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Identification of Micropolymers in Gray water

Primary Supervisor: András Sebők

Assistant researcher

Author: Orekoya Adedapo

CFO83P

Institute of Environmental Sciences

Szent István Campus, Gödöllő

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List of Abbreviations

MP - Microplastics

KOH – Potassium hydroxide

H_2O_2 - Hydrogen peroxide

HNO_3 - Nitric acid

CH_2COOH - Acetic acid

PETE / PET - Polyethylene terephthalate

HD-PE - High-density polyethylene

PE-LD - Low-density polyethylene

PP – Polypropylene

NOAA- National Oceanic and Atmospheric Administration

SEM – scanning electron microscope

FT-IR - Fourier-transform infrared spectroscopy

PY-GC – MS - Pyrolysis–gas chromatography–mass spectrometry

UV - Ultraviolet

PCBs - Polychlorinated Biphenyls

PAHs - Polycyclic Aromatic Hydrocarbons

MSFD - Marine Strategy Framework Directive

EDS - Energy Dispersive X-ray-Spectroscopy

ATR - Attenuated Total Reflectance

WWTP - Wastewater Treatment Plant

CHAPTER ONE

I. Introduction

Plastic material demand and production have surged as a result of industrialization over the past century (Rillig, 2012)**.** However, improper recycling and the resulting fragmentation of plastics have led to the creation of microscopic plastic particles which are also described as microplastics (MPs). Microplastics (MPs) can be referred to as "synthetic solid particle or polymeric matrix, with regular or irregular shape from 1 µm to 5 mm size of either primary or secondary manufacturing origin" (Hara et al. 2020). They are now a reported problem globally, with reports of their occurrence in all components of the environment (Beaurepaire et al. 2021)**.** The primary concern regarding the threats posed by MPs is their impact on organisms and human health, which can be ascribed to their small size, high surface area to volume ratio, and their capacity adsorb or release pollutants (Jahnke et al. 2017a). These resulting microscopic plastics cause biotic interactions because they are widely distributed in freshwater, marine, and terrestrial ecosystems worldwide. Although these MPs do not instantly impact living things, prolonged exposure to them has a number of harmful effects through a variety of methods, including the ingestion of poisonous chemicals, inflammation caused by the sharp edges of the MPs, and subsequent blood flow changes (Jingyi Li et al. 2018). In order to comprehend their existence in the environment, MPs monitoring is crucial. To extract, identify, and quantify the particles dispersed in environmental matrices for microplastic investigations, multiple methodological approaches are needed. However, to eliminate it and release particles into environmental systems with a high organic matter content, a chemical digestion process is necessary. During the density separation stage, organic residues may coalesce with MPs due to their comparable densities, creating challenges in collecting and quantifying particles (Q. Li et al. 2019). Treatments for organic digestion may use oxidizing agents, acids, fundamentals, or enzymes (Löder et al. 2017). Although enzymatic digestion is effective, it has a significant drawback related to the expensive cost of enzymes, which could be a limiting factor even for small-scale operations. Therefore, it requires post-treatment such as alkaline to eliminate any remaining organic matter (Ribeiro-Claro et al. 2017). It is crucial to note that not all procedures can effectively remove organic matter from polymers without causing damage. In addition to efficiently destroying biogenic organic matter, the applied protocols must leave synthetic polymers unaffected in terms of their weight, volume, shape, and

color if necessary. However, despite their widespread use for chemical or pretreatment digestion, the impacts of various chemical agents such as alkaline, bases and acids on the chemical characteristics of MPs have not been extensively investigated. By conducting extensive research on the changes induced in the chemical structures and morphologies of MPs there is potential for enhancing the detection and analysis of MPs by incorporating pre-treatment methods in the future. This research study aims to investigate the effects of oxidative, acidic, and alkaline agents on the morphology and chemical characteristics of MP samples.

Problem Statement:

Despite research on chemical digestion methods for microplastic (MP) analysis, there is no consensus on the most effective method or standardized procedures. Consequently, it is important to minimize the impact of the chosen method on the MP particles being isolated for identification and characterization. The study aimed to evaluate the effects of four treatment agents (hydrogen peroxide, acetic acid, nitric acid) on four types of MP particles. The research examined the ability of each treatment to recover a known quantity of particles and the impact on identifying polymer type using a microscope.

Research Questions:

- Has our protocol caused degradation or fragmentation?
- How many particles were overlooked?
- How much have we falsely identified?

Objectives:

- Effect of digestion procedure on the properties of Microplastics.
- Establish a uniform method for examining microplastics extracted from environmental samples.

CHAPTER TWO

II. Literature Review

Due to its cutting-edge technology, low cost, and wide range of applications, Plastics are among the most commonly utilized substances globally (D'ambrières, 2019). Estimates indicate that the production of plastics will reach 33 billion tons by the year 2050. (Barrows et al. 2017). The global plastic production surged by a factor of 25 in 2015, rising from an estimated 331 million tonnes to 7.82 billion tonnes (Geyer et al. 2017). According to current forecasts, the amount of plastic produced has been growing exponentially and will triple by 2050 (Geyer et al. 2017). Plastic is used in practically every facet of modern life today, including packaging, technology, clothing, and transportation. Packaging is the most common use for plastic, making up more than 40 percentage of plastic manufactured (Lusher et al. 2017a). Regrettably, a significant proportion of plastic products we use are disposable, and the necessary regulations to promote a circular economy and halt their unrestrained growth have not been enforced (Barnes et al. 2009).

Plastics are a diverse group of synthetic and semi-synthetic materials that exhibit qualities such as strength, low cost, light weight, and resistance to corrosion. They also have excellent electrical and thermal insulating capabilities (Laist, 1987). Because of these qualities, plastics have been a priceless resource for civilization with a wide range of uses (Andrady, 2017). However, several features of plastics that contribute to their versatility also allow them to persist and accumulate in the natural environment (Laist, 1987). The widespread use of disposable plastics, inadequate recycling, and improper disposal of plastic waste exacerbate their resistance to decomposition, which is a direct result of the durability of plastics (Cole et al. 2011).

Plastic pollution can harm wildlife once it enters the environment by posing physical hazards (such as entanglement and ingestion) as well as chemical hazards (for example desorption of pollutants) (Li et al. 2016; Wright & Kelly, 2017). Because of this, the general people, scientists, and the government have expressed worry. Around the world, reports of plastic pollution have been made in a variety of environments, such as Arctic seas (Lusher et al. 2015) and ice (Obbard et al. 2014), tropical regions (Lima et al. 2014) and deep-sea sediments (Claessens et al. 2013). During beach clean-up efforts across the globe, commonly discovered types of plastic debris include macroplastics - larger items like plastic bags and water bottles. (Cole et al. 2011). Furthermore, to being unsightly, plastic garbage can have negative economic effects on the environment, including

increased costs for cleanup, decreased fish stocks, and decreased tourism (Lusher et al. 2017a). Additionally, there may be detrimental ecological effects that researchers are still trying to fully comprehend (Browne et al. 2007; Wagner et al. 2017). Microplastics are less obvious but readily available for ingestion by a larger range of organism than macroplastics, which are more obvious (such as strewn plastic bottles and bags) (Hantoro et al. 2019). Because of this, current attention has focused on the possibility that microplastics could enter the food chain (Jiana Li et al. 2016).

Plastic materials have the potential to be transported through various means such as wastewater discharge, surface water runoff, river systems, and atmospheric deposition as aerosols, claim Kooi et al. in 2016. Thus, one of the main sinks for waste plastic trash has been the ocean (Andrady, 2017). According to Floyd & Floyd (2016) the amount of plastic waste entering the ocean annually is rapidly increasing, with estimates ranging from 4.8 to 12.7 million metric tons. The waters may contain more plastic than fish by the year 2050. These debris are common in the maritime environment and pose a serious threat to the ecology, according to Kroon et al. (2018). Smaller bits of plastic, however, may have a variety of as-yet-unknown consequences on aquatic ecosystems, despite the recent focus on large plastics (Cole et al. 2011).

II-1. Sources of Microplastic (MP)

Direct release of microplastic particles into the environment is possible, and the first transit method is dependent on the source (Birch et al. 2020). *Figure 1*. displays the major global sources that have been discovered. Among them are synthetic plastic pellets (0.3%), microbeads (2%), marine coatings (3.7%), road markings/dust (7%), city dust (24%), tire wear (28%) fabrics (35%). (Boucher & Friot, 2017). Microplastic contamination originates from both point and non-point sources, just as other aquatic contaminants. Wastewater treatment plants (WWTP), industrial facilities, and cracking plants are examples of point source pollution sources (Birch et al. 2020).

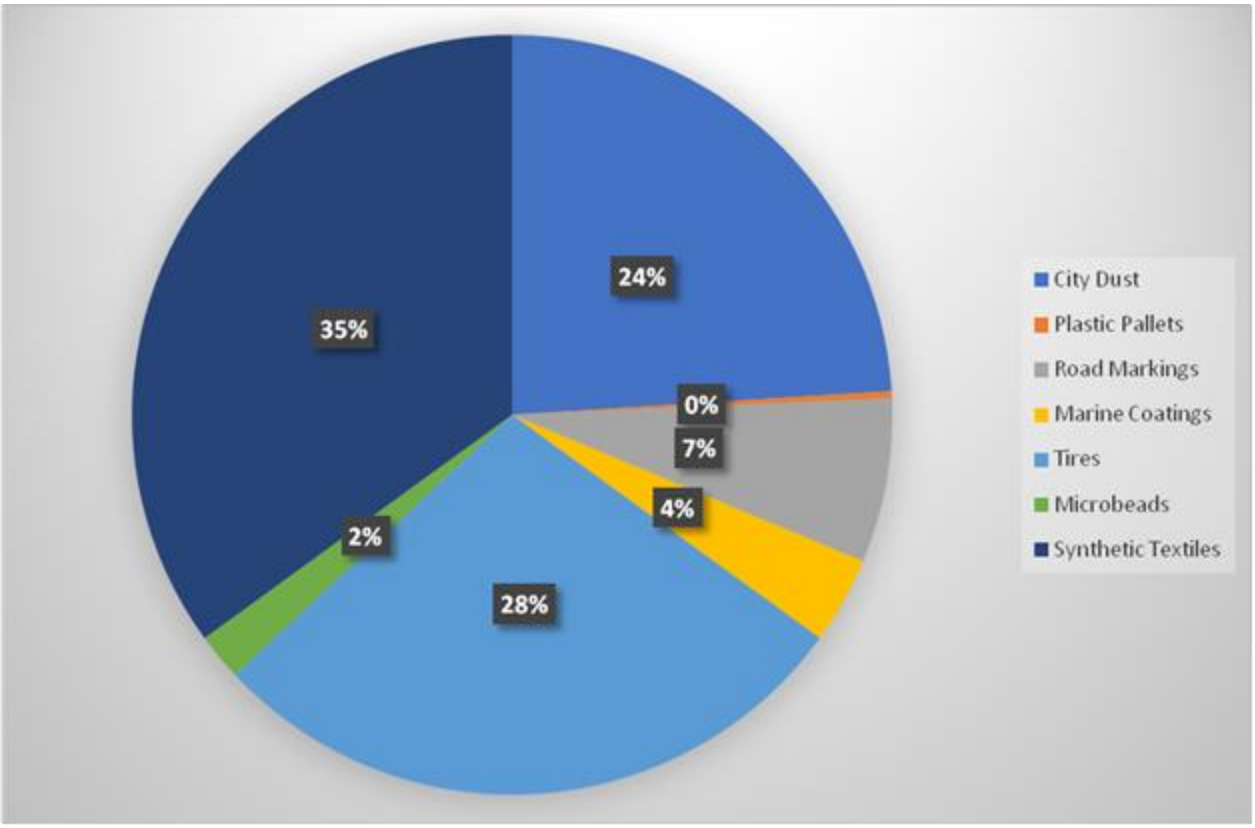

Figure 1: Major global sources Microplastics (Birch et al. 2020).

