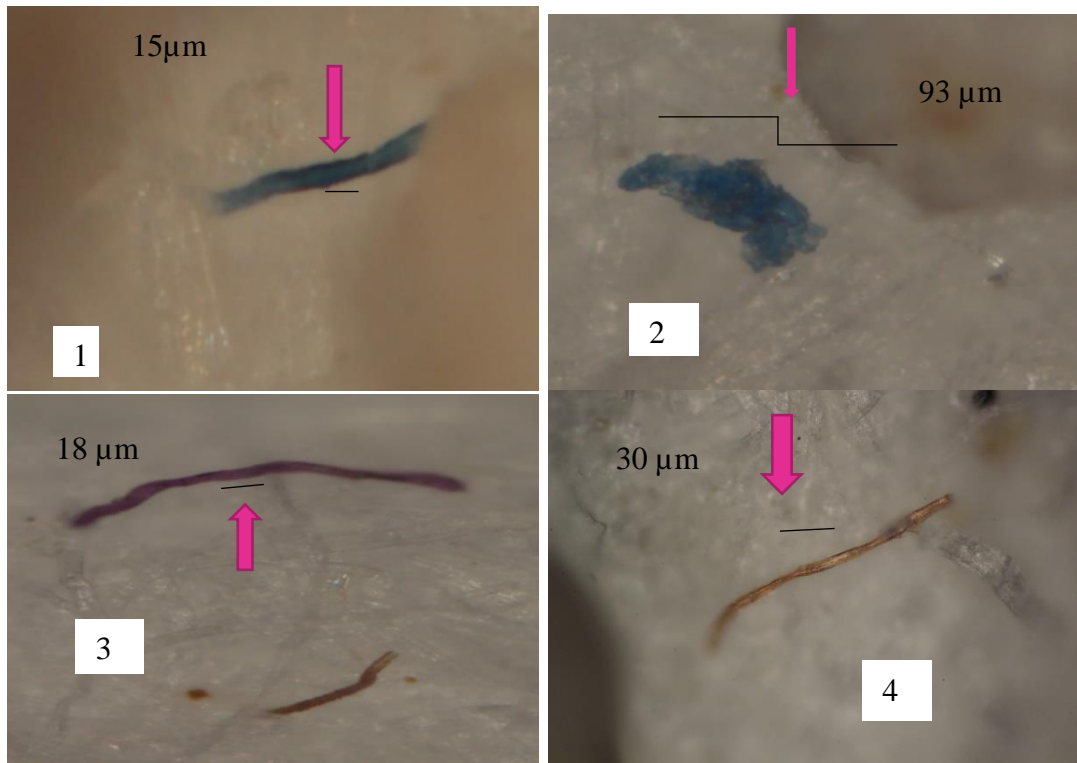


Figure 5-1: (1) Blue color fiber of size 15µm, (2) Blue color MPs of size 93µm, (3) two distinctive color fibers of size 18µm, (4) yellow color fiber of size 30µm.

- Sample 2: Lilaste beach (Gulf of Riga), Latvia-tidal zone.

Samples from tidal zone of Lilaste beach were examined in this step. Results obtained after microscopic examination are given below. From the below results, it can be seen that three distinctive color fibers with one MPs was found. In figure (1) a long fiber was obtained under 10× magnification with dark field, Figure (2) a green colored fiber was found under 50× magnification with bright field, Figure (3) a red colored fiber was found under 50× magnification with dark field, Figure (4) a white colored MPs with some organic matters was found under 20× magnification with dark field.

