Experimental analysis of microplastics in beach sediment samples by density separation and microscopic examination

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Abstract:
This thesis mainly deals with microplastics, its sources and experimental approach to investigate the presence of microplastics in environment. Microplastics are threat to environment in recent years. Its adverse effect is directed towards aquatic animals. This thesis will provide the information about the sources of microplastics and how it ends up to the marine environment. The main objectives of this thesis project are to review the methods to analyze microplastics, develop methods to collect microplastic samples from local terrestrial environment and the procedure to analyze them in Arcada's laboratory. Sediment samples collected from 4 different locations were examined using microscopic analysis. Two different methods were employed to analyze collected sediments samples. Sodium chloride was used as a density separator to extract microplastics from sample. Results obtained from microscopic analysis showed the presence of colored microplastics and fibers in all the collected sediment samples.

Keywords:	Microplastics, Wet peroxide oxidation, NOAA, Microscopy, Density separation
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Abbreviations

NOAA: National oceanic and atmospheric administration

MPs: Microplastics

PP: Polypropylene

PE: Polyethylene

PVC: Polyvinyl chloride

PS: Polystyrene

$H_2O_{2:}$ Hydrogen peroxide

$ZnCl_2$: Zinc Chloride

NaCl: Sodium chloride

WPO: Wet peroxide oxidation

FTIR: Fourier transform infrared spectroscopy

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1. INTRODUCTION

1.1. History & background

Plastics are synthetic organic materials which consist of long chain polymers and have high average molecular weights. They are made up of either synthetic or natural polymers, also called as resins. Plastics are cheap, lightweight, durable, which make them perfect choice for any applications. The same features that make plastic as one of the most usable material also contribute to become a serious pollution threat. Recent studies suggest that 4.8 to 12.7 million metric tons of plastic were disposed to ocean in 2010 (Herrera et al., 2018). Plastics production is accelerating in developing countries and they are now used to the use-and-dispose culture of plastics.

Plastic litter in the marine environment is a recent concern from a global environmental perspective. Plastics is the main waste which can be found in the marine environment and 70% of marine debris is plastic, where it accumulates and persists due to its durable nature (Melissa B. Phillips, 2014). The occurrence of small plastics on marine and coastal environment was first noticed in 1970s but the term microplastics was not used yet then. The effects of large plastics items in marine environment can be noticed as the death and accidents are reported from marine animals due to ingestion, and entanglement of plastics. However, the large portion of plastics found in the marine environment is in the microscopic level. These plastics particles are known as microplastics (MPs). Their size ranges from 1 to 5 mm and belong microplastics group (Kershaw, 2015). Microplastics are synthetic materials with a high polymer content which are insoluble in water and non-degradable in nature. Microplastics have emerged as a recent threat to the global environment.

The main aim of this thesis is to establish the methods of marine microplastic sample collection and methods of examining the collected samples at Arcada's laboratory.

1.2. Objectives

- **To review the methods of microplastics sampling and analysis**
  It includes the literature review of various journal articles, books, research papers which have information on the methods of MPs sampling and analysis.

- **To determine the most suitable method for analyzing microplastics in Arcada by using resources available at Arcada's laboratory**
  Among various methods and analysis techniques for MPs, this thesis will focus on the best suitable method for MPs analysis, considering the resources available at Arcada's chemistry laboratory.

- **To develop the sample collection methods from local environment**
  Thesis will cover the information on how the samples from local environment are collected and what is the best guideline to follow while collecting the samples.

- **To develop the separation methods for collected microplastic samples**
  Collected samples from local environment will be separated in laboratory using best suitable separation methods. Thesis will cover different types of separation methods of MP samples.

1.3. Thesis framework

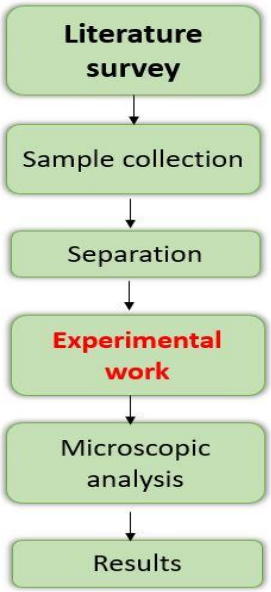


Figure 1: Thesis framework

One of the aims of this thesis was to conduct a literature review of articles published on MP. Therefore, this thesis explained the views of various researchers and authors about MP, their presence in marine environment and experimental methods to analyze MP. Literature review section explained general information on MP, their classification and how they end up in the marine environment. The literature review section is followed by MP in marine environment; which described about presence of different types of plastics in the marine environment, different marine animals which are susceptible to MP and effect of MP on them. Review on sample collection section explained the sample collecting methods from water, sediments and biota and described protocol while collecting each sample. Experimental section described a laboratory examination of sediment samples collected from 4 different beaches around the world using two different methods and lastly the result and analysis section focused on the results from microscopic analysis of the sediment samples.

2. LITERATURE REVIEW

Over the last few decades, microplastics has been a threat to marine environment. In 2012 some of the reviews focused on some areas of microplastics sample collection and analyzing methods particularly from sea water and freshwater (A.B. Silva et al, 2018). During the process of analyzing, the toxicological effects of microplastics towards living organisms has also been thoroughly examined. On the other hand, the presence of microplastics in environment has also been assessed through methods such as extracting the materials from their matrices and their identification is studied through quantitative and qualitative measurement of chemicals presence on them (A.B. Silva et al, 2018).

2.1. Plastics in general

Plastics are made up of long and repeating chains of monomers. A monomer is a base molecule which repeat throughout the polymers. Like wood, paper and wool, plastics are also organic materials. Materials like cellulose, coal, natural gas, salt, crude oil are the main raw materials for plastics. (Anon., ei pvm)

Plastics can be divided into mainly two groups: thermosets and thermoplastics.

- **Thermosets**
  Thermosets are synthetic materials which on heating can strengthen but cannot be remolded or reheated to their initial phase. These types of plastics are crosslinked with narrow chains. Thermosets are hard and brittle. Examples of thermosets are phenolic resins, polyester resins, and epoxies.

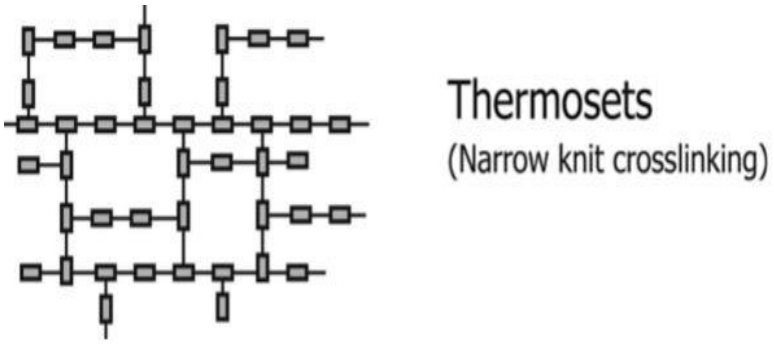


Figure 2: strong crosslinking thermosets (Klein, 2011)

- **Thermoplastics**
  Thermoplastics consists of macromolecules chains without crosslinking between the chains. Thermoplastics are softened when heated and get harden when cooled. Thermoplastics can be changed into different shapes by applying heat, but the chemical composition remains same. (Anon., 2018) The chemistry of thermoplastics

helps it to have a resistance against the environmental effects like UV radiation. (Klein, 2011). Examples of thermoplastics are polycarbonate (PC), polystyrene (PS), polyvinylchlorides (PVC), and polysulphone (PSU).

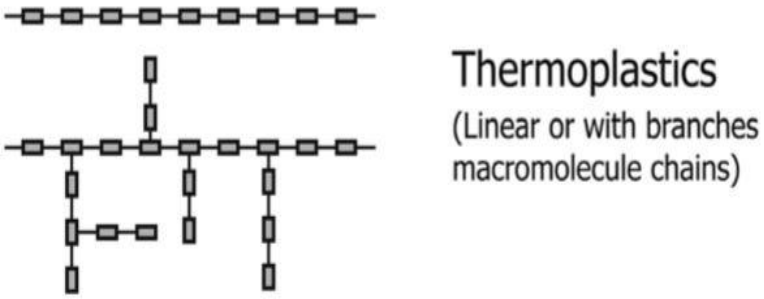


Figure 3: Linear and chained thermoplastic molecules (Klein, 2011).

The main problem with plastics is that they do not degrade easily. It is possible that they may break into smaller pieces but take too much time for degradation. Following table gives clear view on worldwide production of plastics. The mismanaged of these plastic leads to the formation of microplastics.

Table 1: Six types of plastic polymers produced worldwide (Nerlannd et al., 2014).

Plastic type	Abbreviation	Production in tones	(%)
Polyethylene	PE	85	30
Polypropylene	PP	54	18
Polyvinyl chloride	PVC	31	11
Polystyrene Expanded polystyrene	PS, PS-E	21	7
Polyethylene terephthalate	PET	19	7
Polyurethanes	PUR	21	7

2.2. Microplastics

Microplastics pollution is widespread and major threat to the environment in recent times. Microplastics are smaller plastics with ≤5 mm size. There are various ways in which microplastics can enter the environment. The most common way is the degradation of macroplastics. The breakdown of macroplastics can happen through various mechanism.

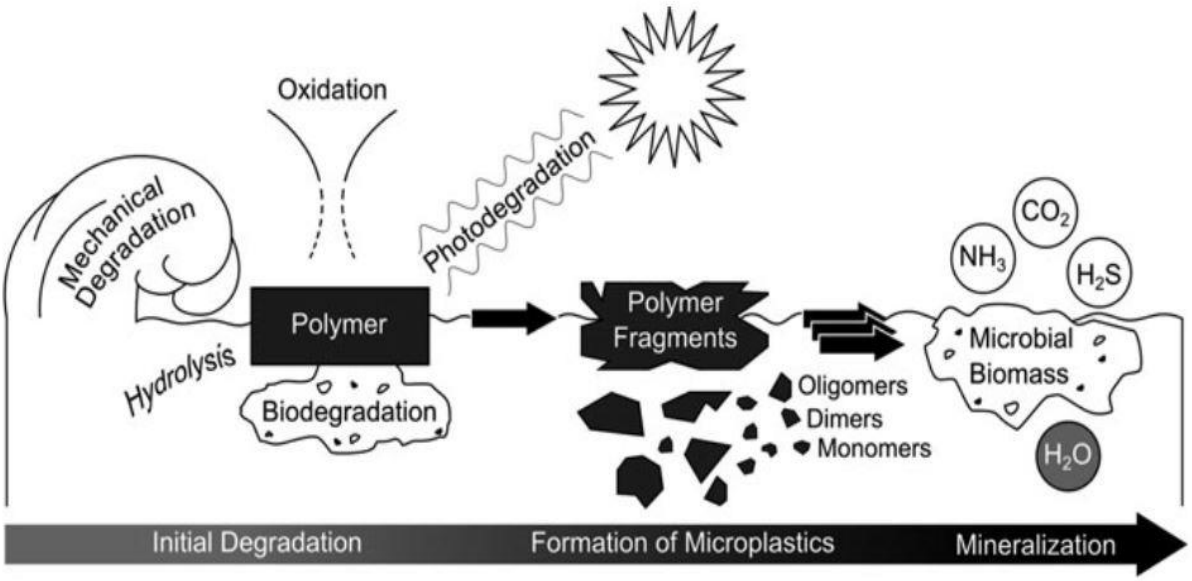


Figure 4:Formation of microplastics by degradation of macroplastics (Wagner, 2017)

