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First approach to identification of microplastics sampled from touristic cave

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Abstract

Nowadays, the ecosystems of natural environment are loaded with great number of polutants. One of them which is becoming increasily hazardous is microplastic. Microplastics are plastic substances within the size range of 1 to 5 mm. They can be manufactured as intended inputs to hygiene products or derive from larger plastic compounds. Their main danger rely on size scope which make them practically inconspicuous. Futhermore, microplastic particles are able to absorb high amount of contaminants as heavy metals or organic polutants. In our study, we operated experiments on soil from the show cave in order to detect and quantify the microplastic particles. We backed our procedures with information from several related research papers and tried to provide a correlation between significant factors. Moreover, we identified the most abundant polymer types and their sources to environment. Accordingly, we were able to investigate the pathways of microplastics and how they affect humans and natural surroundings. Similarly, we point out the most modern techniques for their identification and provided both advantages and drawbacks of each method.Firstly, we started by separating processes to determine the most effiecient way of achieving microplastic from soil matrix. Then, we continued with observed their size distribution and quantity along different locations of show cave. In the end, we recorded the most optimized solutions which worked for us and gave our opinions on future prespective of microplastic polution.

Contents

1. Global perspectives and characterizations of microplastic

1.1. Plastic production and growing trend of waste

Plastics have a high quantity of lucrative properties to both producers and utilizers, which establish being economically convenient, relatively long-lasting and feathery in weight. As a consequence, manufacturing rate grew immensely after bringing in of commercially approachable plastics. The first signs of mass production were approximated to 1950 with the total of just 1.7 Mt. To link with today's figures, it is calculated scope between 322 and 380 Mt only in 2015. (AA Horton, 2017). It has been recorded to be expanded 25 times in annual production since 1970 to 2010, and to reach the total amount of 8300 Mt manufactured worldwide including 2015. (AA de Souza Machado, 2018). Nowadays, nearly 40% of manufactured plastic is used in packaging industry, which is intended to be utilized only once before riddance. This and other factors lead to disposal of about 60% of all plastics produced to landfills and natural surroundings as demonstrated in Figure 1 (AA Horton, 2017). According to other research the trend in plastic waste which ceased to natural environment is increasing enormously, and the figure will indicate around 12 billion tons with compared to value of 4.9 billion tons in 2015 (KD Cox, 2020). In 2014, the European plastic requirement was about 47.8 Mt, although barely 25.8 Mt passed into waste stream management. As comparison with the global recycle, it is even worse, and stands at ratio of 32% of total produced plastics. Furthermore, the investigation is carried out related to manufacturing, utilize and waste control of plastic items demonstrates that 4977 Mt out of total 6300 Mt plastic waste have lead their way into the landfills and environmental surroundings (AA de Souza Machado, 2018).

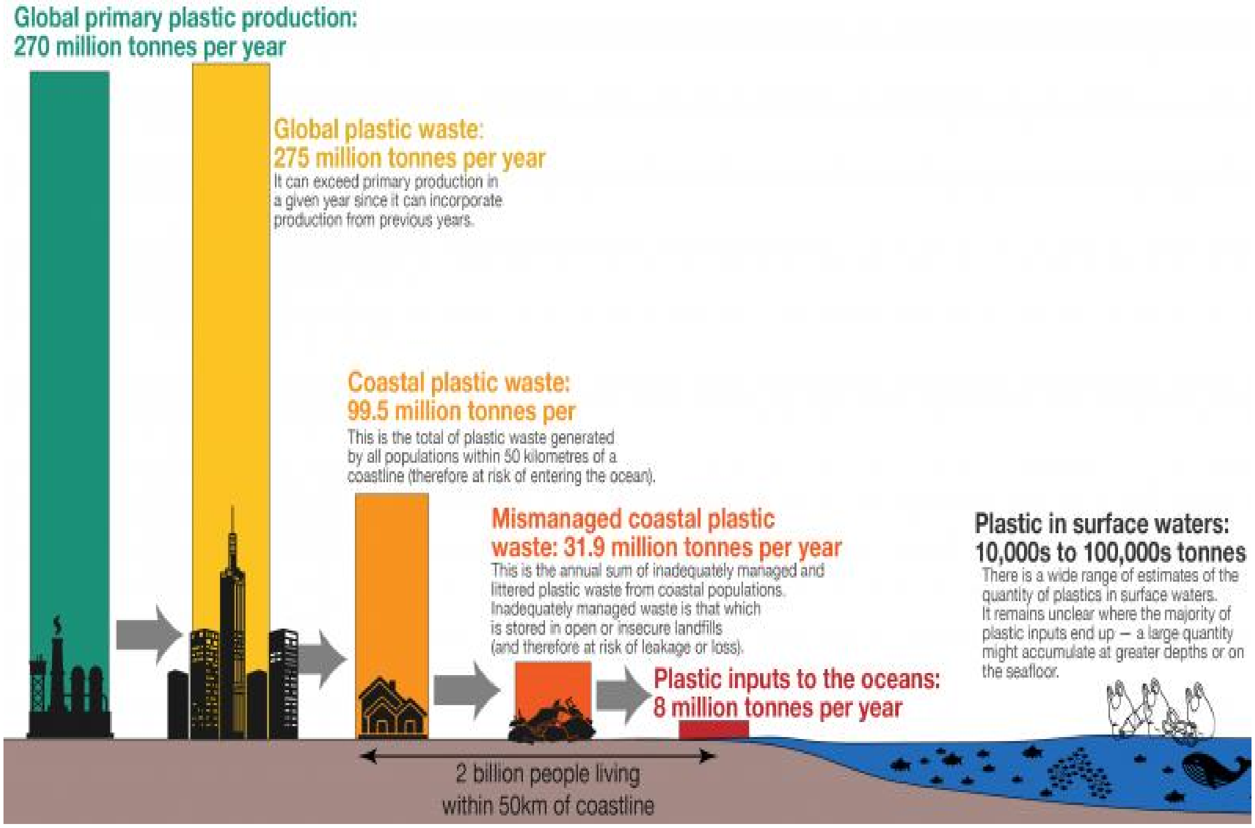


Figure 1. Global view of plastic wastes which are generating across coastlines. If focus on numbers, it can be noted that majority of waste is kept within the terrestrial ecosystems. Photo: (Data, 2015)

1.2. Understanding and classification of microplastics

First of all we should entirely acknowledge the term of “Microplastics” (MPs). Similar to many varieties of other chemical hazards, it is a widely used phrase of chemical compounds which have especial characteristics. Notwithstanding this fact, many scientific papers, journal paragraphs, and broadcastings articles exhibit microplastics such as solely single compound. This type of straightforward approach leads to simplistic surveys and records that can be incompetent to illustrate the origin and the pathway of microplastics, additionally their biological and ecological significances. As instance, surveying the impact of single kind of microplastics with a unique form and dimension will not depict the impacts of microplastics in overall. Furthermore, not distinguishing the multiplicity of components in microplastics sample can disregard the entanglement which is essential to record solid quality analysis and quality control (QA/QC) required in surveying and analytical evaluation methods. For example, several techniques are preferably at regain of certain dimensions, volumes, or class of microplastics (CM Rocman, 2019).

Figure 2. Demonstrates some possible sources for microplastic formation through downsizing of plastic wastes from terrestrial (a), river (b), oceanic (c) and coastal (d) ecosystems. Photo: *(AA Horton, 2017)*

Being a contaminant category, microplastics have a wide range of output kinds which are predominantly categorized as primary or secondary. Primary microplastics are produced in dimension lower than 5 mm. They consist of preproduction pellets which are utilized as inputs in plastic industry, and microbeads which have role of polishing agent in cosmetic and hygiene products (see Figure *3*). On the contrary, secondary microplastics are tiny fragments of plastic which are not manufactured on purpose but are the outcome of the detachment and splitting of greater plastic elements throughout physical, chemical, and biological activities (see Figure *2*).

Figure 3. Shows clearly distinguishable microbeads in tooth paste which is the one type of primary microplastic origin. Photo: *(Cleanpng, 2018)*

Secondary microplastics can be created while usage of plastic items or during direct disposal to environment. Microplastics can be found as well like tailings during production of construction items, outfit clothes, furniture, agricultural tools, and food packets (CM Rocman, 2019). Resin pellets and microbeads which are linked to manufacturing disposals, and the utilization of cosmetics are raw materials for formation of secondary microplastics through influence of degradation activities like UV radiation and physical abrasion. The different origin shows up from synthetic clothing, and one synthetic item of outfit can set free up to 1900 fibres after each washing series (L Van Cauwenberghe, 2015). Environmental plastic rubbish experiences the process of senescence via performance of physical, biological and chemical forces. . Latest studies support the idea that majority of plastics which classified as biodegradable are actually more inclined to decomposition path than natural degradation (see Figure *4*). Consequently, macroplastic litter create and turn into small-scale plastics which are also addressed to microplastics (AA de Souza Machado, 2018).

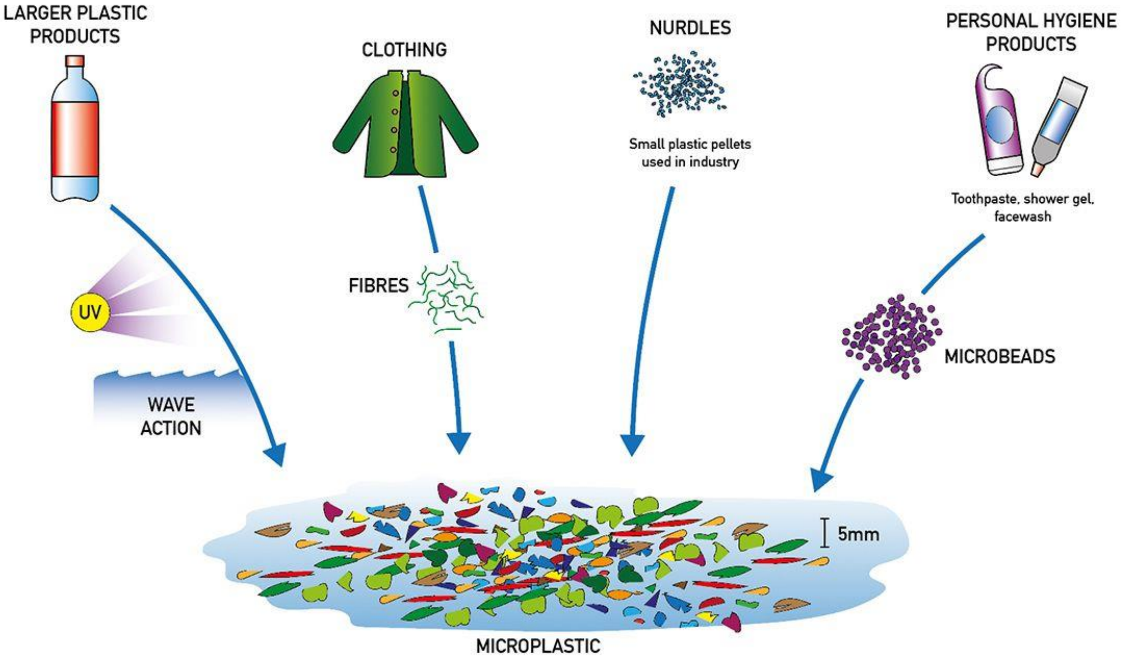


Figure 4. Illustrates the most popular pathways of microplastic formation from the consumer-used plastic product. Photo: *(Encounteredu.com, 2020)*

Microplastics are synthesized from a broad scope of polymer kinds (see Figure *5*). Accordingly, plastic polymers are composed of iterative monomers, which structure the fundamental of the polymer. This fundamental pattern is the essential distinction between polymer varieties, enlightening the chemical and physical properties of a plastic. The most widely manufactured and spent polymer kinds are polypropylene, low-density polyethylene (LDPE), high-density polyethylene (HDPE), polyvinyl chloride (PVC), polyurethane, polyethylene terephthalate (PET), and polystyrene. This multiformity of polymers is vital to accomplish the numerous employment of plastics. As instance, LDPE is too brittle to be applied in water bottles, thereby it is switched to side of PET. PET is also used to manufacture fibers for clothing industry, and LDPE for food packets and shopping bags. Plastics split up to 2 main categories: thermoplastics and thermosets. Thermoplastics are tend to melt when heated up and solidify when refrigerated. The most noted ones in this group are PET, polycarbonate, LDPE, HDPE, elastomers, PVC, polyether ether ketone and etc. Thermoset plastics experience chemical alterations when heated. Polyurethane, phenolic resins, vinyl esters, and urea-formaldehyde are widely used polymers of this type. Consequently, microplastics are not composed of a single matter; yet, they are generated from a multipurpose classes of various chemical materials (CM Rocman, 2019).

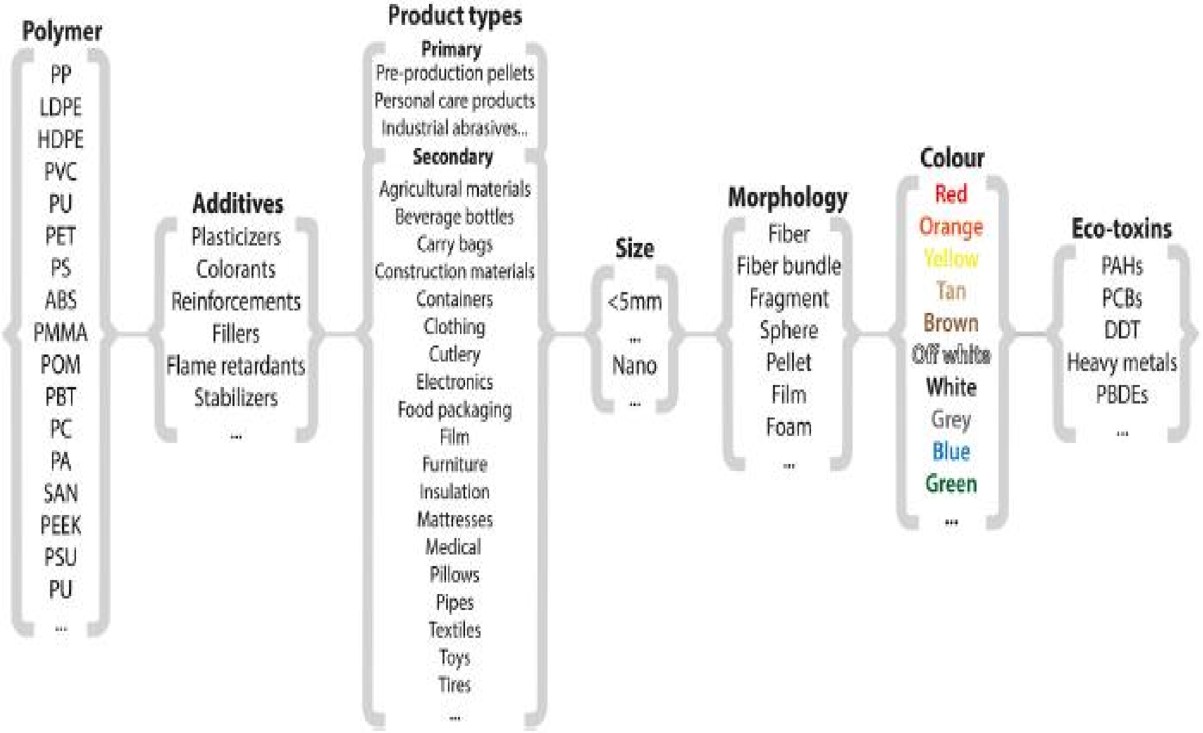
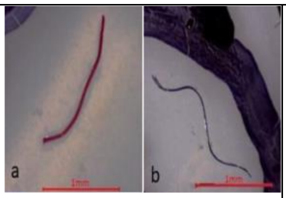


Figure 5. Provides detailed mixture of plastic compositions. Furthermore, their additives for additional physical properties and which kind of contaminants they can absorb. Photo: *(CM Rocman, 2019)*

Microplastics encircle a wide variety of forms and colors. The form of microplastic usually helps to determine allocate it to a familiar type, which indicate to the source. Mainly, there are 4 to 7 diverse types marked by the form or morphology, which encompass fiber, fiber bundle, fragment, sphere (bead), pellet, film, and foam. Table 1 provides a wider description on dimension types of microplastics. To process the origin identification, we are aware that particular forms are mostly separated from dissimilar items, and that leads to signs of where microplastics in environment can arise. As instance, fibers and fiber bundles are prone to detach from clothing, pellets are primarily linked to industrial feedstock, spheres can be microbeads of hygiene products or commercial scrubbers, and foam generally related to polysrtyene foam commodities like insulation or food packets (CM Rocman, 2019). Spherical resin pellets were predominant class in vicinity of manufacturing fields and dense mass of fragments and foams were found near fishing ports. Microbeads from personal care items had highest concentration in domestic sewage and waste water treatment plants. The asymmetrical form and sharp edges of microplastics which detected in landfill leachate represented the process of decomposition of larger plastic products. Microplastics were indicated in both operating and shut landfill leachates. A research was carried out in order to study the dispersion of microplastic shapes in landfills. 5 samples were taken from different depth and spatial positions of landfill. Flakes and fragments were detected in all samples, pellets were detected in samples number 2 and 4. Foams were recorded in samples number 2 and 5. The total ratio of microplastics in form of lines, flakes, fragments, pellets and foams were 14.81%, 22.87%, 58.62%, 0.64% and 3.06% respectively. The highest portion of microplastic (99.36%) was consist of line, flake, foam and fragment. (P He, 2019).