The term "city dust" denotes a set of widespread sources observed in urban regions, including microplastics generated through wear and tear, weathering, and shedding. These sources comprise microplastics arising from infrastructure like household dust, synthetic turf, and building coatings, as well as microplastics produced during abrasive blasting and powder handling. City dust also includes microplastics originating from the abrasion of synthetic footwear soles, plastic utensils, and infrastructure (Birch et al. 2020).

The release of Mps into rivers is caused by variety of sources, such as tire wear and tear, washing synthetic clothing, city dust, Industrial wastewater and the runoff from urban areas (Prata, 2018). Household garbage has the potential to contribute to microplastic pollution when it is released as wastewater. This liquid waste is transported via pipes to sewage treatment plants located underground; however the treatment procedure does not always completely remove all microplastics (Mason et al. 2016). Microplastic pollution from WWTP effluent consequently gets into the waterways. Microplastics, due to their small size and durable, lightweight materials, have the ability to travel long distances through wind and water currents. The common plastics which

can be found in freshwater are polypropylene (PP), polyethylene (PE), and polystyrene (PS). Microplastics have been discovered in all oceans, as well as in every Great Lake. Furthermore, rainwater frequently causes sediments to be carried into rivers, with biosolids containing microplastics being applied to the soil as a significant source. (Dris et al. 2015). Similar to other types of microplastics, biosolids that contain microplastics can release harmful metals into the environment in trace amounts. (Wijesekara et al. 2018).

II-2. Classification of Microplastics

Microplastics are tiny plastic particles that have a size less than 5.0 mm and are made of polymers. (Law & Thompson, 2014). These smaller plastics could be the result of UV light, weathering, corrosion, and breakdown into microplastics in the water (*Figure 2*.). A primary source of microplastics is industrial production, which also produces exfoliating face scrubs, toothpaste and resin pellets. Total surface area grows as weathering and fragmentation of MP particles (Schwarz et al. 2019). MP surface area can regulate biofouling rates this, in turn, impacts the density of microplastics and the rate at which they settle in sediments (Jahnke et al. 2017b). Although the rate at which additives released from the particle or at which contaminants sorb to the particle may both be accelerated by increased surface area (Bouwmeester et al. 2015). The chemical composition of the MP particle can differ significantly from the original microplastics because of the absorption and release of hydrophobic molecules and additives from the surrounding environment.

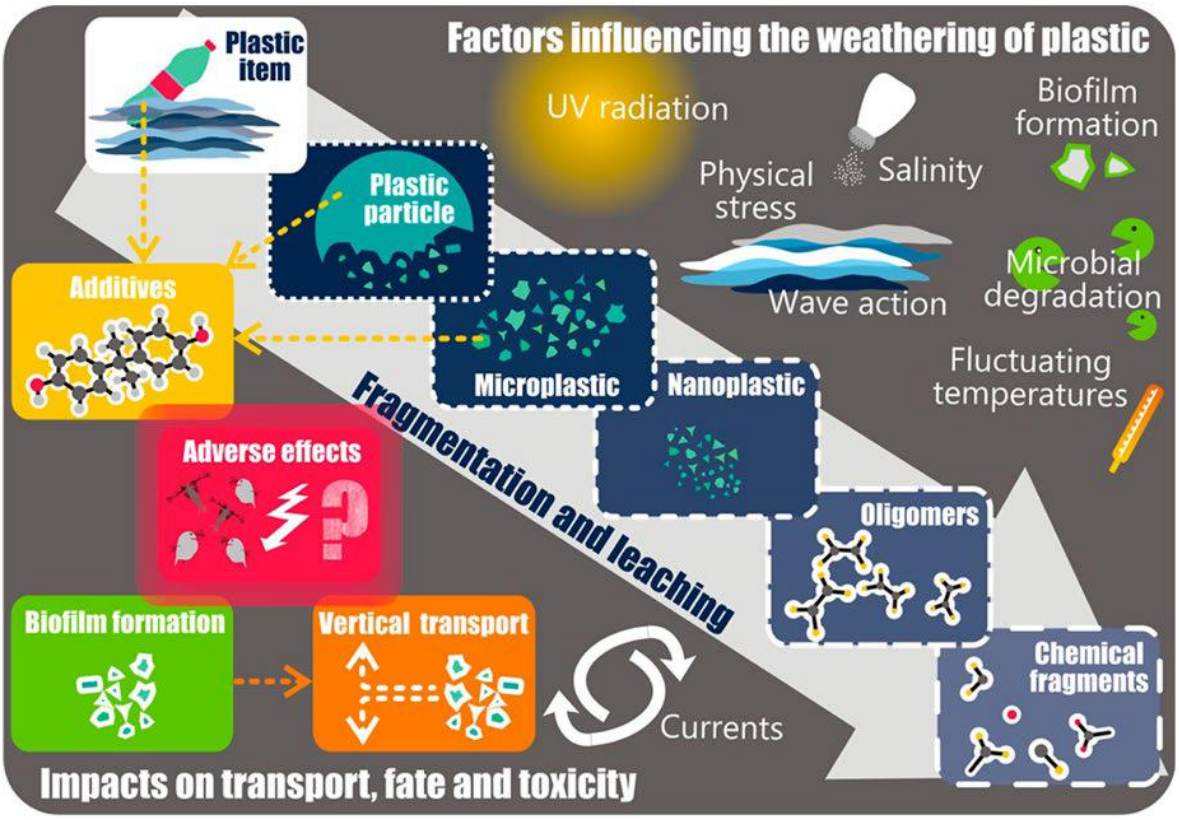

Figure 2: Factors that influence the weathering of plastic in the marine environment (Jahnke et al. 2017b).

II-2.1. Based on Sources

Microplastics can be classified into two types, primary and secondary, based on their source. Primary microplastics are plastic particles that are intentionally produced at the microscale for use in consumer products. Until 2018, when the Microbead-Free Waters Act was passed in the US, microbeads were commonly used as exfoliants and abrasives in personal care products such as face shampoo, scrubs and toothpaste (Schwaferts et al. 2019). Pre-production plastic resin pellets, also known as nurdles, are considered as primary microplastics. These are plastic particles that are created at the microscale specifically for use in various consumer goods (Zbyszewski et al. 2014). Additional uses for primary MPs include sandblasting media (Eriksen et al. 2013). Primary nanoparticles (NPs), despite being smaller, are also utilized in several diagnostic procedures, research settings, and cosmetic items. In general, primary particles are any manufactured particles that are released into the environment as MPs or NPs (Fox, 2021).

However, secondary MPs are created when bigger plastic products are continuously degraded in the environment by mechanisms that alter the structure and reactivity of the polymer (Gigault et al. 2016). Polymer degradation can result from a variety of processes, including hydrolysis, photodegradation caused by UV exposure, abrasion, temperature changes, as well as biological and chemical degradation. The effects of several stressors may coexist. As an illustration, extended exposure to UV can make polymers more brittle due to structural changes (such as the development of carbonyl), which accelerates mechanical breakdown (Mattsson et al. 2015). Certain stresses can affect some polymers more than others. More susceptible to UV breakdown are popular polyolefins like PE and PP as well as PS (Song et al. 2017). Secondary MPs can be produced in high quantities by polymer weathering; subtropical gyres have been observed to contain up to 106 MP particles/km^2 (Jambeck et al. 2015; Law et al. 2010; Law & Thompson, 2014). Although primary MPs usually contain PS, PE, and PP, secondary Microplastics mostly contain more than one polymer (Birch et al. 2020; Browne et al. 2007).

II.2.2. Based on Size

Plastics can be classified based on their size as nanoparticles (100nm), microparticles (0.0001–5mm), mesoparticles (5.00–25mm), and macroparticles (>25mm) (Fok et al. 2020; Windsor et al. 2019).

II-3. Ecological effect on Organism

Microplastics affect the biota in several ways, which includes through consumer ingestion, the delivery of toxins to species and the creation of novel habitats. Because microplastics are tiny in size, they can be ingested by various organisms of different sizes and positions in the food chain. (Barnes et al. 2009; Wright et al. 2013). Direct consumption refers to the act of a prey item intentionally or unintentionally ingesting a microplastic particle (Pradit et al. 2021; Usman, 2007). Misidentification on the other hand occurs when fish consume microplastic that appears similar in size or color to their natural prey. (Pradit et al. 2021). Meanwhile, biocontamination or biofouling occurs when organic debris and microbes accumulate on plastic particles, resulting in changes to the material's appearance and chemical composition. In any case, MPs are likely to be mistakenly recognized when biofouling is present (McCormick, 2015).

At an individual level, consuming microplastics can directly harm organisms due to the physical presence of microplastics and the potential attachment of pollutants. Microplastic fibers can clog filter-feeding systems and lead to suffocation in organisms (Grigorakis & Drouillard, 2018). Microplastics are materials that cannot be digested, and if they are large enough in the stomach, they can create a sense of fullness that is not appropriate. Additionally, particles may cause blockages or expansion of the intestines. (Grigorakis & Drouillard, 2018; McNeish et al. 2018; Norland et al. 2021). Moreover, obstructions and damage to the digestive system may result in less efficient energy absorption, which could affect how the organism allocates its energy and grows (Grigorakis & Drouillard, 2018). Microplastic particles with a size smaller than 150 μm can potentially penetrate the intestinal barrier and enter the bloodstream, where they may accumulate in other tissues. (Rochman et al. 2014; Zipp, 2022).

Microplastic also has ecological implications due to its function in the transportation and release of pollutants (Teuten et al. 2009). Although microplastic polymers are generally considered biologically inert, they can be associated with biologically active compounds. Plastic production often involves the use of chemical additives such as phthalates, bisphenol A, and chemical dyes to modify the material's properties, such as its flexibility or color (Hahladakis et al. 2018). When microplastics degrade in water or sediment, the additives used in their production are released into the environment. These chemicals, such as phthalates, bisphenol A, and dyes, which are added to plastic during manufacturing to alter its properties, can be very harmful when ingested and can seep into the digestive tract (Engler, 2012; Rochman et al. 2014).