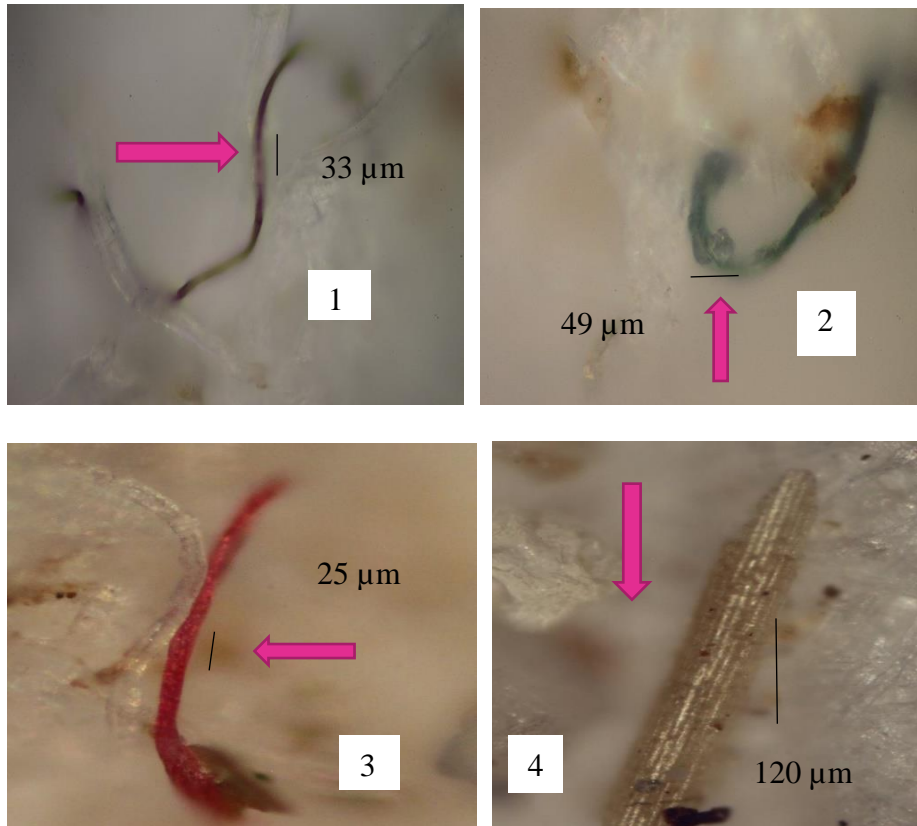


Figure 5-2: (1) Long non uniform fiber of size  $33\mu\text{m}$  , (2) Green colored fiber of size  $49\mu\text{m}$ , (3) red colored fiber of size  $25\mu\text{m}$ , (4) white colored MPs of size  $120\mu\text{m}$  with organic matter.

- Goa morjim beach, (India)

Sand samples far from tidal zone were taken in this step. Results after microscopic examination are given below. It can be seen that, various colors of fiber were found where there was no any MPs found. In figure (1), thick blue colored fiber was found under  $10\times$  magnification with bright field, figure (2) has red colored fiber under  $20\times$  magnification with bright field, figure (3) has black colored fiber under  $10\times$  magnification with dark field and figure (4) has long black fiber under  $10\times$  magnification with bright field.

