UNIVERSITI PUTRA MALAYSIA

ASSESSMENT OF DENSITY SEPARATION METHODS FOR MICROPLASTICS EXTRACTION FROM SOIL

CHE THAQIF BIN CHE MUSTAFHA

ASSESSMENT OF DENSITY SEPARATION METHODS FOR MICROPLASTICS EXTRACTION FROM SOIL

BY CHE THAQIF BIN CHE MUSTAFHA

Thesis submitted in fulfilment of the requirement for the degree of Bachelor Science (Environmental and Occupational Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

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ABSTRACT

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Introduction: For the separation of microplastics from soils, many density separation methods utilizing a variety of density separation solutions have been developed, however their application to tropical soils is limitedly being investigated. **Objective:** The aim of this research was to validate the performance of selected density separation solutions and to select the best density separation solutions in a single stage method that can deliver consistently high recoveries for different microplastic polymers 1 mm and is suitable for tropical soils **Methodology:** Zinc chloride ($ZnCl_2$), sucrose, sodium sulphate (Na_2SO_4), sodium carbonate (Na_2CO_3), and coconut oil were used to evaluate the selected microplastics (LDPE, HDPE, PVC, PS and PP) from post-consumer product. The recovered microplastics identified in terms of size and color via microscopy technique. **Results:** As anticipated, a general pattern of increased microplastic recovery with increasing solution density was found, with greater rates of microplastic recovery in $ZnCl_2$, sucrose, and Na_2CO_3. The size and density of microplastics have been shown to affect the recovery rates and should be considered when selecting a density separation solution. **Conclusion:** The necessity for density separation recovery experiments to verify the use of density separation solutions for microplastic recovery is apparent from this research. This is the most comprehensive validation of density separation solutions for the density separation of microplastic from soils involving tropical soils.

Keywords: ***microplastics, soils, ,density, separation, morphology***

ABSTRAK

PENILAIAN PENGETAHUAN KETUMPATAN KAEDAH EKSTRAK MIKROPLASTIK DARI TANAH

CHE THAQIF BIN CHE MUSTAFHA

Pendahuluan: Untuk pemisahan mikroplastik dari tanah, banyak kaedah pemisahan ketumpatan yang menggunakan pelbagai penyelesaian pemisahan ketumpatan telah dikembangkan, namun penerapannya ke tanah tropika hanya sedikit diselidiki. **Objektif:** Tujuan penyelidikan ini adalah untuk mengesahkan prestasi penyelesaian pemisahan ketumpatan terpilih dan memilih penyelesaian pemisahan ketumpatan terbaik dalam kaedah satu tahap yang dapat menghasilkan pemulihan yang tinggi secara konsisten untuk polimer mikroplastik yang berbeza 1 mm dan sesuai untuk tanah tropika. **Metodologi:** Zink klorida ($ZnCl_2$), sukrosa, natrium sulfat (Na_2SO_4), natrium karbonat (Na_2CO_3), dan minyak kelapa digunakan untuk menilai mikroplastik terpilih (LDPE, HDPE, PVC, PS dan PP) dari produk pasca pengguna. Mikroplastik yang dipulihkan dikenal pasti dari segi ukuran dan warna melalui teknik mikroskopi. **Hasil:** Seperti yang dijangkakan, pola umum peningkatan pemulihan mikroplastik dengan peningkatan kepadatan larutan ditemukan, dengan kadar pemulihan mikroplastik yang lebih besar pada $ZnCl_2$, sukrosa, dan Na_2CO_3. Ukuran dan ketumpatan mikroplastik telah terbukti mempengaruhi kadar pemulihan dan harus dipertimbangkan ketika memilih penyelesaian pemisahan kepadatan. **Kesimpulan:** Keperluan untuk eksperimen pemulihan pemisahan kepadatan untuk mengesahkan penggunaan penyelesaian pemisahan ketumpatan untuk pemulihan mikroplastik jelas dari penyelidikan ini. Ini adalah pengesahan penyelesaian pemisahan ketumpatan yang paling komprehensif untuk pemisahan ketumpatan mikroplastik dari tanah di tanah tropika hingga kini.

Kata kunci: ***mikroplastik, tanah, ketumpatan, pemisahan, morfologi***

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LIST OF ABBREVATIONS

PET	Polyethylene terephthalate
HDPE	High-density polyethylene
PVC	Polyvinyl chloride
LDPE	Low-density polyethylene
PP	Polypropylene
PS	Polystyrene
OM	Organic Matter
LOI	Loss of Ignition
USDA	United States Department of Agriculture
MPs	Microplastics

CHAPTER 1

INTRODUCTION

1.0 Background

Nowadays, the world is facing huge pollution and one of the contributors is plastic. Plastics become one of the products that are being used as everyday consumer products including packaging, mobility, building, construction, and agriculture. Since the advent of plastics mass production in the 1950s and consequently, the global plastic production has increased exponentially from 2 Mt in 1950 to 359 Mt in 2018 (Geyer et al., 2017). Polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) are the majority of plastic being produced (Geyer et al., 2017). Within the next 20 years, at the present rate of growth, plastics production is estimated to double (Lebreton et al., 2019). According to Hoornweg et al., (2013), projected increase in future plastic use will result in a concomitant increase in post-consumer plastic waste and for instance, by 2025 the global urban population is estimated to generate more than 6 Mt of solid waste daily.

As a global player in plastic production with currently 1300 active plastic manufacturers, Malaysia has been ranked eighth among the top ten countries with mismanaged plastic waste around the world. It was being reported that 0.14 to 0.37 million tons of the total 0.94 million tons of mismanaged plastic wastes have been washed into the oceans (Ibrahim et al., 2020). Plastic packaging, carry bags, straws – all of which intended to be used only once before they are thrown away are the most commonly single-use plastics. This will result from the 20th century invention, planet Earth as the only habitable planet at the moment is dying a slow death by degradation (New Strait Times, 2015).