	Fibers are flexible, with the uniform thickness across the board and clean-cut, pointy, or frayed ends. They are usually tensile and resistant to breaking. The durability of materials varies depending on their level of deterioration. Fibers come in a variety of hues, which may vary from particle to particle owing to bleaching.

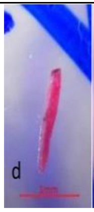
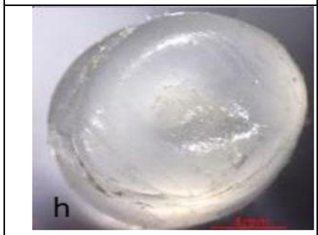
	Fiber bundles are made up of 20 or more individual threads that are tightly coiled into a mass that is impossible to disentangle. Fiber bundles should only be used when quantifying individual fibers is difficult or when untangling the bulk might result in individual fiber breaking. The appearance of the fibers in bundles should be constant.
  	The structure of fragments is hard, and their form is occasionally uneven. They come in all shapes and sizes, including round or angular. They aren't usually uniformly thick and might seem twisted or curled. Plastic production shavings, droplets, and seams fall under this group. Fragments can be any color or color combination.
	Spheres have a smooth surface and are spherical in form. As a result of breaking during production, use, or weathering, spheres may also appear as hemispheres. They are normally between 100 μm and 2 mm in size.
	Pellets (sometimes known as "nurdles") are similar to spheres but are bigger, ranging between 3 and 5 mm in diameter. The form of pellets is frequently spherical or cylindrical. Colors for spheres and pellets are unlimited.
	Flat, thin, and flexible are the characteristics of films. Films can be folded or creased, but they do not easily break apart.Films come in a variety of colors and are often partly or entirely transparent.
	Foams are cloud-like, soft, and compressible. They're generally white and/or opaque, however they come in a variety of colors.

Table 1. Fraying (a) or clean-cut (b) endings can be found on fibers (b). There is no way to disentangle the fiber bundles (c). It is important to note that the fragments are both stiff (d, e) and uneven (f). Spheres (g) have a smooth surface and are circular. It is common for pellets (h) to be round or cylindrical in shape. It's easy to work with thin, flat films (i). It is possible to compress foams (j). Photo: *(CM Rocman, 2019)*

The density and form of microplastic fractions will have essential influences on their carriage and preservation in soil and sediments. Despite the fact that greater number of plastics have low densities and float on water, there are still many others which have higher density value than water, and will finally drown into water. PVC and PET are among the most wide used dense plastics. Unfortunately, the density of plastic is variable related to their surroundings. Thus, the biological activity on plastic fractions can lead to rise of microalgae (biofouling) which will result in increase of total density and force plastic to sink bottom and cover the basin sediments. Having diverse physique also effect its behavior in natural environment. As instance, round shaped particles have a faster and simpler settling mechanism confronted with ones which have asymmetrical form. In addition, round fractions have a greater chance to arrive back to bank from river flows, rather than sinking down like the asymmetrical microplastics (AA Horton, 2017).

In the view of fact that plastic is usually represented as a rigid substance because of its bulky molecular structure, each fragment of plastic holds a multiplex chemical mixture of monomers, oligomers, and additives (see Figure 6). Sometimes, chemical additives can reach the point of having the highest portion in plastic composition. The main types of additives are plasticizers, colorants, reinforcement, flame retardants, and stabilizers. Phthalates, which have a role of transforming hardened and glassy plastics to tender material, are associated to plasticizers. Colorants are added to set the color of final product. Reinforcements magnify the mechanical characteristics of plastic by reinforcing it. Flame retardants are necessary for special cases, when plastic is manufactured to be used in construction items and electronics. And finally, stabilizers grow characteristic of the durability and strength. Samples which were retrieved from natural environment, had microplastics which contained sorbed contaminants. Microplastics store organics matters and heavy metals from the surrounding habitats. Contaminants like polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and DDT are among the most regularly sorbed ones. The key factors, which determine the amount of chemicals or metals that plastic can accumulate, are diffusivity, crystallinity, hydrophobicity, and surface area of polymers (CM Rocman, 2019).

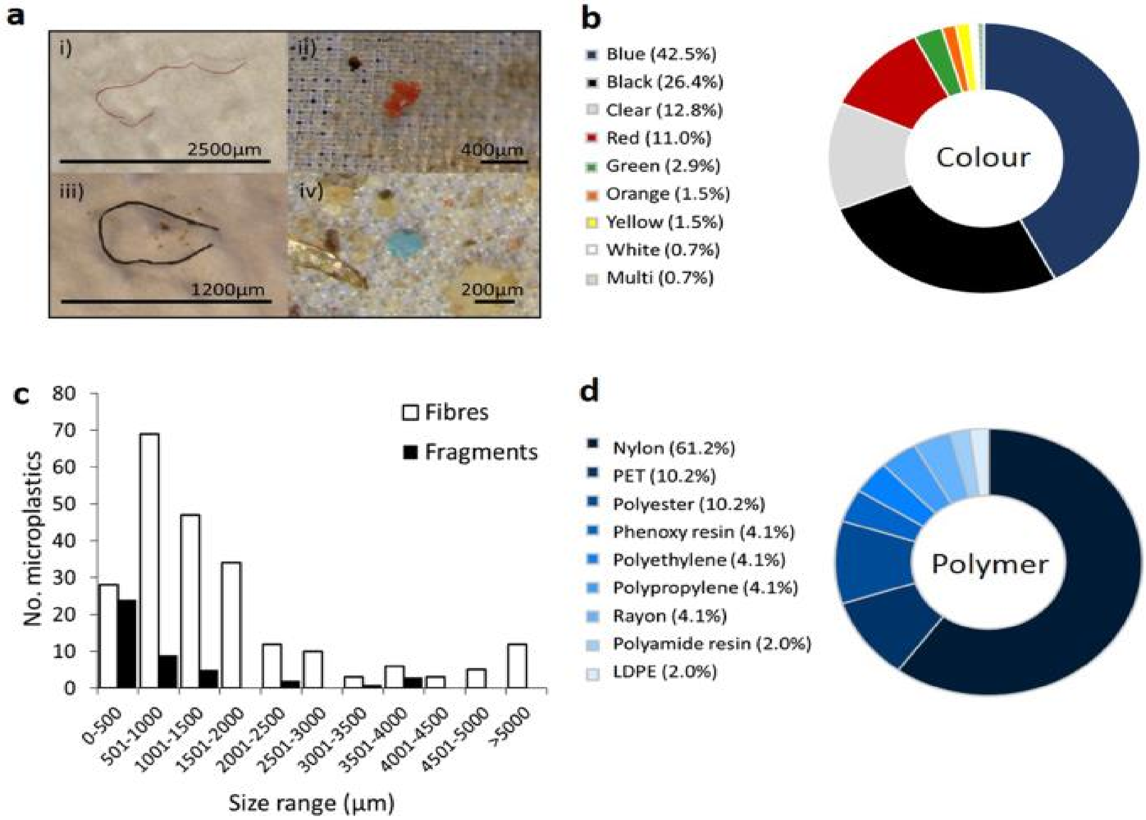


Figure 6. Shows the dimension of microplastic particles (a), the color classification among the plastic production industry (b), number of detected particles (c) and their chemical composition (d). Photo: *(Nelms, 2019)*

1.3. Microplastic transportation and cycle in natural environment

Nowadays, microplastics are so widespread through all environmental systems that we encounter to change the way we interpret them. A modern concept to redefine the microplastic pollution is to introduce the view of a “Plastic Cycle” (see Figure 7). There are countless ways that microplastics are able to circulate among natural surroundings. Having said that, yet the main pathway trend of microplastic transportation is from soil to aquatic systems, it is not certain if microplastics arrive to seas and oceans that they will retain there, since there are many reports that illustrate the retrieval of microplastics back to land via strong tides and storm occasions. The records of high microplastic concentration in coastal areas accompanied by storm events is not only argument for this idea, but the reports that show the abundance of microplastic even in islands where no human activity is present (AA Horton, 2017). The prevention measures that are taken into account are strongly connected to microplastics source and the transportation ways to surroundings. The main problems that stand for environmental pollution are unsuitable waste management and irresponsible human activity. Microbeads that are processed as input for cleansing hygiene products and cosmetics, unmeant loss of plastic pellets during production and conveying, as well fiber loss from textile washing are among most common dumped items to natural habitat (P He, 2019).

There is a globally acceptable belief that the oceanic water and basins stand for the substantial amount of microplastics, as the land and rivers play a necessary role in conducting origins and

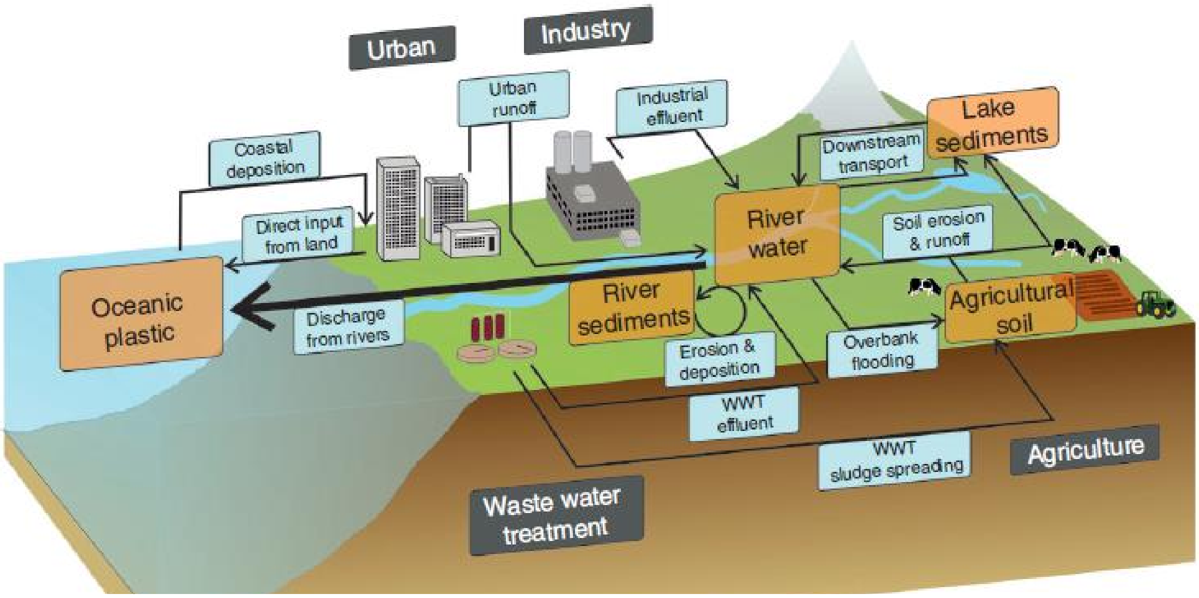


Figure 7. Illustrates majority of possible microplastic sources and their pathways among waterway streams which doesn’t accumulate at certain point. Photo: *(AA Horton, 2017)*

paths for microplastics to oceans and seas. It is also important to consider the air currents as key transport agent, which function both within and between the aquatic and terrestrial systems. Many studies demonstrated the rivers as one of the basic sources for microplastic pollution in oceans which are mainly produced on land. Nevertheless, it is starting to be acknowledged that freshwaters and soils act not only as transporter medium, but depositing higher portion of microplastic waste within their patterns (AA Horton, 2017). Newly, microplastics were found even at the great depth of 5000m in oceanic basins and are calculated nearly 2000 pieces per each m^2. In addition, sediment core analysis was carried out to show the multiply of microplastics by 3 times in Belgian beaches (L Van Cauwenberghe, 2015).Wherever, microplastic fractions are subjected to marine motions, they will act as the other transport particles among river like sand and silt. Clearly, while the velocity of river is high, all particles will flow and no precipitation will occur. However, even buoyant microplastics can sink and deposit in basin bottom sediments of river channels. As reason for that is the slow-moving part of river, where flow energy drops dramatically and thus particles remain in suspension or become submerged (AA Horton, 2017).

A greater number of plastic pollution that arrives to oceans has been manufactured, utilized, and usually released on overland, where it goes through many natural manners which identify its final destination and impact on environment. Consequently, it is estimated that microplastics primarily function with the biota inside terrestrial ecosystem, likely to vary chemical and physical characteristics and leading to possible environment hazards.On contrary, majority of researches are mainly focused on oceanic microplastic pollution rather than in terrestrial systems. Whereas, hazards related to microplastic concentration on land are somewhere between 4 and 23 times higher than aquatic medium. As a matter of fact, the part of soil which correspond to agricultural activities preserve more microplastics than in ocean sediments. The main aspect that lie behind importance of microplastic pollution in oceans is that it is ingestible nearly for all living organisms of aquatic systems. On other hand, terrestrial system is involved more in transportation of microplastics, and possibility of physical damages to surrounding habitat. The sophisticated morphology of microplastics makes a huge influence on soil chemical and physical characteristics, varying its texture and structure which essential for water and air circulation in terrestrial systems and plant-soil bond (AA de Souza Machado, 2018).

Microplastics do not carry out only its self-contained hazards, but also stands for the transport of other adsorbed pollutants which consist of toxic metals, polychlorinated biphenyls (PCBs), aromatic hydrocarbons (PAHs) and polyfluoroalkyl (PFAS). Microplastics can function as carrier of micropollutants to the natural environment and induce many destructions to both human and natural livings. It was calculated that presence of contaminants in microplastics is 6-70 times higher than on average of natural environment (H Golwala, 2021). According to another study, contaminants which are hydrophobic are more prone to adsorption in microplastics. As instance, the accumulation of PCBs on waste polypropylene in Japan was approximately 106 times higher than nearby water networks. Lately, it was explained that the modification of pellets dimensions caused by natural erosion is leading for growth in pollutant affinity. To support this idea, experiments were done both on fresh made pellets and destructed ones, and the increased area of damaged pellets resulted with high polarity and thus with greater efficiency of sorption (L Van Cauwenberghe, 2015).

Microplastics are tend to be more spread in urban areas (see Figure 8). The research was carried on 18 sediment samples from 6 different countries. It was found to have a positive trend between the amount of microplastics and the human settlements. As expected, microplastics were recorded to be in high quantity along the North Sea, Mediterranean Sea and in coastal area of Brazil. On beaches with great number of visitors, microplastics are estimated to be up to 3.3% of the soil by weight, on contrast to average value of 0.12% plastic on all monitored beaches (L Van Cauwenberghe, 2015). Another study was done on surface of manufacturing fields in Sydney, and it is calculated to bear up to 7% of microplastics by weight. The discharge of nonvolatile contaminant like organochlorine which was recorded to be 300 times more than inorganic chloride in area of interest, cause to wide range of modifications in geological parameters. Even some investigators conclude that about 60% of industrial areas are consist of microplastics. Eventually, as these particles emerge down to soil, they get rid of light and oxygen which can result in 100 years of presence (AA de Souza Machado, 2018). Environmental regulations related release of microplastics from industrial factories are not efficient as planned. As example, an Austrian manufacturing company with the production rate of 100 L/s dispose to Danube river approximate amount of 94.5 tons of microplastic each year. Therefore, just one manufacturing plant is able to provide up to 6% of total microplastics which Danube river deposit to Black Sea, and is evaluated to be nearly 4.2 tons per day (AA de Souza Machado, 2018).