The most common ways are: chemical degradation, tire abrasion, physical weathering of macroplastics. (Boucher, 2017)

2.2.1. Types of microplastics

The main basis on which microplastics are categorized is whether the particles are originally manufactured to be that size or they break down in smaller pieces. Mainly two types of microplastics are contributing the contamination in marine life. They can be divided as:

- **Primary microplastics**
  The main sources of primary microplastics are cosmetic and personal care products like cleansers, scrub (as skin exfoliators) shower gels, toiletries agents. They can also be generated

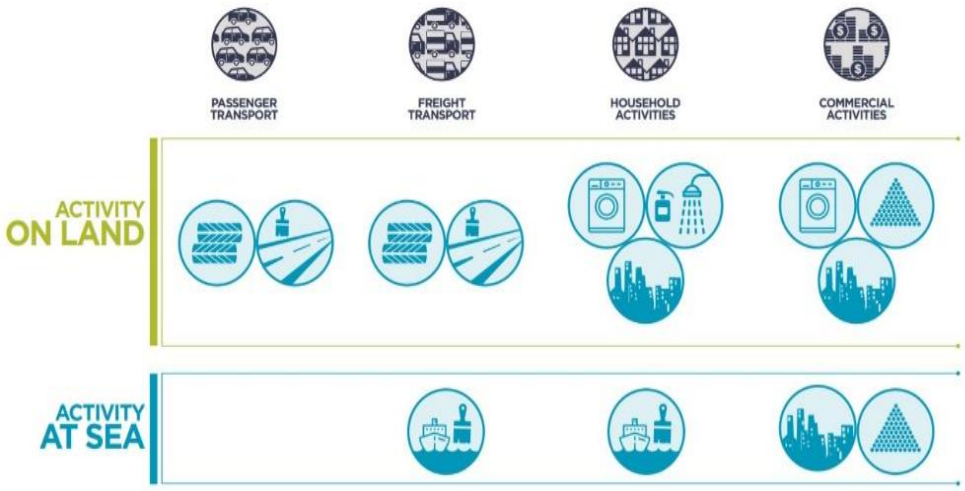


Figure 5:Primary sources of MPs (Boucher, 2017)

through other various means: erosion of tyres while driving, abrasion of synthetic textiles during laundry, road markings, marine coatings, and city dust.

- **Secondary microplastics**
  The secondary sources of microplastics are the larger plastics which break downs into smaller pieces through the process of photo degradation which are caused by ultraviolet rays from the sun and other mechanical forces. This happens to mismanaged waste like used large plastics bags and fishing nets (Boucher, J et al., 2017).

Figure 6:MPs from break down of macroplastics (Nerlannd et al., 2014)

3. MICROPLASTICS IN THE MARINE ENVIRONMENT

Growing evidences suggests that MPs are rapidly entering the environment. MPs have been a major concern of debate among the scientists, politicians and public around the world. It is scary to see how fast MPs are entering to the environment.

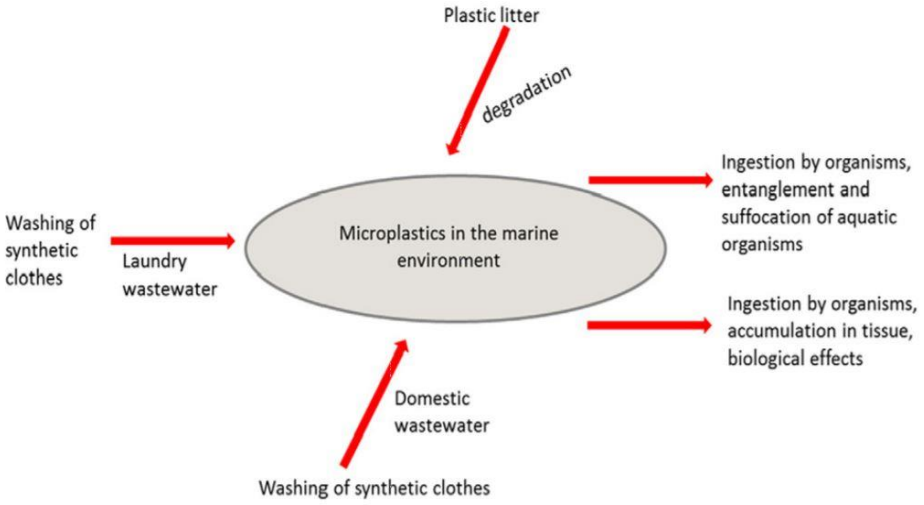


Figure 7: Ways of MPs ending up in marine environment (Stolte, 2014)

MP particles have been found widespread in numerous sites: from costal region to remote offshore areas (Hidalgo-ruz et al., 2012). The occurrence of MPs depends on the nature and location of sources and the environmental conditions. The sources of MPs can be unmanaged plastic waste on land or ocean, leakage from transport of goods, fishing equipment, waste water treatments plants etc. Several variables are responsible for MPs' distribution in the environment.

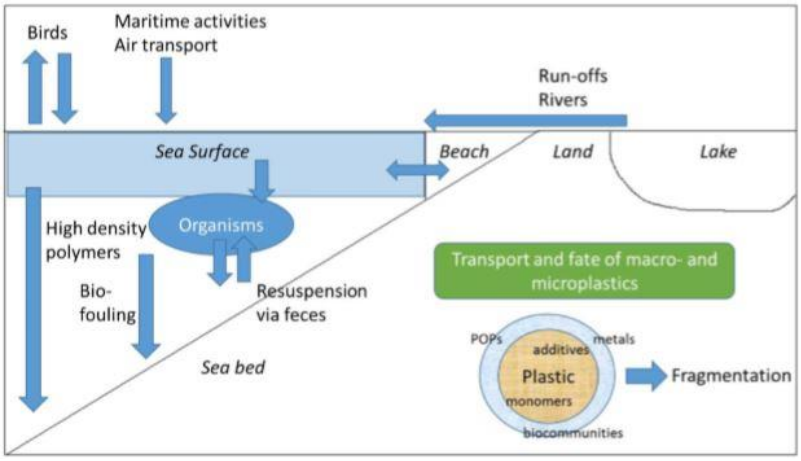


Figure 8: Transport of MPs in environment through various process (Kärrman et al., 2016)

One factor is the buoyancy of plastic polymers; for instance, PE and PP are float on the water surface as they have lower density than the water. In same way PVC and PET have higher densities than water, hence that is why they found below the water surface (Kärrman et al., 2016). MP distribution in the environment depends on the particle size of MPs. As Kärrman et al. suggested macro particles are capable of being transported in water longer distance in comparison to the micro particles (Kärrman et al., 2016).

Occurrence in the water column

Microplastics are widely distributed in the world's oceans. In the case of water column sampling they have been found in both the Atlantic and the Pacific oceans (Nerlannd et al., 2014). From the samples collected worldwide research studies have shown that the presence of MP is 60% in the northwest Atlantic, 61% of Portugal, 74% in Corsica in western Mediterranean, 89% in the Celtic sea and 97% in an estuary on the North Sea (Nerlannd et al., 2014). The most abundantly found MPs are fiber, granules and films. There is no record of research done in deeper water regarding the presence. Following table shows the presence of different types of plastics in water column and their origin.

Table 2: Types of plastic in marine environment (Andrady, 2011)

Plastic Class		Specific Gravity	Production worldwide	Products and typical origin
Low-density polyethylene	LDPE LLDPE	0.91–0.93	21%	Plastic bags, six-pack rings, bottles, netting, drinking straws
High-density polyethylene	HDPE	0.94	17%	Milk and juice jugs
Polypropylene	PP	0.85–0.83	24%	Rope, bottle caps, netting
Polystyrene	PS	1.05	6%	Plastic utensils, food containers
Foamed Polystyrene				Floats, bait boxes, foam cups
Nylon	PA		<3%	Netting and traps
Thermoplastic Polyester	PET	1.37	7%	Plastic beverage bottles
Poly (vinyl chloride)	PVC	1.38	19%	Plastic film, bottles, cups
Cellulose Acetate	CA			Cigarette filters

Occurrence in beach sediments

The occurrence of MPs in sediments were reported back in the late 1970s. The early observations were made in various countries such as Spain, New Zealand, Canada, Bermuda, and Lebanon. This helps to understand the MPs distribution worldwide from the late 1970s (Van Cauwenberghe Lisbeth, 2015).

The main way of MPs entering the aquatic system is through the plastic litter from various sources such as municipal wastes, activities in shores, illegal dumping of domestic and industrial wastes, and from sewage treatment systems. Kärrman et al. suggested that the plastic waste generated from coastal countries worldwide in 2010 was approximately 192 tons and out of it 2–5% was mismanaged which later end up in ocean (Kärrman et al., 2016).

Various reports suggested that in the UK, MPs and fibers were found in 23 out of 30 submerged sediments samples (Stolte, 2014). This indicates that how MPs can be effectively transported from water column to sediments. The macroplastics loads in beaches can affect in the presence of MPs. For example, a high number of MPs were detected in Hawai'ian beaches and in Greek beaches (Stolte, 2014). Similarly, the accumulation of litter on German Baltic Sea and North Sea beaches is dominated by plastics comprising of 59% of whole litter.

The main sources of MP accumulation in sediments are local plastic industry, large ports, large number of pellets and wastes from recreational activities on shore (Hidalgo-ruz et al., 2012).

Effect of MPs on aquatic organisms

When MPs through various means of carriers end up in the aquatic environment they will interact with their surroundings and their biological fate and mobility depends on their size, shape and other different properties. Aquatic organisms are hugely affected by the MPs present in water. The main pathway is through ingestion of MPs by aquatic animals. Since MPs can be found in sediments, thus the detritus feeding animals are likely susceptible (Wright, 2013). Recently, several controlled laboratory experiments have been performed to find out the reason behind the ingestion of MPs by marine biota. The main reason of ingestion of MPs particles in many cases can be taken as an accident because often the MPs is mistaken as food by marine organisms.

The consumption of MPs can cause harm on marine organisms in physical and chemical ways. It may affect the mobility through attachment of polymer in external surfaces. It can also clog the digestive tract and can cause inflammation (Auta, 2017). From the laboratory experiments, it has been observed that the MPs ingestion is found in variety of marine organisms.