The endocrine system, for instance, has been discovered to be disrupted by styrene which is a monomer contained in several popular plastic types, including polystyrene. The functioning of an organism's immune system and reproductive system depends on the endocrine system (Rochman et al. 2014). Behavioral changes and weight loss have also been connected to polystyrene, a kind of styrene (Besseling et al. 2013; Smith et al. 2018). Fish have been identified as "sensitive indicators" of endocrine disrupting chemicals (EDCs) in laboratory investigations because exposure to these chemicals can lead to changes in gonadal development, gonadal degeneration, sex-specific gene protein activation, and the prevalence of intersex in these animals (Rochman et al. 2014). Being a stress multiplier, microplastics are a problem in terms of ecotoxicology. In

addition to their negative consequences, they also pass along a cocktail of toxins to the fish (Rochman et al. 2014).

Microplastics can attract and accumulate other hydrophobic compounds due to their own hydrophobic nature. According to Rochman et al. (2013), microplastic is linked to more than 78% of priority pollutants. Hydrophobic substances such as persistent organic pollutants, organochlorine insecticides, polybrominated diphenyl ethers, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), heavy metals, pharmaceuticals, and petroleum hydrocarbons can be absorbed by microplastics. (Grigorakis & Drouillard, 2018; McNeish et al. 2018; Rochman et al. 2014). After microplastics with attached hydrophobic chemicals are consumed, the chemicals may detach in the digestive tract and then be assimilated by the animal's tissues (McNeish et al. 2018)

The process of chemical sorption and release into an organism's body depends on both the type of pollutant and the body composition of the organism. Moreover, the characteristics of the microplastic particles, including their size and polymer type, also influence the chemical sorption and release process. These factors play a role in the kinetics of chemical sorption and release into the body, both when microplastics are ingested and when biofilms form on the plastic surface (Rummel et al. 2017). The toxicity of aquatic species to microplastic exposure can be attributed to many of these additives being harmful (Groh et al. 2019; Johnson, 2021; Lithner et al. 2012).

II-4. Effects on Humans

The harmful consequences of MP exposure on humans have also been studied. At present, humans primarily come into contact with microplastics through ingestion and inhalation (Browne et al. 2007). An estimated 11.000 MP particles are consumed annually by humans. According to Heddagaard & Møller (2020), a significant portion of the Microplastics that are consumed are thought to be caused by food contamination, especially seafood, during preparation, processing, and shipping. Some estimates suggest that human exposure to microplastics could be as high as 121,000 particles per year when taking into account the inhalation of microplastics in the ambient air (Cox et al. 2019).

Microplastics can contaminate the air in enclosed spaces due to the breakdown of synthetic textiles and fibers. Therefore, it is evident that most people today consume huge amounts of microplastics,

the effects which are mostly unknown. Given the large amount of MP particles that people consume annually, it is imperative to assess the danger of exposure to the human tract. Although few, non-human mammal in vivo MP exposure studies may be crucial for establishing the toxicological risks that MPs represent to human health (Heddagaard & Møller, 2020).

Although human cells exposed to MPs and NPs in vitro showed decreased cell viability, it is still unclear to what extent these findings suggest a risk to the general health of humans (Barboza et al. 2018; Cox et al. 2019). Moreocer, there are limited controlled studies on the impacts of plastic particles on human health, toxicity related to plastic particles is observed in health data from accidental inhalation in industrial environments and patients who were injected with NPs during therapeutic procedures as drug delivery vehicles. (Kögel et al. 2020).

Similar to this, it has been shown that persons who use plastic prostheses accumulate plastic particles in their lymph nodes (Kögel et al. 2020). There is still much to learn about the danger that plastic particles, particularly NPs in the human gastrointestinal system represent to human health, and plastic particle toxicity is now a developing area of toxicology (Fox, 2021).

Microplastics can pose a potential threat to human health and other living organisms due to the release of plasticizers, antioxidants, and other compounds. Furthermore, microplastics can absorb organic carcinogens such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and organochlorine insecticides from the environment (Lehner et al. 2019). After being ingested, these chemicals and additives can become detached from plastic particles, turning MPs and NPs into potential entryways for carcinogens and other hazardous materials. Overall, microplastics might still turn out to be safe, but making that assumption before we understand how they interact with all physiological systems is improper and wrong (Fox, 2021).

II-5. Sampling Technique:

For years, studies on microplastics have been carried out, but there is no standardization of methods for sample collection, pre-treatment, quantification, and identification. (Jingyi Li et al. 2018). Following consideration of the matrix type (water, sediment or biota) to be tested for the presence of microplastics, various sample techniques may be used (Selected sampling, bulk sampling and volume-reduced sampling) (Razeghi et al. 2021).

II-5.1. Selective sampling

Selective sampling entails the systematic collection of observable items from the environment. Furthermore, visually identifying microplastic particles in the environment, collecting them, and separating them from the media in which they are found. Usually used on top of coastal sands, this approach is most effective for large microplastics (1–5 mm) (Razeghi et al. 2021). The removal and collection of anthropogenically created coastal debris for subsequent characterization and laboratory analysis would be an example of selective sampling. Additionally, there are several issues with selective sampling. Standardizing a methodology for selective sampling may be difficult because researchers may apply it differently (Fox, 2021). Consequently, the count of microplastic particles with certain characteristics like size, shape, or color could be lower than the actual count in a specific aquatic environment due to the lack of standardization in methodology. Reproducibility issues, peculiar researcher behavior, and undercounting should all be reduced by effective sampling approaches (Fox, 2021).

II-5.2. Bulk sampling

Bulk sampling is the procedure of collecting a sizable amount of environmental material from suspected microplastic-containing environments (such as sediment, water, air, and organisms) (Fox, 2021). When utilizing this approach, there are no field-applied separation or selectivity methods. Bulk sampling involves collecting a large volume of water, such as one cubic meter, for subsequent laboratory analysis. However, when it comes to testing microplastics in water, bulk and selective sampling are not commonly used. Moreover, bulk sampling of water may not be practical for certain applications because collecting and transporting such a large amount of water can lead to errors in statistical counting (Karlsson et al. 2020). Nonetheless, collecting, storing, and transporting large volumes of water for later study can be a challenging task. (Fox, 2021).

II-5.3. Volume-reduced sampling:

Volume-reduced sampling is a method used to separate and collect microplastics from the environment. It involves reducing the volume of the sample that will be evaluated in subsequent stages. (Razeghi et al. 2021). One way to perform volume-reduced sampling is to use a manta net that filters the surface water and collects particles above a certain size for further analysis. This is a common method for collecting microplastics from aquatic environments. Manta net trawls and

neuston net trawls are commonly used for surface water sampling, while bongo nets, plankton nets, continuous plankton recorders, and near-bottom trawls are commonly used for water column sampling (Wang & Wang, 2018).

Volume-reduced sampling is already a common method in MP research (Cózar et al. 2014; Eriksen et al. 2013) because to the extensive and productive usage of manta nets and neuston nets. The choice of method for a study is influenced by several factors, such as cost, feasibility, accessibility, precision, accuracy, and the type of media from which microplastics need to be extracted (Rochman et al. 2017). Pumps for water intake have also been employed for water column sampling among these methods (Karlsson et al. 2020). These methods of volume-reduced sampling allow the filtration of large volumes of water, while simultaneously collecting possible microplastic particles from the water column without any selection bias. (Fox, 2021).

II-6. Separation Techniques

II-6.1. Filtering or sieving

To separate microplastics from water samples and plastic-containing supernatants from sediment density separation, filtering is the most used method. Filters and sieve mesh have varying pore sizes, which determine the size of the solid separated. The filters employed in microplastic sample collection have pores ranging from 0.2 m to 300 m (Syakti et al. 2018). The common practice for filtering involves the use of a setup consisting of a funnel, a filter membrane, and a vacuum pump. (Crawford & Quinn, 2017). The size of microplastics detected is determined by the size of the mesh or pores, but using small pores or mesh sizes could result in clogging due to organic and mineral materials. Fabric can break down cellulose and synthetic fibers, turning them into potent pollutants that could pass through the filtering membrane. Microplastics may be lost when transferring the filter from the filtration apparatus to the petri dishes. Nylon filter paper is another possible contaminant, so there is a risk of underestimating or overestimating the amount of microplastics detected during the filtration process. (Tirkey & Upadhyay, 2021).

II-6.2. Visual sorting

The visual separation and identification of microplastics are crucial steps in their analysis as they facilitate the removal of non-microplastic detritus from the sample. The detritus comprises natural biological remains like shell pieces, seaweed, and wood, as well as human-made pollutants like

paint, metals, and oil residues. This is achieved either through naked-eye observation or with optical microscopy and tweezers to reduce the number of particles requiring analysis, thereby reducing the likelihood of errors. However, this process is time-consuming and may lead to misidentification since distinguishing microplastics from other materials is difficult. (Tirkey & Upadhyay, 2021; Wagner et al. 2017)

II-6.3. Density separation:

This technique removes microplastic from sediments and other inorganic materials that have not undergone chemical or enzymatic degradation (Stock et al. 2019; Tirkey & Upadhyay, 2021). Targeted microplastics can be distinguished from sample matrices by their various densities. It is possible to separate the microplastics for processing because of the significant density disparity that causes clastic particles to sink and microplastics to float (Fok et al. 2020). In this procedure, the sample is thoroughly combined with a saturated salt solution or a salt with a high concentration before being subjected to density-based separation for a predetermined period of time (Fok et al. 2020; Löder & Gerdts, 2015).

The Marine Strategy Framework Directive (MSFD) technical subgroup (European Commission, 2013) and National Oceanic and Atmospheric Administration (NOAA) both recommended NaCl as one of the salts that is most frequently used for density separation because of its widespread availability, low cost, and environmental friendliness (Nuelle et al. 2014). Due to its low density, the use of NaCl is not effective in separating high polymers such as polyvinyl chloride and polyethylene terephthalate, resulting in an overestimation of their concentrations in the environment (Gago et al. 2019; Tirkey & Upadhyay, 2021). To increase the recovery of microplastics, especially of heavier polymers, using higher density solutions such as NaI, which has high density, is safe, and can be reused, along with separation columns or the use of oil, is recommended instead of the low-density NaCl solution (Prata et al. 2019).

Elutriation is an alternative technique for density separation that involves introducing a liquid, such as water, from the bottom of a column to separate lightweight microplastics from settling organic debris and silt. (Kedzierski et al. 2017). Microplastics are collected using the mesh of the column and separated using dense solutions such as NaI (Claessens et al. 2013; Kedzierski et al. 2016). Elutriation has the advantage of removing microplastics from large amounts of sediments

efficiently and affordably, improving environmental representativeness and lowering the volume of the sample undergoing density separation (Kedzierski et al. 2016). However, this method requires at least an hour for each sample and needs to be pre-sieved by size range (Kedzierski et al. 2016; Prata et al. 2019).