Plastic debris can be broken down into smaller plastic fragment that is normally sorted by size as macroplastics (> 2 cm), mesoplastics (5 mm - 2 cm), microplastics (<5 mm), and nano plastics or micro-mini (<1 mm). (Liu et. al., 2018). Plastics that are directly being delivered into the environment as a small form of particulates or micro-size particles are called primary microplastics. Examples of primary microplastics include scrubbing agents and beauty care (e.g shower gel). Other than that, large plastics that are being abraded during manufacturing, use, or support, for example, the erosion of tires during driving and synthetic textiles during washing can result from primary microplastic. Secondary microplastics are microplastics that begin from the degradation of bigger plastic things into more modest plastic pieces once exposed to the marine environment which occurs through photodegradation and other weathering cycles of mishandled waste. (Boucher and Friot, 2017). Microplastics are recognized as having a ubiquitous distribution in the environment and it is currently an emerging pollution (Pagter et al., 2018). Microplastics are currently present in all environmental matrices including seawater, sediments, rivers, soil or even the air (Prata et al., 2018). Concern on microplastics pollution is increasing and has been listed as the second important scientific issue in the field of environment and ecology (Horton et al., 2017). Microplastics can be present in soil thus it is being considered as an emerging threat to terrestrial ecosystems, where soils may represent a larger reservoir for plastics than seas (Hurley et al., 2018).

Tropical soils range from remaining soils which are regularly soils shaped by in-situ enduring of parent rocks to soft clays and organic soils, including peat (Huat, 2010). Tropical regions are the area where tropical soils are found and these soils are distinctive to their area due to natural factors, such as the ocean, wildlife and decomposing minerals found in the earth around the equator (Punke, 2017). Malaysia lies in an area with tropical climate and soil found in Malaysia can commonly be named as tropical soils (Huat, 2010). Soil in tropical regions is different from temperate regions. Tropical soils have a slight difference in characteristic of the soil such as kaolinite (clay) soil that is commonly found in tropical regions has very good drainage compared to temperate regions. Other than that, tropical soils are more likely to be acidic in nature rather than alkaline due to having a low base saturation and tropical soils have low cation exchange capacity due more to the lack of nutrients since the soil itself is composed of quality clay (Punke, 2017). Figure 1 shows a simplified map of the soil distribution of Peninsular Malaysia, Sabah, and Sarawak.

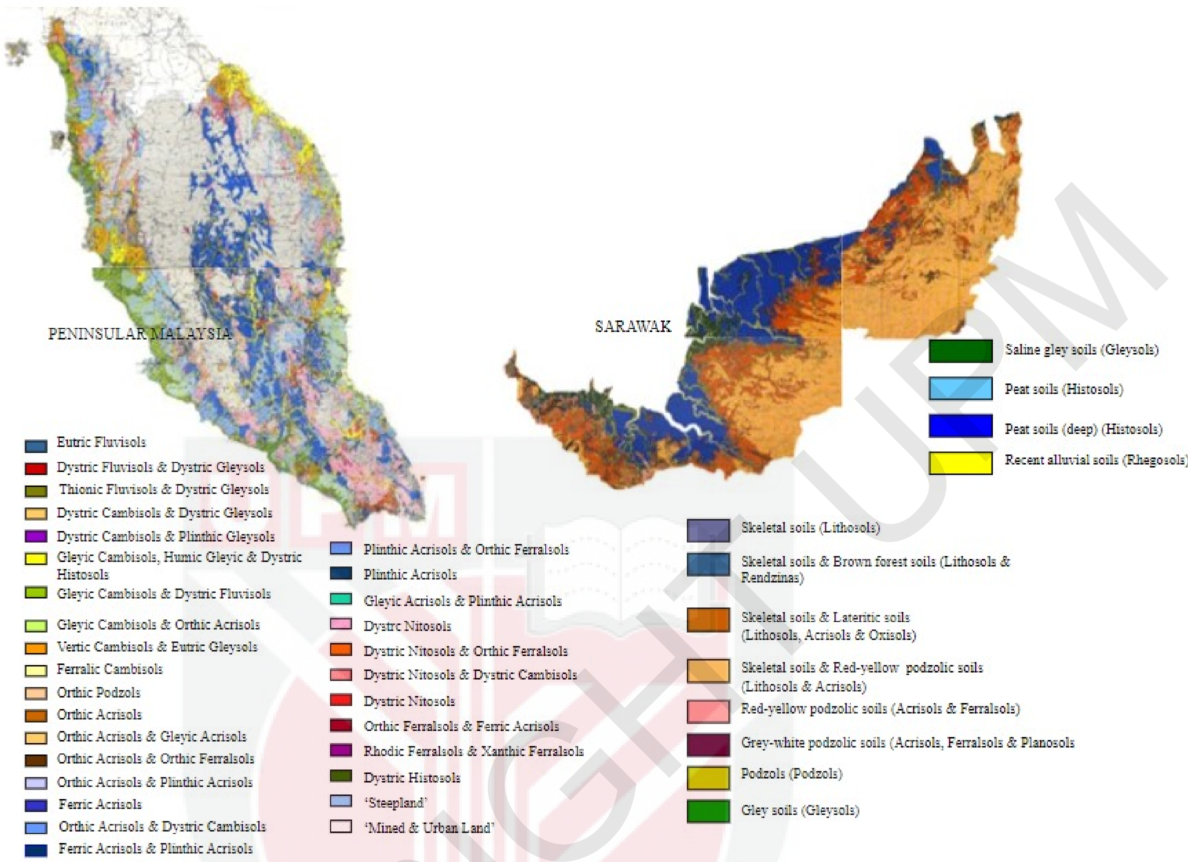


Figure 1.1: Simplified map of the soil distribution of Peninsular Malaysia, Sabah and Sarawak referred from Hayne (2016).

Although the topic of plastic and microplastic pollution in aquatic ecosystems (marine and freshwater) has gotten a lot of coverage, the issue of plastic contamination in the terrestrial environment has gotten a lot less attention. While plastic and microplastic contamination is more visible in the oceans, more than 80% of the plastics found in marine environments were created, consumed, and discarded on land. As a result, land-based plastic pollution is a concern in terms of contamination and disruption to terrestrial ecosystems, as well as transfer to aquatic systems. Microplastic pollution levels on land have been found to be high, ranging from 4 to 23 times higher than in the oceans (Machado et al., 2017, Horton et al., 2017). Moreover, fibrous, and fragmentary microplastics have been detected in large numbers in soils all over the world, according to limited reports (Zhou et al., 2020). van den Berg et al. (2020) recently discovered fragmented-dominated microplastics in agricultural soils, indicating that sewage sludge application contributes to microplastic accumulation in agricultural soils (van den Berg et al.,2020). Microplastics can be untaken by plants and passed down the food chain until they accumulate in soil (Guo et al., 2020). Of necessity, toxins adsorbing on microplastics are included. Microplastics can enter soil through a variety of routes, including the use of sewage sludge and compost (Huerta Lwanga et al., 2017b; Li et al., 2019), irrigation (Blasing and Amelung, 2018), plastic mulching (S. Zhang et al., 2020), littering (Akdogan and Guven, 2019), and atmospheric deposition (Akdogan and Guven, 2019). (Allen et al., 2019).