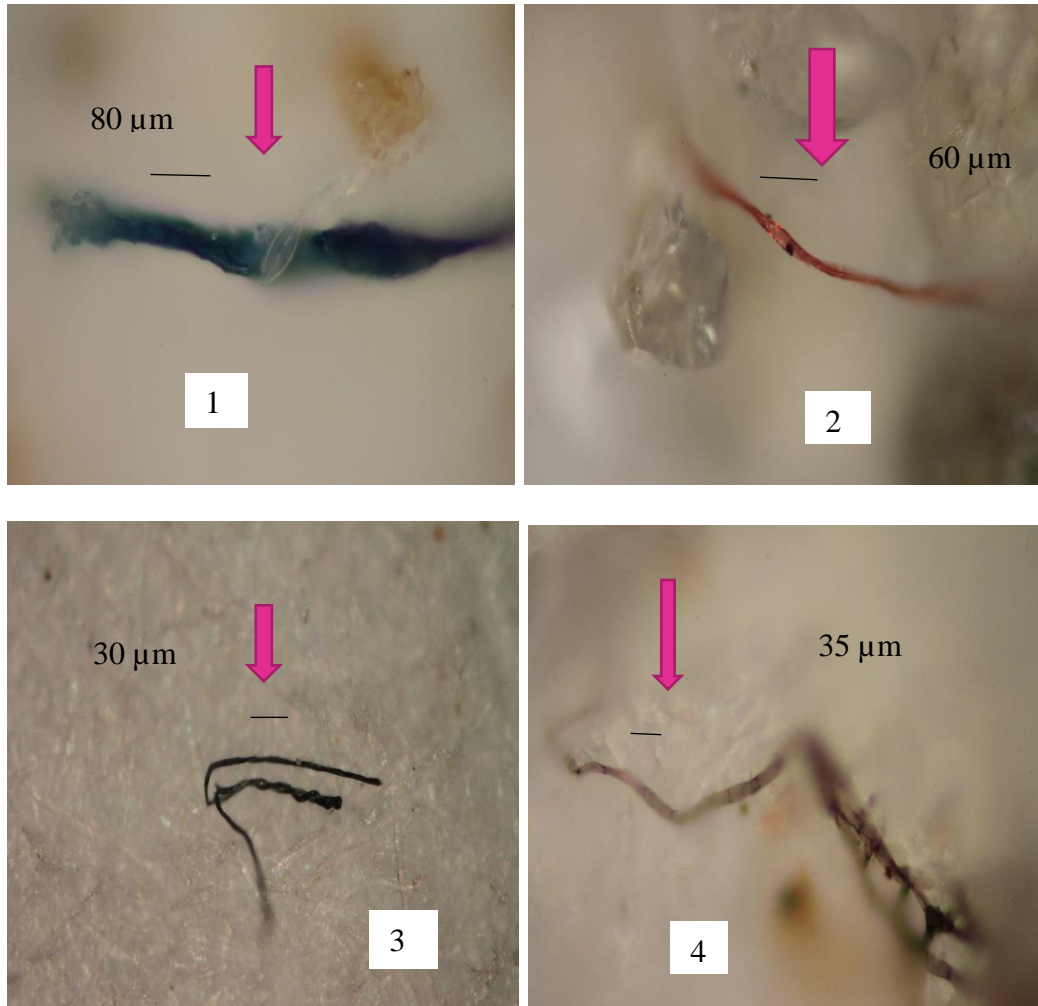


Figure 5-3: Figure(1) blue colored fiber of size 80 $\mu$ m, Figure(2) red colored fiber of size 60 $\mu$ m, Figure(3) black colored fiber of size 30 $\mu$ m, Figure(4) Black and white colored non uniform fiber of size 35 $\mu$ m.

- Sample 4: Läänemeri (Kuressaare) beach, Estonia.

Sand sample was taken near tidal zone from Beach of Estonia. During, first microscopic examination no any MPs or fibers were found so, density separation process was done again from which one fiber was found with some organic matters and some undissolved salt crystals as shown below. The examination was done under 20 $\times$  magnification with dark field.



*Figure 5-4: Fiber of size 57μm with organic matter and NaCl crystals.*

- Sample 5: Balangan beach, Bali Indonesia.

Sand samples from Balangan beach was taken far from tidal zone. Figures after microscopic examination were obtained as shown below where, figure (1) has fiber with MPs under 10× magnification with bright field, figure (2) has non uniform fiber under 20× magnification with dark filed, figure (3) has short fiber under 20× magnification under dark field, figure (4) has white colored fiber under 20× magnification with dark field.