For example: Blue Mussel, copepods, amphipods, barnacles and lugworms represents some of the most omnipresent zooplankton which are highly susceptible species in ocean (Stolte, 2014). A recent study from the Baltic Sea examined the presence of 10 µm PS spheres by zooplankton (Jönsson, 2014)

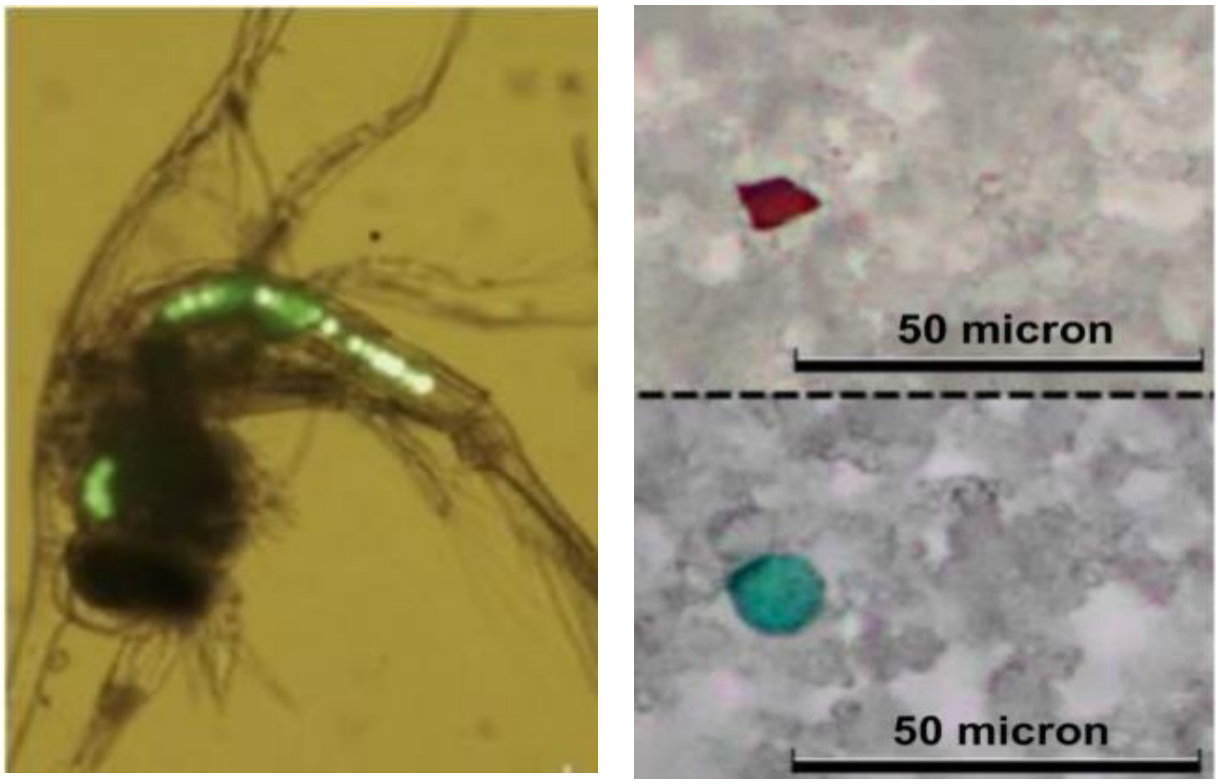


Figure 9: Fluorescence marked MP ingested by copepods (left panel); Ingested MP particles in mussel tissues produced for human consumption (Right panel). (Stolte, 2014)

A wide range of toxic chemicals can be present in MPs, for example some MPs are covered with heavy metals such as aluminum (Al), copper (Cu), silver (Ag), zinc (Zn), and lead (Pb). These toxic chemicals contaminants have an adverse effect of marine biota if they end up inside their body through ingestion. It may lead to cancer and endocrine disruption, birth effects, and immune system problems (Auta, 2017).

Many studies have reported that the same chemicals which form plastics have been found in fish tissues; this implies that the transfer of MPs plastics is very threatening and scary. The main reason which enhances the transfer of toxic chemicals is the predator-prey interaction within the marine biota (Andrady, 2011).

Recent research conducted in North Sea found that the presence of MP ranging from 0.2 mm to 4.8 mm detected in fish species (Cedervall, 2015). Polystyrene microspheres of 10 µm size can be ingested by marine organisms like echinoderms and bivalves and these particles can translocate through epithelial membrane of the gut into tissue (Cedervall, 2015).

The following table below shows which marine organisms are susceptible to MPs ingestion and their leading pathways to the MP ingestion.

Table 3: Organisms susceptible to MPs and the pathway led to ingestion (Wright, 2013)

Species	Encounter pathway
Marine algae e.g. *Scenedesmus* Grazing microzooplankton e.g. the marine ciliate *Strombidium sulcatum*	Adsorbs Nano plastics, especially when positively charged. Size-based selectivity indicates potential to ingest microplastics of appropriate size.
Benthic deposit feeders e.g. the polychaete Arenicola marina and the *holothurian floridana*	The sea bed is a sink for high-density microplastics; size-based, deposit- feeding strategies adopted by A. marina indicate potential to ingest microplastics of appropriate size; H. Floridana selectively ingests plastic particles, showing a preference for fibrous shapes.
Benthic scavengers e.g. the crustacean *Nephrops norvegicus*	Fibrous microplastics have been found to accumulate in marine sediments; gut content analysis has shown plastic microfibers are being ingested in the environment; ingestion is passive via food it scavenges or sediment.
Mesozooplankton e.g. echinoderm larvae, calanoid copepods, chaetognaths	Low density microplastics present on the sea surface with greatest abundances in gyres and industrial harbors; size-based selectivity indicates potential to ingest microplastics of appropriate size.
Benthic suspension feeders e.g. the bivalve *Mytilus edulis*	Susceptible to sinking microplastics; have been found to ingest microplastics despite low qualitative value.

4. REVIEW ON SAMPLE COLLECTING METHODS

This section explains the various methods on sample collection employed by many researchers during their experimental work. MP sample collection and sample handling process is a very delicate process. Therefore, it is very important to identify the sources of contamination. The material like synthetic fibers, gears, clothing and other unwanted can contribute to the contamination of microplastics samples. To prevent these risks of contamination, the sample collecting equipment should be cleaned thoroughly, samples should be covered between use and polymer free clothing should be worn while performing tests.

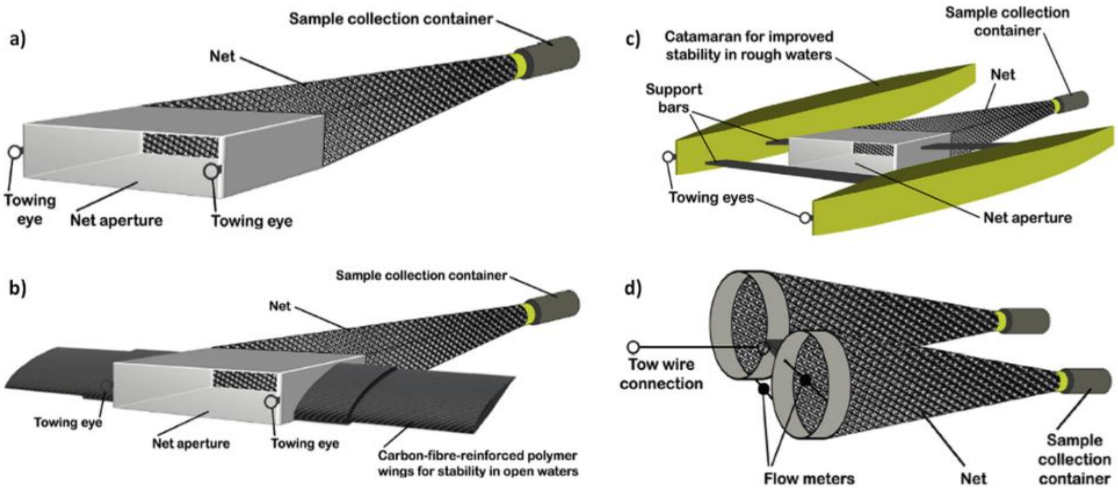
The microplastics samples can be collected in following ways

Table 4: Sampling equipment used to collect MPs (A.B. Silva et al, 2018)

Matrix	Equipment
Water	
Surface:	Collection with trawl having rectangular opening and a net which relates to net or a bag. Collection using bongo nets
Mid water level	
Sediments	Collection with a box corer
Bottom sediments	Collection with iron spoons or non-plastics spades
Surface samples	
Seabed samples	Collection with core or bottom trawl
Biological tissue	Dissection marine animals, egestion.

Water sample

Water sample is collected using different kind of nets. Most widely used nets are neuston net, manta trawl, bongo nets. These nets have sample collection container at their tail end. The size of nets varies from 53 µm to 3 mm, which influence the volume and the types of microplastics samples obtained during sampling process (A.B. Silva et al, 2018). These nets are mostly used for large scale sample collection. Whereas, laboratory sample collection bottle can be used for small scale sample collection procedure. The collected sample should be labelled with the GPS location, collected date, project name and the name of a person who collected the sample.



a) *Figure 10:Equipment used during sample collection process is: a) neuston net, b) is manta trawl, c) is a catamaran which is used for mid water level sampling and d) is a bongo net* (A.B. Silva et al, 2018).

Manta nets and trawls are recommended for the large-scale sample collection for surface water sampling in lakes and seas. Due to the large surface areas of seas and lakes neustonic nets are used for surface water sampling at depths of 0–5 m (Lei Mai et al, 2018).

4.1. Sampling of sediments

This is the method of sampling where the microplastics are collected from coastal beaches or from the lake bottom. The sample collection in beaches is easier and more convenient. There is not one specific way to be followed during sampling process. It solely depends on individual and the place of sampling. Most researches prefer to use tidelines, sampling depth to collect samples from beaches (Lei Mai et al, 2018).

The procedure of sample collection is simple as it is done by using stainless steel tools like a shovel or spoon. Using latex gloves and cotton clothes during the sampling process helps to minimize the contamination in samples.

Sediment sample collection process can be done in following way: First try to find the high tide line, where the debris that washed away with tide get accumulated. Then randomly select the location tide line and place the quadrat with the tide line as shown in the figure below. After selecting the location make sure to remove large pieces of debris from the selected location. At last use shovel or stainless-steel spoon to pick up the sample from the top 3 cm of sand. Then the collected sample should be placed in sample collection bottles and marked with the label having information on date of sample collection, place, GPS location.

Figure 11: High tide line (Sartain, 2018)

Figure 12: Using spoon to get sediment sample (Sartain, 2018)

4.2. Biota sampling

Biota samples can be used to determine the range of microplastics present in the aquatic environment since microplastics can be ingested by aquatic animals like fish, sea turtles, seagulls and many other planktons. The samples are collected from the digestive tracts of animals (Lei Mai et al, 2018). The marine animals are dissected in laboratory to collect the samples from their digestive tract.

Figure 13: Plastics litter ingested by fish (Mytilineou, 2015)

4.3. Sample separation and purification

4.3.1. Flotation

Sample separation and purification can be done in many ways. The most widely used in microplastics sampling is density separation. It is used to isolate the particles which have low-density from higher density particles like sand, mud, and sediments. Microplastics such as PP, PE, have lower density in the comparison to sea water (1.10 g/cm^3) (Lei Mai et al, 2018). In the case of higher densities microplastics like PVC (1.40 g/cm^3) or greater than that, different kinds of density solutions are used to separate microplastics from them. Saturated NaCl is mostly used during this process, because of its nonhazardous characteristics. It is also cheap and quite easily available in market. There are also some disadvantages of using NaCl solution as a density separator. In the case of sample which have higher density (PVC) may not be completely extracted during the process.