II-7. Digestion

The use of volume-reduced sampling methods for field filtering can result in the collection of not only microplastics but also other organic and inorganic materials. Such materials may include zooplankton, phytoplankton, organisms, and biofilms, all of which may act as contaminants (Wang & Wang, 2018). To eliminate the unwanted organic material in the sample, an organic matter digestion step is commonly used. The objective of each digestive reaction is to remove undesirable organic matter while preserving the physical and chemical properties of MP particles. Depending on the type, size, shape, and specific reaction conditions of the plastic, the MP particles may or may not be affected during the digestion process. (Fox, 2021). The oxidizing, acidic, alkaline, or enzymatic approaches could be used to accomplish this.

II-7.1. Oxidizing Method

Organic material in the sample is selectively broken down by organic matter digestions, leaving the MP particles mainly untouched. Numerous reactions, such as Fenton's reagent, which is a mixture of sulfuric acid (H_2SO_4), ferrous iron (Fe (II)), and hydrogen peroxide (H_2O_2) at a 30 percent concentration, have been used to successfully digest organic materials (Fox, 2021; Wang & Wang, 2018). For the elimination of organic waste, H_2O_2 is a reliable and well-known oxidant (Tirkey & Upadhyay, 2021). When 30% H_2O_2 is introduced to a sample, the organic matter is digested within 7 days with minimal impact on the plastic polymer. The polymer undergoes a slight change in texture, becoming smaller and thinner as a result of the H_2O_2 treatment during digestion (Liebezeit & Dubaish, 2012; Nuelle et al. 2014). By producing hydroxyl and hydroperoxyl radicals, which can add to unsaturated organic molecules or remove hydrogen atoms from organic matter, the reaction progresses. Both of these processes produce alkyl radicals that can react with oxygen, leading to the complete oxidation of organic matter into water and carbon dioxide. (Chen et al. 2020). Fenton's reagent can rapidly oxidize organic matter and has been

demonstrated to have minimal effects on the chemistry or size of microplastics. (Fox, 2021; Tagg et al. 2017).

II-7.3. Acid Method

Another method of digesting organic material, and more especially, digesting biological tissue, uses either perchloric acid (H_2SO_4), nitric acid (HNO_3), or hydrochloric acid (HCl) (Wang & Wang, 2018). However, certain polymers, such as nylon, PET, and polyethylene terephthalate, are more vulnerable to acid degradation, especially under conditions of high temperature and concentration. (Birch et al. 2020). HNO_3 has been demonstrated as the most effective reagent for acid digestion of biogenic compounds found in the sample during microplastic analysis studies. (Fox, 2021). Although HNO_3 is effective for acid digestion of biogenic materials in microplastic analysis studies, it can have some negative effects. Specifically, it may leave oily residues or tissue fragments, cause yellowing of certain polymers, melt PET, HDPE, LDPE, and PS, and cause nylon to degrade. (Rochman et al. 2013). Nonetheless, HCl treatment causes changes to the surface of polyethylene terephthalate and polyvinyl chloride, and the effectiveness of acid digestion using HCl is inconsistent and inadequate for digesting biogenic compounds. (Cole et al. 2014; Foekema et al. 2013; Karami et al. 2017).

II-7.4. Enzymatic Method

Samples of organic material can be cleaned using enzymatic digestions (Löder et al. 2017; Wang & Wang, 2018). Enzymatic digestion is less dangerous because it doesn't require a fume hood and is less likely to cause microplastics to become damaged (Maes et al. 2017a). However, the type of organic material present in the sample will affect the enzyme's effectiveness (Andrady, 2017). Studies have shown that using a combination of proteases, cellulases, and chitinases is an effective method for removing as much as 98% of organic matter from samples. However, enzymatic digestion is a time-consuming process, and each enzyme has specific requirements for pH and temperature that must be carefully monitored and maintained during the experiment. (Lusher et al. 2017b; Miller et al. 2017; Stock et al. 2019; Tirkey & Upadhyay, 2021).

II-7.5. Alkaline Method

Utilizing bases like NaOH or KOH solution is another method of digestion. 1M NaOH has been shown to have an efficiency of 90% by (Cole et al. 2011). More efficient digestion took place when the temperature and molarity were raised. North Sea fish were examined by (Foekema et al. 2013) who also added a 10 M KOH solution to the sample. After two to three weeks, they saw that the organic materials had completely vanished. In a different study, numerous plastic fragments from beaches and marine waste were examined, and it was shown that most of the polymers (with the exception of cellulose acetate) were resistant to the use of KOH (Kühn et al. 2017).

This is also the rationale for the suggestion that KOH be used for microplastic research by (Dehaut et al. 2016). Research has shown that using a high-performance technique involving the presence of 10% KOH and incubating it for 48-72 hours at 40°C is effective in digesting plastic polymers. by Karami et al. (2017). Scientists have also experimented with 10 M NaOH for alkali digestion. However, NaOH causes the degradation of both organic matter and plastic polymers like polyethylene terephthalate, polyvinyl chloride, polycarbonate, and cellulose acetate, making it unsuitable. As KOH was found to be very effective in breaking down mussels, crab, and entire fish without altering the characteristics of the microplastics, it is recommended for the processing and analysis of microplastic samples (Dehaut et al. 2016; Tirkey & Upadhyay, 2021).

II-8. Analytical techniques for identification of microplastics

After sampling and a lengthy process, microplastic identification and detection is the next stage. This process verifies whether the isolated particle is a true microplastic or not (Tirkey & Upadhyay, 2021). Various techniques such as SEM, FTIR, and Raman Spectroscopy are employed for identification and chemical composition determination of microplastics. These techniques have their own specific features, advantages, and disadvantages.

II-8.1. Visual identification

The most common method for quantifying microplastics is through visual counting and sorting into various categories based on polymer type, size, color, and shape. This approach is utilized in approximately 79% of studies on microplastics, according to estimates (Renner et al. 2018). The majority of samples retrieved from surface waters using manta nets are examined visually (Hidalgo-Ruz et al. 2012). The use of optical microscopes in visual counting and sorting of

microplastics is preferred due to its simplicity, affordability, and non-destructive nature. This technique can be used as a preliminary step before employing more advanced and complex analytical methods (Fox, 2021; Renner et al. 2018).

The method of visual counting has several limitations when it comes to accuracy. Since the size range of plastics in the environment is vast and non-plastic particles can be mistakenly identified as plastic, visual counting can lead to significant overestimations or underestimations of plastic content (Al-Azzawi et al. 2022). For instance about 20% of particles that were initially identified as microplastics through visual inspection were later discovered to be aluminum silicate from coal ash when using a scanning electron microscope (SEM). (Schwaferts et al. 2019).

The accuracy of microscopic detection of microplastics may be affected by various factors. One of the main issues is the incomplete separation of sample particles, which can make it difficult to distinguish between microplastics and other particles. Additionally, the presence of sediments and biological materials can make it challenging to visualize microplastics under a microscope, even after chemical degradation. Another difficulty is differentiating between synthetic and natural fibers, as interference from other particles can make it challenging to distinguish between them using a microscope alone (Fox, 2021).

II-8.2. Fluorescence microscopy

Fluorescence microscopy is a technique that is similar to optical microscopy. In this method, microplastics (MPs) are subjected to a lipophilic dye, such as Nile Red, during the sample preparation process and before microscopy. The dye selectively stains the MP particles without staining other organic impurities present in the sample (Maes et al. 2017a). After filtration, the sample is observed through a fluorescence microscope to identify the MP particles that have been stained with the dye, which are visible due to their fluorescence (Fischer et al. 2016; Fox, 2021; Maes et al. 2017b).

Compared to optical microscopy, fluorescence microscopy has the potential to detect smaller microplastics (MPs) as even the tiniest, transparent or hard-to-find particles can be stained and made fluorescent. However, the challenge lies in choosing appropriate solvents and staining procedures for the lipophilic dyes as they tend to precipitate in water-based solutions, making it difficult to stain the targeted MPs effectively. Optical identification of small particles is difficult,

and different researchers may have varying approaches to particle testing, which can lead to inconsistent results for the same sample. If protocols are not agreed upon and meticulously followed, what one researcher may identify as a polypropylene fragment could be considered a mollusc shell fragment by another (Fox, 2021). Another obstacle to visualizing MPs is the chemical additives used during production, which can also impact their fluorescence properties. (Piruska et al. 2005). For example, additives may possess luminescent properties that can interfere with fluorescence measurements at a microscopic level (Lee et al. 2020). Therefore, proper pre-treatment is required to remove these contaminants as much as possible (Elert et al. 2017).

There is a general consensus that relying solely on visual identification is not sufficient, and that it should be complemented with more advanced techniques that involve chemical characterization to reduce any possible ambiguity in particle classification (Renner et al. 2018).

II-8.3. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) is a microscopic technique that can generate high-resolution images of the surface state and offer details about the morphological surface structure of MPs. To produce images in SEM, a high-intensity electron beam is utilized to irradiate the sample. Signals that provide information about the sample's morphology and topography are generated using secondary electrons that result from the interaction between the sample and the electron beam. (Tirkey & Upadhyay, 2021). Moreover, SEM can be equipped with Energy Dispersive X-ray Spectroscopy (EDS) detectors, which can furnish data about the chemical makeup of materials. EDS can provide information on the elemental composition and useful details such as the types of stabilizers, antioxidants, additives, and colors that were utilized to manufacture the particle under examination (Fox, 2021).

To generate an image, primary electrons must traverse through a solid object, which leads to several scattering processes, both elastic and inelastic. These scattering processes produce signals that are captured by various detector systems (Bogner et al. 2007). As the secondary electrons travel through the specimen, their intensity increases, providing information about the material's topography and contrast based on the atomic number (Z). Meanwhile, the secondary electrons produce a detailed image that assists in comprehending the morphology of the objects. (Campagnolo et al. 2021).

One advantage of SEM is its ability to produce high resolution imaging, reaching as low as 10 nm, and three-dimensional imaging. Because of its extremely fine spatial resolution, SEM can be utilized to visually scan suspected MP particles and determine whether they are made of plastic or not (Silva et al. 2018). By visually examining the polymer's surface for fissures, cracks, and pitting, SEM can also be used to measure the degradation of MP particles (Wang & Wang, 2018). Similarly, the high resolution provided by SEM makes it an excellent method for determining particle morphology. (Zobkov & Esiukova, 2018). To determine the chemical composition of the particle in question by calculating its elemental makeup, Energy Dispersive X-ray Spectroscopy (EDS) must be used alongside SEM, since SEM only produces a microscopic image (Li et al. 2016). The enormous effort required for sample preparation is the main drawback of SEM imaging of suspected MP particles (Renner et al. 2018; Silva et al. 2018). Sample preparation for SEM is time-consuming, which makes high throughput experiments or the analysis of anything beyond a limited subset of the sample's particles impossible (Fox, 2021).

II-8.4. Raman spectroscopy

Raman spectroscopy is a spectroscopic technique that utilizes the interaction between a sample and monochromatic light photons to provide structural information about plastics, which can be analyzed to determine their polymer type (Dehaut et al. 2016). This method uses laser radiation to interact with the vibrational movements of molecules and re-emit light at wavelengths unique to specific atomic groups. Inelastic scattering of photons from incident radiation by the sample's molecules produces a Raman spectrum (Ribeiro-Claro et al. 2017). Raman spectroscopy uses non-destructive chemical analysis along with microscopy, comparable to the Fourier transform infrared (FT-IR) spectroscopy approach (Alimi et al. 2018).