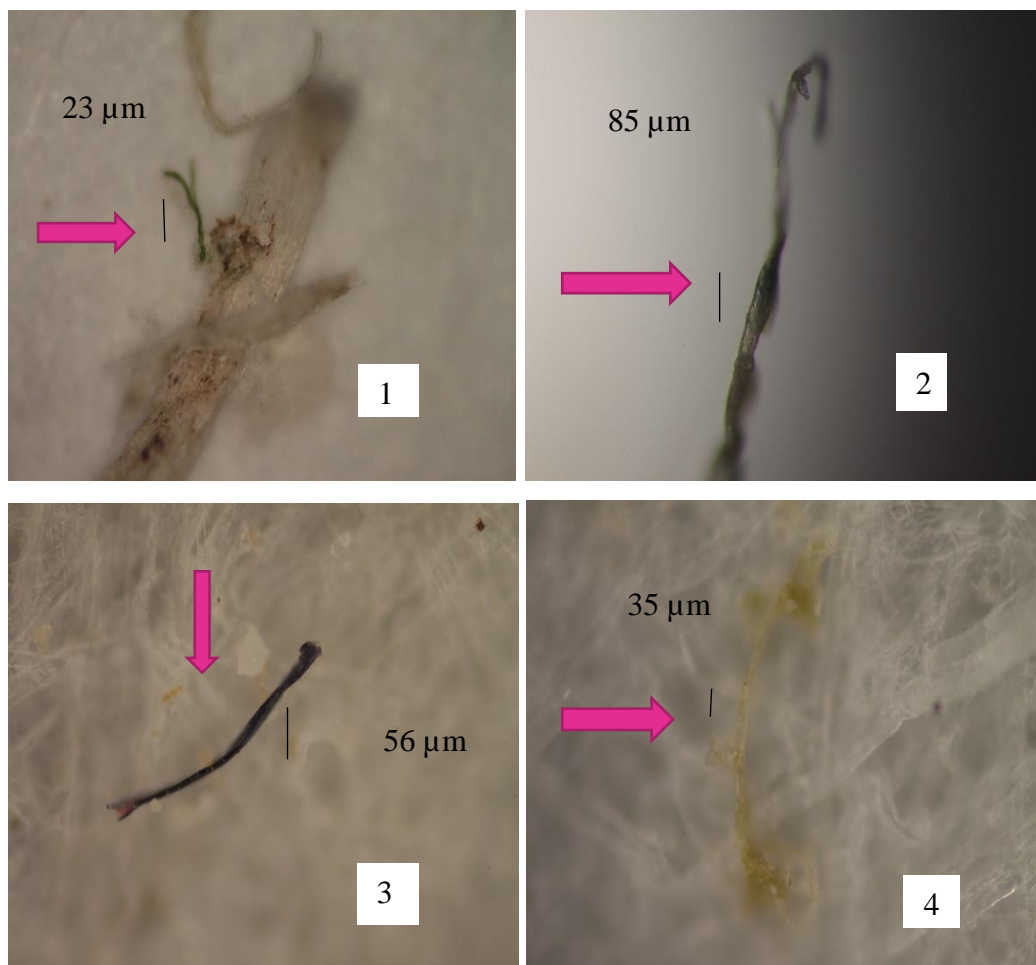


Figure 5-5: (1) Fiber of size 23μm with MPs , (2) long black colored fiber of size 85μm, (3) short black colored fiber of size 56μm, (4) white colored fiber of size 35μm.

- Sample 6: Praia da Boa Viagem (Rio de Janeiro).

Sand sample was taken next to tidal zone from this beach. After, microscopic examination various MPs and fibers were found as shown below. In figure (1) MPs was found under 20× magnification with bright field, figure (2) has two MPs with some organic matter under 20× magnification with dark field, figure (3) has blue colored MPs with organic matter under 20× magnification with bright field, figure (4) has long fiber under 20× magnification with dark field.