1.1 PROBLEM STATEMENTS

Common density separation solutions that being used are zinc chloride ($ZnCl_2$), zinc bromide ($ZnBr_2$), sodium chloride (NaCl), sodium iodide (NaI) and sodium bromide (NaBr). However, each density separation solution has limitations and disadvantages in extracting microplastics from soil samples. According to Thomas et al., (2020), $ZnCl_2$ can cause harm to the environment and expensive. Similarly, $ZnBr_2$ is also expensive and severely hazardous to the environment. Quinn et al., (2016) stated that the need of using NaCl in high amount and having low efficiency is the limitation of using this solution. NaBr in the other way having low efficiency (Thomas et al., 2016) and required more wash for microplastic recoveries (Quinn et al., 2016). Other than that, Quinn et al., (2016) also stated that there will be difficulty in isolating microplastics when NaI being used and it is also an expensive solution to be used (Thomas et al., 2020).

Microplastics (up to 55.5 mg kg) have been found in 90 percent of floodplain soils in Switzerland, according to past studies (Scheurer et al., 2018). Zhou et al., (2018) showed the abundance of microplastics in coastal beach soils (Shangdong, China) ranged from 1.3 to 14712.5 number kg. Recent study showed microplastic and meso plastic pollution in farmland soils in suburbs of Shanghai, China (Hurley et al., 2018). Up to the available reading resources, currently there is a lack of studies being performed in tropical regions especially in Malaysia, thus this study would be aiming in detecting microplastic in soil in this tropical region.

1.2 JUSTIFICATION OF STUDY

This study will be able to provide a suitable density separating solution to extract microplastics from tropical soil. Suitable density separating solution is need for tropical soil, as this soil has difference properties including organic matter content which made different density separation solution is need for extraction process.

1.3 RESEARCH QUESTIONS

1. What are the suitable density separation solution that can be considered for microplastic solution?

2. What is the best density separating solution for microplastic extraction involving tropical soil?

1.4 RESEARCH OBJECTIVE

1. To evaluate the performance of selected density separation solutions (zinc chloride, sucrose, sodium carbonate, sodium sulfate and coconut oil) for microplastic extraction in tropical soil.
2. To select the best density separation solution for microplastic extraction involving tropical soil.
3. To test the best density separation solution performance for microplastic extraction in field sample testing.

1.5 HYPOTHESIS

Ho : There is a difference between each density separation solution on extracting of microplastics from tropical soil.

1.6 CONCEPTUAL FRAMEWORK

Figure 2 shows the conceptual framework of the study. This study focuses on density separation of microplastics from tropical soil and density separation solution for microplastics extraction from tropical soil. Microplastics pollution can be classified into primary and secondary microplastics. All this source of pollution either primary or secondary source of microplastics will eventually lead into microplastics pollution in soil. This microplastics pollution in soil will be extracted through a density separation method using different types of density separation solutions. The result will be identified into size, shape and total particles and proceed with characterization process which based on plastic polymer.