Another widely used solution during the flotation process is $ZnCl_2$, which is very helpful to extract almost all microplastics with different densities. The predominant materials like PP and PE have a specific low density than the $ZnCl_2$ and $CaCl_2$ solutions (Stolte, 2014). The drawback of using $ZnCl_2$ is that it is toxic in nature. Various other flotation solution is used as a density separator, but the overall procedure is quite same.

Table 5: Specific densities and solubility of crystalline salts at room temperature (Stolte, 2014)

Salt	Specific density g/cm^3	Solubility g/ml
NaCl	2.17	1.20
$CaCl_2$	2.15	1.47
$ZnCl_2$	2.91	2.14

The process of density separation begins with mixing a salt solution with a sample and shaking it properly to homogenize the slurry (Lei Mai et al, 2018). Then it is allowing to settle down for few hours, which let higher densities particles (sand) to settle down on the base. The solution above the sediment is separated using filtration and in this way extraction of microplastics can be done using flotation method.

- **Centrifugal density separation method**
  Recent research on MPs suggest that many researchers use the intensive density separation methods to extract synthetic polymers from the sand grains. The centrifugal density separation method is one of them. The first step starts with air venting of the sample, using high density solution, preferably aqueous $ZnCl_2$ at 1.4–1.6 g/ml densities (Stolte, 2014). Air venting is applied for several hours before the light weight plastics material are observed on the surface.

4.3.2. Sieving and rinsing microplastics sample

This method of microplastics separation is applicable for many common plastics like PE, PP, and PVC. Microplastic samples with size ranges from 5 mm to 0.3 mm can be analyzed using this method (Masura et al, 2015). The size of sieve may change according to sample. For instance: water samples and beach samples, generally a 5 mm sieve is used but for bed samples a 0.3 mm sieve is used. The sieving process is done by using stainless steel sieves rather than plastics ones to avoid contamination of microplastics samples. The drawback of this method is clogging of sieve aperture and length of sample processing time.

4.3.3. Sample purification

Sample purification is done to obtain reliable data about microplastics present in samples. Much organic matter is attached to the surface of microplastic samples which are obtain from various parts. These samples need to be purified before going for identification processes. To do so, a solution of 30% H_2O_2 has been frequently used (Lei Mai et al, 2018). Many other digestion techniques are also employed to take the organic matter away from the sample. Most of those techniques are reliable to extract the microplastic samples from tissue of zooplankton (Herrera et al., 2018). A recent study suggested that the presence of vegetal material like algae, seagrasses and along with various small residue is abundant in microplastics samples obtained from beaches. These materials can be sorted by naked eyes or using sieving, but the other small residue cannot be sorted with naked eyes. To overcome these residues from microplastics sample, an efficient procedure is discovered. which is less time consuming and likely to extract all the small vegetal residual from microplastics sample (Herrera et al., 2018). The procedure is based on a 5-digestion protocol using chemicals like HCL, NaOH, KOH, and H_2O_2 treatments along with density separation process using 96% ethanol. The digestion process is very useful to eradicate the vegetal contamination present in microplastic samples.

4.4. Review on identification process

After sample preparation, microplastics can be identified from various techniques. Among all the microplastics identification techniques, visual identification is the most common approach to identify microplastics. Which is then followed by confirmation through the chemical composition by using optical and spectroscopic techniques (A.B. Silva et al, 2018).

4.4.1. Visual identification

This is the first step toward the microplastics identification process. In case of large microplastics, it can be sorted out directly. While in the case of small sized microplastics there is a need for further observation under a microscope. Visual identification is not applicable to the particles size <500 µm because the chances of misidentification is very high (Bergmann et al., 2015). Therefore, (Hidalgo-ruz et al., 2012) suggested that the particle size limit is 1 mm for the visual identification process. This method is specially employed for large sized microplastics; therefore, the need of other techniques can be avoided in this case. Which finally can save time and resources throughout the process.

The drawback of this method is the size limitation of the sample, for instance: particles below certain size cannot be differentiated. Another drawback is that it depends on the individual involved during the counting process, therefore if something goes wrong than it directly effects on data collection procedure.

4.4.2. Identification by chemical composition

This method, with the help of molecular composition of plastic polymer can determine their polymer origin (Bergmann et al., 2015). Furthermore, this process gives clear way to identify samples using polymer identification methods like FTIR, Raman analyses, pyrolysis along with GC and MS.

- **Pyrolysis- GC/MS**

  This analytical method to identify microplastics from environmental sample is used in combination with gas chromatography and mass spectrometry.

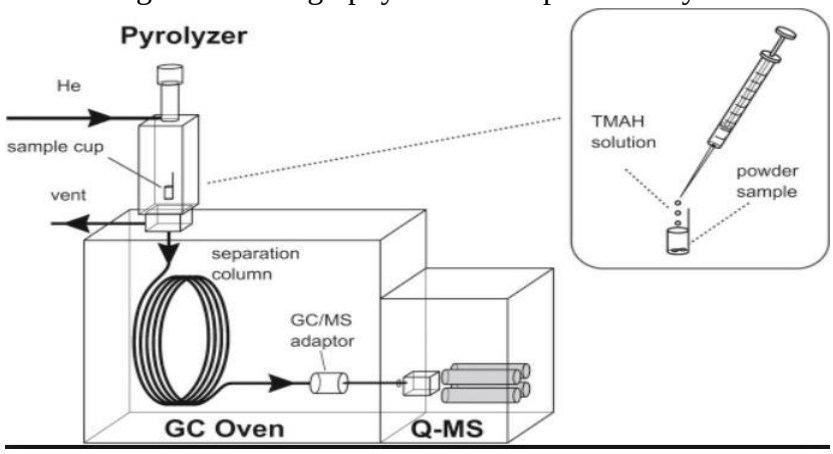


Figure 14: Schematic diagram of pyrolysis process. (Wampler, 2014)

It helps to assess the chemical composition of microplastics particles by thermal degradation (Bergmann et al., 2015). The main advantage of this approach is that it can simultaneously analyze both the polymer type and organic additives contained (Lei Mai et al, 2018). The disadvantage of this technique is that it only allows one particle to pass

through pyrolysis tube at one time and hence more time consuming and lengthy process. It has also size limitation and cannot be used for large size samples.

- **FTIR spectroscopy**

This method is suitable to determine the polymer origin and composition of microplastics particle in samples (Bergmann et al., 2015). In addition, it offers the chance of exact identification of particles accordingly their characteristic IR spectra. The main advantage of using this technique is that it excites the molecular vibrations while interacting with sample. In the case of a plastic sample, it makes easier with this technique to obtain highly specific IR spectra with distinct band patterns. Furthermore, FTIR spectroscopy also provide information on weathering of sampled plastic particles by detection level of oxidation (Bergmann et al., 2015). The other optimized technologies such as attenuated total reflectance (ATR) FT-IR and focal array detector based micro FT-IR imaging are also used to study about microplastics sample (Lei Mai et al, 2018). In case of microplastics samples with irregular shapes, ATR FT-IR stand out as best technique due its ability to obtain spectra more clearly than any other technique. To obtain high quality data and to reduce the measurement time, a resolution of 8 cm^{-1} is suggested (Bergmann et al., 2015). The main drawback of this technique is the size limitation of sample. There is still problem to analyze particles size < 1 µm.

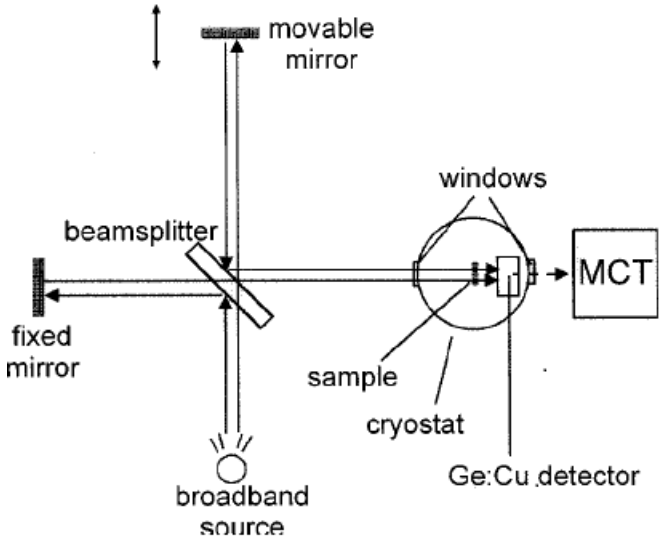


Figure 15: Schematic representation of FTIR (Mccluskey, 2000)

- **Raman spectroscopy**

This is one of the best analytical technique available to determine the chemical composition of unknown plastic fragments with high reliability (Hidalgo-ruz et al., 2012). The main advantage of Raman spectroscopy is that it can examine small size sample (1 µm) and it has also better response toward non-polar functional groups in comparison to other analytical methods (Lei Mai et al, 2018). The process begins with the exposure of sample to monochromatic laser source, which helps to irradiate the sample. The wavelengths available for laser process are within the range of 500 and 800 nm (Bergmann et al., 2015). When the sample come under the interaction of laser light then it starts to vibrate and results in the spectra, which is also called Raman shift. It is also a surface technique to analyze microplastic samples, the large amount of visually sorted MPs samples can be analyzed along with microscopy (Bergmann et al., 2015).