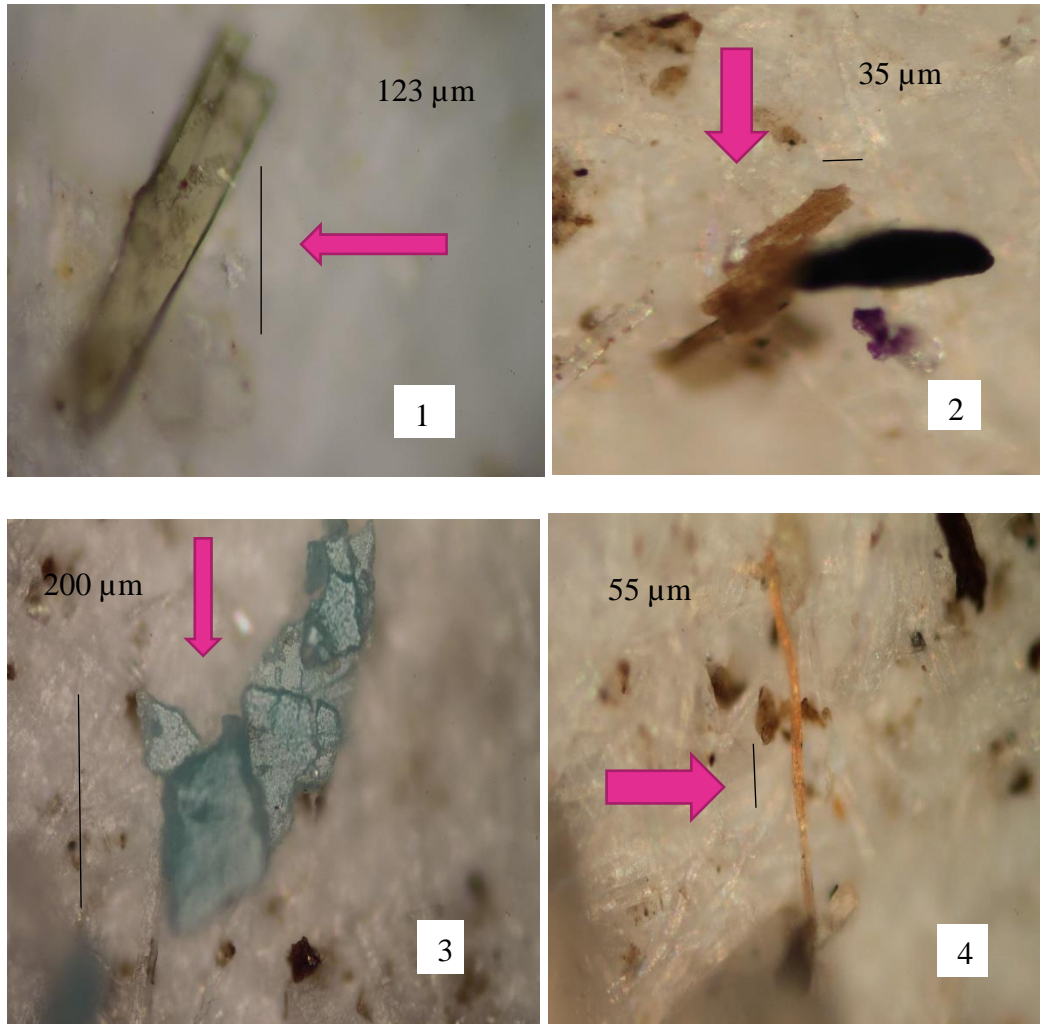


Figure 5-6: (1) MPs of size 123 $\mu\text{m}$ , (2) two microplastics of size 35 $\mu\text{m}$  with organic matter, (3) MPs of size 200 $\mu\text{m}$  with organic matter, (4) Long uniform fiber of size 55 $\mu\text{m}$  with organic matter.

- Sample 7: Praia da Barra, Brazil.

Sand sample were taken from next to tidal zone from this beach. Various fibers were found with less MPs from this sample as shown in figures below. Figure (1) has blue colored MPs with some sand crystals, which was examined under 20 $\times$  magnification with dark field, Figure (2) has red colored fiber examined under 5 $\times$  magnification with bright field, Figure (3) has blue colored fiber examined under 10 $\times$  magnification with bright field, Figure (4) has white colored fiber examined under 10 $\times$  magnification with bright field.