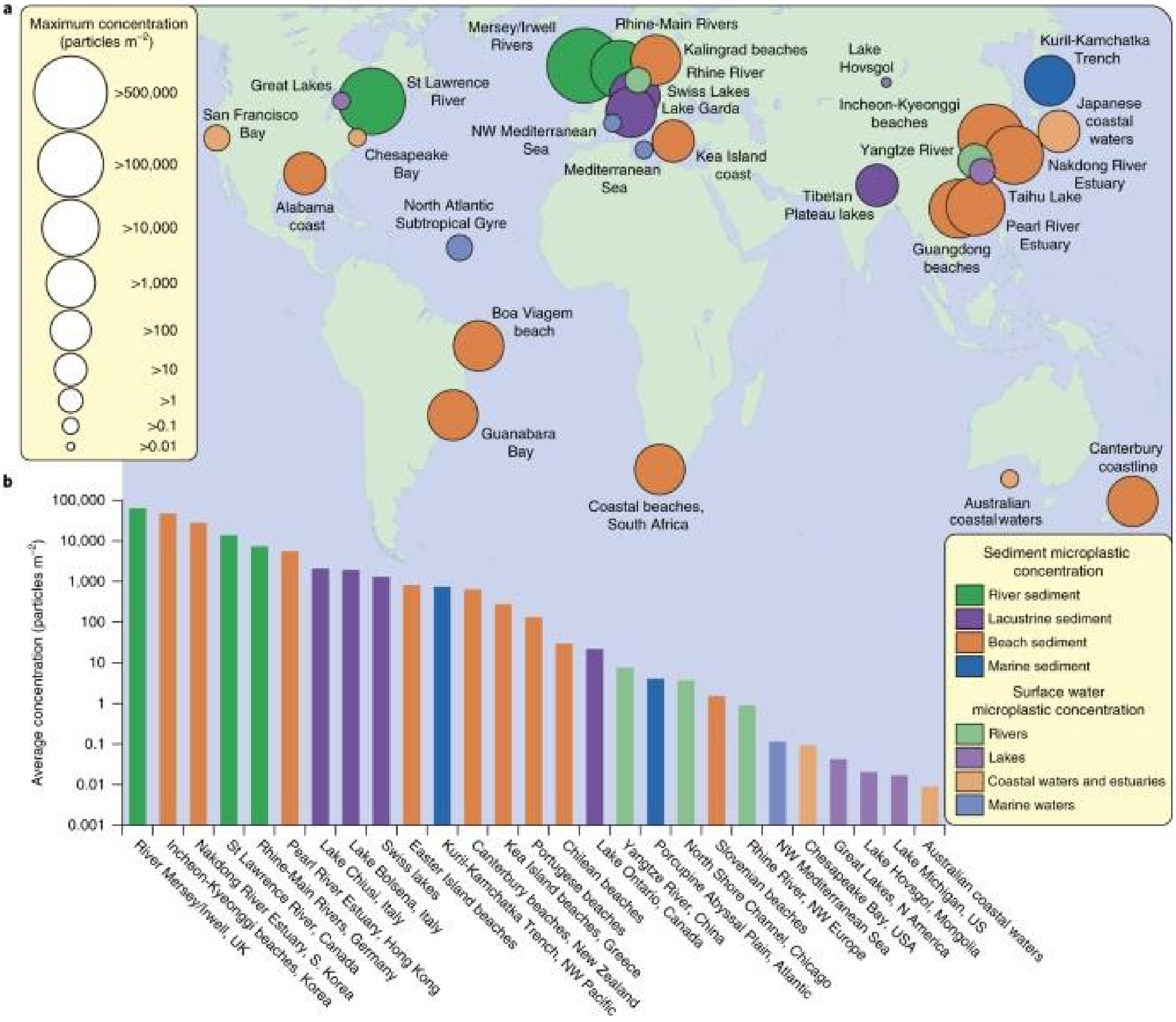


Figure 8. This figures provide average concentration of microplastic particles in determined points with higher abundance and disposal of plastic consumer-used products. Photo: *(Hurley R, 2018)*

1.4. Waste water treatment and landfilling systems

Sewage treatment plants is another global source of microplastics. Fibers and microbeads are most dominant types that come from textile and personal hygiene products. Around 80% to 90% of arriving microplastics are preserved in the sludge. Despite of treat processing, a high amount of microplastics is still remained in the sludge depending on operation type (AA de Souza Machado, 2018). Sludge microplastics accumulation is directly linked to location, because of diverse population numbers and waste management processes (see Figure 9). Obviously, more people means more flow sewage water and personal hygiene products. As example, 271,700 particles/kg of sludge was recorded in China, where only 301.4 particles/kg was found in Finland (H Golwala, 2021).

Waste water treatment plants is also considered as a significant source for microplastics pollution due to its complex composition of very poorly degradable polymers as cosmetics, facial scrubs and clothing. Outcome of any operation units differs noticeably with application of physicochemical and biological treatments. Introductory phase which include screening and skimming is referred to separation of 35-58% of microplastics in settled sediments. Whereas, primary and secondary operations are able to increase that value up to 97.8%. In addition, when we can reach even efficiency of higher than 99% with implementation of tertiary treatments (H Golwala, 2021). On contrary, it should be mentioned that the removed microplastics will not be degraded by mean of any chemical, biological or physical methods. It simply shift the allocation of microplastics into raw leachate. Denser microplastics will sink down to equalization bottom, and eventually enter the treatment where they will pass in sludge or again to sewage waters (P He, 2019). The sewage sludge can be applied completely to soil by the mean of fertilizers for agricultural purposes. A research was made on the agricultural land after being received sewage sludge 15 lastly years ago, it was found microplastic fibers are still present within soil texture. It also demonstrated the possibility of microplastic abundance at the depths higher than 25 cm, where they have been accumulated over a long period of time. It was calculated in Europe that a huge amount of around 125-800 tons sewage sludge is applied every year to agricultural lands. In addition of 473,000 to 910,000 tones microplastic wastes that generated annually both in soil and aquatic systems (AA Horton, 2017). Actually, even plastic additives are among most detected contaminants in environmental surroundings that was caused by human behavior. Compounds like phthalates and bisphenols are sampled in great amount and possibly was the outcomes of sludge addition in agricultural motives (AA de Souza Machado, 2018).

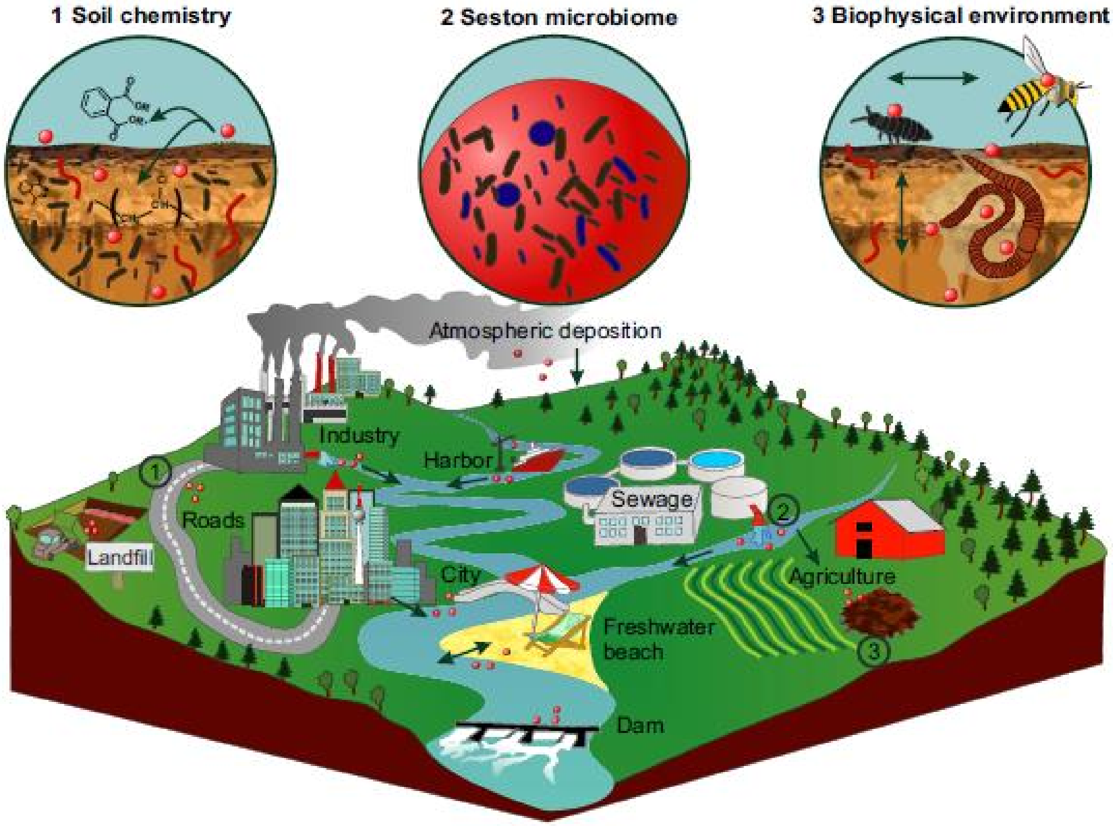


Figure 9. As mentioned, landfills and sewage systems are on the top of list for the microplastic inputs to environment. Microplastic additives for agriculture purposes also have drawbacks as they strongly change the characteristics of soil. Photo: *(AA de Souza Machado, 2018)*.

Landfills are one of the most wide spread methods in disposal management. They contain nearly 21-42% of globally circulated plastics. Landfills also have an immense effect on plastics by disintegrating them into secondary microplastics via combination of biochemical and physical factors. As mentioned before, the crashed and asymmetrical forms of microplastics depict the process of breakdown into smaller particles, thus accumulating microplastics from primary microplastics or high dimension plastics. However, primary microplastics are also transported straightly to landfills by sludge. Landfills also dominant in concentration of microplastics among other soils, sewages, and agricultural lands. It was estimated to contain 20,000-91,000 items/kg on average of all landfills. Despite of leachate application, the microplastic accumulation remains higher than was detected in leachate treatment, demonstrating the low efficiency of its operation. The presence of microplastics in agricultural lands has both advantages and negative sides on vegetation and soil. By adsorbing the surrounding contaminants in soil, microplastics contributes to creation and functioning of soil enzymes. Furthermore, enzymes strengthen the solution of necessary nutrients like organic carbon, nitrogen and phosphorus. In addition, accumulation of contaminants in microplastics separates their hazards from plants. On other hands, concentration of beneficial nutrients on certain points can lead to lack of distribution in overall. Although microplastics are not transferred to plants, further breakdown of microplastics can create nanoplastics which were found inside fruits and vegetables (H Golwala, 2021)

1.5. Microplastic impacts on humans and natural living organisms.

By each year, microplastics are tend occupy more and more space in environment and increasing the probability of facing both aquatic and terrestrial organisms. Vast number of aspects like dimension, structure, charge, color, and ubiquity define the effects on broad variety of global food chain elements (L Van Cauwenberghe, 2015). Microplastics have a potential to spread to almost every layer of food webs, however there is not still an accurate study on whenever particles more concentrated on land or in aquatic systems (see Figure 10). As example, microplastics were detected in digestive canal of 94% of all dead earthly birds in China. Nevertheless, there are also records of microplastics in guts of marine mainland birds. The main source is apparently linked to agricultural processes and the way microplastics are used as fertilizers (AA de Souza Machado, 2018). In many studies, mytilus edulis is singled out due to its global abundance and the fact that they are sedentary and infiltrate a huge quantity of water. In all last 4 researches that were made on them, microplastics were detected in different numbers. The mussels that was collected in Europe accommodated nearly 0.2 to 0.5 microplastics/g damp weight. On contrast, the ones which were studied in Canada contained up to 34-178 microplastics in every mussels (L Van Cauwenberghe, 2015). It was demonstrated that greater plastics are causing thresholds in the maintaining of gases and compounds that could influence natural fitness and lead to livings complexity. On other hand, tiny fractions are directly ingested or inhaled and build foundation for obstruction of digestive systems and exasperation of mucosa (AA de Souza Machado, 2018). An exact hazard was found in means of microplastics deal damage on reproduction system of earthworms which are essential agents for land nutrient cycle and ventilation. This factors are strongly connected to land productivity and vegetation and play an important role for agricultural purposes. The final chemicals of breakdown mechanism like additives can have a huge difficulties towards a high agricultural efficiency (AA Horton, 2017). Two main organisms like springtails and earthworms were recorded to carry out microplastics within land in all spatial directions. As for earthworms, microplastic activity was related to textural modifications in their

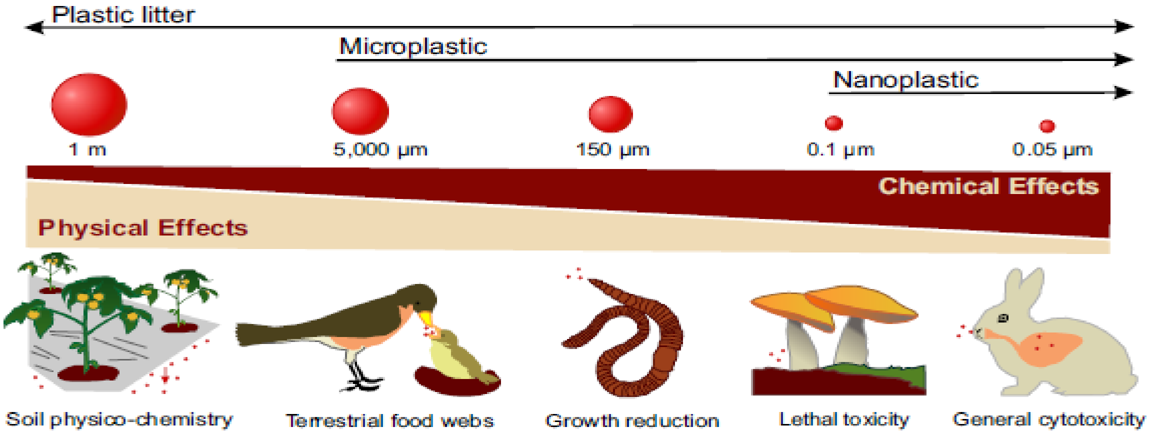


Figure 10. As the size of microplastic particle reduces, it becomes more hazardous to natural habitats and plants, some which can be consumed by humans. Photo: *(AA de Souza Machado, 2018)*

burrows, a final stage that is straightly interconnected to soil aggregation and operation. Likewise, springtails trigger impacts in their gut microbiomes as biophysical environment tends to alter (AA de Souza Machado, 2018).

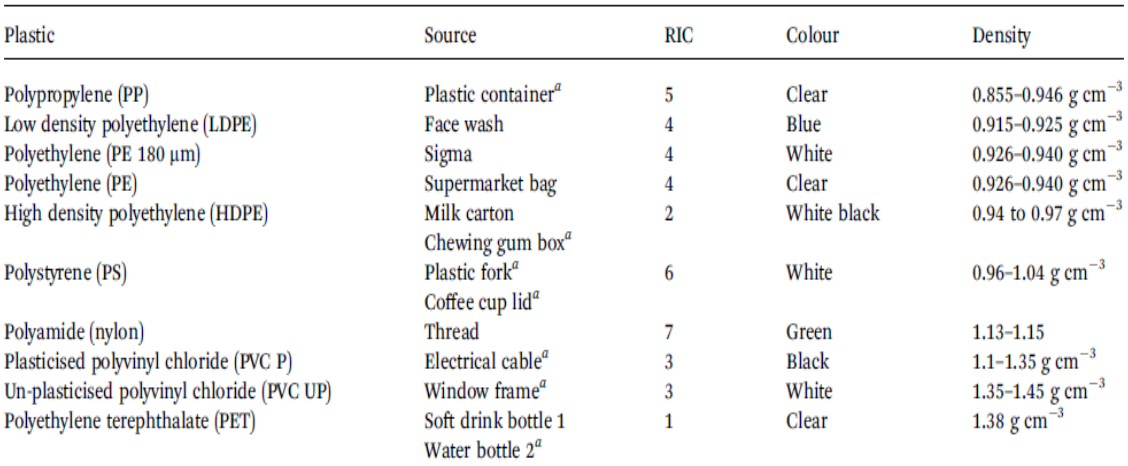
2. Separation Methods

2.1. Density separation for the rapid recovery of microplastics from sediment

Nowadays, microplastics can be detected in all natural surroundings and are separated from water, soil and organic specimens all around globe. Majority of studies are carried out on marine environment which are categorized as higher portion of microplastic contamination with the limit values of 3.3 % by weight in the most polluted areas. In order to identify and determine amount of microplastics, several separation techniques were introduced for both organic and inorganic spheres. During some occasions, the specimen can be brought down in the sampling region by filtration and density separation, however generally the specimen would be carried to laboratory in whole form for microplastic inspection. Visual sorting, filtration, sieving, density separation, elutriation, flotation and chemical digestion are among the most used methods for division of microplastics from soil. Likely to many microplastic studies, the fast progression of topic and absence of standardized rules brought to unpredictability of investigation and quantification in microplastics, especially the ones in sediments. Although, protocols and regulations were established by organizations in order to try determining the appropriate methods of microplastic separation from environmental tests. This study mainly focus on comparison and identification of some brine solutions for better density division of sedimentary microplastics.

Physical properties of both microplastics and surroundings like dimension, form and density play an important role in this technique. Visual sorting is mainly applied to samples which contains bigger microplastics, because of being simple and practical. On contrast, separation of tiny particles is more challenging, specifically when soil is consisted of fine sediments which coupled with microplastic form strongly affect separation potential. Methods as elutriation was introduced which stands for separation of particular dimensions, forms and densities by applying flow of gas or liquid in against way to precipitation. Similarly, flotation is also used for this purpose by taking advantage on correlative buoyancy and hydrophobic properties of microplastics. Notwithstanding, separation on density variations is still the most well founded and conventional technique among the researchers.

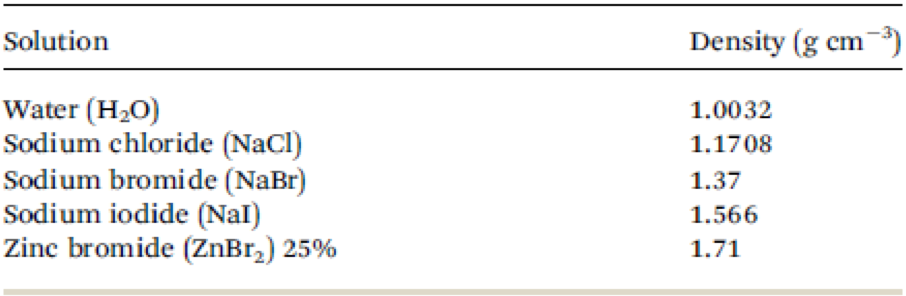
Density separation is simply the process of transferring the specimen into liquid of medium density and leaving the density variation to settle down the denser sediment part and float the microplastics to the surface. Modification of salt solvents can lead to higher density gaps which in the order increases the power of solution. Not surprisingly, in almost 65% of all studies related to microplastic separation from sediments, the density separation technique was used. The most prevalent brine solution is composed of sodium chloride (NaCl) which has specific gravity of 1.2 g/cm^3 and is able to influence microplastic particles with less density from a common sediments having density of 2.65 g/cm^3. The biggest upper hand of NaCl is being cheap, environmentally neutral and large availability in markets. There are several microplastics with a density of 1.2 g/cm^3 which can or cannot be separated by this method. The most spread microplastics of this type are polypropylene (PP), polyethylene (PE), polystyrene (PS) and polyamide which is also known as nylon. Moreover, more solid microplastics as polyvinyl chloride (PVC) and polyethylene terephthalate (PET) put together more than 17% of whole plastic industry (see Figure 11).