Raman spectroscopy has the advantage of detecting signals from non-polar, symmetric bonds and being essentially unaffected by spectral interference from water (Fox, 2021). Furthermore, non-contact Raman spectroscopy has the advantage of preserving the microplastic sample both before and after the analysis for possible future investigations (Woo et al. 2021). One of the primary disadvantages is that it is challenging to identify the specific polymer type of the target through Raman spectroscopy because the presence of additives and pigments in microplastics can affect the Raman signal (Dowarah & Devipriya, 2019). Inaccuracies can also arise from the curved surface of microplastics that have undergone wear and tear. Furthermore, Raman spectroscopy,

like FT-IR, has the disadvantages of being costly and necessitating specialized analysis (Woo et al. 2021).

II-8.5. Fourier transform infrared (FT-IR) spectroscopy.

In material characterization, the technique of infrared spectroscopy, also called IR spectroscopy, is commonly used to explore chemical bonding. This absorption spectroscopic method involves the absorption of an infrared photon by a molecule, which causes it to move from its initial vibrational state to a higher, excited vibrational state (Campagnolo et al. 2021).

FT-IR spectroscopy is a method used to determine the chemical structure of a substance by examining the vibrational modes of its molecules using IR radiation. The extent of these vibrational shifts gives information about the vibrational modes present in the sample. This interaction between the sample and the IR radiation causes the energy of the incident radiation to be either increased or decreased. Only molecule vibrations that result in dipole moments changing can be detected using FT-IR spectroscopy, which is also prone to contamination of the spectrum by moisture in the surrounding environment. However, this sensitivity enables FT-IR to be effective in identifying compounds that contain polar groups (Fox, 2021; Silva et al. 2018).

Micro-FT-IR is a single platform that combines both features and allows for the microscopic imaging of small plastic particles before their spectroscopic identification, making the identification of microplastics more straightforward (Morgado et al. 2021). In addition, the study of microplastics using FT-IR spectroscopy can utilize various modes, including transmittance, reflectance, and attenuated total reflectance (ATR) (Hendrickson et al. 2018). Unlike the transmission mode, both the reflectance and attenuated total reflectance (ATR) modes do not require any sample preparation steps for thick and opaque microplastics. Additionally, ATR mode can produce consistent spectra even on uneven surfaces of microplastics. Theoretically, the minimum detectable size of microplastics is limited by the aperture size of the IR beam in the ATR probe, typically around 10 µm.

Micro-attenuated total reflectance Fourier-transform infrared spectroscopy (micro-ATR-FT-IR) is currently a useful method for identifying microplastics in environmental samples as it combines the capabilities of microscopic identification of plastic particles with subsequent chemical identification through spectroscopy (Morgado et al. 2021). However, it is often difficult to obtain

a clear and identifiable spectrum for microplastics that are smaller than 50 µm. Additionally, since ATR-FTIR analysis involves contact with the surface, the pressure exerted by the ATR probe can potentially damage fragile microplastics (Silva et al. 2018). Small plastic particles can be difficult to detect due to electrostatic interactions with the probe tip or adhesion. Moreover, the process of individually identifying each particle using the ATR mode can be time-consuming. Additionally, the use of micro-FTIR devices can be costly. In environmental samples, microplastics are often weathered, have complex chemical compositions, and may exist as composite materials, making it challenging to obtain a clear spectrum that can be correctly interpreted. Therefore, skilled operators are required to obtain an accurate and reliable spectrum for microplastic identification. (Woo et al. 2021).

II-8.6. Pyrolysis-gas chromatography-mass spectrometry (py-GC/MS)

By examining the thermal breakdown products of possible microplastic particles, pyrolysis-gas chromatography (GC) and mass spectrometry (MS) can be utilized to determine the chemical composition of the particles (Fries et al. 2013). Plastic polymers undergo pyrolysis, which generates distinctive pyrograms that make it easier to identify the type of polymer. After extracting and visually classifying microplastics from sediments, this analytical strategy is already applied.(Qiu et al. 2016).

This method depends on pyrolysis, or thermal degradation, of a small portion of the material. Usually, just 50–500 g are needed (Nguyen et al. 2019; Wang & Wang, 2018). A gas that does not react chemically is employed to move the sample that has turned into vapor through a column in chromatography. The column separates the various gases by using a combination of the molecules' polarity, solubility, and vapor pressure. The byproducts in gaseous form are then separated from the column and pass through a mass spectrometer where they are ionized, propelled through an electromagnetic field, and divided into fragments based on their mass-to-charge ratios, generating a mass spectrum. The sample's chemistry and the presence of any organic plastic additions can be determined from the resulting mass spectra (Fries et al. 2013).

To avoid damaging the sample, it is recommended to initially study potential MP particles using optical microscopy, to document characteristics like size, shape, color, and malleability. This is because py-GC/MS is a destructive method. While py-GC/MS can be applied to a very small

quantity of the sample, the particles analyzed in MP research must be of sufficient size to be extracted from a filter and transferred to a GC vial for analysis (Wang & Wang, 2018). If the plastic is not uniformly distributed at the nanoscale, the limited amount of analyte utilized in py-GC/MS may not accurately represent the larger plastic, which could have a negative impact on the measurements (Dümichen et al. 2015). Another disadvantage of py-GC/MS is the time required to analyze each sample. Typically, it takes around 30 to 100 minutes to analyze one sample, which makes it impractical to analyze numerous samples due to the time-consuming nature of the process (Wang & Wang, 2018). Furthermore, it is crucial to maintain all particle data during visual inspection before pyrolysis since py-GC/MS is a destructive technique. In general, py-GC/MS is an excellent method for identifying particle chemistry because it is not affected by particle size, shape, or thickness, unlike many other spectroscopic methods (Wang & Wang, 2018). Nonetheless, it is most effective when used in combination with a preceding visual examination stage where characteristics of the particles other than their chemical composition can be observed and recorded (Fox, 2021).

II-9.0. Classification of Plastics.

II-9.1. Polyethylene (PE)

PE, an abbreviation for Polyethylene, is a lightweight and versatile plastic produced by polymerizing ethylene, a type of artificial resin. PE is classified as a member of the polyolefin resin family and is the most widely used plastic in the world. Its uses include a wide range of applications such as transparent food packaging, shopping bags, detergent containers, and car fuel tanks. Additionally, it can be transformed into synthetic fibers through slitting or spinning or modified to display rubber-like elasticity (Ghatge et al. 2020).

II-9.2. Low-density polyethylene (PE-LD)

The manufacturing process of PE-LD, also known as low-density polyethylene, involves the use of gaseous ethylene at high temperatures (up to 350°C or 660°F) and high pressures (up to 350 megapascals or 50,000 pounds per square inch), along with oxide initiators. As a result, the polymer structure contains both long and short branches, which prevents the polyethylene molecules from closely packing together in rigid, crystalline arrangements, thus creating a highly flexible material. LDPE has a melting point of approximately 110°C (230°F). It is primarily

utilized for packaging film, garbage and grocery bags, agricultural mulch, wire and cable insulation, squeeze bottles, toys, and household items (Dey et al. 2020).

II-9.3. High-density polyethylene (HDPE)

To manufacture HDPE, which stands for high-density polyethylene, low-temperature and low-pressure methods are employed, using either Ziegler-Natta and metallocene catalysts or activated chromium oxide (also known as a Phillips catalyst) (Olesik et al. 2021). The absence of branches in its structure enables the polymer chains to pack closely together, producing a dense, highly crystalline material that has excellent strength and moderate stiffness. It has a melting point more than 20°C (36°F) higher than LDPE, making it capable of withstanding repeated exposure to temperatures of up to 120°C (250°F) and can also be sterilized (Tabish Wani et al. 2020). HDPE is used to produce a variety of products, such as milk and household cleaner bottles made through blow-molding, grocery bags, construction film, and agricultural mulch made through blow-extrusion, as well as pails, caps, appliance housings, and toys created through injection-molding (Olesik et al. 2021).

II-9.4. Polypropylene (PP)

Polypropylene (PP) is a thermoplastic with high versatility that has a broad range of applications worldwide. It is utilized in plastic packaging, plastic parts for machinery and equipment, as well as fibers and textiles. This rigid, semi-crystalline thermoplastic was first polymerized in 1951 and has been extensively used in various industrial and domestic applications. The global demand for polypropylene is increasing rapidly and is estimated to be approximately 45 metric tons (Hisham A. Maddah, 2016). Polypropylene is a versatile material that has a unique texture that makes it suitable for various purposes, including plastic furniture and low-friction machinery applications. It is highly resistant to chemical corrosion, making it an excellent option for packaging cleaning products, bleaches, and first-aid supplies. Additionally, it is known for its durability, toughness, and excellent fatigue resistance and elasticity. Its high insulation properties make it a safe option for electrical goods and cable casings.

Apart from being used for tote bags, polypropylene finds extensive use in other products such as ropes, twine, tape, carpets, upholstery, clothing, and camping equipment, primarily due to its waterproof properties. The automotive industry widely uses polypropylene for battery casings,

trays and drink holders, bumpers, interior details, instrumental panels, and door trims. The medical industry values the waterproof and flexible strength properties of polypropylene, as well as its resistance to mold, bacteria, and chemical corrosion. It can be sterilized through steam sterilization methods and is used in medical applications such as syringes, medical vials, Petri dishes, pill containers, and specimen bottles.

II-9.5. Polyethylene terephthalate

PET or PETE is a type of polyester polymer that is strong and rigid. It is formed by combining ethylene glycol and terephthalic acid and is widely used in the packaging of food and beverages, including carbonated drinks, water, and juice bottles. The raw materials are synthesized to create a polymer chain, which can be melted and shaped into different forms through extrusion or molding. PET can also be stretched into a thin sheet, forming PET film, which is utilized in video, photo, and packaging applications. Due to its durability and transparency, PET is a preferred choice for packaging health, beauty, and cleaning products. PET is considered safe for use in various industries, including food, beverage, personal care, pharmaceutical, and medical, as confirmed by global health regulatory bodies (Khairul Anuar et al. 2022).

CHAPTER THREE

III. Methodology

III-1. Research Location.

The study was conducted at the Soil Science and Agrochemistry Research Group Laboratory of the Institute of Environmental Science, which is part of the Hungarian University of Agriculture and Life Science (MATE). The Department of Soil Science, located within the Institute, is responsible for providing education, research, and related services in the field of soil science. The department focuses on soil classification, mapping, surveying, fertility management, improvement, and conservation, and engages in various national and international research projects. Its research areas include investigating the potential of soils to capture carbon, implementing modern soil survey technologies, and developing soil information systems in Europe, Africa, and Asia. The research group is headed by Erika Csákiné Michéli DSc., a Professor and Doctor of Hungarian Academy of Sciences.