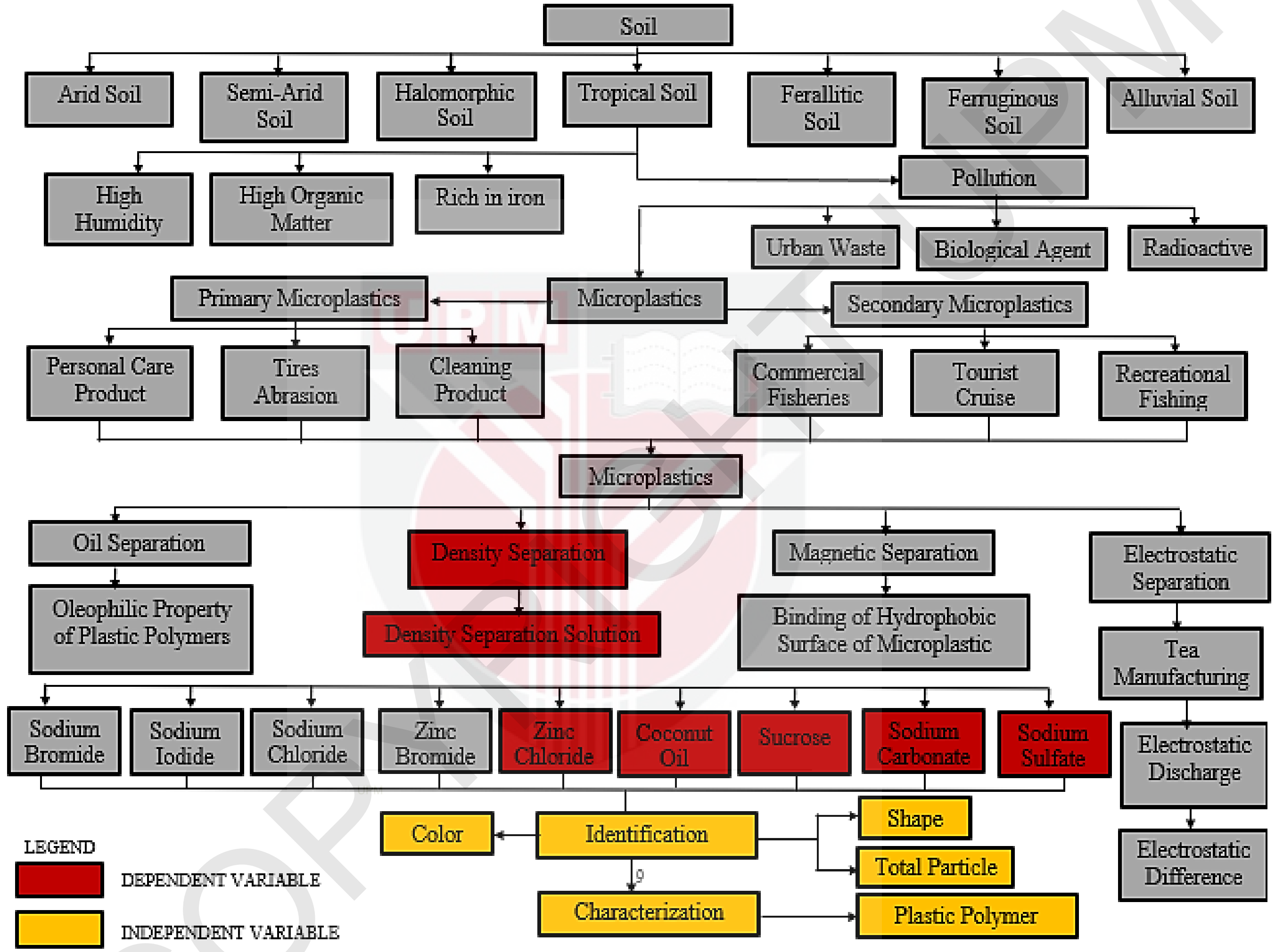


Figure 1.2 : Conceptual framework involving density separation of microplastics

1.8 DEFINITION OF TERMS

Microplastic

Microplastics are plastic particles smaller than 5.0 mm in size (Arthur et al., 2009)

Primary Microplastics

Primary microplastics consist of made raw plastic material, for example, virgin plastic 1pellets, scrubbers, and microbeads that enter the sea by discharge from land. (Masura et al., 2015)

Secondary Microplastics

Secondary microplastics are the results of degradation of bigger plastic materials, from mechanical or photo-oxidative pathways. (Pico & Barcelo, 2019)

Particle Size Analysis

It is a method for determining the physical proportions of three sizes of primary soil particles using a hydrometer to determine their settling rates in an aqueous solution. The hydrometer method of determining particle size analysis (sand, silt, and clay content) is based on dispersing soil aggregates in a sodium hexametaphosphate solution and then measuring changes in suspension density. (Sheldrick et al., 1993)

Organic Matter- Lost of Ignition Method

This approach calculates soil organic matter using gravimetric weight changes related with organic matter combustion at high temperatures. With a technique detection limit of 0.05 percent, the percent weight loss during the ignition stage is recorded as organic matter-loss of ignition (OM-LOI) (percent weight loss). The organic carbon computation assumes that organic matter has a carbon content of 58 percent. (Nelson et al., 1996)

CHAPTER 2

LITERATURE REVIEW

2.1 Microplastic extraction method

Quinn et al. (2016) stated that various methods and techniques have been conducted using many density separation solutions to extract microplastics from soil and extracting microplastics from soil is dependent by the physical characteristics including their size, density, and shape. Based on He et. al (2020) stated that the most common method of extracting microplastics in soil is density separation which is based on the difference of density between the density separation solution and the microplastics. According to Thomas et al. (2020) stated that the density separation method utilizes the buoyancy of plastic particles in solutions with a higher density than that of plastics (ρ = 0.9–1.6 g cm−3), while the soil mineral fraction (for example silica, ρ >2.0 g cm−3) stays at the base. Until now, there is no standardized density separation step and procedure for microplastic extraction from soils. Next, He et. al (2020) also stated the other method that is being used to extract microplastics from soil is oil separation and this method is dependent on the oleophilic property of plastic polymer. According to them as most plastics have hydrophilic characteristics, microplastics can be extracted via the water-soil interface. Oil-based separation, on the other hand, has not been widely used in the detection of environmental microplastics. Its limitations, according to He et. al (2020), are mostly owing to its low efficiency and accuracy, which fluctuates depending to the different qualities of environmental samples. Because some contaminants in samples can stick firmly to the surface of microplastics, the lipophilic characteristics of plastic polymers may be changed. As a result, samples must be digested to various degrees prior to separation. Crichton et al. (2017) used detergents and reagent alcohol (including 90 percent ethanol, 5 percent methanol, and 5 percent isopropanol) to effectively clean oil on the surface of microplastics, and FTIR spectra were unaffected following treatments. Overall, oil separation is a timesaving and environmentally beneficial process that deserves to be developed further as an alternate separation method (Mani et al., 2019).

Other than that, Silveira et al. (2018) also mentioned electrostatic separation is also one of the methods to extract the microplastics from soil and this innovation has been utilized in different fields of industry and horticulture for example tea manufacturing. Initially, electrostatic discharge was used to recycle plastic trash by separating it from industrial or municipal garbage. According to Hu et al., (2020), this technique has been used in the detection of microplastics in the environmental area in recent years. The idea behind this method is that solid matrices like soil and silt are normally charged or corona because to their conduction qualities, while (micro)plastic is typically non-conductive. As a result of the electrostatic differential between conductive media and non-conductive microplastics, discharges from electrostatic generators may accomplish high-efficiency separation. Electrostatic separation has several benefits, including automation, ease, and high accuracy. According to simulated testing on environmental samples including various amounts of organic matter and microplastics particle sizes, this strategy may simplify their separation while eliminating up to 99 percent of the original sample mass and preventing microplastics loss (Felsing et al., 2018). Even for microscopic microplastic particles as tiny as 63 m, the recovery rate of microplastics remained extremely high (> 99%). Moreover, He et al., (2020) also stated that the other method that is being used to extract microplastics is magnetic separation method which is microplastics hydrophobic surfaces may be magnetized by binding hydrophobic Fe nanoparticles. Following that, due to magnetic characteristics, Fe-combined microplastics may be isolated from ambient media in an extra magnetic field, which is the mechanism of magnetic separation of microplastics (Grbic et al., 2019; Xu et al., 2011). Simulated trials revealed that this method might even separate microalgae attached Fe_3O_4 nanoparticles in situ in a short amount of time (Xu et al., 2011). Rhein et al. (2019) found that magnetic filtering efficiently removed tiny microplastic particles from dilute solutions. According to research on microplastics of different polymer types in sediments, recovery rates for medium-sized MPs (0.2–1 mm) varied from 49 percent to 90 percent, although recovery rates for fine MPs (0.2–20 m) reached up to 90 percent (Grbic et al., 2019). However, the effectiveness of this magnetic screening approach on microplastics is reliant on the pH of environmental samples, as well as various kinds of magnetic nanoparticles and nanoparticle quantities attached to microplastics (Rhein et al., 2019). Finally, He et. al (2020) also stated that the other method that is being used to extract microplastics is solvent extraction separation method which is natural solvents which can be used to extricate plastic polymers from ecological media under specific physicochemical conditions. This methodology is created from traditional extraction techniques, for example, Soxhlet extraction, bubbling under reflux, and disintegration followed by reprecipitation.