Plastic	Source	RIC	Colour	Density
Polypropylene (PP)	Plastic container[a]	5	Clear	0.855–0.946 g cm^{-3}
Low density polyethylene (LDPE)	Face wash	4	Blue	0.915–0.925 g cm^{-3}
Polyethylene (PE 180 µm)	Sigma	4	White	0.926–0.940 g cm^{-3}
Polyethylene (PE)	Supermarket bag	4	Clear	0.926–0.940 g cm^{-3}
High density polyethylene (HDPE)	Milk carton	2	White black	0.94 to 0.97 g cm^{-3}
Polystyrene (PS)	Chewing gum box[a] Plastic fork[a] Coffee cup lid[a]	6	White	0.96–1.04 g cm^{-3}
Polyamide (nylon)	Thread	7	Green	1.13–1.15
Plasticised polyvinyl chloride (PVC P)	Electrical cable[a]	3	Black	1.1–1.35 g cm^{-3}
Un-plasticised polyvinyl chloride (PVC UP)	Window frame[a]	3	White	1.35–1.45 g cm^{-3}
Polyethylene terephthalate (PET)	Soft drink bottle 1 Water bottle 2[a]	1	Clear	1.38 g cm^{-3}

Figure 11. Represents colors and densities of the most spread polymer compounds. Photo: *(B. Quinn, 2017)*

In view of this problem, many other brine solutions with higher density were introduced as sodium polytungstate (1.4 g/cm^3), zinc chloride (1.5-1.7 g/cm^3), calcium chloride (1.30-1.35 g/cm^3) and sodium iodine (1.8 g/cm^3). Lately, the 5.4 M solution of lithium metatungstate (1.62 g/cm^3) was also accepted as appropriate for high density separation (see Figure 12). Generally, all solutions with density higher than 1.45 g/cm^3 were categorized as applicable for separation of every type of microplastics. Nevertheless, their disadvantageous sides as being too costly and hazardous to the environment made them less workable, and usually they are not considered to use in majority of experiments.



Solution	Density (g cm^{-3})
Water (H_2O)	1.0032
Sodium chloride (NaCl)	1.1708
Sodium bromide (NaBr)	1.37
Sodium iodide (NaI)	1.566
Zinc bromide ($ZnBr_2$) 25%	1.71

Figure 12. Describes density values of the utilized solutions during separation experiments. Photo: *(B. Quinn, 2017)*

Although, the implementation of many brine solutions was carried out in numerous researches, just in the few cases their performances were accurately recorded. Some studies used small round microplastics less than 1 mm to determine their effectiveness, but unfortunately this trend is little bit optimistic. Operating the density separation gave microplastic yield of 91-99% for the dimensions around 1 mm, but with decreasing size the yield percentage could reduce to value of 40. This very study is concentrated to investigate all brine solutions potential and water for separation of environmentally found consumer disposed microplastics having the dimension spans of 200-400 µm and 800-1000 µm.

In this particular study four brine solutions as sodium chloride (NaCl), sodium bromide (NaBr), sodium iodide (NaI) and zinc bromide ($ZnBr_2$) were examined. To obtain complete saturation, NaCl, NaBr and NaI were dissolved in a 1 L of distilled water and magnetic stirrer were used to provide the full solution. Brine salts were added progressively to the bottom of the beaker until any excess was formed. On other hand, the saturated $ZnBr_2$ has of 4.2 g/cm^3, thus only 25% saturation was need to acquire 1.7 g/cm^3. It was recorded that for this purpose exact amount of 1125 g/L of $ZnBr_2$ was used and no excess was remained.

In order to find out the recovery values of brine solutions and water, several experiments were carried out using 66.66 g of cleaned sediments and 0.066 g of microplastics which were put together in a 400 mL glass beaker. The mixture was stirred at the power of 300 rpm for 3 minutes. Then, the duration of 10 minutes were given for mixture to settle down the sediment part and provide the floating or the suspension of microplastics. Further, the vacuum suction was applied to collect particles from surface of the mixture. Vacuum system was composed of glass tube which went through the surface of mixture and particles were accumulated in the flask with three barriers which have a role of trap. Additionally, the rubber and glass tube were totally rinsed to prevent any possible microplastic lost. To deliver a better accumulation of plastics on surface, a little bit of soap were periodically dropped to the mixture. Later on, the gathered microplastics from the trap went through filter paper by operating a Buchner funnel. When filtration was finished, additional washing of flask was run to secure the collecting of all microplastics and then the filter paper was place in a watch glass for drying out in oven for 10 minutes in the temperature of 70 °C. Finally, the sample was completely ready for calculation of recovery percentage. Moreover, for the denser solution as NaI and $ZnBr_2$ only washing once was performed, as for the lower ones the repetition number was determined as three.

With an eye to keep out any contamination from sample, preventing protocols were applied in each stage of experiment. Tidy laboratory coats consist of pure cotton were used and no synthetic material was present in outfit of experiment operators. The preventing protocol refers to three main stages. First is to clean all needed equipment and tools with 70% ethanol and distilled water to be sure of riddance of any microplastic particles. Second is to inspect all working areas and to detect any possible contamination by taping method. This step is done in both before and after the procedure. Third is also to control the contamination level in atmosphere by implementation of taping method. In this case, the final filter papers left from procedure were placed in Petri dishes for further contamination control.

Another aspect that was aimed during the process is to minimize the operating time of separation experiments, because of excess air subjection which can lead to effects of contamination. Further, Petri dishes were used for this purpose and to preserve for future activities. Density separation experiments were done with Petri dishes instead of sediments for ensure that no microplastic is present on glass surface.

Despite the fact that various separation techniques are implied to separate microplastics from sediments, not enough records were taken on exact numbers of microplastic recovery. The outcomes of tests with the size range of 200-400 μm evidently demonstrates the link between higher solution density and better recovery as NaI and $ZnBr_2$ solutions gave higher proportions of recovery for all samples (see Figure 13). Clearly, experiments which are processed with tap water showed the worst results, because of having the lowest density. Accordingly, PET being the denser particle, was found to have recovery around 70% which is very poor outcome. On contrary, nylon fibres showed recovery higher than 95%. Hence, it should be point out that microplastics of fibre type were dominated when researches were made on marine sediments. Another factor of better separation of fibres with water is that their large area to volume proportion and form provides a better sustainability in the surface tension layer.

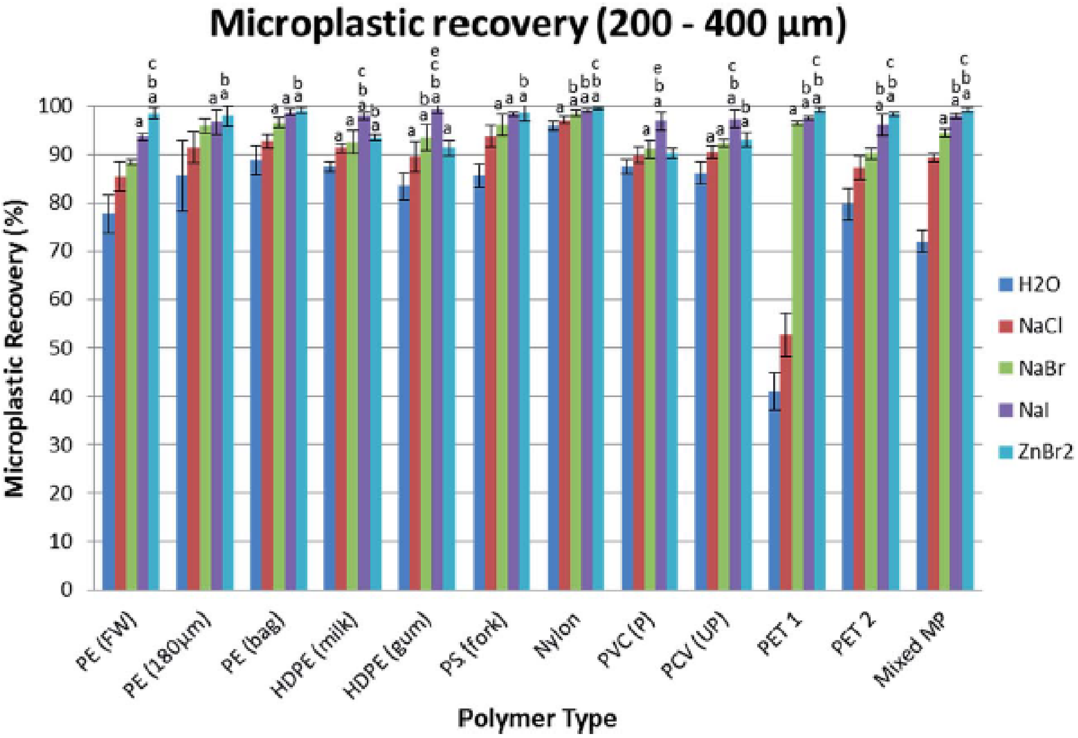


Figure 13. This graph represents the yield concentration of microplastic specimens (200-400 µm) related to each separation agent. Photo: *(B. Quinn, 2017)*

Meanwhile, among the brine solutions, NaCl demonstrated the lowest recovery results for 200-400 µm with being less than 90% and having higher variability which means no microplastic of particular type was outlined by this solution. Surprisingly, just 4 types: nylon, PVC, and both type of HDPEs out of total twelve specimens were found to have greater recovery than tap water. This fact makes NaCl very appropriate as it has almost the same density with tap water. Additionally, NaCl is most widely used brine solution for certain reason as being cheap, globally available and environmentally safe. Despite of being very ineffective for separation of PET, it was revealed that PET from different post-consumer products showed interestingly dissimilar results. PET 1 sample was obtained from carbonated beverage bottle and PET 2 was from usual water bottle. The reason that stands behind is probability of plastics to interface with various additives. Moreover, recent studies on microplastic separation lead to introducing of specific wetting agents which work better with each particular polymer type and lead to a greater recovery.

In this laboratory work, the NaBr solution was introduced as density separation medium for first time in any microplastic study. Because of having relatively higher density (1.37 g/cm^3) than tapped water and NaCl solution, its results substantially large amount of recovered particles. Whereas, only the lighter PE and PET 2 specimens had near the same recovery, the others nine out of twelve showed a better separation with NaBr. Moreover, it was demonstrated that for the microplastic scope of 200-400 µm there are not many essential reasons to replace simple NaCl with it. For investigation of marine source particles, the solution of sodium polytungstate which has the close density of 1.4 g/cm^3 was implied.

The certain size target of 800-1000 µm showed best outcomes with application of NaI solution (see Figure 14). It was proved to have a better recovery values than water, also nine out of twelve samples comparing to NaCl, and three out of twelve comparing to NaBr. Furthermore, four out of all microplastic test, NaI solution performed considerably better than $ZnBr_2$, particularly with PVC. Meanwhile, the separation yields of microplastics with the size of 1 mm which are made of PE, PP, PVC, PET, PS and polyurethane (PUR) was found to be near 91-99%.

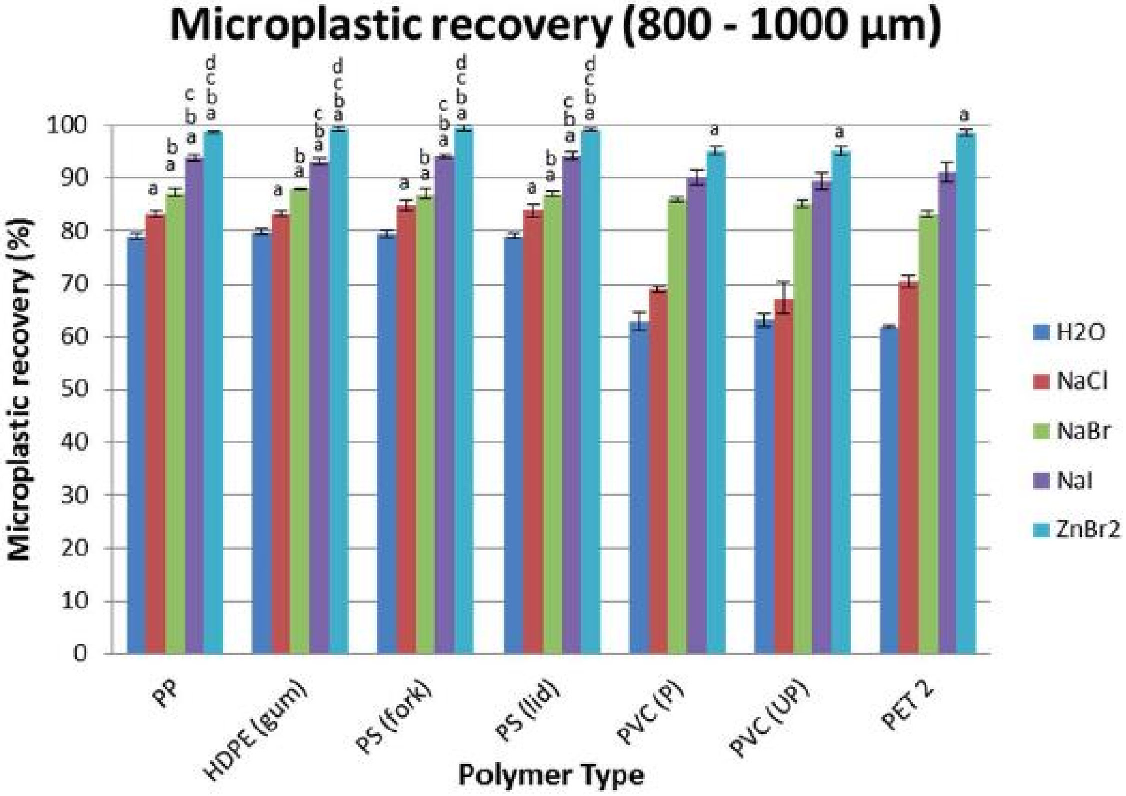


Figure 14. This graph represents the yield concentration of microplastic specimens (800-1000 µm) related to each separation agent. *(B. Quinn, 2017)*

This research is unique in a way that to firstly use the solution of $ZnBr_2$ for the inspection of microplastics from soil matrix. As the reason of having the density of 4.5 g/cm^3 when it is completely saturated, only 25% of saturation was aimed to have the reasonable density value of 1.71g/cm^3. Consequently, $ZnBr_2$ demonstrated the superior outcomes for eight out of twelve samples within the dimension scope of 200-400 µm. (B. Quinn, 2017).

2.2. Froth Flotation

After many years of application in mining industry as separation technique, froth flotation is proved to be also very effective in separation of plastic materials from different matrixes. Although the process is among the cheapest methods, it has a very high results in recovery of defined plastics. In particular cases, it is applied to differentiate as well plastic from plastic. Amongst the most spread plastics that are produced in industrial fields are polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyethylene (PE) and polypropylene (PP). Only the manufacturing rate of PVC is about 4 million tons per year. Dissimilar to other ones, incineration of PVC generates a highly toxic gas hydrogen chloride which is intolerable for presuming of surroundings. The growth of PVC made materials in fields of electronics and automobiles forced to come across with a method which would distinct it from others. The progress of flotation technique gave new opportunities to distinguish countless kind of plastics which was due to mismanaged dumping and reduce the hazards to environment. While there is abundance of PVC and PET in solid matrix, the separation is more complicated. Despite of PVC and PET are both comfortably diversifiable from HDPE and PP by simple water flotation, they are denser than water and practically impossible to separate with application of just water flotation. Additionally, the majority of plastics which are manufactured for packing purposes irreconcilable with each other and especially PVC-PET couple introduces the worst scenarios of cross-contamination hazards.

Flotation was primarily advanced for mineral separation in early 20th century. The implementation of this technique for plastic distinguish is apparently novel. The first studies related to this topic were done in the beginning of 1970s. The concept of application flotation method to separate plastics was originated from ore flotation records, which illustrated that the surface characteristics of diverse substances can be varied preferably by the mean of surfactant adsorption. Flotation process contains all three forms of substance: liquid, solid and gas. The combination of rigid fractions and water (pulp) is placed in flotation apparatus and then provided by the gas phase. The concept which lies under flotation method is producing selectively hydrophobic substance surfaces to get in touch with gas sparkles, thereby establishing the chance to diffuse gaseous bubbles into the hydrophobic fractions. Final bubble fragment aggregates are moved to froth stage which mainly develop on the upper section of flotation devices. The outcome of this process includes bubble fragment aggregates transferred out by mean of froth. The remained pulp in device is removed by suitable system. Elective flotation is carried out parallel by elective wetting of desired substance. Depressants and surfactants are the mechanisms which allow us to perform wetting of hydrophobic surfaces. The key principle to separate plastics by flotation is to apply bubble particles to stick to substance which we are focused to obtain like a final result. Thus, there is must be an adequate variance in the surface wettability of components. Being that almost all plastics are hydrophobic in natural condition, selection of appropriate wettability among substances is essential for the separation. A suitable reagent mixed together with water increases the effect of the wetting process, as plastics are referred to low energy-surface particles. Proper plastic surfactants are consist of ability to adsorb on plastic covers, similarly as of molecular categories that provide fraction hydrophilic. Accordingly, the basic obstacles in plastic separation by flotation is that it needs a careful analysis to find out the most fitting wetting agents depending on reagent concentration, pH, duration of operation and component dimension.