III-2. Sample Collection

For the experiment, real wastewater could not be used because of the permission and life threating disease that was associated with it. Moreover, wastewater treatment plants usually have hazardous chemicals and strict environmental regulations that must be followed to protect the environment and public health as visitors may unintentionally introduce contaminants or pollutants, which could lead to violations of these regulations. So artificial wastewater was created by mixing water and fragmented plastics into water. The plastics were categorized into PET, HD-PE, PE-LD, and PP only according to the Resin Identification Code (RIC) system (Figure 3) shredded to MP size and properly labelled (Figure 4**)**. The plastics were picked from used plastics material of different properties. The plastics were easily identifiable from the properties described on them. They were then fragmented into very small sizes ranging from 2-5mm (Figure 5 and Figure 6). The shredded microplastics were then washed with distillated water to remove contaminants. Different letters were assigned to the fragmented plastic.

Figure 3: Resin Identification Code (RIC) system (Gunasekar et al. 2022)

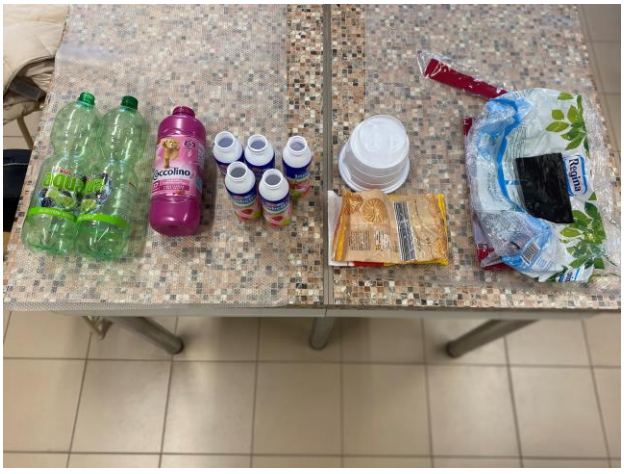

Figure 4: Collection of Plastic

Figure 5: Shredded Particles

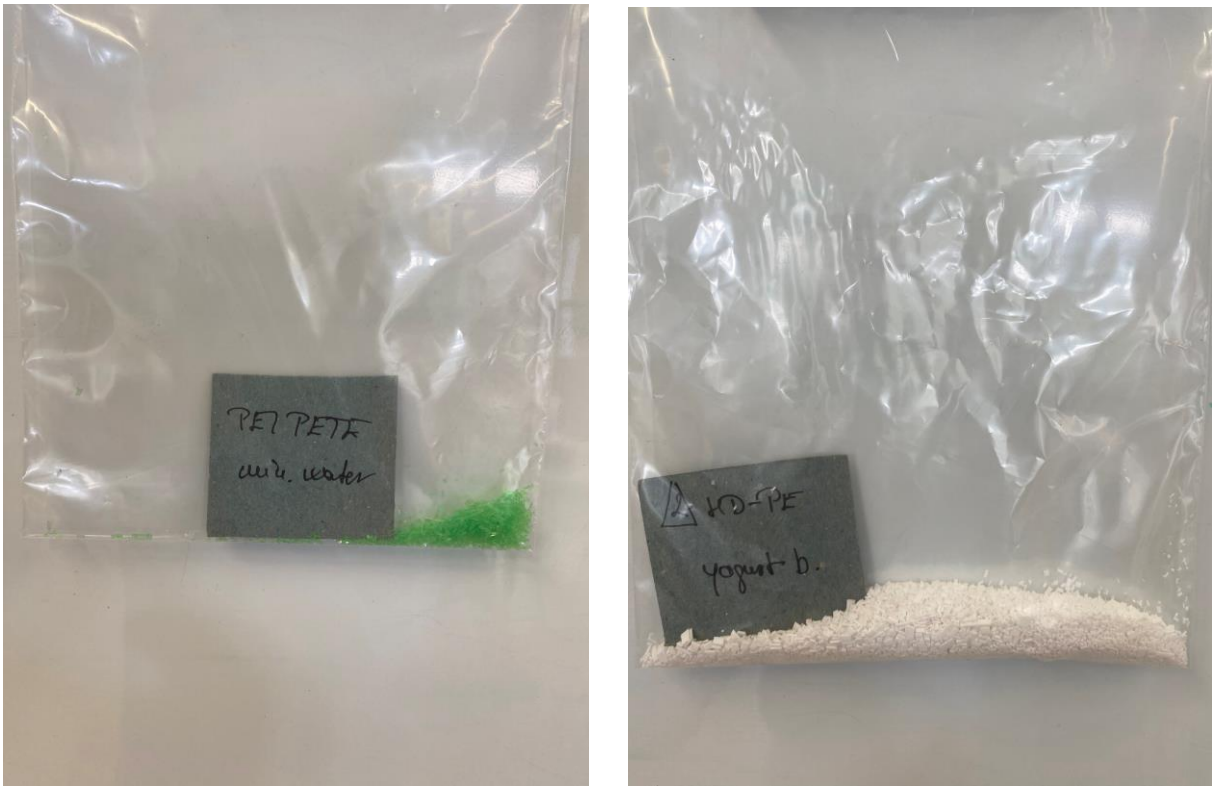

Figure 6: Well labelled Microplastics Plastics

III-3. Materials

Further into the experiment is digestion. Digestion is essential to isolate microplastics and free it from organic matter. This will ease the identification of microplastics. The reagents used for the

digestion include nitric acid (HNO_3), potassium hydroxide (KOH), hydrogen peroxide (H_2O_2) and acetic acid (CH_3COOH) as shown in Figure 7.

Figure 7: Chemical Reagents used for digestion.

The BTC BIM 312T microscope (shown in Figure 8) was used for the microscopic analysis of physical properties. This microscope is ideal for both amateur and semi-professional use and is specifically designed for biological applications. The microscope is equipped with two eyepieces, which provides greater convenience during observation. Additionally, it features five semi-plane lenses that are housed in a five-point revolver head, offering a range of magnification options (4x, 10x, 20x, 40x, 100x). The lenses are free of most optical defects and are designed with improved wind correction capabilities. A micro-focusing button is included, in addition to the course focusing mechanism. The Abbe condenser (with an NA of 1.25) can be adjusted precisely in all directions. The lighting unit is a full-Köhler system, and the LED intensity of the light source can be adjusted using a potentiometer brightness control. This allows for precise lighting adjustments,

even when performing phase contrast or dark field examinations. The display table of this model is larger than usual and includes integrated fine movement capabilities. Unlike simpler models, the 312 microscopes come equipped with PL eyepiece pairs, which provide better imaging. The eyepieces have a diameter of 30.0 mm. To facilitate the transfer of microplastic images into digital and transferable formats, the microscope was equipped with a TuopCam digital camera (Figure 9).

Figure 8: BTC BIM 312T microscope

Figure 9: ToupCam digital camera

III-4. Sample Preparation:

Each category of MPs was measured on an analytical balance (Figure 10) and then 5ml chemical reagent was added inside test tube. The concentration of the 5ml solutions are as follows.

Table 1: Concentration of Reagents

Solution	Chemical Name	Concentration
HNO_3	Nitric Acid	35%
KOH	Potassium hydroxide	10%
CH_3COOH	Acetic Acid	5%
H_2O_2	Hydrogen Peroxide	30%

Figure 10: Analytical Balance

After this treatment, the samples were separated into two groups: one group was kept in room temperature (20 -23 ^{o}C) and given minimum 1 day standing time and one group was kept between 50 - 60°C and minimum 1 hour standing time and covered with foil paper (Figure 11) to prevent extraneous particle contamination.

Figure 11: Grouping samples into room and heated temperature.

Thereafter the samples were filtered through a filter paper (Figure 12) and thoroughly washed with a deionized water to wash off reagents as well as organic particles around the microplastics to isolate it (Figure 13).

Figure 12: Filtered Microplastics Samples

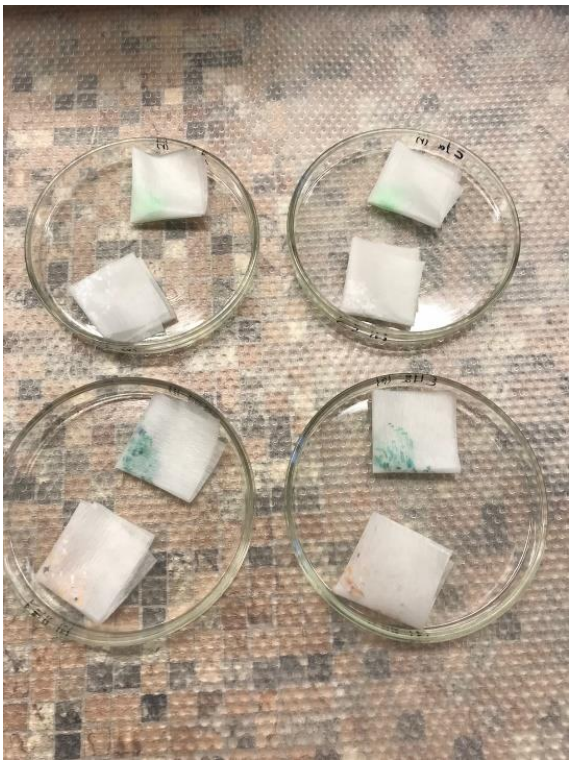

Figure 13: Already Filtered MPs samples.

The samples were then dried in an oven at a temperature of about 60 ^{o}C to obtain dried particles which was then analyses by the microscope to detect physical changes.

III-5. Quality Control

To prevent contamination from synthetic clothing, a coat made of cotton was worn during the laboratory procedures. Glass instruments were thoroughly washed three times using deionized water and then covered with aluminum foil. All the analytical steps were conducted within a laminar flow cabinet to prevent any contamination caused by airborne particles in the laboratory (Figure 14).