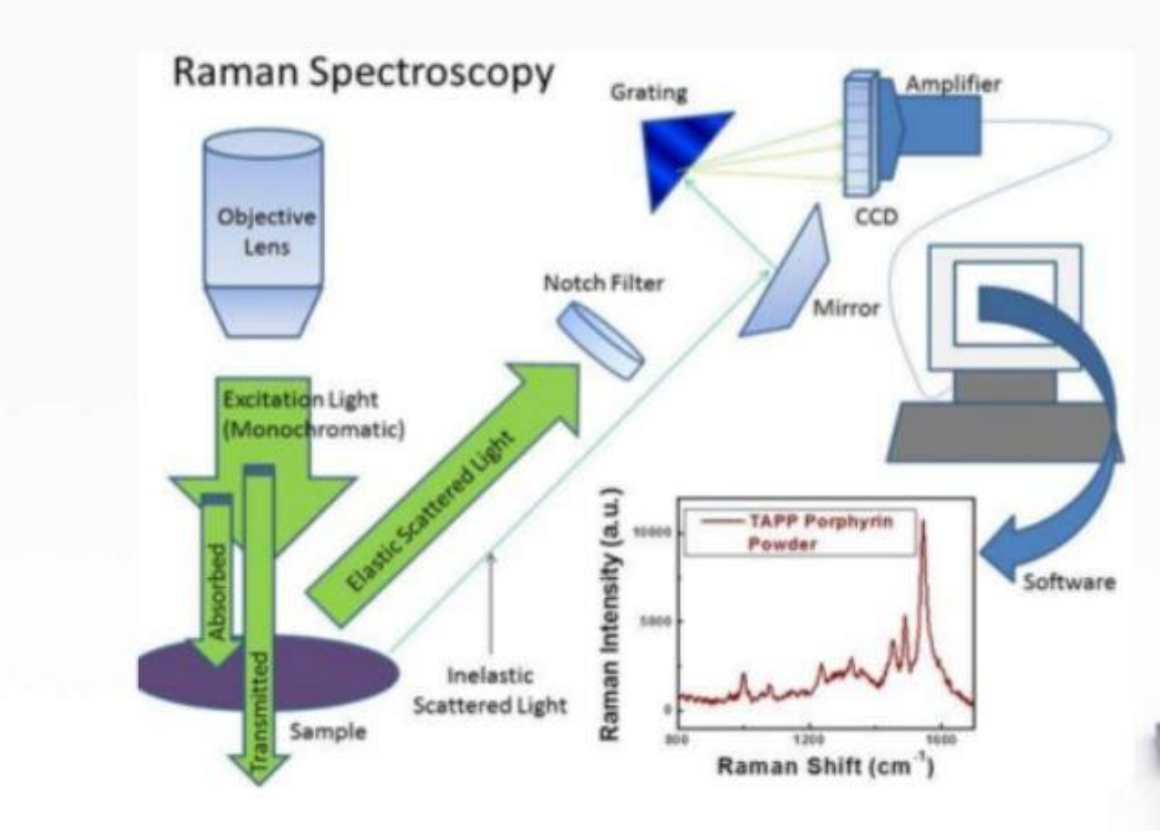


Figure 16: Schematic representation of Raman process (Jefriyanto, 2016)

4.4.3. Identification using laboratory methods

The process of microplastic identification in laboratory can be done in various ways. There are different approaches to identify them in laboratory and it depends on sample source. The methods are different for each sample. The National Oceanic and Atmospheric Administration (NOAA) has categorized the process as follows:

- **Analysis method for water samples**

  The main aim of this method is to analyze the presence of plastic debris as suspended solids in water samples. The process begins with collecting samples from various places using manta net. The collected samples include hard plastics, foams, films, fibers and sheets (Masura et al, 2015). Samples are filtered using 0.3 mm sieves. The obtained material is taken for drying process which helps to determine the exact weight of solid material present in collected sample. In the presence of Fe(II), wet peroxide text is carried out to digest the organic matter. The further treatment is carried out in presence of aqueous NaCl to isolate plastic debris using filtration (Masura et al, 2015). A density separator is used to separate denser undigested material form floating solids. Furthermore, the floating solids are collected using a 0.3 mm filter and then air dried. Then the plastics material is removed and proceed further to determine the exact weight of microplastics present in sample.

- **Analysis method for beach samples**

  This method is used to analyze plastic debris which are collected from beaches. The method starts with sieving the dry beach samples using 5 mm sieve to remove large size plastics. The plastics debris analyzed using this method ranges from 0.3 mm to 5 mm. The process starts with preparing sample and let it to dry at 90^0 C till the sample get dry. Then the process is followed by density separation using aqueous lithium metatungstate (d = 1.62 g/mL) solution or NaCl solution and taken for visual observation under microscope at 40X power (Masura et al, 2015).

- **Analysis method for bed samples**

  This method is used to determine very common plastics like PE, PP, PVC and PS. The sample are collected from bed sediments, which includes films, soft and hard plastics, fibers, sheets. The process starts with disaggregating the dried sediments and then it is sieved using 5 mm and 0.3 mm sieves. Wet peroxide oxidation is done to the MPs collected in 0.3 mm sieves in the presence of Fe (II) catalyst which helps to digest organic matter present in the sample (Masura et al, 2015). Then the process is followed by density separation using NaCl(aq) to isolate the remaining plastic waste through floatation. At last, the floating plastics debris is collected using a 0.3 mm filter and then air dried and taken for examine to determine the MPs concentration.

5. EXPERIMENT AND METHODOLOGY

In this chapter the sampling of beach sediments is introduced, and the methodology applied for density separation, WPO for digestion of organic materials present in MPs samples is described. The author follows the suggestion of (Hidalgo-ruz et al., 2012) in general steps like sieving, filtration, and density separation.

The experimental processes were carried out at Arcada's chemistry laboratory. Two different methods were followed during the experimental process:

1. **Following NOAA guidelines (Wet peroxide oxidation process)**
2. **Using saturated NaCl solution for density separation and microscopic examination.**

Prior to the experimental work, various articles, journals and books related to MPs identification were studied. The guidelines recommended by NOAA marine debris program were followed during the WPO process, filter design process and microscopic examination process.

The reason behind choosing NOAA guidelines to do the experimental work is because it covers all the necessary information regarding MP sampling and experimental processes. It is easy to follow the pattern and format which are listed in NOAA guidelines. Most of the researchers also follows the same NOAA guidelines in their research articles.

5.1. Materials and equipment

The materials used in experimental process were restricted to glass to avoid the contamination of plastics. The following are the materials used during the experimental process and which came direct in contact with the samples.

- Glass beakers: 800 mL and 500 mL
- Weighing balance precise to 0.1 mg
- Drying oven
- Chemicals: NaCl, Iron Fe (II) solution (0.05 M), $FeSO_4.7H_2O$ (278.02 g/mol)
- 30% Hydrogen peroxide: **To digest the organic matter present in sample.**
- Stir bar: **It helps in stirring process and solution get mixed easily.**
- Watch glass
- Laboratory hot plate
- Bottle contacting distilled water
- Sodium chloride
- Microscope
- Metal forceps
- Various size sieves
- Glass slides
- Double – sided Tape
- Funnel

5.2. Sample and sampling location

- Aberdeen beach UK
- Tigaki beach, Greece
- Kardamena beach, Kos island, Greece
- Lara beach, Turkey.

Figure 17: Beach sediment sample collected from different beaches around the world for microplastic examination.

Aberdeen beach UK

It is renowned for receiving the resort seaside award in 2013. It is on the western side of the North Sea and is bound by two rivers: The Don in the north and the Dee in the south. It is short distance away from Aberdeen city center and it is recognized as most popular recreational and sports area to attract many visitors throughout the year. Samples were collected at 0-2 cm depth at high tide zone.

Figure 18: Aberdeen beach (Visitscotland.com)

Tigaki beach Greece

It is one of the popular holiday destination which attract many tourists each year. It is located 11 kilometers away from Kos town and 7 kilometers away from north of Asfendiou village. Samples were collected at 0-2 cm depth at storm line.

Figure 19: Tigaki beach, Greece (www.kosinfo.gr)

Kardamena beach

It is one of the busiest beach in Greece. It is most popular tourist destination; the beach is covered with soft sand and stretches for over 3 km. Samples were collected at 0-2 cm depth at high tide zone.

Figure 20: Kardamena beach, Greece (Tripadvisor.com)

5.3. Filter design process

Four different size of filters were designed for sample filtration process. Steel mesh and PVC pipes were used in the process. The available mesh sizes were 25 µm, 50 µm 130 µm and 300 µm. The reason behind designing 4 filters with various sieve sizes was to trap the MP with different sizes by passing the prepared sediment solution through each filter.

PVC pipe with 75 mm diameter was cut down into pieces and attached with connector with the help of glue. Then Aluminum mesh was attached at one end of pipe using super glue. In this way four filters with different mesh size were designed.

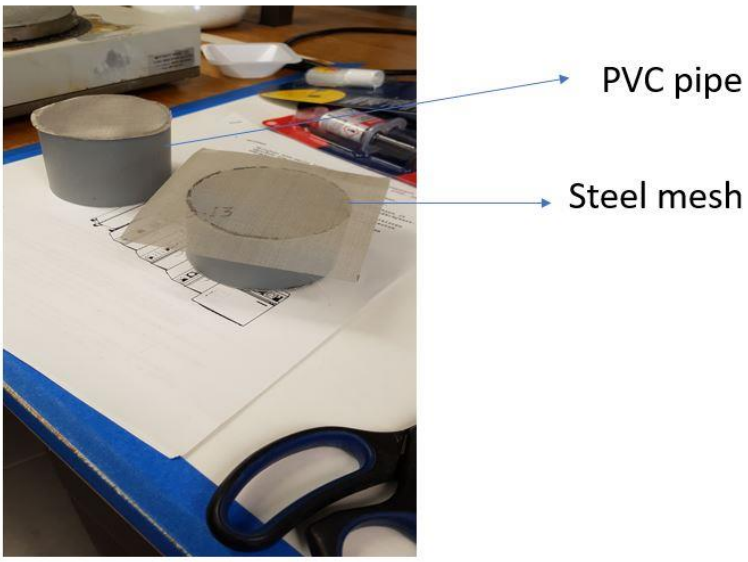


Figure 21: Filter making process

Figure 22:Filters with various mesh size

5.4. Beach sample preparation process

Sample preparation started with weighing 100 g of wet samples by putting them in 800 mL beaker. Prior to the further analysis, the test samples were dried in standard hot- air drying oven at 90^0 C overnight. Then the samples were left to air dry for overnight.

Now, the final weight of sample was calculated by subtracting the final weight of sample from initial weight.

Sample 1: Aberdeen beach UK.
Calculations:
Weight of wet sample taken = 100 g
Final weight of sample after drying = 97 g

5.5. Density separation

Density separation methods include use of NaCl to extract the largest fraction of MPs from the samples.

1.25 M (d = 0.073 g/mL) of NaCl was added into 800 mL of distilled water and volume was adjusted to 1 L by adding distilled water in it.

Weight of NaCl used = 73 g

Metal spatula was used to stir the sand and water vigorously. It helps to float MPs in the surface.

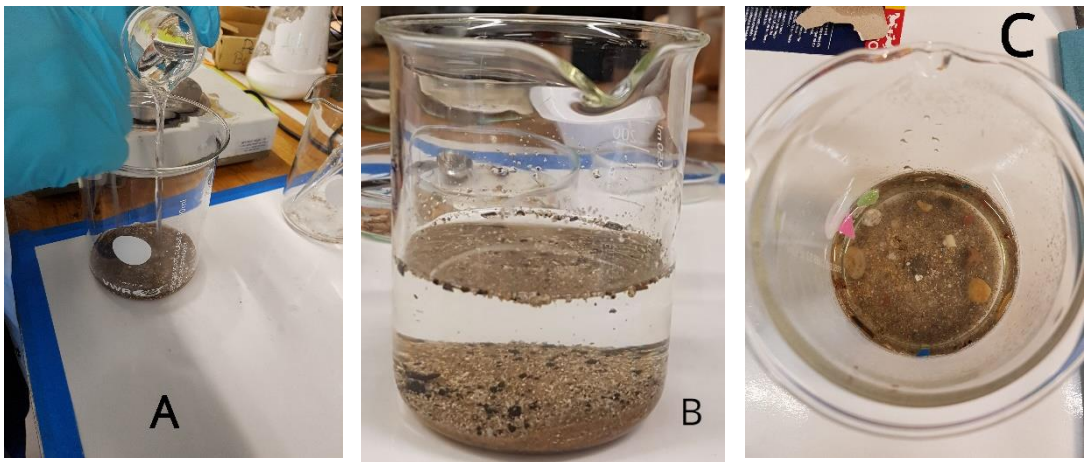


Figure 23: (A) Adding distilled water in sample, (B) After vigorous stirring, (C) MPs after density separation.