2.2 Density separation solution

Microplastic (MP) in soil will become a part of the complex mixture of all different kinds of mineral and organic particles. Thus, it is a specific challenge to separate MPs from soil (Bläsing and Amelung, 2018; De Souza Machado et al., 2018b; Rillig, 2018; Huerta Lwanga et al., 2017; Rillig et al., 2017). According to Quinn et al. (2016) currently, several density separation techniques using numerous solutions have been developed for the separation of microplastics from sediment and the method used for the separation of microplastics from sediment is influenced by the physical characteristics (size, density, shape) of both the sediment and the microplastics. However, microplastics has been separated using a variety of reagents, including sodium chloride (NaCl), calcium chloride ($CaCl_2$), zinc chloride ($ZnCl_2$), and sodium iodide (NaI), with separation efficiency being proportional to the density of those solutions (Crichton et al., 2017; Quinn et al., 2017). To select the most appropriate chemical, however, the performance of the procedures, the environmental implications, and the cost effectiveness must all be addressed (Scheurer and Bigalke, 2018; Peng et al., 2017; Matsuguma et al., 2017; Mintenig et al., 2019). Five solutions that are commonly being used are zinc chloride ($ZnCl_2$), zinc bromide ($ZnBr_2$), sodium chloride (NaCl), sodium iodide (NaI) and sodium bromide (NaBr). According to Thomas et al., 2020), $ZnCl_2$ can cause harm to the environment while it is also expensive. This same goes to $ZnBr_2$ where it is also expensive and severely hazardous to the environment. Even though NaCl is an inexpensive and safe solution to be used and not harm the environment as same as NaI and, it is being reported that NaCl to be less efficient in separating microplastic from the sample and a high amount is needed while for NaI, the solution being reported to have difficulty in isolating the microplastic that have high density plastics like PET or PVC (Quinn et al., 2016; Liu et al., 2018; Scheurer and Bigalke, 2018; Zhou et al., 2018). $CaCl_2$ has a higher separation effectiveness than NaCl, however calcium ions can react with organic matter (Scheurer and Bigalke, 2018), and the density may be too low to separate all types of MP (Liu et al., 2019). Furthermore, sodium bromide (NaBr) has been studied as a non-toxic, non-corrosive, and inexpensive sediment separating reagent (Quinn et al., 2017), however it was not used to extract soil microplastics (Liu et al., 2019). Therefore, there are limitations related with density separation solutions that were used in previous studies due to their disadvantage in each solution.

Other than the five most common solutions that are used for separation of microplastic used in previous studies, coconut oil is also one of the choices of solution that can be used. In some tropical and subtropical countries, coconut is an important food, with the coconut tree being referred as the 'tree of life' which according to DebMandal and Mandal, (2011), stated that coconut and its products (milk and oil, among others) are used in daily life by the general population for several purposes, such as cooking, hair and skin treatment, food ingredient, and folk medicine. Noureddini et al. (1992) mentioned coconut oil under 37.8°C having the density of 0.903 g/ml. Coconut oil has a little higher density than the microplastics that will be extracted in this investigation (LDPE, HDPE, PVC, PS and PP). Coconut oil is mostly made up of saturated fatty acids (approximately 94 percent), with a significant amount of medium-chain fatty acids (about 62 percent). Lauric acid is a kind of fatty acid found in coconut oil. Crichton et al. (2017) coined the term "oleophilic interaction" to describe the attraction between the oil's long chain fatty acids and the polymer backbone. The non-polar lipophilic component of the fatty acid molecules in coconut oil has a stable attraction to the non-polar lipophilic carbohydrate surface of synthetic polymer fragments (microplastic), allowing the microplastic to be removed from the soil. Coconut oil also has one of the greatest viscosities among natural plant oils, allowing a thick oil coating to develop around the polymer particles. In this study, coconut is viewed as a more environmentally friendly fluid than other separation solutions.

Meanwhile, sucrose is being used for the density separation approach, even though it is not a common solution for extracting microplastics from soils. Sugar is a frequent name for sucrose. Sucrose is a crystalline or powdered white odorless substance that is denser than water and very soluble in water. (1998-1999) (Lide) , It has been reported to have a density of 402.9 g/ml in a 35 percent sucrose concentration (Lane, 2006). This density is high enough to extract microplastic, which has a significantly lower density. The density of sodium carbonate (Na_2CO_3) is 2.5 g/ml (Haynes, 2014), making it an appropriate solution for density separation. In water, sodium carbonate is easily soluble, but in ethanol, it is insoluble.

2.3 Morphology of plastics polymer

The definitions and terms of microplastics was formally initiated and founded in 2004 by Thomson et. al (2004). As indicated by their shapes and morphology, the microplastics are arranged into pellets, foams, fragments, flakes, films, fibers, and sponges. The trait of pellets is hard, regular, disc-, ovoid-or round or cylindrical-shaped plastic particles. The foams are lightweight, white Styrofoam and the sponges are yellow, lightweight, and permeable. The fragments are hard, rugged, and unpredictable plastics, and the chips are level sheets of plastic. The films are transparent, soft, and slender. The fibers are usually found in fishing lines and clothing (Zhou et al, 2018). Meanwhile, Liu et al. (2018), shows that there are a few shapes of microplastics that are found in shallow and deep soil in the studies which are fiber, fragment, film, and pallet. Next, the distribution of microplastics color is different in shallow and deep soil and the colors found are black, green, transparent, red, and blue Liu et al. (2018). Polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) are most of the plastic being produced (Geyer et al., 2017). The primary polymers were polyethylene (PE) and polypropylene (PP) pellets and fragments, a polymer mix of both PE and PP on account of fibers, polypropylene (PP) flakes and films, polystyrene (PS) foams, and polyurethane (PEU) sponges. Zhou et al (2018). From Moharir and Kumar (2019), water bottles are one example of using polyethylene terephthalate (PET) in their products. The density of PET is ranging from 1.37- 1.45 g/ml and PET can last in this world until up to 20 years. They also mentioned that low-density polyethylene (LDPE) usually can be found in plastic bags and squeeze bottles and the density of LDPE is ranging from 0.917- 0.930 g/ml. Meanwhile, high-density polyethylene (HDPE) is one of the mediums used in detergent bottles. The density of HDPE is ranging from 0.93- 0.97 g/ml and HDPE can stay in the ground for more than 28 years. In addition, the common plastics that commonly being found in previous literature is PVC that is usually being used in engineering and technician works; pipes, electric cables, and clothing for example and the density of PVC is ranging from 1.20-1.45 g/ml and its lifespan is 140 years. Furthermore, PP can come from stoppers and clothing and contain 0.89-0.94 g/ml in density. Finally, the density of PS is 1.04-1.11 g/ml and PS is usually being used in ready-to-eat food.