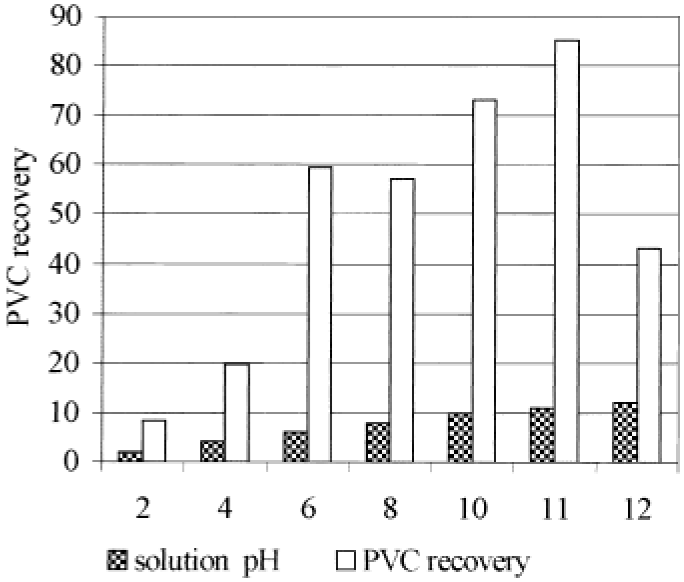


Figure 15. Demonstrates recovery rates of PVC with modification of solution pH. Photo: *(G.A. Marques, 2000)*

The specimen includes two dissimilar types of plastics, pipes and beverage bottles were used to gain particles of PVC and PET. Every plastic fragment was minimized utilizing screening device and cutting mil. The screening dimensions which selected to use in flotation were 4.7-3.36 mm and 3.36-1.70 mm (where both size categories are related to microplastics). The sample placed in flotation apparatus composed of a mixture both plastic kinds which have 10 g of weight each. In addition, PVC was colored black and PET transparent in order to faster differentiate the final result and inspect the specimen concentration. Lignin sultanate having 300 mg/l of concentration is added as wetting agent for elective depression. Adsorbed depressant molecules have a role of affecting the affinity of sparkles for various kinds of plastic during adequate conditions. Pine oil and methyl isobutyl carbinol (MIBC) are added to accelerate frothing. The used amount changed from three to five linked to each operation. The froth flotation processes were performed in a column with 1000 mm of height and 100 mm of diameter. The advantage how high column is that the depressed plastic particles tend to be further from the surface. As bigger the column, the wider the selection spectrum. In the base of the flotation tank was placed a permeable plate for sparkling air. It was feasible to reach 0.3 mm of bubble diameter, increasing the chance of bubble-fragment interaction. The final stage was to carry out froth discharge and to recover plastic particles from it by use of sieves. Four liters of solution was made by adding surfactant having 300 mg/l to tap water. Operation duration was 20 minutes and HCl and NaOH were used to modify the pH of solution. Color dissimilarity was the most obvious tool to determine to which kind the plastic is related by applying handheld distinction.

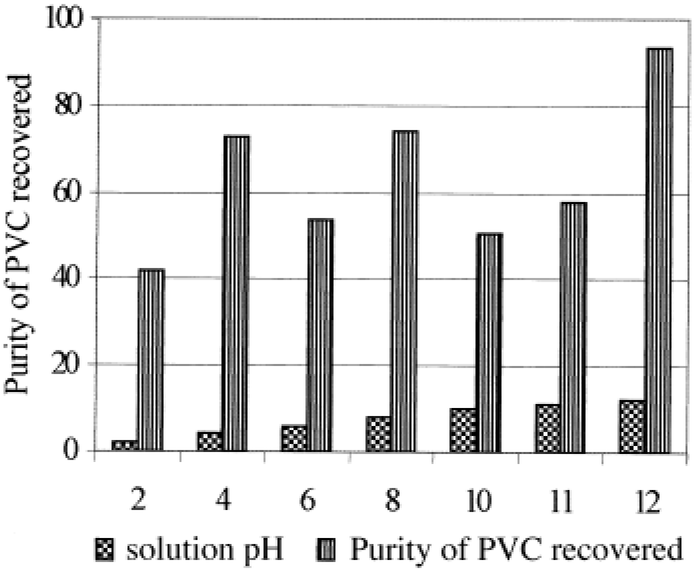


Figure 16. Provides values of PVC purity with modification of solution pH. Photo: *(G.A. Marques, 2000)*

Because of being hydrophobic, it was possible to implement flotation on both samples. Thus, selection of proper surfactant was essential factor in order to obtain feasible results. Another important aspect was pH which differed the final outcomes. Relying on provided experiments, more reasonable results were obtained with solution having pH 12 and utilizing MIBC as frothing agent. The separation of PVC reached point of 43.3% and a purity of 93.5% (see Figure 15). On contrast, while utilizing pine oil as frothing agent, almost no separation was achieved. The reason which stands behind is that pine oil introduces a charge into solution which muddles the result. Furthermore, the PVC yielding reduces with reduction of pH value. Just the pH value 12 showed irregular trend, whereas the purity of PVC increased at the pH values of 4, 8 and 12.

Moreover, the operation period differs the wettability of plastic area. Four operation timings were used which are 10 min, 30 min, 1 h and 2 h. MIBC was added as frothing agent and pH of solution was set to 12. After testing several times, the most appropriate operation period for PET was maintained. In the operation timing of 2 h the surfactant effected both PVC and PET, depressing step of the PVC which was not intended. In operation timings of 10 and 30 min the yield of PVC was high, but the additive of PET was higher than 30% (see Figure 16). Altogether, the operation timings listed above were not advantageous for PET fragments. Therefore, the perfect duration was detected to be 1 h. The surfactant effected just PET particles and the PVC was nearly fully extracted. The fragment dimensions of 4.7 mm and 3.36 mm were not the most suitable size class for this testing, because recovery figures were not high as expected. These fragments demonstrated that air sparkles join to their cover area, but even though several particles were not able to rise to top because of its heavy weight. Another option for better outcome was to increase the gas flow input on the bottom of the tank, but it lead to faster turbulence and higher byproduct of PET additives. Moreover, the growth of water input also elevated the froth part and the concentration level which directly decrease the yield. On other hand, fragments of 1.70 mm showed a great selective separation, and the air flow was more productive which lead to a lower values of left inside the tank. (G.A. Marques, 2000)

3. Microplastic identification techniques.

3.1. FTIR

The properties of microplastics split to chemical and physical operations. Physical observation is provided by utilizing a microscope and the fragments are classified according to dimension and color. Optical experiment is obligatory in order to identify microplastics that are detached from matrix and this process is carried out in majority of studies. Introductory recognition among plastics and non-plastics can be stated from primary visual inspections, particles cannot contain organic substances, fibers should show constant thickness along its longitude and the color must remain same throughout the sample (see Figure 17). Apart from the application of spectroscopy for demonstrative identification, scanning electron microscopy is a method to examine the

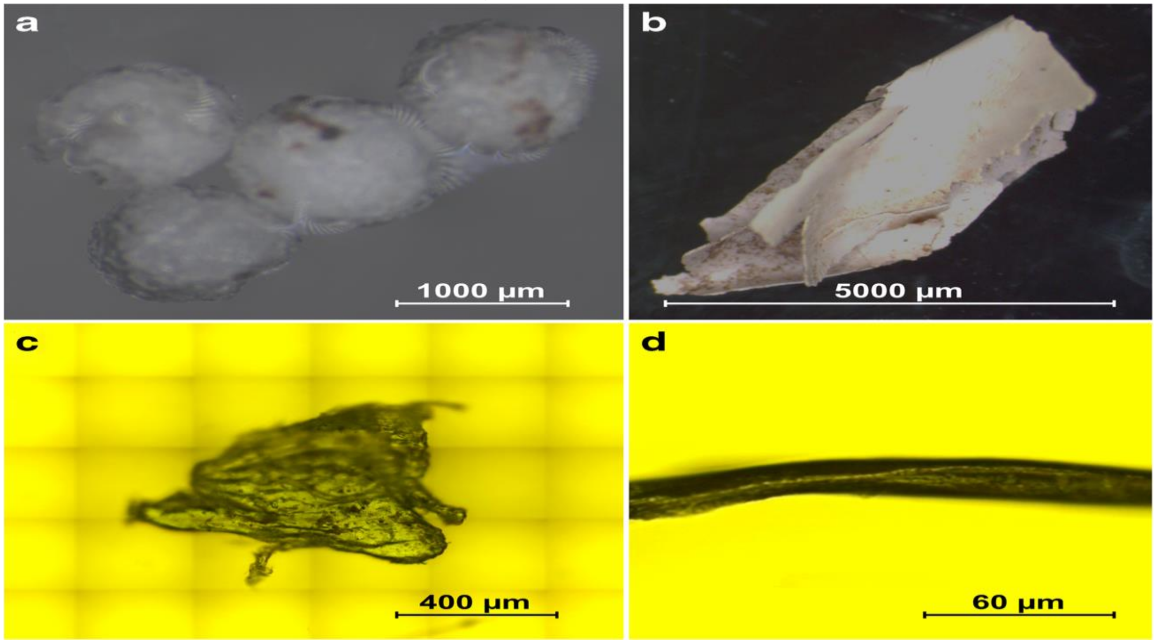


Figure 17. Demonstrates different microplastic shapes and colors under optical microscope inspection. White spherical particle (a), white fragment particle (b), blue foam particle (c) and black fiber particle (d). Photo: *(A Käppler, 2016)*

accidence of fractions. Owing to the option of providing high-resolution figures, scanning electron microscopy can be used towards pointing out presence of microplastics and its additives. Notwithstanding the rigorous rules that are implemented to microplastic identification, the possibility of error occurrence increases dramatically with reduction of sample sizes. As example, the fiber fragments of cotton and polyester have almost the similar appearance and it is impossible to find out which one is microplastic without observing their chemical characteristics. Consequently, analysis techniques like chromatography, FTIR or Raman spectroscopy are used to define accurate chemistry of samples. These methods have a crucial role in discrimination between plastic and non-plastic substances, as well detecting even the impurities (see Figure 18). This data is essential for becoming aware of which microplastics are disposed from surrounded community activity (C Bretas Alvim, 2020).

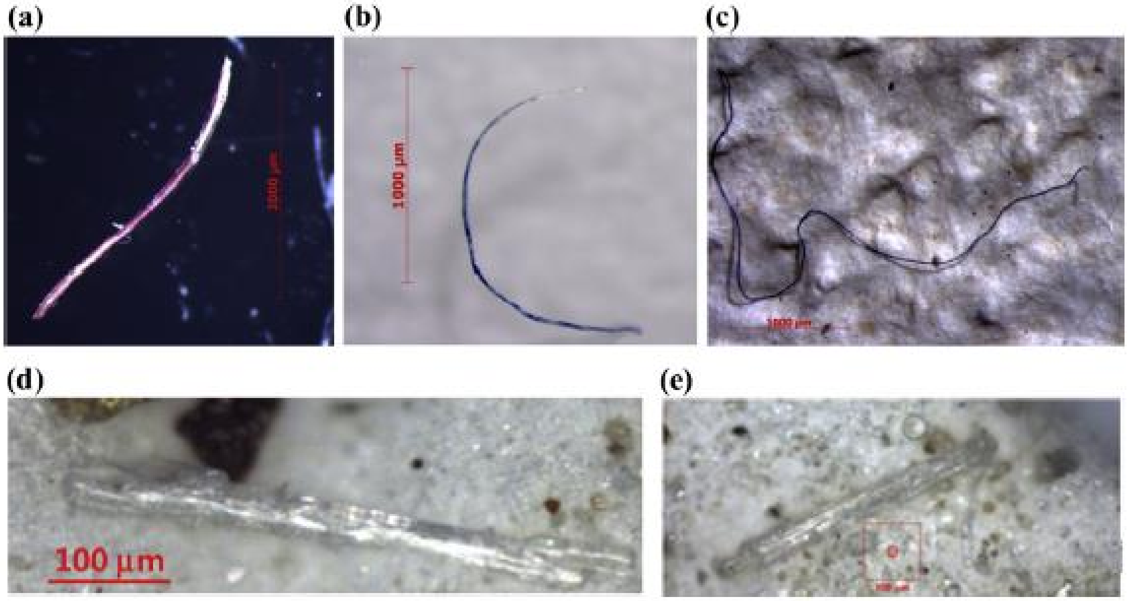


Figure 18. Provides images that some of them can be related to microplastic particles, but in closer examination they can be concluded as apart substances. Organic matter (a), cotton (b) and rayon (c) are non-plastic objects. The samples (d) and (e) are polypropylene particles. Photo: *(YK Song, 2015)*

The pollution of microplastics in marine and terrestrial systems is demonstrated via the several researches and negative effects have been proven. FTIR and Raman spectroscopy are involved as the main methods for chemical distinction of microplastics. Fragments and fibers that are less than 500 µm in dimension were obtained from beach soil and examined through both FTIR and Raman spectroscopy. The outcome shows reasonable information that either techniques are suited for microplastic identification in natural surroundings. Nevertheless, in some unique samples with specific colors, application of both techniques was required to declare precise chemical structure. Then, the specimens which are holding fractions in size less than 400 µm were inspected by Raman imaging and FTIR transmission imaging. The outputs were listed according to dimension, amount and class of founded microplastics like as spectra degree, measuring period and processing. It was illustrated that FTIR imaging has a drawback of microplastic identification in the dimension scope less than 20 µm, and having a huge misleading around 35%. On other hand, time consumption of Raman imaging is significantly higher than of FTIR imaging (A Käppler, 2016). Each method is vibrational spectroscopy, which provides molecular stimulation of the test and consequently the production of specific spectral fingerprints. Along having reliable spectral database, it is feasible to obtain the exact identity of sample. FTIR functions as irradiation agent by exercising IR light (see Figure 19). Relying on molecular composition, portion of radiation is captivated by testing substance and then exhibit in reflecting pattern. The main concept is that

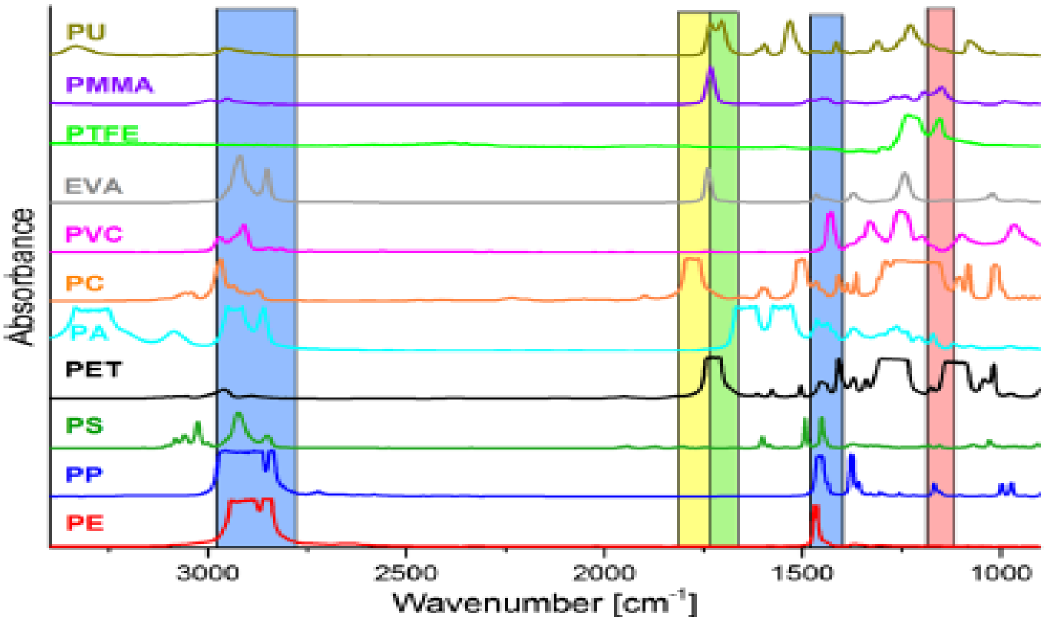


Figure 19. Illustrates the fingerprints of the most abundant polymer types for FTIR identification. Photo: *(A Käppler, 2016)*

every compound has characteristic chemical connection and that lead to producing of individual spectrums which are then matched with records of database.

FTIR has two implementable ways for microplastic identification: transmission and reflection modes, involving the attenuated total reflectance optimization, where the relation between test and crystal must be taken as touchable. There is a number of dissimilarities between Raman and FTIR techniques, but the key underline is that the spectrum produced by FTIR is controls the differentiation in continuous dipole moment of the chemical bonding, whereas the Raman is mainly concentrated on the variation of chemical bonding polarization. Therefore, as FTIR spectroscopy generates the prevalence of IR light, Raman spectroscopy utilizes a monochromic laser and produced energy is consumed by the testing substance prior to triggering a spectrum. It is also proposed to implement the combination of methods in order to come with more fixed and exact decisions. Operationally, Raman spectroscopy is able to generate more data on non-polar composition which leads to a better realization on the fraction details. Regardless of high precision rate of Raman in investigation of fractions that are less than 20µm, it experiences a limitation in analyzing of impurities and additives of microplastics, which creates uncertainty of plastic spectrum and resulting in accurate identification. Thereby, it is possible that the impurities are carried out in matrix and cover the original fingerprints of main microplastics and lead to estimation of greater amount. Another barrier of Raman spectroscopy is the fluorescence. Color additives and pigments can seriously absorb fluorescence and preventing the recognition of compound spectra. Additionally, Raman spectroscopy strongly related to combined tools, every modification in equipment may result in totally different outcomes (C Bretas Alvim, 2020).