After vigorous stirring It was observed that the MP particles were floating on the surface. Using 0.3 mm size sieve all the solids were transferred to another beaker. Particles with size more than 0.5 mm were removed. The collected solids were left for oven drying at 90^0C for 24 h.

- **Mass of collected solids after using 0.3 mm sieve = 0.28 g.**
- **Weight of sample after drying = 0.23 g**

5.6. Wet Peroxide oxidation (WPO)

WPO is a highly reactive. One should follow the laboratory safety and policies before starting the test. The WPO is very important test in the whole MPs analysis process. The main aim to perform this was to reduce the organic matter presents in the sample. To perform the WPO test on sample following procedure was followed:

20 mL of aq. 0.05 M Fe (II) was added to beaker which contained the samples. 0.05 M of Fe (II) was prepared by adding 7.5 g of $FeSO_4.7H_2O$ to 500 mL of distilled water and 3 mL of concentrated sulfuric acid.

20 mL of hydrogen peroxide was added in the beaker and the mixture was kept in room temperature for 5 minutes before following the next step. A stir bar was added in the beaker and the beaker was covered with a watch glass. The beaker was placed on a hotplate; maintaining the temperature of 75 °C. Temperature was kept below 75^0C to avoid overflow of solution.

The whole process was done inside fume hood to minimize the potential chemical risk.

After few minutes of heating on hotplate, still natural organic matters were visible in the beaker, so to digest the organic matter 20 mL of 30% hydrogen peroxide was again added to the beaker.

To increase the density of solution 6 g NaCl was added. Then the mixture was left to heat on hot plate until the added salt dissolved.

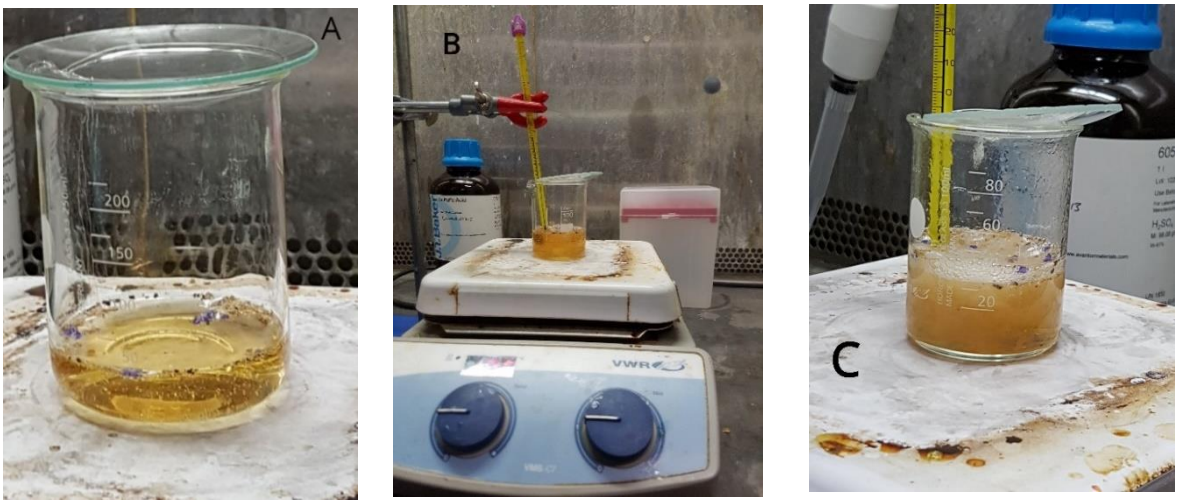


Figure 24:WPO: (A) Beaker is placed on hot plate; (B) After 20% hydrogen peroxide added, (C) Bubbles observed after the temperature reached at 75 °C.

5.7. Using saturated NaCl solution to extract MPs

Saturated NaCl solution was used as a density separator to extract MPs from samples. Excess of NaCl was mixed into distilled water to make saturated NaCl solution. The solubility of NaCl in water is 35 g/100 mL at 20^0C. Therefore, 100 g of NaCl is enough in 200 mL of distilled water.

This was the second method followed in this thesis to analyze the MPs. It is simple and straight method where saturated NaCl solution was mixed with sample and the whole mixture was sieved using filters with various mesh sizes. Then the double-sided tape was used to extract MPs particles from filter surface and the tape was attached on glass slides; which was further used for microscopic examinations.

Four samples were used in this process;

- Lara beach sample, Antalya turkey
- Aberdeen Beach sample, UK.
- Tigaki beach sample, Greece
- Kardamena beach sample, Greece

General procedure

36 gm of NaCl was mixed in 100 ml of distilled water to make saturated NaCl solution. The solution was properly mixed and poured into the beaker which contain 50 gm of beach sample. The whole mixture was swirled with the help of metal spatula. When the sample and saturated NaCl solution get properly mixed then it was passed through the filters with various mesh sizes.

Figure 25: 50 µm sieve is used to filter the solution.

After the solution was passed through the filter, MPs particles get trapped in the mesh. The particles more than 5 mm size should be removed, and the filter was left for dry until couple of hours. Double-sided tape was used to get the MPs particles less than 5 mm size from the mesh

surface when the mesh gets dried. The tape was now attached on the glass slide and taken for microscopic examination.

Figure 26: MPs particles in filter

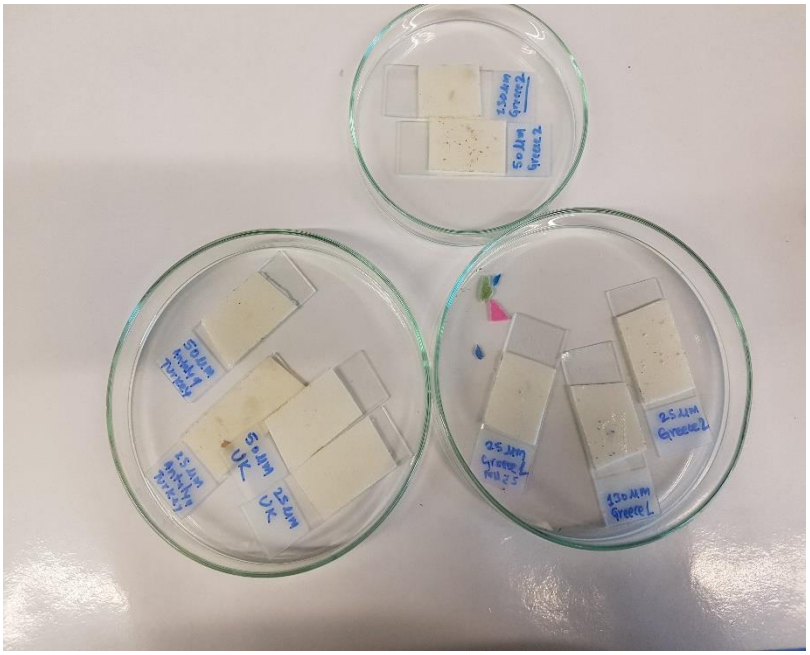


Figure 27: Slides for microscopic examination

Same process was repeated for other 3 remaining samples. The slides were named according to the filter size used and the location from where sample were collected.

6. RESULTS AND ANALYSIS

6.1. Results of wet peroxide oxidation process (WPO)

The author followed the NOAA guideline during first experimental part while analyzing MPs sample. The process of sieving, sample collection, filtration is same in both methods. NOAA guidelines suggests using WPO method to digest the organic materials present in sample.

Before beginning the WPO, the author was expecting to extract MP less than 5 mm size from the sample. NOAA guidelines also suggested that the use of 30 % of hydrogen peroxide will digest all the organic matters present in the sample. But, in this case it didn't work as the organic matters were still visible in the sample. Unfortunately, the expected results were not obtained at the end of the WPO. However, process was repeated more than 5 times using various samples.

As shown in Figure 23, only chitin -based crustacean or insect shells were found in the sample along with some organic material, but no traces of MPs were observed.

Figure 28: Results from WPO showing organic matters and insect shells.

Results from density separation method (using saturated NaCl solution)

After not getting the expected results from WPO. The author followed the direct method of using saturated NaCl solution for density separation suggested by (Hidalgo-ruz et al., 2012). Saturated NaCl solution is used as a mean to separate lightweight particles from the sediments in 50 g of sample.

Beach samples from 4 different beaches around the world were examined in this process. The result obtained from this process are as follows:

Results of Aberdeen beach sample

Following pictures are obtained from microscopic examination of MPs sample:

Sieve used: 25 µm and 50 µm.

Pictures obtained from examination of Aberdeen beach sample showed the number of colored MP and fibers along with some organic materials on it. Fibers detected in the test samples showed the uniform structure and diameter, which suggest being from synthetic origin (Figure B). Nevertheless, fibers were very difficult to distinguish under the microscopic examination and needs advance technology to determine their origin and characteristics.