2.4 Effect of microplastics in soil ecosystem

MPs particle can form clumps and aggregations in soil in a variety of ways: loosely in fragment-types, and more tightly in linear-types (de Souza Machado et al., 2018a; Zhang and Liu, 2018). Microplastics have been shown to cause soil toxicity and harm the environment (de Souza Machado et al., 2018a, 2018b; Liu et al., 2017). Soil organisms can transform plastic waste into microplastics by their biological processes (Chae and An, 2018). For example, earthworms have been known to ingest microplastics. Microplastics can be ingested and excreted by earthworms by the burrowing insects. They are transported vertically from shallow to deep soils by the anecic earthworms (Cao et al., 2017; Huerta Lwanga et al., 2016; Rillig et al., 2017a). Later, earthworm casts containing concentrated microplastics can be eaten by soil microarthropods. Microplastics are likely to migrate from soil through the actions of rodents and certain plant species that ingest them (Maaß et al., 2017; Rillig, 2012). In a study by Zhu et al (2017) demonstrated that the ability to transport and distribute small plastic objects in soil was enhanced by the presence of a predator-prey bond. Furthermore, microplastics can also alter soil water dynamics and cause changes in a variety of physiological photosynthetic efficiency indicators, implying possible repercussions for plant performance (de Souza Machado et al., 2019; Faucon et al., 2017). Microplastics have also been found to change soil permeability and water retention, affecting water evaporation (de Souza Machado et al., 2018a; Wang et al., 2015a). Because humic-like materials can increase soil stability, water holding capacity, and nutrient availability, the accumulation of high molecular-weight humic-like materials induced by microplastic addition could imply that MPs may play a role in enhancing soil quality (Liu et al., 2017; Schnitzer, 2000).

Lastly, MPs has the potential to alter soil enzymes, which can function as a marker for determining soil fertility and recreated a significant role in the control of soil nutrient cycle for nutrients such as carbon, nitrogen and phosphorus (Allison and Jastrow, 2006; Trasar-Cepeda et al., 2008). Soil enzyme activity may provide information on microbial activity as well as the availability of substrates for microbe uptake. A high concentration of microplastics (28 percent by weight) was shown to considerably enhance the buildup of DOM and promote the release of soil nutrients such as dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and dissolved organic phosphorus (DOMP) (DOP). Microplastics can also have an impact on the soil's physical characteristics and minerals. Changes in the soil physical environment, particularly soil aggregation, which has been observed to integrate linear microfibers, are predicted to have a different impact on microbial development than changes in the soil physical environment of non-microfiber-structured soil (Rillig et al., 2017b, 2018). Furthermore, when changes in soil porosity and soil wetness occur because of the presence of microplastics, the flow of oxygen in the soil can be disrupted, resulting in a shift in the relative distribution of anaerobic and aerobic bacteria (Rubol et al., 2013). Furthermore, the alteration of pore spaces induced by microplastics has the potential to result in the loss of microhabitats and the extinction of indigenous microbes (Veresoglou et al., 2015). Aside from this, microplastics considerably altered the composition and structure of the microbial community, while the rates of substrate-induced respiration (SIR) were dramatically reduced, showing that microplastics were responsible for alterations in soil microbial activity (Judy et al., 2019).

2.5 Transportation of Microplastics into Soil Ecosystem

Although primary microplastics are a major source of litter in both marine and freshwater habitats, it has also been found in agricultural environments as a result of sewage sludge containing microfibers and microplastics being deposited to agricultural land (Horton et al., 2017). The use of sewage sludge from urban wastewater treatment plants as fertiliser for agricultural land is a common practice that is a significant source of primary microplastics pollution in soil. Europe and North America have producing about half of all sewage sludge from agricultural use. Annual microplastics additions to agricultural land in Europe are projected to be between 125 and 850 tons per million inhabitants. This equates to an annual microplastics input of 63,000-43,000 tons in European and 44,000-300,000 tons in North American farmlands, respectively (Nizzetto et al., 2017). Meanwhile, secondary microplastics are released during the collection, sorting, transportation, and landfilling of municipal solid waste. Furthermore, wind contributes to the spread of microplastics through land, as well as from land to water and vice versa. In soil, agricultural plastics like polytunnels, silage bailing, and plastic mulches are related to secondary microplastic pollution. Containers, packaging, and netting are examples of additional plastic materials used for agricultural purposes and thus represent possible sources of microplastic pollution in soil. On ground, fragmentation is aided by sunlight, which has a greater effect on these plastics than it does in the sea (Horton et al., 2017).

CHAPTER 3

METHODOLOGY

3.1 Soil Sampling

Soil samples were collected randomly from an undisturbed agricultural area. A topsoil with an area of 3x3cm2 with depth of 25 cm (3x3x25cm) had been sampled. The soil sample was weighed by used analytical balance and placed into a beaker. The weighted soils were spiked with a known number of microplastic particles of six different polymers (HDPE, LDPE, PP, PVC, PET, PS) in January 2021 and kept in an undisturbed environment for three months.

Figure 3.1 : Soil sampling site in an undisturbed agriculture area.