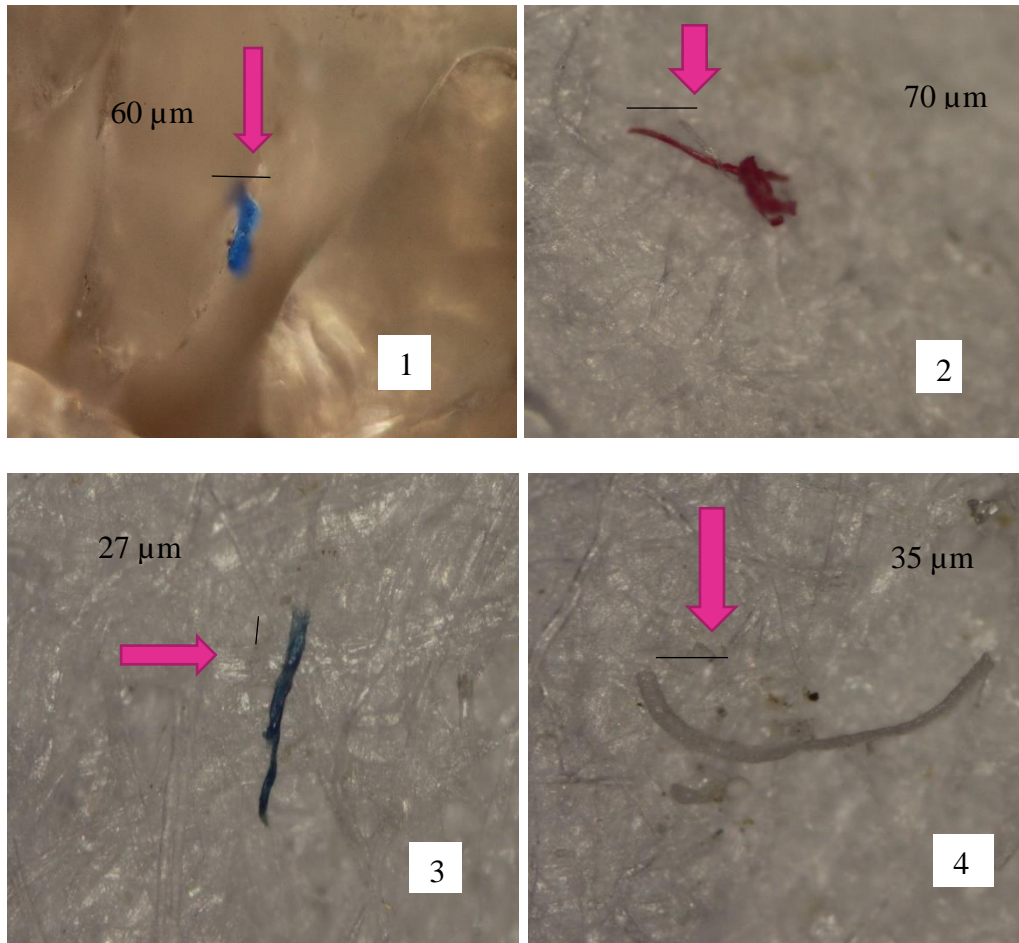


Figure 5-7:(1) blue colored MPs of size 60 $\mu$ m, (2) Red colored fiber of size 70 $\mu$ m, (3) Blue colored fiber of size 27 $\mu$ m, (4) white colored fiber of size 35 $\mu$ m.

## 5.2 Analysis of the result obtained from all sand samples.

### Microscopic analysis of the samples

The microscopic measurement of all the samples were carried out using magnification size of 5×, 10×, 20× and 50×. During, microscopic analysis samples were taken in a filter paper and observation was done directly through it. Overall, comparatively higher amount of fibers was found than microplastics for example in sand sample of Goa Morjim beach and kuressaare beach from Estonia no any MPs were present. Higher amount of blue colored fiber and MPs were found in each sample as shown in the figures above.

Some fibers and MPs were found to be attached with organic matters for example, figure 4 of Lilaste beach (tidal zone), kuressaare beach Estonia, figure 2,3 and 4 of Praia da Boa Viagem beach. Some samples were found to be uniform in structure and some were non-uniform in structure from which it can be concluded that those uniform structure fibers could be synthetic fibers.

## **6 DISCUSSION AND CONCLUSION**

This section contains discussion and conclusion about results obtained and the process applied in whole thesis.

### **6.1 Discussion**

The main aim of this thesis was to extract microplastics from beach sediments. Sediments from 7 different beaches around the world were taken to examine the presence of microplastics. All the sand sampling locations are explained briefly in section 4.4. Density separation method with sediment microplastic isolation unit was used to separate the lighter particles from the sand samples. Sediment Microplastic Isolation unit was constructed in arcada laboratory as explained briefly in section 4.2 and 4.3. During density separation, magnetic bar with some magnetic pulses were used to mix the sand and NaCl solution properly. Separated lighter particles were vacuum filtered and extracted filtered samples were transferred properly into a petri dish and covered with a parafilm to prevent from external contamination.

Filter paper containing extracted samples was taken under microscopic examination and results was obtained like, mostly presence of fibers was counted compared to microplastics. Fibers and Microplastics of various colors were obtained where, blue colored fibers counted the most. Along with blue color fiber and MPs various other colors such as, red, white, yellow was also found.

### **6.2 Conclusion**

Throughout this thesis, various methods and techniques of identification of microplastics from marine environment are explained briefly. Detail information about effects of microplastics on aquatic environment are also described briefly. Sand samples after density separation were taken into microscopic examination from where, only the presence of microplastics and fibers were detected. To find out the origin of the microplastics some

advanced analytical laboratory techniques such as micro-FTIR (Fourier Transform Infra-red) or micro- Raman spectroscopy are used but due to lack of those methods only microscopic analysis of the samples was done in this thesis.

### **Limitation and Further Research**

- During, experiment some MPs were lost because they were attached on wall of SMI unit, some were lost during transferring of extracted samples into the vacuum filter and some during microscopic analysis of samples directly through filter paper.
- Density separation of the sand sample was done using NaCl solution in this process but some other denser salts like  $ZnCl_2$  and NaI could be used to get more better results.
- For further examination to identify the origin of MPs, advanced analytical laboratory methods like: FTIR or Raman spectroscopy could be used.

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