Firstly, examined substances of aquatic system were sampled from coast of the Geoje Island which touches with marine water discharge from Nakdong River in South Korea. The location of substances was 10 different spatial positions and realization was in May and July of 2012. Metallic sieve was utilized to gain samples below 150-400 µm of water surface. The size of mesh sieve was 2 mm and was able to obtain 3.14 m^2 of testing space in each location. Final sample volume was in scope of 2.2-2.8 L and isolated in 1 L polyethylene bottles. Similarly, soil samplings were extracted from 6 different coastal areas of Geoje Island and 10 spatial locations were spontaneously picked in every beach zone. The dimension of sampling was 0.5 m both in width and length, but just 5 cm in depth to obtain reasonable volume of 12.5 L. Received samples were dried in order to get rid of any humidity and to clear out all pores. Finally, they were also placed in 1 L polyethylene bottles (YK Song, 2015).

The water substances were filtered by mean of filter papers and then dried at temperature of 60 ℃ and preserved for future operations. The identification and quantification of filtered samples were run by stereomicroscope. Further, each detected microplastic was classified according to its size and kind. Damaged version of polystyrene which are less than 1 mm in size was categorized as additional kind by reason of its wide spread in shoreline zones of Korea. Regarding to soil tests, 50 ml portion was taken from each storing bottle and added to new entire sample of 500 mL. Next step was to include 330 mL of saturated NaCl solution in order to achieve a better microplastic distinction. After 1 minute of mixing, sample was kept for precipitation around 10 minutes. This process was repeated 3 times and then the collected liquids of every round were transferred to 1 L beaker. The final measures were to keep beaker at around 1 day static position and afterwards operate the filtering (YK Song, 2015).

FTIR spectroscopic microscope was used towards quantification and identification of both samplings from water surface and beach sediments. Fractions were estimated from 5 occasional parts of filter paper which made quarter of total sampled area. Thermo Nicolet 6700 was selected as FTIR and tooled with a liquidized nitrogen-cooled mercury cadmium telluride sensor and attenuated total reflectance containing germanium crystals by declining mean angle of 25°. The spectral recordings were in medium of 128 scanning in spectra scope of 650-4000 cm^{-1} at resolution accuracy of 8 cm^{-1} and an opening of 100 µm (YK Song, 2015)

The ubiquity of microplastics in water and soil samples was inspected and further procedures were carried out to recognize any possible differences between observing with stereomicroscope and FTIR. The trend of microplastic prevalence in sense of kind was alike in the beach soil and from water. The average presence of fragments and damaged polystyrene was higher in FTIR than in microscope, whereas the average presence of fibers was greater in microscope method (see Figure 20). The particle distribution of fragment existence was 127±111 pieces/L in microscope and 206±117 pieces/L from FTIR analysis for surface water sample. Following investigation of fragments in beach soil demonstrated 1192 pieces/L using FTIR and 152 pieces/L from microscope. It goes without saying that fragment abundance in surface water and beach sediment was considerably greater from utilization of FTIR technique rather than stereomicroscope. Records of damaged polystyrene were 0.1±0.2 piece/L in microscope and 0.4±1.8 pieces/L from FTIR for surface water sample, and 631 pieces/L and 1521 pieces/L respectively from beach soil. Surprisingly, the prevalence of fiber particles noticeably poor with FTIR and reported to be 4.5±4.1 pieces/L in water surface and 8 pieces/L in beach soil, along with having 13±15 pieces/L and 29 pieces/L in water surface and beach soil respectively from microscope observation. Very low amount of sheets was found from microscope procedure, yet nothing from FTIR. The entire microplastic ubiquity was counterbalance connected to varying figures among microscope and FTIR techniques. In conclusion, the amount of microplastic detected by FTIR was higher comparing to microscope for almost all sampled locations and intervals (see Figure 21). Similarly, presence of fragments of every dimension was greater from FTIR analysis. It was clearly established that FTIR displayed a growing figure of microplastic amount against length reduction, whereas the trend of quantity from microscope reached its summit in the dimension span of 50-100 µm. There was a big gap between these methods in the quantity of fragments which are less than 50 µm in size. Additionally, no fiber was found in dimension of <50 µm by utilizing both techniques. However, the presence of microplastics in range of 200-1000 µm were identified reasonably greater by microscope in comparison with FTIR. Moreover, it was not possible to determine any statistical figure of size distribution by FTIR analysis, but microscope illustrated an increasing relationship between size and quantity. Microscope was able to detect several amount of fiber in range of 50-200 µm, while FTIR did not show any. Another microplastic type which was almost distributed same was sheet. Finally, FTIR dominated in length >200 µm of damaged polystyrene, but it was microscope method which detected more in size less than 200 µm (YK Song, 2015)

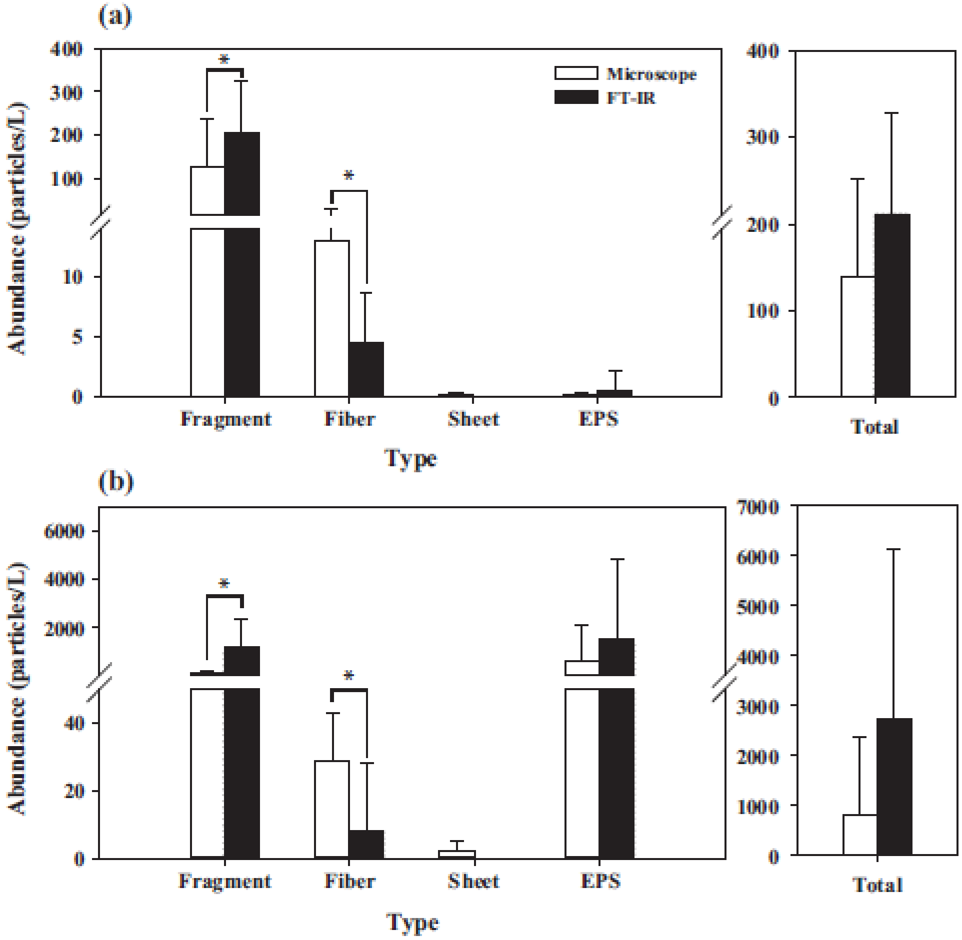


Figure 20. A stereomicroscope and a Fourier transform infrared spectroscope were used to identify the amount of microplastics by type in the sea surface microlayer (a) and coastal area (b). Photo: *(YK Song, 2015)*

The fragment was the primary microplastic type that was abundant in all sampling locations from beach soil and surface water. Regarding to the period, fibers was highly detected by microscope comparing to FTIR during May, whereas the opposite happened in July. Additionally, the variation of microplastic quantity which was identified by both methods varied through each month, yet the rate of differentiation was higher for surface water samples along May. The average thickness of microplastics which were detected by FTIR was around 10-35 µm in diameter and colorific, while some colorless fibers also were identified with diameter of 10-70 µm. For the beach sediments, the identified quantity of microplastics by FTIR was greater than by the microscope method, only not including Wahyun beach. The peak ratio reached to 52 during comparison fragment identification by FTIR to microscope. If implying the microscope technique, fibers were abundant in all stations. On contrary, FTIR was able to find fibers only in Heungnam beach and it was even 1.5 times greater than quantity detected by microscope. Moreover, fibers demonstrated a constant trend of having diameter around 20 µm. Lastly, the damaged polystyrene particles dominated during identification by FTIR in all sediment samples (YK Song, 2015).

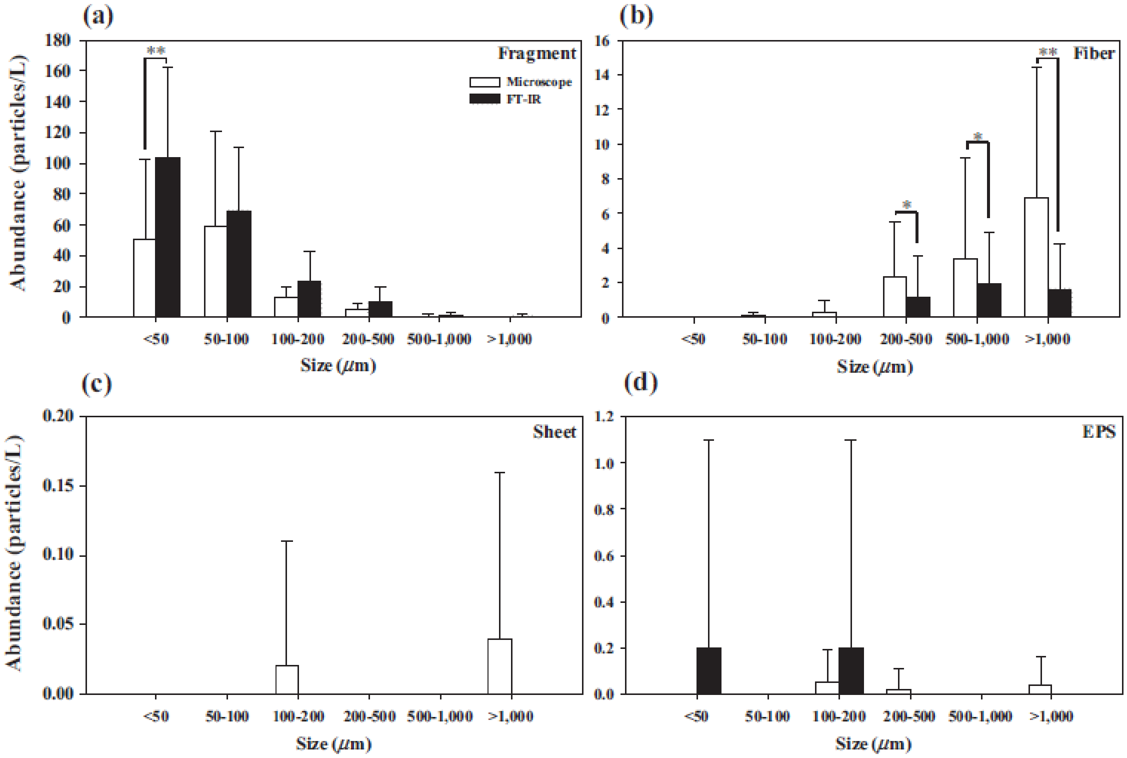


Figure 21. Represent size distribution of each microplastic types as fragment (a), fiber (b), sheet (c) and EPS (d) under inspection of both FTIR and stereomicroscope. Photo: *(YK Song, 2015)*

The main aspect that come to the mind when commenting these techniques is that FTIR consumes much more time than microscope identification. On other hand, there is a high probability that microplastics less than 1mm in dimension will be undetected with application of microscope, thus it is more adequate to identify microplastics which are higher than 1 mm.

Regarding to visional inspection, it is very easy to miscount non-plastic fractions like minerals and organic matters as plastics and overlook microplastics which are colorless. Consequently, it forms many misread estimation where it is impossible to fact anything accurately. On contrary, the FTIR spectroscopy technique was more precise and was able to minimize the underestimation and overestimation of microplastics. In fact, fine microplastic particles which are less than 1 mm length showed a regular trend, even the particles less than 50 µm. As was mentioned before, it is well known that the amount of microplastic grows with decreasing of length, thereby FTIR plays a significant role to study microplastics extensively. Furthermore, the key characteristic of FTIR is that it is able not only detect but also provide data about microplastic polymer composition. It is a crucial step in order to define the source, transport pathway and future state of micropalstics. As instance, absorption and adsorption magnitudes of hazardous pollutants vary significantly for every kind of chemical structure of polymers. Unfortunately, it is not economically convenient to investigate every particle and to determine either it is microplastic or not, well as it needs vast amount of time per each sample. Besides it, not only one trial per each sample is acceptable, because spectral images can alter due to sampling condition, dimension and kind, thus sometimes it can require numerous inspections before making a solid decision. Generally, up to 4-5 hours are spent on each case, which is almost 8-10 times more time than needed for microscope technique. So it stands as main reason for surveying only 25% of whole filtering area which is going to pass through FTIR. This is still more suitable technique than trying to examine entire filtering paper, because there is not a reasonable variation in presence of widely spread microplastics, yet the amount limited particles can be underestimated (YK Song, 2015).

3.2. RAMAN SPECTROSCOPY

Raman spectroscopy is one of the tools which utilizes a vibrational spectroscopy method relied on the elastoplastic dispersion of light that produces a vibrational array of data needed to identify the composition of microplastics. It is relatively a modern technique, but which gets high attention from many studies due to its superior precision. The Raman spectrum is similar to a footprint of chemical structure and may be used to describe the characteristics in a sample (see Figure 22). FT-IR methods share some of the advantages of Raman spectroscopy, such as non-destructiveness, minimal sample volume requirements, high throughput screening capability, and not being harmful to the environment. Furthermore, vibrational spectroscopy is a popular system for determining MPs, and the European Union expert group on marine litter proposes that all suspected MPs in the 1-100 µm size scope have their polymer identity validated thorough spectroscopic evaluation. Raman methods outperform FTIR spectroscopy in terms of spatial resolution (smaller than 1 µm versus 10-20 µm), wavelength coverage, sensitivity to non-polar binding sites, decreased water disturbance, and shorter spectral domains. On the flipside, Raman spectroscopy is susceptible to fluorescence interference, has an intrinsically poor signal-to-noise ratio, or might induce specimen heating owing to the application of a laser as a light source, resulting in secondary emission and possibly polymer degradation. Given the numerous benefits, the detection of MPs using Raman spectroscopy has yet to achieve the reputation of FTIR approaches, despite the fact that the number of articles is steadily growing (A Käppler, 2016).

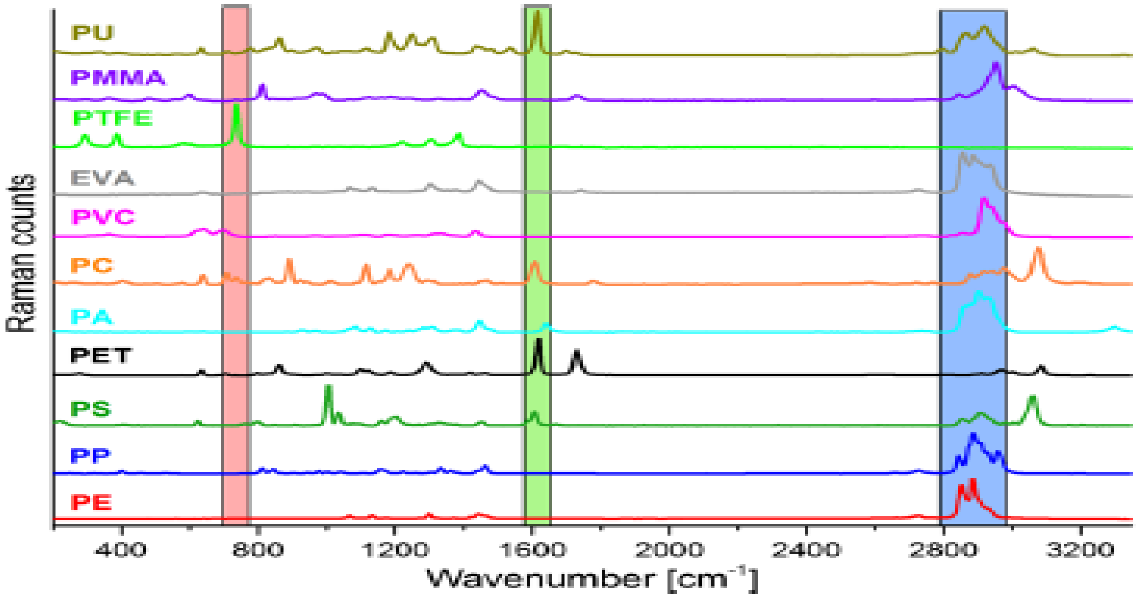


Figure 22. Provides the footprint array of the most abundant consumer-used plastics. Photo: *(A Käppler, 2016)*