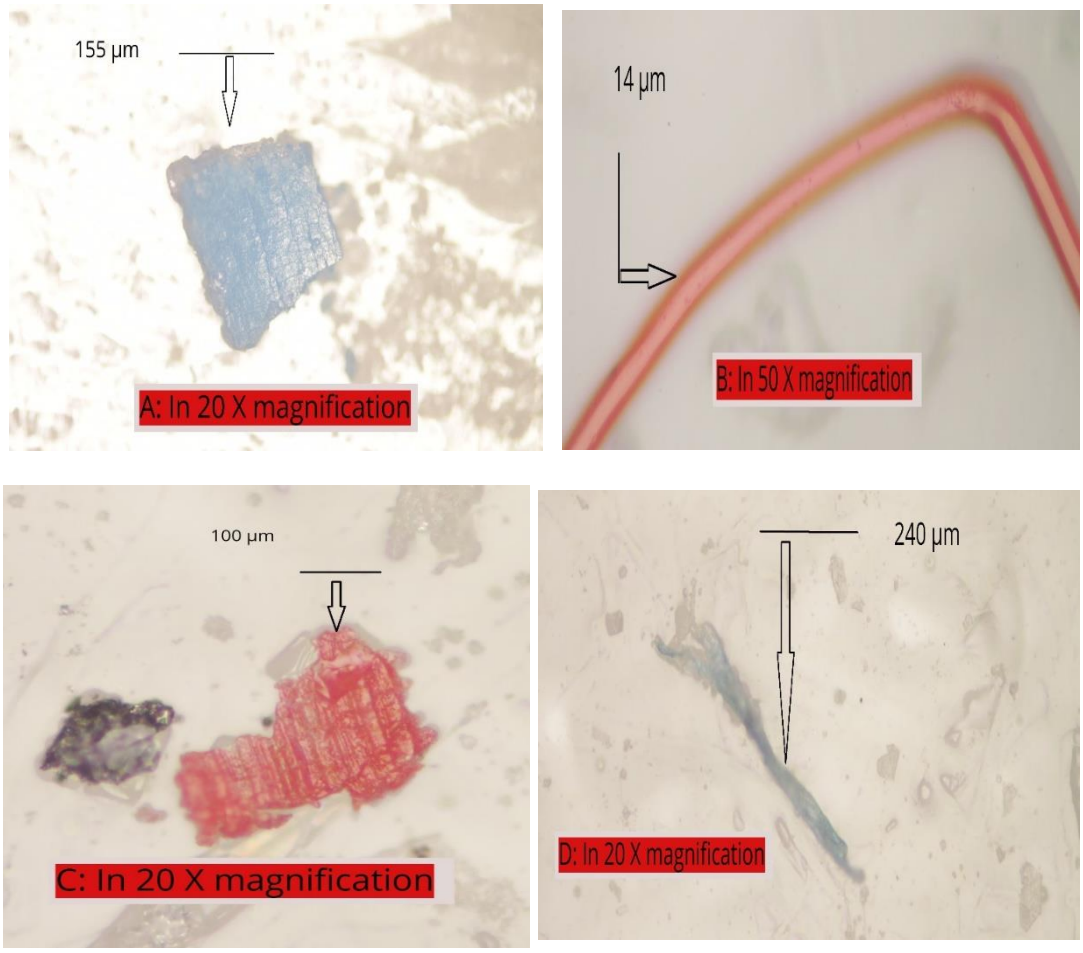


Figure 29: A: Blue colored MP 25 µm size sieve, B: Red colored fiber from 50 µm size sieve C: Red colored MP and organic material from 50 µm size sieve; D: Blue colored MP from 25 µm size sieve.

Results of Kardamena beach sample, Greece

Two different filters with sieve size 130 µm and 25 µm were used to filter the MP sample obtained from Kardamena beach. In figure A: two blue colored fibers can be seen along with some undissolved NaCl crystals. In figure B: MP with some organic matters can be seen. Figure A and B were obtained by using smaller magnification. While figure C and D were obtained using higher magnification and bright field microscopy. Distinct blue colored fibers can be seen in figure C and D. In Kardamena beach samples the presence ratio of colored fibers is high in comparison to the colored particles.

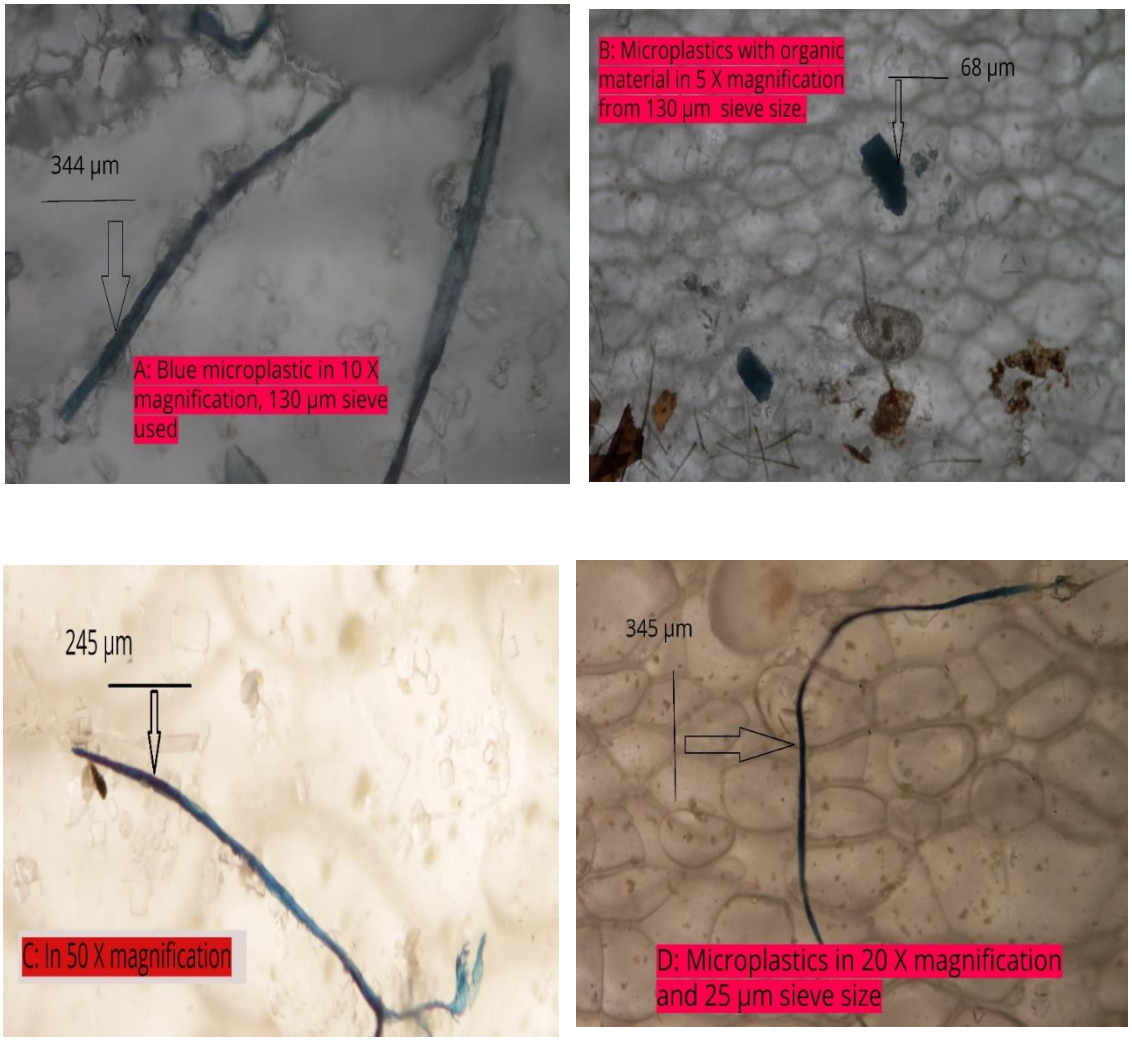


Figure 30: A: Blue colored MP along with some undissolved NaCl crystals, B: Blue colored MP and some organic matters from 130 µm size sieve; C: Blue colored fiber from 25 µm size sieve; D: Blue colored fiber (likely cotton) from 25 µm size sieve.

Results of Tigaki beach sample, Greece

The visual inspection of Tigaki beach samples showed that the presence of distinct colored MP particles and fibers along with organic matters. The detection of colored particles and fibers can be seen in picture below. Like in other sediment samples, Tigaki beach samples also showed the colored fiber count is higher than the particles present in the sample.

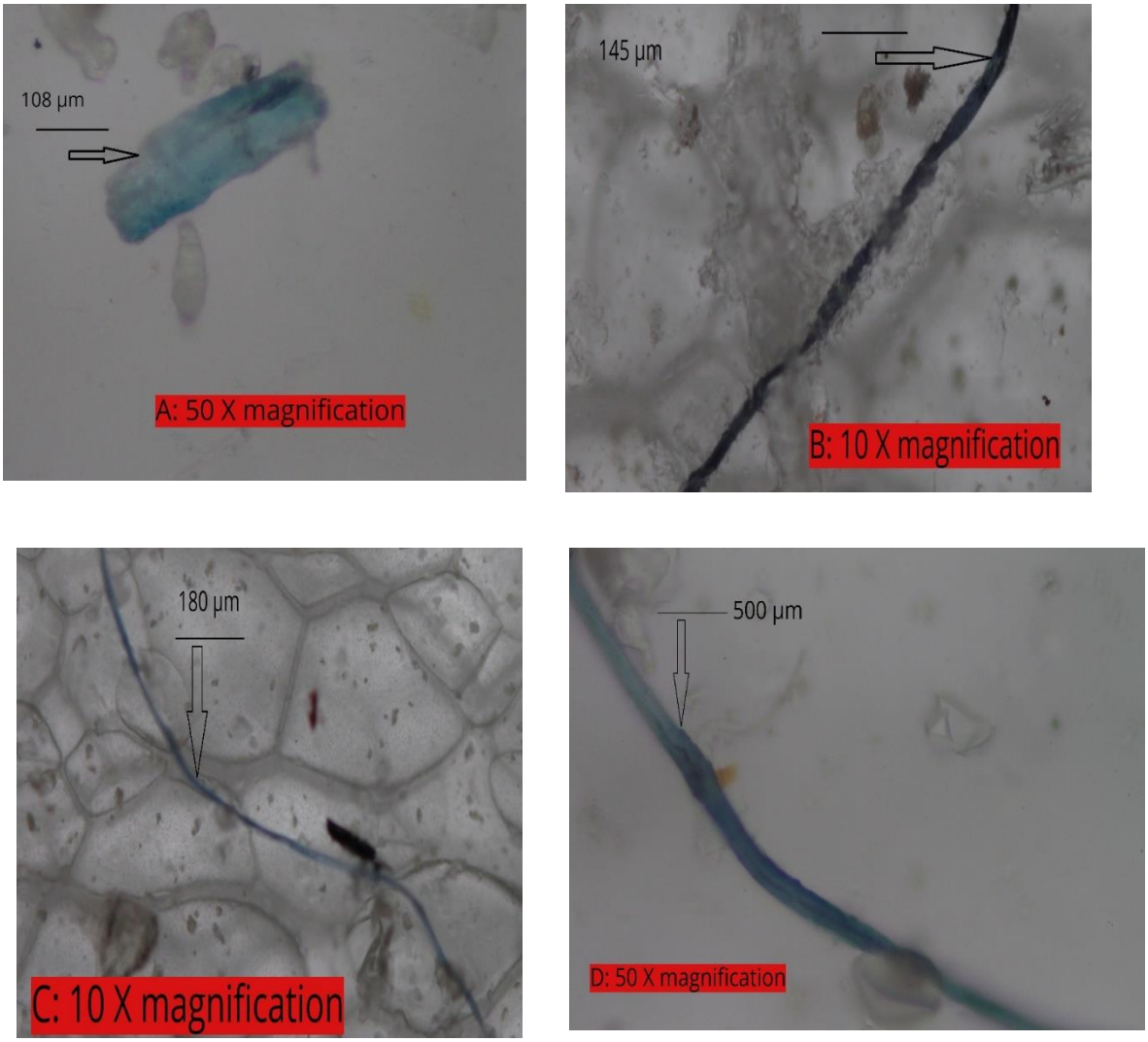


Figure 31: A: Blue colored MP from 130 µm size sieve; B: Blue colored fiber and some undissolved NaCl crystals from 25 µm size sieve, C: Blue colored fiber along with some organic matters from 50 µm size sieve; D: Blue colored fiber and some salts crystals from 25 µm size sieve

Results of Lara beach sample, Turkey

Visual inspection of Lara beach samples showed the presence of uniform size blue colored fibers. The fiber size ranges from 210 µm to 500 µm. The MP particles found in this sample were very few in compare to the colored fibers. In figure B: the fiber looks like from synthetic origin because of its uniformity in structure. Some undissolved salt crystals and sand particles can be seen in figure from lower panel.