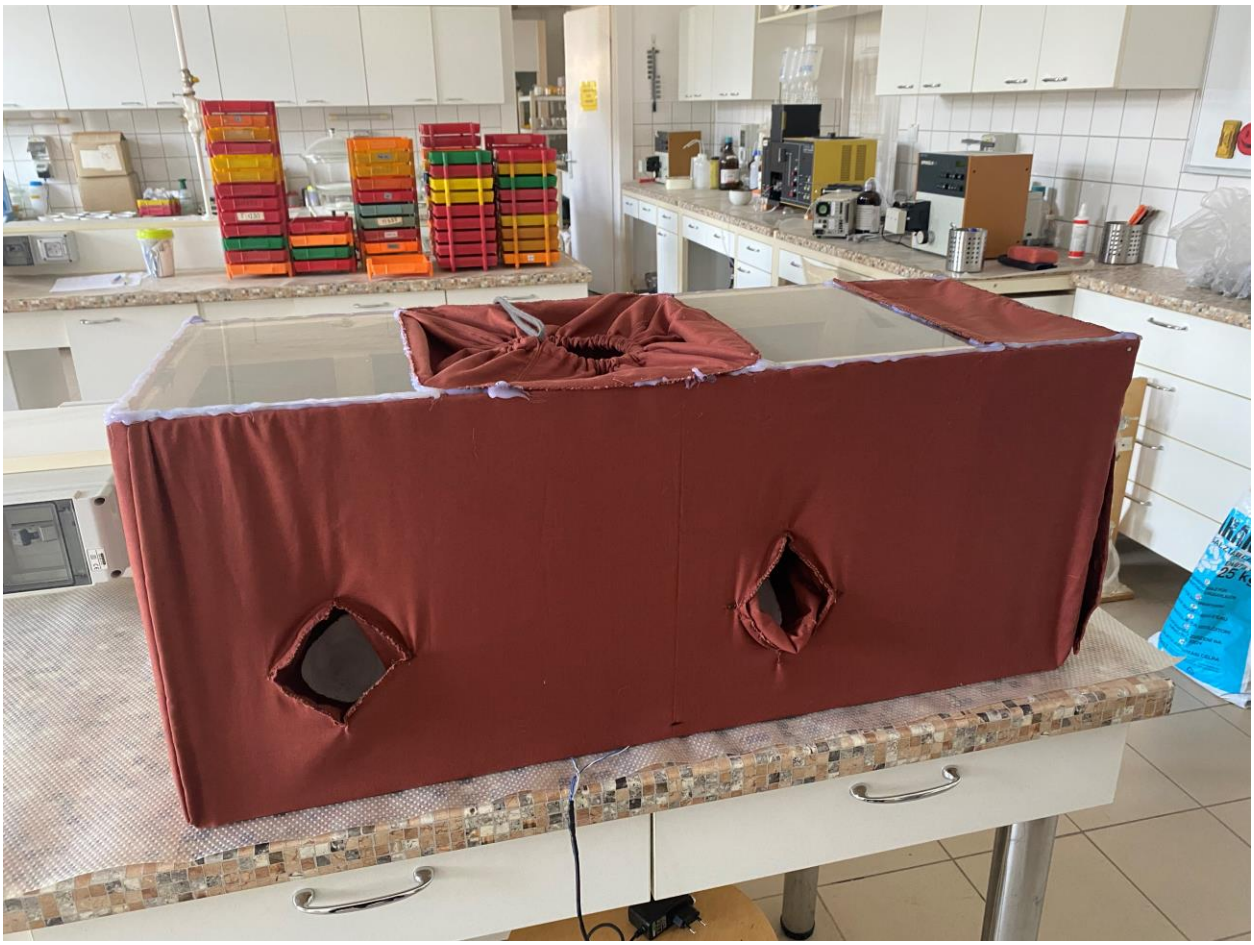

Figure 14: Laminar flow cabinet

III-6. Analysis of Result

Due to technical issues at the laboratory as the university goes on a major reconstruction, the next stage of the thesis which ought to involve major work and creation of replicates of samples couldn't be achieved. Hence minimal statistics, percentage recovery value (Equation 1) and the pictorial output of the microscope to make comparison. This will serve as a basis for further work on this research.

$$\% \text{ Recovery} = \frac{M1-M2}{M1} \; x \; 100\% \qquad \text{Eq. 1}$$

M_1 = Mass before Digestion

M_2 = Mass after Digestion

CHAPTER FOUR

IV. Results

Table 1 and 2 show the percentage recovery of each plastic as tested with different solutions at each of the studied temperature. The micro-plastic degradation value at room temperature ranges from 0.500% to 24.55%, while it ranges from -5.051% to 6.087 in the heated temperature.

Table 2:Estimated values of degradation and percentage degradation at room temperature (20-23 ^{o}C) for 24 Hours

Reagent	Plastic Type	M_1 [g]	M_2 [g]	Δ M [g]	% Recovery
HNO_3	PET/PETE	0.0993	0.0988	0.0005	0.504
	HDPE	0.1000	0.0995	0.0005	0.500
	PE-LD	0.0113	0.0112	0.0001	0.885
	PP	0.0112	0.0090	0.0022	19.643
H_2O_2	PET/PETE	0.0996	0.0990	0.0006	0.602
	HDPE	0.0968	0.0960	0.0008	0.826
	PE-LD	0.0118	0.0109	0.0009	7.627
	PP	0.0101	0.0098	0.0003	2.970
KOH	PET/PETE	0.0986	0.0978	0.0008	0.811
	HDPE	0.0982	0.0976	0.0006	0.611
	PE-LD	0.0096	0.0076	0.0020	20.833
	PP	0.0106	0.0102	0.0004	3.774
CH_3COOH	PET/PETE	0.0997	0.0989	0.0008	0.802
	HDPE	0.0979	0.0969	0.0010	1.021
	PE-LD	0.0110	0.0083	0.0027	24.545
	PP	0.0135	0.0131	0.0004	2.963

Table 3:Estimated values of degradation and percentage degradation at heated temperature (50 - 60 ^{o}C) for 1 hour

Reagent	Plastic Type	M_1 [g]	M_2 [g]	Δ M [g]	% Recovery
HNO_3	PET/PETE	0.0997	0.0997	0.0000	0.000
	HDPE	0.0997	0.0989	0.0008	0.802
	PE-LD	0.0099	0.0104	-0.0005	-5.051
	PP	0.0115	0.0108	0.0007	6.087
H_2O_2	PET/PETE	0.0993	0.0986	0.0007	0.705
	HDPE	0.0992	0.0991	0.0001	0.101
	PE-LD	0.0113	0.0107	0.0006	5.310
	PP	0.0104	0.0099	0.0005	4.808
KOH	PET/PETE	0.0938	0.0931	0.0007	0.746
	HDPE	0.0959	0.0954	0.0005	0.521
	PE-LD	0.0113	0.0107	0.0006	5.310
	PP	0.0114	0.0112	0.0002	1.754
CH_3COOH	PET/PETE	0.0990	0.0989	0.0001	0.101
	HDPE	0.0963	0.0957	0.0006	0.623
	PE-LD	0.0119	0.0113	0.0006	5.042
	PP	0.0138	0.0133	0.0005	3.623

M_1: Mass before digestion; M_2: Mass after digestion.

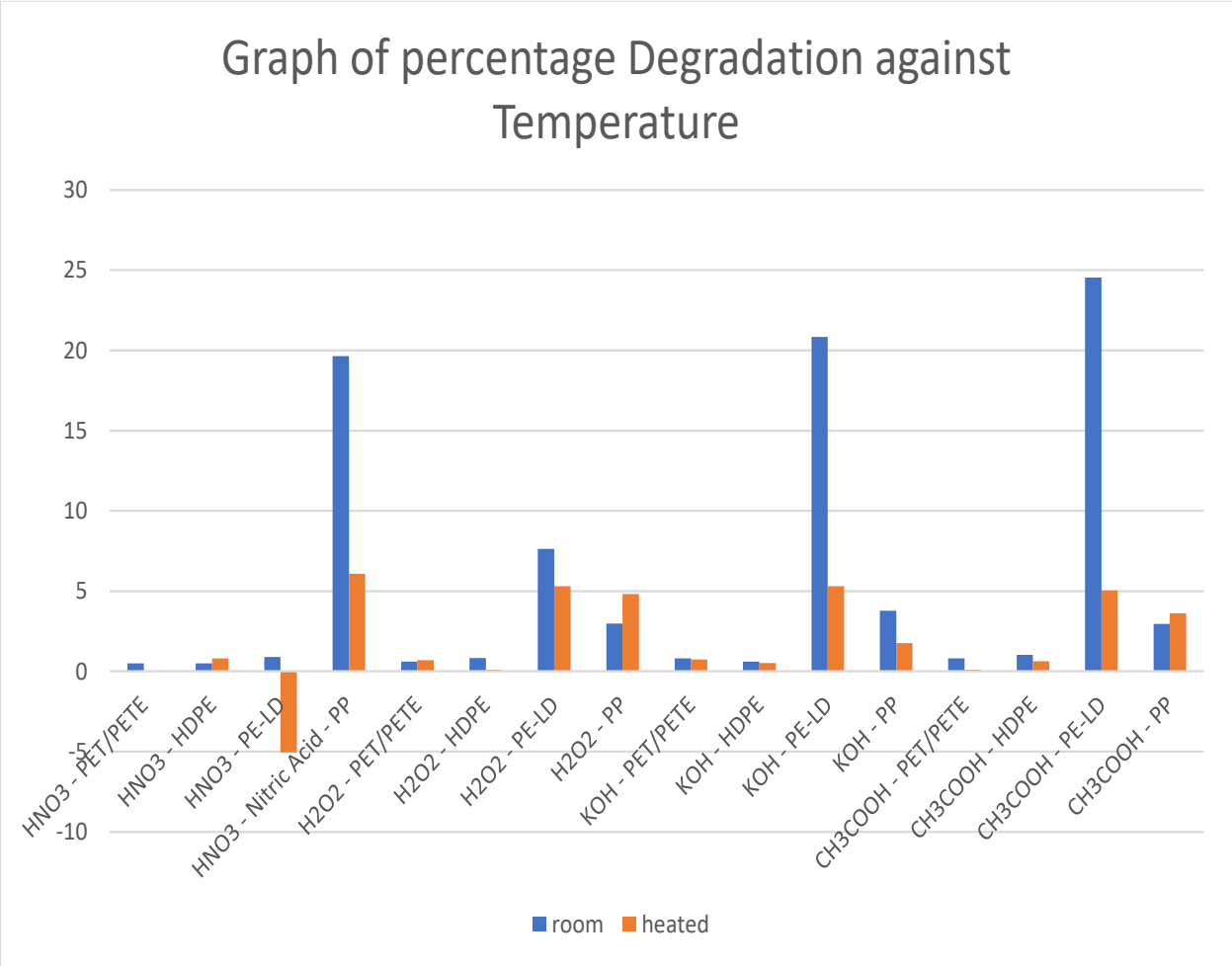

Figure 16: Degradation efficiency in terms of temperature.

CHAPTER FIVE

V. Discussion

V-1. Acidic Digestion: Nitric Acid

Despite the fact that this method can potentially cause plastic degradation and is considered a destructive technique for plastic isolation, derivatives of it have been recommended by the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) as a monitoring protocol (Dehaut et al. 2016). As a general class of materials, polyethylene (PE) and polypropylene (PP) are recognized for their excellent resistance to harsh chemical environments according to the chemical resistant chart. However, in this study, both at room temperature for 24 hours and heated temperature for 1 hour, PP showed the highest degradation as the recovery rate was 19.643% and 6.087% respectively. The cause of this is either prolonged exposure to room temperature or a brief period of high temperature in an oven. Due to the enhanced mobility of many chemical molecules and the accelerated rate of reaction at high temperatures, exposure to temperature would typically be a significant effect. As temperatures rise, so does the ability of solvents to diffuse into polymers. The amount of stress placed on a part can frequently quicken the rate of chemical or solvent attack. Lastly, the resistance of the polymer typically declines as the chemical concentration or strength increases (Lee et al. 2020). Furthermore, according to the research, HD-PE resulted in the lowest degradation for room temperature while PET/PETE didn't show any sign of degradation. This may be as a result of the high ductility and tensile strength (Laiwang et al. 2020).