It could be said that, in comparison to m-FTIR, the most appealing aspect of m-Raman is better image quality, which is notably useful for spotting extremely tiny microplastics (<20 µm). As described in this study, there is a critical need for a convenient and simple monitoring solution process of tracking smaller microplastics. Plastic particles subjected to environmental factors which make them to undergo repeated downsizing (photo-, thermal-, and biodegradation), resulting in a sharp rise in the number of particles for lower sizes. However, quantification analyses have widely ignored the lower size portion of MPs. There were 41 inspections of estuarine MPs in which plastic waste was captured via neuston meshes. Around 80% of these studies utilized nets with a very wide mesh size (300 mm), which completely missed the lower MP share and resulted in a significant miscalculation of the real MP concentration in aquatic habitats. Similarly, a three-decade assessment on MP abundance in the Baltic Sea employed a bongo net with a mesh size of 150 mm and reported no considerable rise in MP abundance between 1987 and 2015. For MP portions under 150 mm, the outcomes may have been varied. According to a research, 64% of tiny MPs (under 40 µm) gathered from the Atlantic Ocean and identified by m- Raman are under 40 µm in length, and their prevalence in the 10-100 µm range reflects a rate equation with a growth exponent of 1.96. These figures are backed up by a research that employed m-Raman to detect MPs (<400 µm) from the surface water of Plymouth Bay (UK), concluding that MPs smaller than 40 µm make up about half of the entire quantity (see Figure 23). In one experiment, MP pollution was discovered in all 38 types of bottled water (plastic, carton, and glass containers), with 80% of the particles detected in the 5-20 µm size category. The effectiveness of m-Raman for identifying tiny MPs is demonstrated by a contrast of FTIR and Raman methods for MP characterization. Since both approaches were perfectly capable for items bigger than 20 mm, m-FTIR had a low recognition performance level for smaller particles. MPs as tiny as 5 µm were identified by µ-Raman, while µ-FTIR overlooked every particles in the 5-10 µm size category and 40% of MPs in the 11-20 µm range of sizes. Furthermore, while µ-FTIR was successful in detecting certain MPs in the 11-20 µm range, the spectra's quality degraded owing to a poor signal to noise ratio, which was a direct result of the particle's size nearing the device's diffraction threshold. By examining the quantity of MPs present in wastewater samples collected upstream and downstream from the filtering station, the performance of an inventive post-filter system installed in the wastewater treatment plant of Oldenburg (Germany) was examined. MPs with a size more than 500 µm had a treatment efficiency of 100%, whereas those with a size less than 500 µm had a disposal efficiency of 93%. Considering that the filtering system is made out of pile material with a mesh size of 10-15 µm and that FTIR imaging cannot identify particles smaller than 20 µm, a considerable number of extremely tiny MPs may be slipping through unreported. As a result, m-Raman imaging tests should be used in conjunction with FTIR screening tests to effectively account for MPs in the lower length scale. Nevertheless, because to its complexity and long measurement duration, µ-Raman is not employed in the majority of MP identification investigations (14 percent according to a recent survey). (C.F. Araujo, 2018)

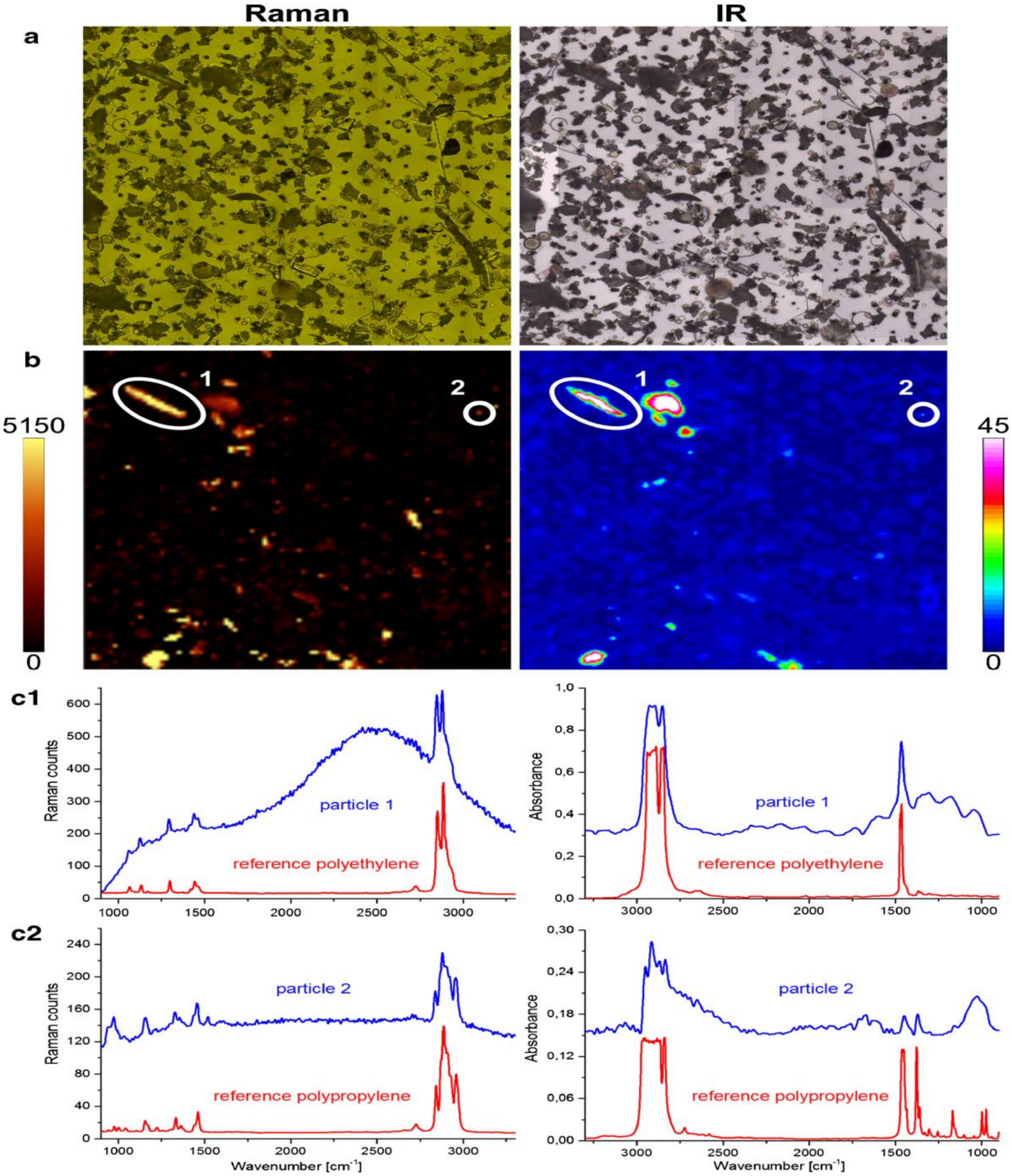
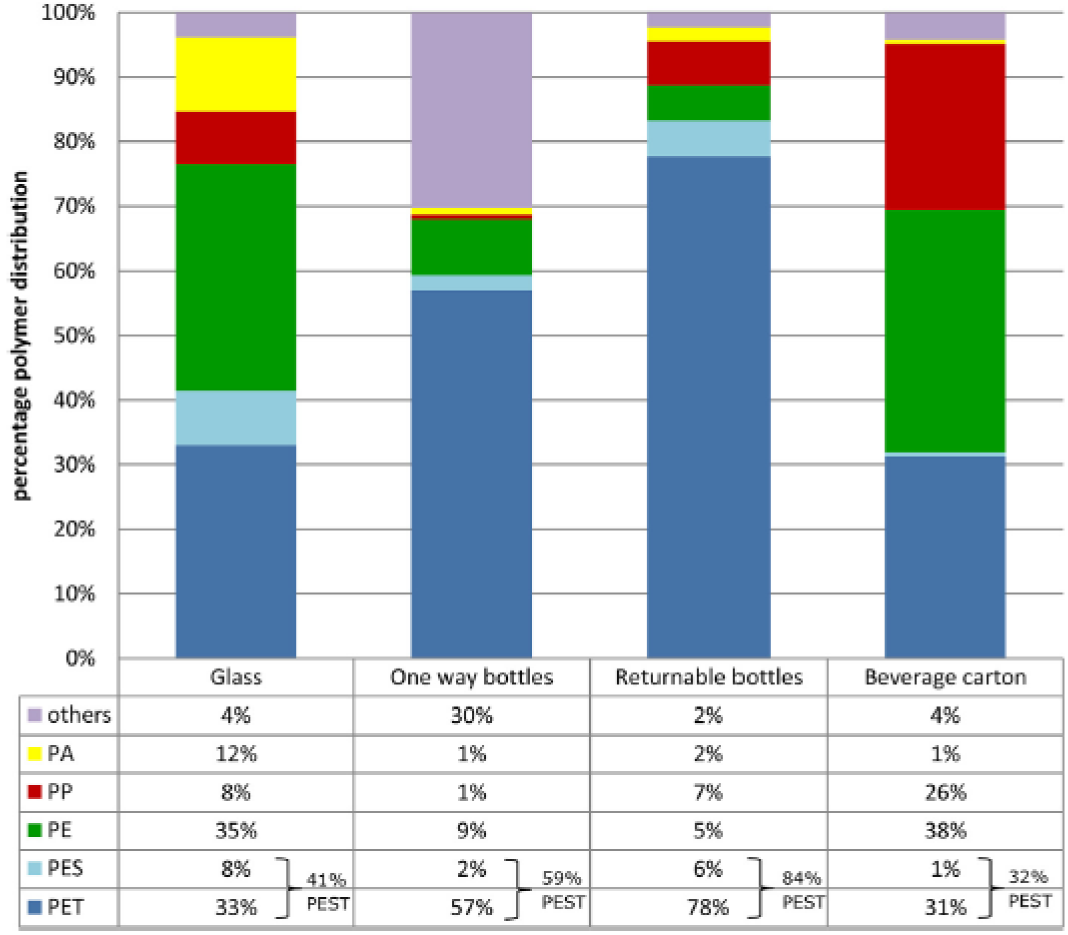


Figure 23. Demonstrates comparison between Raman and FTIR by examining two microplastic particles from costal area with size < 4mm. As seen from the images, Raman spectroscopy has more corresponding fingerprints to the referenced substances. Photo: *(A Käppler, 2016)*

Bottled water research is uncommon, and most experiments have relied on micro-Fourier Transform Infrared Spectroscopy (μ-FT-IR). This method's effort to discern particles smaller below 20 μm is a serious drawback. Micro-Raman spectroscopy, on the other hand, can identify much smaller particle sizes. As a result, it was demonstrated that the approach utilized in this work is very effective in detecting plastic particles in drinking water with size of particles in the low micrometer scope. The examination was about the outcomes of drinking water supplied in plastic bottles, glass bottles, and beverage cartons in this analysis (see Figure 24). Particles may be identified down to a length limit of 20 μm using μ-FT-IR spectroscopy. Because of its higher definition of up to 1 μm, μ-Raman spectroscopy may detect extremely fine microplastic particles that were previously undetected. The identification of extremely tiny microplastic particles, particularly in food inspection, is critical due to the potential effects on the body. Smaller particles are thought to be more easily swallowed by the digestive system than bigger ones. We examined the water's microplastic concentration after collecting it from German super markets in 22 distinct re - usable and single-use plastic bottles, 3 beverage cartons, and 9 glass bottles. In every kind of water, researchers discovered microscopic plastic particles as tiny as 50 micrometers and as little as 1-50 μm. A surprising 80 percent of the microplastic particles detected had a particle size between 5 and 20 μm, making it impossible to identify them using the analytical procedures previously employed in the research of concern. There were 118 ± 88 particles/l on mean in returnable bottles, but only 14 ± 14 particles/l in single-use plastic bottles for microplastics. Only 11 ± 8 particles/l of microplastics were found in the beverage cartons. Plastic particles in glass bottles were found to be much higher than expected among some (vary 0-253 particles/l, average 50 ± 52 particles/l). On contrary, μ-FT-IR has not been able to identify the majority of the microplastic particles since they are so tiny (less than 20 μm in diameter). (D. Schymanski, 2018)



	Glass		One way bottles		Returnable bottles		Beverage carton	
🟪 others	4%		30%		2%		4%	
🟨 PA	12%		1%		2%		1%	
🟥 PP	8%		1%		7%		26%	
🟩 PE	35%		9%		5%		38%	
🟦 PES	8%	41% PEST	2%	59% PEST	6%	84% PEST	1%	32% PEST
🟦 PET	33%		57%		78%		31%	

Figure 24. Shows the percentage distribution of polymer kinds which were found in various typed of water containers by application of Raman technique. Photo: *(D. Schymanski, 2018)*

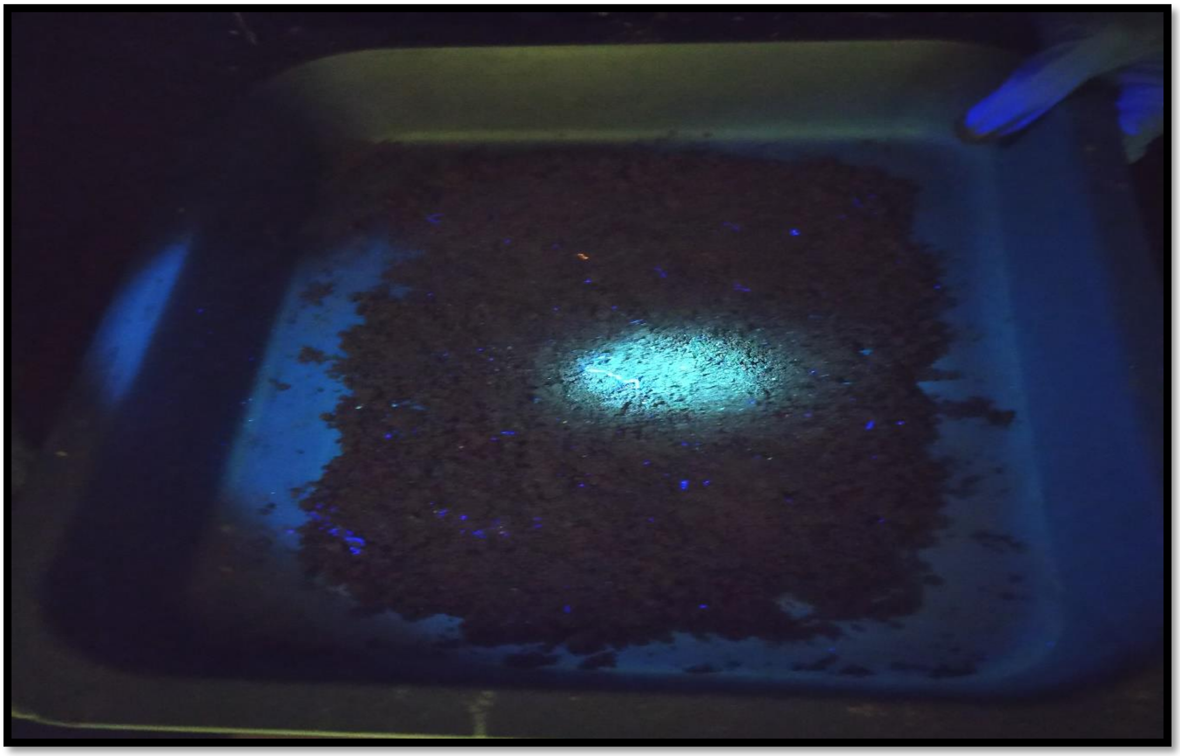
4. LABORATORY WORKS

4.1. Material and methods

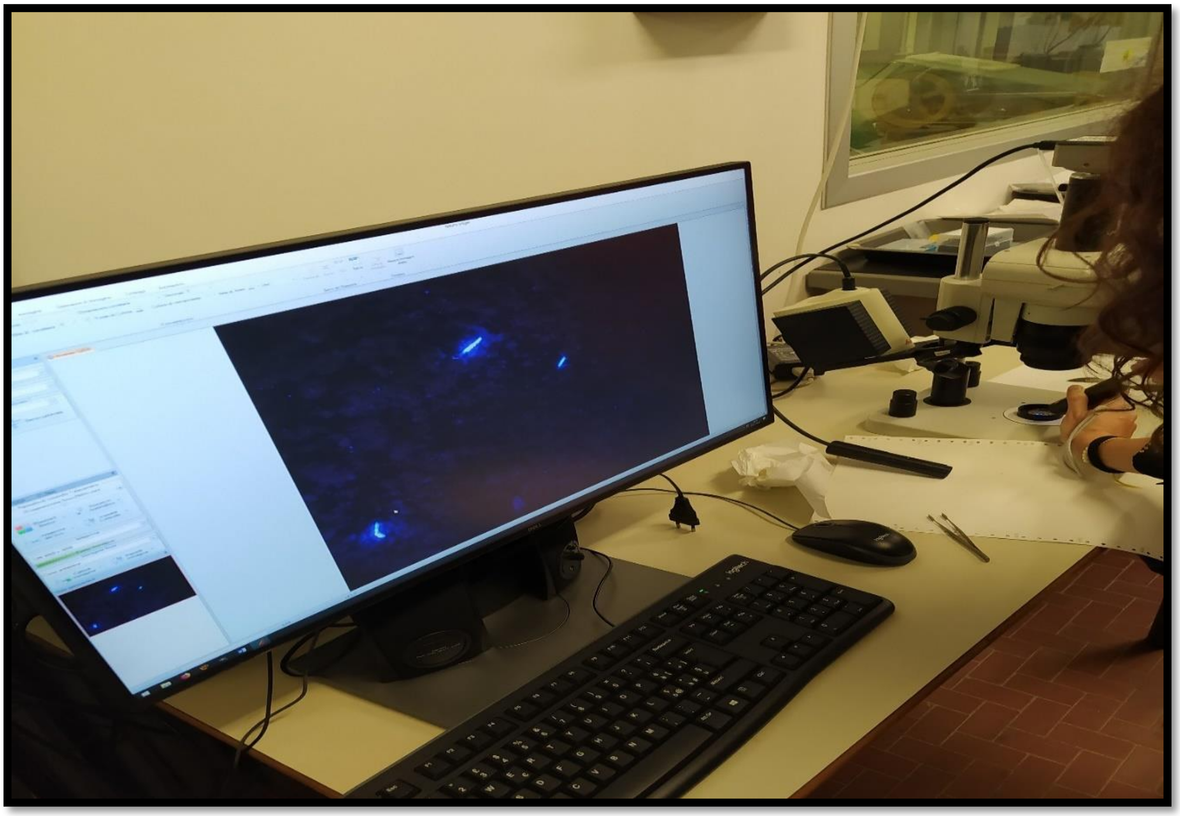
The sampled soil was taken from the Bossea show cave which is situated in Frabosa Soprana district of Piedmont region. It is a reserved natural site and the attendance can reach to 12000 tourists each year. There is only entrance to the cave and its premises are 2800 m long with a slight upward slope. There is also located so called “Bear Room” which depicts marks and remainings of bear (R.Bellopede, 2022). The used material in our experiments was both from the entrance and from the “Bear Room” section. Firstly, the sample was inspected by utilizing of microscope. Then, separation techniques as density separation and froth flotation were operated to acquire microplastis from soil matrix for the next stage. And finally, the outcomes of separation were investigated by microscope and its quantity and typology were reported.