Figure 3.2 : The area of soil sampling site

3.2 Microplastics Preparation

6 different types of plastic polymer were taken from 6 different post-consumer products (listed in Table 3.1) that were broken down to secondary microplastics. For each plastic sample, the color and reference density were recorded. However, the Fourier transform infrared spectroscopy (FTIR) analysis was unable to be undertaken due to situation of SARV-Cov 2 (Covid-19) to confirm the correct sample identification. Following mechanical breakdown, the polymers was sieved and separated into size classes used a mechanical shaker, with the 200–400 mm and 800–1000 mm fractions being examined. These secondary microplastics might been classified as fragments, despite their various shapes.

Table 3.1 : The source, colour and density of plastic polymer that has been tested

Plastic	Source	Colour	Density
PET	Plastics Bottle	Clear	1.37- 1.45 g/mL
HDPE	Laundry Bottle Plastics	White	0.93- 0.97 g/mL
PVC	Bubble Plastic Sheet	Clear	1.20-1.45 g/mL
LDPE	Shisha Tips	Blue, Green, Yellow, Red	0.92- 0.93 g/mL
PP	Water Bottle Lid	Blue	0.89-0.94 g/mL
PS	Plastic Spoon	White	1.04-1.11 g/mL

3.3 Density Separation Solution Preparation

Each density separation solution was made freshly based on the method by from Claessens et al. (2011) which is with a ratio of 3:1. Zinc chloride (R&M Chemicals) was prepared by dissolving 600g $ZnCl_2$ in 500 mL of distilled water. Next, sucrose (R&M Chemicals) was prepared by mixing 250g of sucrose with 200g of $NaCl_2$ (Bendosen) and dissolved in 500 mL of distilled water. After that, sodium sulfate (Na_2SO_4) (R&M Chemicals) was prepared by dissolving 732g Na_2SO_4 in 500 mL of distilled water. Meanwhile, for coconut oil was brought from Country Farm Organics. Finally, sodium carbonate (Na_2CO_3) (R&M Chemicals) was prepared by dissolving 1270g Na_2CO_3 in 1100 mL of distilled water.

3.4 Density Separation Process

The mixture had been stirred used a stirrer at 2000 rpm for 30 minutes and left undisturbed for 24 hours. The supernatant had been left for 8 hours. The lighter floating plastic particles to float had been taken manually and particles that accumulated on the surface of the solution had been filtered used filtration, Whatman no. 42 filter papers. The filter paper had been transferred to a petri dished and placed in an oven (50°C) for 20 min. The chosen density separation solution as an independent variable was zinc chloride, sucrose, coconut oil, sodium sulfate, and sodium carbonate. Savva et al. (2016) stated that coconut oil was removed from the kernel of developed coconuts harvested from the coconut palm and was rich in saturated fats. According to Noureddini et al. (1992), the density of coconut oil in 37. 8°C was ranging from 0. 9033 g/ml. Next, lane (n. d) stated that the density of 35% sucrose solution was 402. 90 g/mL and according to Larranaga et al. (2016) the density of sodium sulfate solution was 2. 671 g/mL. Thomas et al. (2020) stated that the density of zinc chloride ($ZnCl_2$) are ranging from 1.5-1.7 g/mL. Finally, the density of sodium carbonate solution was 2. 5 g/cm³. Haynes (2014). Table 3.2 listed the density of the selected density separation solution used in this study.

Table 3.2 : The density of density separation solution used.

Density Separation Solution	Density (g/mL)
Coconut Oil	0.903
Zinc Chloride	1.500-1.700
Sodium Carbonate	2.500

Sodium Sulfate	2.671
Sucrose	402.900

3.5 Identification and Characterization

For microplastics identification and characterization had been done by used the Nikon Eclipse E200LED MV RS coupled with best scope international limited camera and the image would also been analyze used an open-source particle-analysis software. The identification of particles from each sample preparation stepped was further analyzed to identify the total particles, size, and shape of these polymers.

3.6 Data Analysis

The composition of microplastics had been classified according to size, shape, total particles, and undergo characterization process which had been classified based on polymer typed. The dried and recovered microplastic particles had been counted and the percentage recovery calculated following the equation:

$$\textbf{\% Recovery} = \frac{\textbf{Final number of microplastic particles}}{\textbf{initial number of microplastic particles added}} \times 100$$

3.7 Organic Carbon Analysis

Organic Carbon Analysis is a technique for determining a soil's organic matter content. To oxidize all the organic matter in a dried sample of soil, it is heated to a high temperature in a muffle furnace. The organic matter content is determined by the weight loss after cooling (Money,2021). The detailed information of organic carbon analysis is given in Appendix I.

3.8 Soil Particle Analysis

The size distribution of individual particles in a soil is measured via particle-size analysis. The soil texture is defined by the particle-size distribution (i.e., their percentages) in the soil. Unfortunately, the texture of a soil is classified differently by the several soil categorizations schemes now in use. The USDA (United States Department of Agriculture) devised one of the most frequently recognized and utilized soil categorization schemes, which is seen in Figure 3.1 In this scheme, soil particles and their size ranges are clay (2 µm), silt (2-50 µm), and sand (2-50 µm) (50-2000 µm). Sung and Talib (2006). The purpose of particle-size analysis is to figure out how the different grain size fractions of the mineral component of the soil are distributed (by weight). The soil organic matter is removed using a hydrogen peroxide solution. The breakdown of calcium carbonate and distribution of cement (amorphous sesquioxides) that link the colloidal fraction is achieved by treating them with hydrochloric acid followed by distilled water washing. A 50 µm sieve is used to remove the very fine fraction from sand while screening under water. Dry sieving with the proper sieve sizes is then used to separate the distinct sand fractions. Sung and Talib (2006)