In addition, there was an increase in the mass of PE-LD. This is possible due to the absorption of acid into the plastic material. This can cause the plastic to swell, which may result in an increase in mass. Additionally, some types of plastics can undergo chemical reactions with certain acids, leading to the formation of new compounds that contribute to the increase in mass. Another possibility is that the acid can cause the plastic to break down or degrade, leading to the release of small molecules or fragments that become trapped in the plastic and contribute to an increase in mass. It is important to note that not all plastics will necessarily experience an increase in mass when reacted with acid, as the behavior of a specific plastic will depend on its chemical composition and the specific acid used.

In terms of morphological changes, while others didn't show visible physical changes both at room and oven temperature, PP showed the most significant changes such as deep surface cracking and embrittlement as shown in Figure 15. This tells that HNO_3 will not only affect chemical but also physical properties of microplastics. Nitric acid, despite its disadvantages, may be the easiest and most affordable method of processing large quantities of samples fast, making it valuable for routine bio-monitoring projects (Desforges et al. 2015)

Figure 15: Cracking and embrittlement effect on PP.

V-1. Acidic Digestion: Acetic Acid

Unlike PP showing the highest recovery rate during initial acidic degradation with nitric acid, PE-LD showed the highest recovery rate (5.042% at room temperature and 24.545% at 60^{o}C) meanwhile, PET/PETE showed the lowest recovery rate (0.802% at room temperature and 0.101% at 60^{o}C). Since the highest recovery rate was in room temperature, it shows that low temperature at a prolonged time would affect PE-LD. This is evident likewise under microscope as shown in Figure 16.

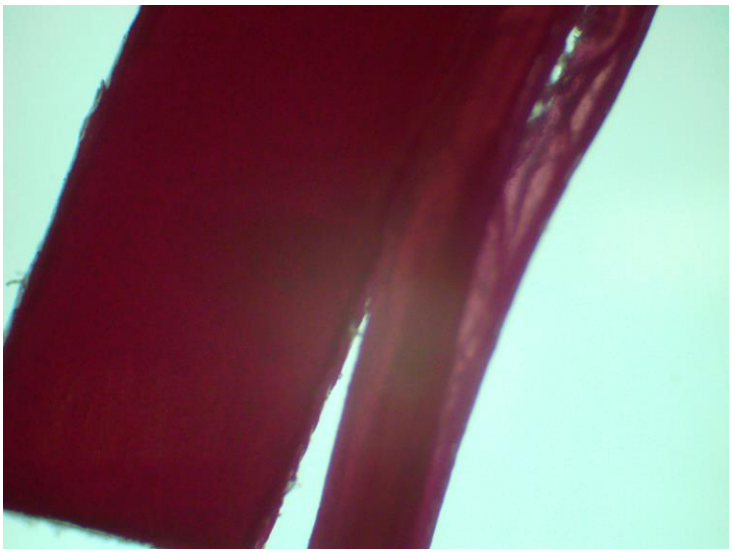

Figure 16: Cracking and embrittlement effect of acetic acid on PE-LD at room temperature

V-2. Oxidative Digestion:

Oxidation digestion was achieved by reacting H_2O_2 (Hydrogen peroxide) with microplastics. Both at room and oven temperature, PE-LD showed the highest percentage recovery with recovery rate of 7.627% and 5.310% respectively. Although at room temperature with a standing time of 24 the degradation was less than that of the oven temperature with a standing time of 1 hour. The means temperature and time influence the degradation of PE-LD although little. On the other hand, the reaction didn't show significant physical changes on PET/PETE and HD-PE both at room and heated temperature as both recovery rate was slightly above 0.5%. Morphologically, both at room and oven temperature there were no physical changes and cracking on all the plastic types as shown in Figure 16.

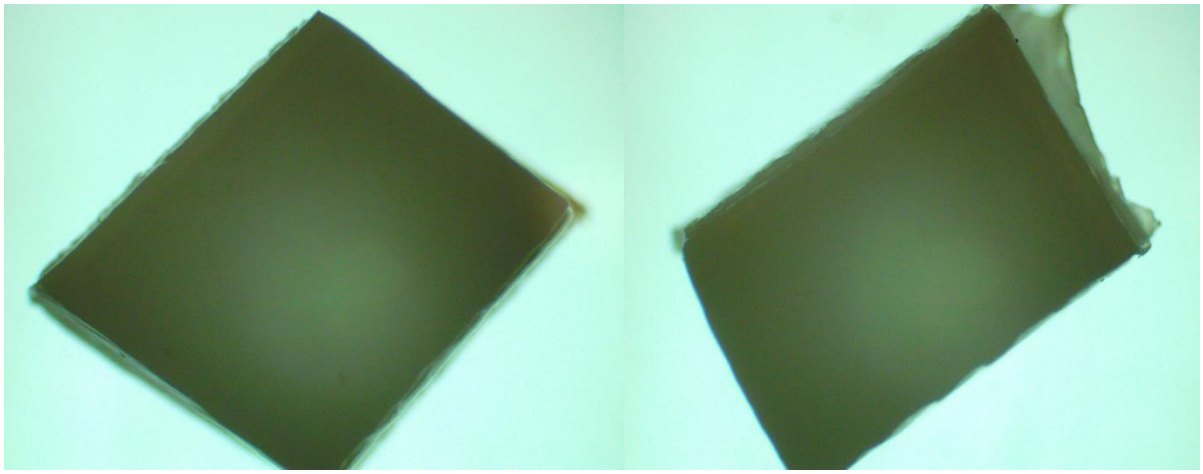

Figure 17: Absence of physical damage of microplastics when reacted with Hydrogen Peroxide

V-3. Alkaline Digestion

Alkaline digestion protocol was achieved by reacting KOH with microplastics. Considering both temperatures, PE-LD had the highest percentage recovery (5.310 at 50 - 60^{o}C and 20.833 at 20-23^{o}C) while HDPE had the lowest (0.521 at 50 - 60^{o}C and 0.611 at 20 - 23^{o}C). This shows that change in temperature has limited effect on the reaction of this reagent with plastic materials. Under microscopic observation, even though temperature shows no effect, alkaline showed cracking, and embrittlement effects on PE-LD (Figure 17).

Figure 18: Cracking and embrittlement effect of alkaline on microplastic

Chemical digestion is a crucial process in the analysis of microplastics (MPs), especially in environmental samples that contain a lot of organic matter. This is because the removal of natural debris is necessary to effectively extract MPs from the sample. However, it is important to choose a digestion protocol that is not only effective but also preserves the integrity of the polymers, as it could otherwise Impact the identification and measurement of particles. The focus of this study is on the impact of different digestion protocols, such as acidic, alkaline, and oxidative, on the recovery and integrity of MPs. The study investigated the suitability of commonly used chemical reagents such as KOH, H_2O_2, HNO_3, and CH_2COOH for the digestion of microplastic.

Out of the four substances tested for their digestive properties, it was discovered that H_2O_2 had the smallest impact on the characteristics of microplastics. In contrast, numerous studies utilize HNO_3 and CH_3COOH in chemical digestion techniques due to their potent acidic and oxidizing abilities, which hasten the breakdown of organic matter (Schwaferts et al. 2019; Tanaka & Takada, 2016). Nonetheless, the physical and chemical data indicate that these agents can noticeably impact the plastics, and therefore, it is advisable to exercise caution while using HNO_3. Even though they are generally preferred for digesting biological matter, they should be used with care when attempting to retrieve MPs. Moreover, using these reagents over a prolonged period at normal temperature can cause the degradation of plastic polymers, with this degradation being more common in smaller-sized MPs.

CHAPTER SIX

VI. Conclusion and Recommendation

The purpose of this study was to investigate the effects of different chemical digestion protocols, including acidic, oxidative, and alkaline reagents, on the physical and chemical properties of microplastics. The findings of this study are valuable for researchers who wish to recover microplastics from biological samples without damaging them. Before processing and analyzing samples, it is critical to assess chemical digestion protocols to ensure that microplastics are recovered optimally. Based on the observations of different types of microplastics and methods tested, oxidative digestion at room temperature (20 - 23°C) appears to be the most appropriate for digestion, but it can be destructive over an extended period. Although oxidation remains effective for digesting samples containing plant matter in marine and freshwater samples, it is also necessary to control temperatures during the reaction, avoiding temperature spikes at or below 60°C to minimize the loss of constituent microplastics, especially microbeads from personal care products.

The research showed that some outcomes of the microplastic reactions contradict the chemical resistant chart (HCS, 2020) as in the case of PP and PE-LD. For example, PP reacted poorly with nitric acid (above 70% concentration) at 20°C and 50°C, according to the chemical resistant chart. However, at 35% concentration and a temperature between 20 - 23°C, microplastics degradation was observed. Similarly, the chemical resistant chart showed that PE-LD has excellent resistance to degradation at 50% KOH concentration and 20°C and 50°C. However, significant changes to the physical and chemical properties of microplastics were observed at just 10% KOH concentration at a temperature between 20 - 23°C. Therefore, microplastics tend to behave differently at different temperatures and concentrations of reagents used for chemical digestion. It is thus necessary to update the chemical resistant chart with microplastics' behavior at different temperatures and reagent concentrations.

Additionally, selective processing conditions that eliminate certain materials may result in incomplete assessments of the occurrence, types, sources, and impacts of microplastics. Therefore, it is critical to use recommended digestion protocols to ensure accurate and reliable microplastic analysis in samples. In conclusion, the properties related to digestion can vary depending on the polymer type, so further research is needed on the specific polymer types relevant to each study.

SUMMARY

Identification of Micropolymers in Gray water

OREKOYA ADEDAPO

Agricultural Water Management Engineering, Master degree

Department of Soil Science /Institute of Environmental Sciences

Primary thesis advisor: András Sebők, Assistant researcher, Institute of Environmental Sciences /Department of Soil Science

Microplastics, which are tiny plastic particles, have become a topic of great interest for both scientists and the public due to their status as emerging particulate anthropogenic pollutants that can be found all over the world, including in food and drinking water. To better understand their presence in the environment, it is crucial to monitor microplastics, which involves using multiple methodological approaches to extract, identify, and quantify the particles dispersed in environmental matrices. However, when dealing with environmental matrices that are high in organic matter, chemical digestion treatment is required to get rid of microplastics and release the particles. This type of treatment involves using a range of chemical agents, including acids, bases, and oxidizing agents. Unfortunately, there has been limited research into the chemical resistance of various types of microplastics to these substances. To address this issue, a study was conducted to examine the chemical resistance of four species of microplastics (high-density polyethylene, low-density polyethylene, polystyrene, and polyethylene terephthalate) to hydrogen peroxide, potassium hydroxide, sodium hydroxide, nitric acid, and acetic acid. The study assessed the percentage degradation and physical damage of microplastics using a microscope. The results indicated that acidic and alkaline substances were the most destructive to microplastics, while oxidative reagents resulted in fewer changes to plastic properties. These findings provide valuable insights into the properties of MPs and their response to strong acids, bases, and oxidizing agents, which can serve as a reference for future studies on MP pretreatment. In addition, used as a guideline to update current protocols and ensure that microplastics can be treated without causing damage.

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