4.2. Detection of microplastics

To start our procedures, we firstly wanted to make sure that if our sample contains any microplastic particles. For this purpose, we set up a configuration of microscope and computer. During observation, we applied UV lights to enhance fluorescence properties of microplastics. Consequently, we were able to detect a considerable amount of particles. There was a wide range of both particle sizes and colors. The next step was to operate the selection processes of our sample to choose required amount for further stages. We applied selection technique of dividing the sample to four parts by its volume and then mixing one couple of reverse parts and discharging the other couple. We did it 3 to 5 times until we had desirable final product. Then, we screened the sample to separate any present boulders or gravels in order to operate further only



with soil matrix. When screening was do ne, we put our specimen to oven for approximate of 5 hours to get rid of any hydration. After drying, the soil had better physical characteristics to detect possible microplastics. The weight of our determined specimen was 207.43 g.



4.3. Density separation technique

Our first way of approach was to define applicable separation methods to acquire microplastic particles for their future identification processes. Among the main techniques that stood out were density separation by mean of different solutions which was explained by (B. Quinn, 2017) and froth flotation defined by (G.A. Marques, 2000). We started our work with use of tapped water to separate MP from water by mean of density differences. After observing our sample with UV light we detected a reasonable amount of fibers. As the upper size limit for microplastics stands as 5 mm, we decided to separate our material by seven sieving values with 5 mm being on top. Table below demonstrates obtained acquired results after sieving which was processed by hands for 3 minutes.

5mm	2.46 g
2mm	42.18 g
1mm	46.9 g
0.5mm	52.58 g
0.212mm	34.19 g
0.063mm	22.29 g
<0.063mm	2.87 g

After sieving was finished, we sorted the samples with UV lights and come to conclusion that particles between 0.5mm and 0.212mm have the highest concentration of MP, so we moved forward with that range of material. The sample was placed in metallic pan with high area to height ratio. Next, we added tapped water and stirred the specimen for 3 minutes to allow any microplastic stuck between the soil grains move freely. Unfortunately, separation by regular water did not show any significant outcomes and was reported as having a very low separation efficiency. But the positive outcome was that we came to the common point of using the solutes to increase the water density of future experiments and to have a higher density gap.

4.4. Forth Flotation technique

As mentioned before, the method of using tapped water as a separation medium did not yield in high success, and the greater part of testing material left in the sink. Thus, we decided to apply another separation technique to our specimen which is called froth flotation. We started by drying our sample for excessive period of time in order to be certain that there was no humidity left.

Furthermore, the same division of sieving plates which is utilized for density separation was also selected for this operation. To the contrary, this time sieving machine was used for screening. It functioned 2 cycles of 5 minutes period each, with the operating power of 5 MW.

5mm	0.45g
2mm	23.88g
1mm	27.12g
0.5mm	46.23g
0.212mm	46.07g
0.063mm	46.43g
<0.063mm	12.23g

Sieving machine gave us different weight ratios compared to which we performed by hand, but the MP abundance was nearly similar, having the greater proportion in the samples between 0.5mm and 0.212mm. The contrast was that in this sieving there was also some MP in sample lower than 0.063 (in previous one there was practically nothing). Starting the flotation, we firstly

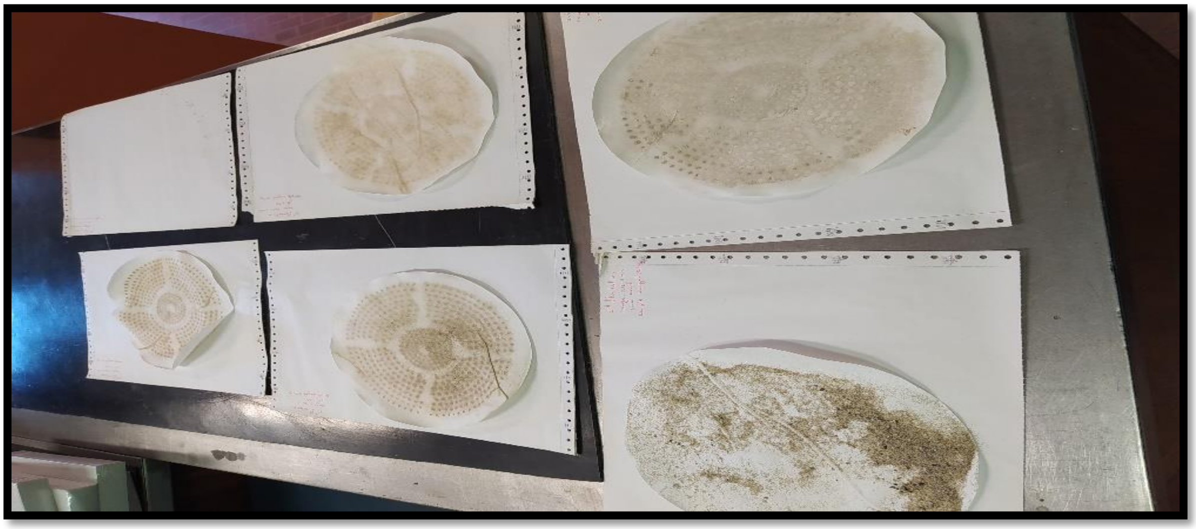
mixed 46.23g of 0.5mm and 46.07g of 0.212m samples, then we added them to beaker having 1L of water. Then we used stirring machine to effectively mix the suspension. Stirring machine functioned 5 minutes with the operating rate of 1300 rounds/minute. We placed our beaker in flotation machine and recorded the value of pH being 7.6 which meant that the solution medium is nearly neutral. After, we operated the flotation machine to generate bubbles into our solution and then added few drops of detergent as separating agent. Several seconds later, foaming process started occurring intensively and we utilized metallic pans to collect the loading excessive foam from top of beaker. In the end of procedure, we acquired 3 pans full of foam which was holding possible separated microplastics. Further step was to filter our samples, thus removing the foam and settling the microplastics on the filter paper. We also filtered the sink sample from the beaker in order to observe the amount of the residual microplastics. Finally, we placed the filter papers of foam and the sink of the suspension in oven for drying. After carefully inspecting both filter papers with UV light, we come to conclusion that the results of flotation was much better than water density separation, but still there was high portion of MP in sink part, and also the final product from foam filtering was not only MP, but it kept within the finest particles of soil as well.

4.5. Flotation technique with pH modification

The first flotation process was operated as trial in order to see if we are able to separate microplastic particles. Consequently, we obtained a considerable amount of desirable outcome.

Furthermore, we decided to enhance the potential of froth flotation technique by modifying its pH number. Since we had a substantial amount of microplastics left in the sink of previous test, we detached the dried sink from the filtering paper and sieved it again with two plates of dimensions 0.5 mm and 0.063 mm. Moreover, we wanted to analyze if the soil dimension effects the efficiency of flotation outcome. Thereby, we started by dimension fraction of 0.5 mm which was weighed as 54.23 g and added it to 1 L beaker full of water. Before operating the flotation process we put our sample in stirring machine for 5 minutes with the operating power of 1300 rounds/minute. Eventually, we performed the flotation with pH value being 7.6 and added a few drops of detergent as separating agent. After we collected all outgoing foam, we started to steadily adding our base agent CaO until the value of pH reached near 11. Similarly, we add a few drops of detergent to initiate the flotation and then collected all outgoing foam to metallic pans. Finally, we filtered foams from each pH recordings and also the residual sink from the flotation tank. Exactly the same process was run for the soil having the dimension of 0.063 mm and all of them were put in oven for drying. After close inspection of all six filter papers we came to conclusion that solutions with higher pH value led to a greater number of microplastics being separated. Additionally, the higher pH number forced more soil grains to move into foam formation as the weights of filter papers were significantly various. In this method we tried to change the values of solution pH in order to observe the differences in MP accumulation. We used CaO as the base agent. Before adding CaO, the pH value was 7.6, and after supplement of 8.36g and 8.11g of it, the pH was around 11. Also we used 2 samples, and did 2 rounds of flotation, because MP distribution was different. The sample was sieved by the plates of dimension 0.5mm to 0.063mm.

	Weight	pH	Sieve size
Filtred foam before adding CaO	0.48g	7.56	0.5mm
Filtred foam after adding CaO	0.27g	11.03	
Sink	54.23g		
Filtred foam before adding CaO	0.54g	7.35	0.063mm
Filtred foam after adding CaO	1.22g	11.06	
Sink	7.23g		



Practically we were not able to measure the weight of MP from the foam, so to distribute the correlation between them. Although, while observing with UV, the outputs with higher pH had greater concentration of MP.

4.6. Density separation and quantification by microscope

Not having the precise results to report from our previous experiments led us to this technique. We started our study by taking new portion of testing soil which was from the entrance of Bossea cave. After its division in four parts and combining the opposites with removing the other couple, we acquired our needed amount to proceed as we did in the first experiment. After drying the material, we screened it with UV lights and come to conclusion that we have enough amount of MP to move forward. We used five beakers having 100 ml of volume for the samples. To increase the density gap of medium, we added NaCl to the tapped water until the solution was saturated. We poured 500 ml to 1 L beaker and started to constantly adding NaCl salt with applying of continuous stirring by hand. As we see from the graph of NaCl saturation in water, we observed that 125 g was required quantity. Then, we transferred our solution to graduated cylinder and used densimeter to record its density. As was mentioned by (B. Quinn, 2017), the density of solution was around 1.2 g/cm^3, as in our case we measured it being 1.22 g/cm^3. The determined amount of soil for each beaker was 10 g. Further, we started to pour 100 ml of our solution to each beaker and stirred every of them for 3 minutes by hand. Onwards, we waited until the precipitation of soil grains was over, after about 24 h, and each sample was inspected by UV light to detect microplastics. Surprisingly, there was a considerable number of microplastic particles that was both on the surface and within the solution. The next step was to filter the supernatant, using a vacuum filtration system. Thus, we utilized the pipette to gather the upper part of solution and then conveyed it to paper filter. After applying this process to each sample, we put the obtained filtered papers to oven for its drying. Additionally, we put 10 g of soil to 1 L beaker and poured 10 ml of 10% HCl strong acid in order to purify the sample and remove all present organic substances. Similarly, we added water and stirred the sample, and consequently filtered it and put in oven with other filtered papers. After counselling with professor, we come to agreement to proceed with the microscopic quantification. The aim was to record every detected microplastic per each displacement of microscope round. Thanks to using the linked computer, we were also able to measure the length of each detected particle. To start the experiment, we divide each filter paper in halves to fully scan every point under the microscope. Then, we accurately used pincers to place filtered paper on microscopic glass kit and strengthen its position by using scotch on each end of glass kit. During the inspection we recorded both the quantity and dimensions of every particle. After processing the filter papers through microscope we defined

many MP with different dimensions. We placed UV lights over our sample during the microscope observation to have concrete determination of MP.

We decided to use 125g of NaCl and 500ml of water solvent to have higher density of 1.22, and to yield more MP because of greater density difference. We took 5 beakers with volume of 100ml and added about 10g of sample to each. After stirring 3 minutes, we set our samples to submerge for 2 hours. After we used pipette to capture the upper part of solution and filtered through the filter papers, and then placed them to oven for drying. We also took additional sample of 10g to purify with 10ml of 36 vol H_2O_2 solution, in order to eliminate all possible organic matters, and then added 100ml of water. After stirring and keeping it for 2 hours of time, we also filtered it and put in the oven.

Sample 1	10.04g	100 ml solution	3 minutes stirring
Sample 2	10.06g	100 ml solution	3 minutes stirring
Sample 3	10.10g	100 ml solution	3 minutes stirring
Sample 4	10.30g	100 ml solution	3 minutes stirring
Sample 5	10.06g	100 ml solutiom	3 minutes stirring
Sample 6 (purified)	10.08g	100 ml solution+10ml HCl	3 minutes stirring

The other aspect that we wanted to investigate was to determine if either there is a link between particular part of cave and the presence of microplastics. Previously, our sampling material was from the entrance of Bossea cave, where the gathering of tours is located, so there is an immense number of people. On contrast, in this experiment we used soil from the middle point of cave. Similarly to preceding operation, we took 6 beakers and put in each around 10 g of material. The solution of NaCl was applied to all of them, and 10 ml of 10% HCl was added to the last sample for purification purpose. After filtering and drying in oven, we processed with the scanning of filter papers through microscope in order to detect and note the amount and size of microplastics.

5. RESULTS AND CONCLUSION.

We started by analyzing the outcomes of material which was sampled from the entrance of Bossea cave. Surprisingly, there was a remarkable number of microplastics, varying in wide size scope. The total quantity of detected microplastics was 100 in just 60 g of soil. The tiniest particle was recorded as 0.1 mm. Although, there were many other small fragments, we decided to take into account only those which had distinct microplastic structures, thus eliminating the possible overestimation of polymers by organic substances. The longest fiber that was present had a length of 2.3 mm. The dominant size scale was the fibers which were longer than 1mm. As expected, application of purification procedure to the last sample resulted in relative reduction of particle number, and dramatic absence of the ones from the finest range.

MP size	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6(purified)	Total MP
0.1mm	6	0	1	1	0	0	8
0.2mm	5	4	1	0	4	1	15
0.3mm	4	3	4	1	2	1	15
0.4mm	4	1	0	1	1	1	8
0.5mm	2	0	2	0	2	2	8
0.6mm	3	2	0	1	0	0	6
0.7mm	2	1	0	0	0	1	4
0.8mm	1	0	1	3	0	0	5
0.9mm	1	2	2	1	2	0	8
1mm	1	0	0	0	1	0	2
Above 1mm	4	4	4	1	6	2	21
Total MP	33	17	15	9	18	8	100

Speaking about the specimen from the middle part of the cave, there was a considerable decline in total quantity of particles, establishing in number of 74 and being lower by 24% than the previous experiment. Once again, the tiniest fragment had a length of 0.1 mm. On other hand, the longest fiber was reported as 5.4 mm which is around the size limit between normal plastic and microplastic. It was clearly demonstrated that in the middle part, there is superiority of the fine microplastics which was recorded as having the total amount of 21 fragments. Even after the purification process, there were still present tiny particles. This trend was totally opposite from which the entrance of cave showed. Likewise, it provides a significant prove to the studies of (CM Rocman, 2019) which noted that plastic materials which enter and carried out among the environmental surroundings are going through the degradation and decreasing in size scale.

MP size	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6(purified)	Total MP
0.1mm	7	6	4	0	0	4	21
0.2mm	1	7	2	1	0	0	11
0.3mm	4	1	2	2	0	1	10
0.4mm	2	2	2	0	1	1	8
0.5mm	2	0	2	0	2	1	7
0.6mm	0	1	0	0	0	0	1
0.7mm	0	1	3	0	1	1	6
0.8mm	0	0	0	0	0	0	0
0.9mm	1	0	1	0	0	1	3
1mm	0	1	0	0	0	0	1
Above 1mm	0	2	0	3	1	2	8
Total MP	17	19	16	6	5	11	76

In conclusion, we were able to detect and separate the microplastic particles from soil matrix. Although, in some cases it was really difficult to differentiate whether it is polymer or organic substance, there were definite particles that illustrated strong fiber characteristics. Another drawback was that every procedure demanded high waiting time, so overall the process period was very high for separation and detection of fairly low amount of soil. Accordingly, it would be really complicated to apply separation techniques for large area purposes. Thus, we should firstly try to prevent microplastic accumulation in natural environments and provide global regulations for both manufacturers and consumers. As it was discussed, the term of microplastic being established as a contaminant to the environment is a relatively novel term, and number of researches and studies regarding to this issue is growing adequately greater. Thus, there is a lack of commonly accepted treatment procedures that then can be handful for compering the result and trying to increase the performance and efficiency of the investigations. We detected a great number of microplastics just from the show cave soil, so it can be predictable that their presence in more visited areas of natural environment sites is ten or even hundred folds higher. It became pretty obvious that the detection of microplastic particles is very complicated, and if this growing trend continues, it can lead to irreversible results both to natural habitats and humans. On other hand, microplastic is not an invisible and unknown enemy anymore, and larger number of communities and organizations starts to rise concerns about its future impacts and possible present hazards.

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