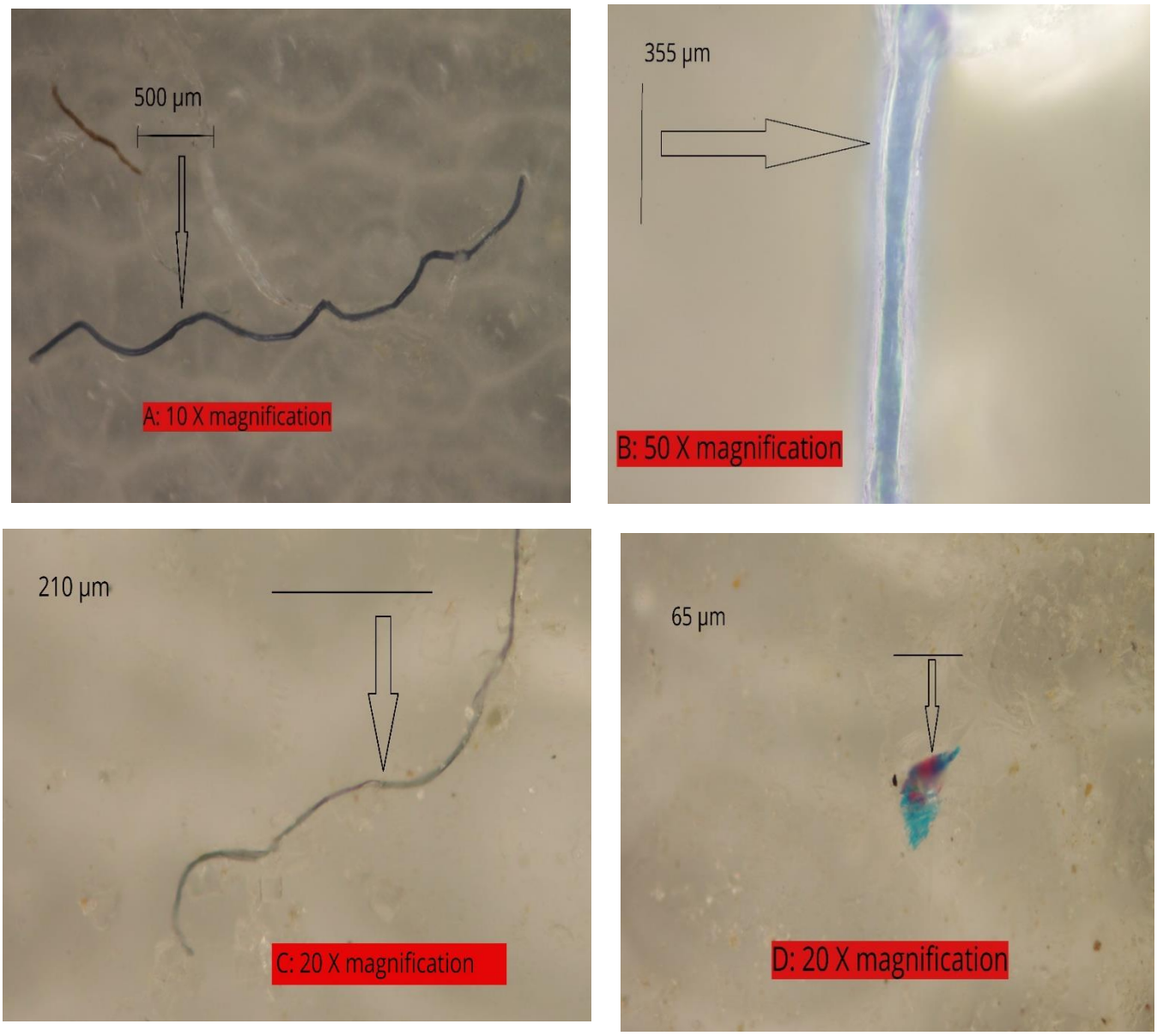


Figure 32: A: Blue colored fiber and organic matter from 50 µm size sieve; B: Blue colored fiber from 50 µm size sieve; C: Blue colored fiber along with some salts crystal from 50 µm size sieve; D: MP from 25 µm size sieve.

6.2. Analysis

Analysis on method used

One of the objectives of this thesis was to develop the method to analyze MPs sample at Arcada chemistry laboratory. This thesis covers two different types of methods to analyze the MPs sample which are collected from various beaches around the world. The first method described in this thesis was the method suggested by NOAA guidelines using H_2O_2 as a digestor. The main reason of following NOAA guidelines while performing the experimental part is because it is the base for all the research articles published till now on experimental methods to analyze MPs in laboratory. Most of the researchers who published the articles has cited the NOAA guidelines as their main source.

It is supposed to digest all the organic material present in the sample. But in contrary, the process did not work as expected and many organic matters remain undigested in the sample. Undigested organic matter can be seen in the figure 34 below. Another unexpected result obtains from this method is that absence of MPs particles less than 0.5 mm size after completion of test. There might be various reason behind the test did not work for this specific sample. One reason could be the use of quantity of sample (100 gm) during the experimental procedure.

Figure 33: Undigested organic matter in beaker after completion of test.

In figure 29, the organic matter is not completely digested even the 30% H_2O_2 was used three times as suggested by the NOAA guidelines. Another method explained in this thesis is the direct method of using saturated NaCl solution for density separation and proceeding the sample for microscopic examination. For microscopic examination of sample, it does not make any

difference whether there is presence of organic matter or not. At the end one can differentiate between microplastics and organic matter present in it.

Analysis of scientific sample collected from 4 different locations

All the samples collected were processed according to the procedure described in section 5.5 and 5.7 using two different methods. While analyzing the pictures obtained from the first procedure one can see that there is no presence of MPs particles (Figure 29) only the undigested organic matter can be seen.

But in microscopic examination of the same samples, one can see that the presence MPs with distinct blue and red color and the presence of blue colored fiber in huge amount in every sample (Figures 32 and 33). During the visual inspection of all the test samples, the presence of colored fibers was higher than the colored particles. Most of the fibers detected showed the uniform structure and diameter. This implies that their origin must be synthetic, but it was very difficult to distinguish through visual inspection.

General observation of the test samples showed the presence of red and blue particles with smooth surface and were frequently detected in all 4 sediment samples.

The colored fibers count was high in every samples than the colored particles except in Aberdeen beach samples. In Aberdeen beach sample the presence of colored MP particles was higher than the fiber (Figure 30). All the test samples contained from minimum of 1-7 colored fibers and 1-5 colored particles. The detection of colored particles and fibers are displayed from figure 30-33 above.

Visual inspection of all the test samples also showed the presence of organic matters like insect's shells, water plants blades (fig 31 B) and sand particles. During the sediment separation process the organic matter clogged the filter surface area. In this experiment, double-sided tape was used to get the microplastic from the filter surface and organic matters get attached to the tape along with the MP particles. This is the reason why an organic material was visible during the visual inspection of samples.

7. CONCLUSION

This thesis has provided an overview on various analytical methods used to analyze MPs sample collected from coastal environments. It also provides information on various methods of MPs sampling techniques, extraction and quantification MPs. Due to use various approach while analyzing MPs samples the data might get different from the existing data. The comparison of MPs contamination worldwide only can be done if the procedures and methodologies got standardized globally. In a nutshell analytical procedure can be explained with the help of following flow chart:

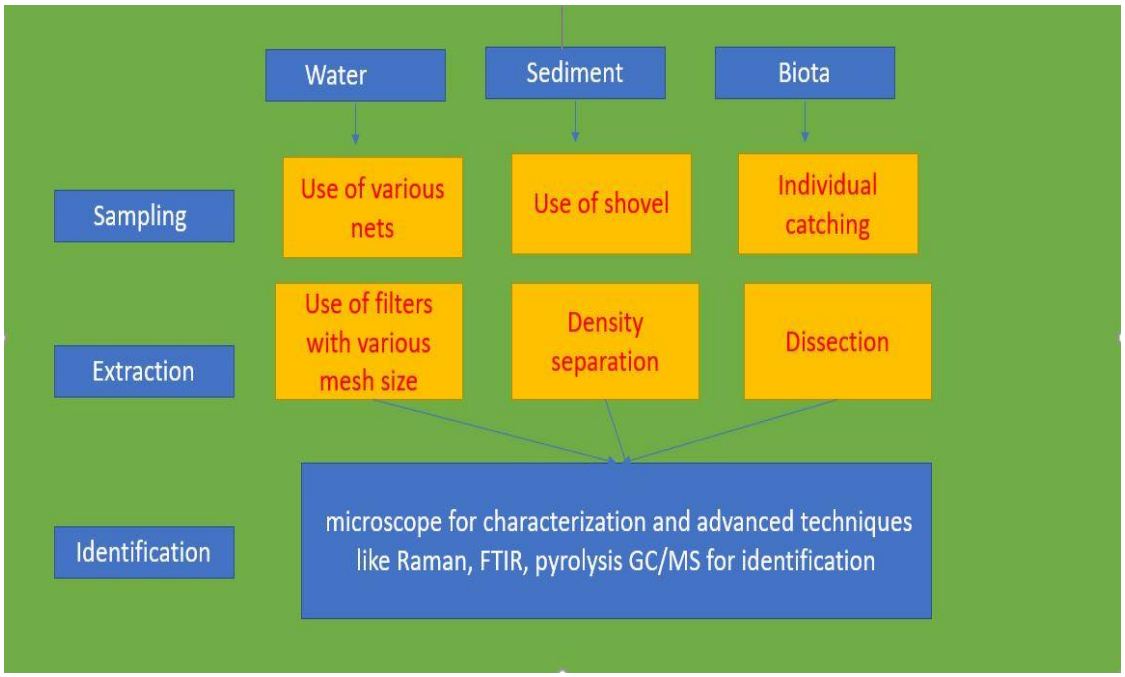


Figure 34: Flow chart explaining analytical procedure of analyzing microplastics sample

In this thesis only, the scientific samples collected from beach sediments were analyzed using microscopic examination method. Due to the lack of advanced analytical methods like Raman spectroscopy, FTIR, and pyrolysis GC/MS, it is difficult to understand the origin of the MPs. Microscopic analysis can only help to characterize the MPs.

As the objective of this thesis was also to do the literature review on experimental methods of analyzing MPs samples, author can give following recommendations based on study:

- For the sediment sampling process selection of the site is highly important. One should have to find the site which can provide the representative of MPs contamination in that specific site.
- Various types of water sample collecting nets described in the literature review section of this thesis can used to collect the water sample in large scale. The small-scale collection can be done with sample collection bottle.
- Density separation is very important during the purification process and is strongly recommended. In this thesis, the author chose to use the NaCl solution in the density

separation process but other denser salts like $ZnCl_2$ and $CaCl_2$ are also highly recommended to get the effective results.

- Wet peroxide oxidation procedure done to digest the organic matter present in the sample did not provide very satisfactory results. Other strong digesting agents can be recommended. But when it comes to microscopic examination, this part can be avoided.

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