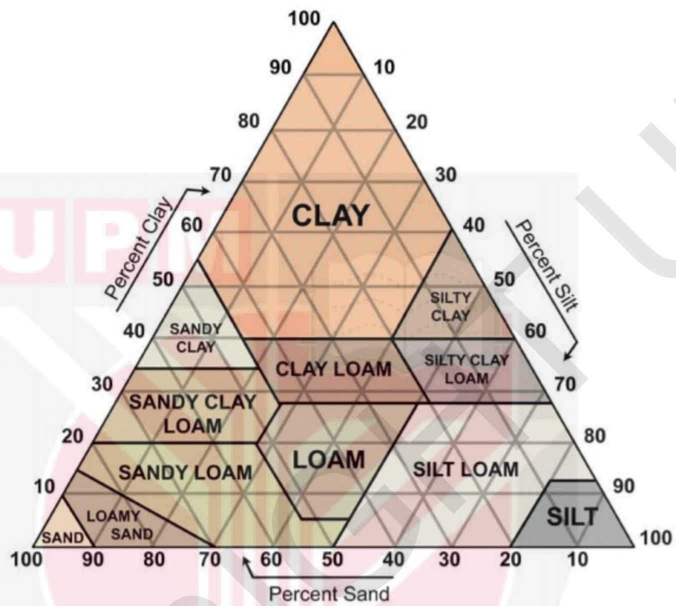


Figure 3.3: The USDA soil classification scheme referred from https://wisconsindot.gov/Documents/doing-bus/eng-consultants/cnslt-rsrces/geotechmanual/gt-03-03.pdf

3.9 Field Sample Preparation

Field sample soil was prepared by spiked a known number of microplastics into an agricultural soil for 3 months. Then the soil was taken into laboratory for extraction of microplastics using the selected density solution that have been conducted on experimental soil. The best density separation solution that has been selected to extract the plastic polymer from the samples was sodium carbonate and sodium sulfate.

3.10 Quality Control and Assurance

At all times, clean white lab coats were worn with no synthetic fibers below. Before usage, all surfaces and equipment were cleaned three times with 70 percent ethanol and distilled H_2O, and the equipment was checked for microplastic contamination using a dissecting microscope. All laboratory benches were checked for particle contamination. This was done before and after all operations, with the tapes being inspected microscopically and using FTIR as needed. Microplastic contamination in the atmosphere was tested using the taping approach and by hygiene filter papers in Petri dishes for the duration of the lab session, which were then tested for contamination. During the density separation technique and after the microplastics were recovered, care was made to restrict the length of time a sample was exposed to air. To avoid contamination, filter papers containing microplastics were maintained in covered Petri dishes. Moreover, blank runs of distilled water were carried out to make sure that the distilled water that has been utilized contain no microplastics fragments. Finally, glassware material has been used throughout the process to prevent any contamination in the soil samples.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Percentage of Microplastics Recovery

Table 4.1 shows the number of microplastics that floated and non-floated in different density separation solutions and the average in which shows that sodium carbonate has able to recover most of the type of microplastics polymer that has been selected in this study. This could be due to the organic matter (OM) in the soil can coat and bind soil mineral particles, forming stable soil aggregates. Microplastics may interact with OM and get enveloped in soil aggregates, making it difficult to separate them from soil particles (He et al., 2018). Sodium carbonate (Na_2CO_3) is a basic substance that may neutralize an organic layer containing trace acids (Reaction Workups., 2021). Because of this characteristic, sodium carbonate can remove the majority of microplastics by decreasing the binding between microplastics OM and therefore minimizing the development of soil aggregates, in addition to having a high density. Moreover, sodium carbonate has been recognized to have adhesive properties, allowing it to bind agents in general. Aside from that, sodium carbonate can operate as an adsorbent, causing molecules to adhere to a surface (National Center for Biotechnology Information, 2021). Meanwhile, coconut oil has the lowest number of microplastics recover (floated) in most type of plastics polymers. Sucrose has the highest density among all the other density separation solutions (402.9g/ml) (Lane, 2006), followed by sodium sulphate (2.671g/ml) (Larranaga et al., 2016), sodium carbonate (2.5g/ml) (Haynes, 2014) and coconut oil are the lowest density. Coconut oil possesses the lowest density which is even lower than all the microplastic being used (0.903g/ml) (Sigma Aldrich, 2003). However, the result shows that coconut oil solution is able to extract microplastics polymer. This could be due to the presence of lauric acid which makes up approximately half of the fatty acids in the coconut oil; likewise, medium-chain triglycerides which contain lauric acid account for approximately half of all triacyl glycerides in coconut oil. These fatty acids enable stable attraction between the non-polar lipophilic component of the fatty acid molecules and the non-polar lipophilic carbohydrate surface of synthetic polymer fragments. Furthermore, coconut oil features one of the highest viscosities of the natural plant oils allowing the formation of a thick oil layer around the polymer fragments (Marks and James, 2016). So, from this study, sodium carbonate is the best solution to be used in density separation solution due to it high density and ability to extract microplastic

Table 4.1: Number of plastic particles floated and non-floated in each density separating solution and the average.

		PET		HDPE		PVC		LDPE		PP		PS				
		S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**	**R1**	**R2**	**Average**
$ZnCl_2$	**Float**	8	7	6	7	4	7	7	7	5	7	4	6	34	41	37.5
	Non-Float	0	0	2	2	0	1	3	3	5	3	3	2	15	11	13.0
Sucrose	**Float**	7	8	5	6	5	3	3	2	4	2	5	6	29	27	28.0
	Non-Float	1	1	5	4	1	1	6	8	6	8	5	4	24	26	25.0
Na_2SO_4	**Float**	0	1	1	3	0	3	2	4	4	6	2	4	9	30	19.5
	Non-Float	3	2	9	6	1	1	8	6	6	4	6	4	33	23	28.0
Na_2CO_3	**Float**	7	0	10	10	1	3	10	10	10	10	10	10	48	43	45.5
	Non-Float	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0.5
Coconut Oil	**Float-Oil**	3	2	8	10	0	0	6	7	9	9	0	3	31	26	28.5
	Float-Water	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Non-Float	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4.2 shows the percentage of plastic polymer recovery in different density separation solutions in soil and the average percentage recovery of different plastics in different each solution. PET has the highest recovery percentage of microplastics on sucrose solution which is 85% compared to the other density separation solution while the lowest recovery percentage of PET is in coconut oil solution with only 25% recovery. Meanwhile, HDPE shows that the highest percentage of recovery is when recovered from sucrose and sodium carbonate with 100% of percentage recovery then followed by sodium sulphate and zinc chloride with 95% and the lowest percentage recovery is from coconut oil with 90%. Next, PVC is the most slightly low in percentage of recovery compared to other plastic polymers among the density separation solutions. The highest percentage of recovery for PVC is from zinc chloride solution with 60%, followed by sucrose, sodium sulphate, sodium carbonate and lastly is coconut oil with 50%, 25%, 20% and 0% respectively. LDPE has the highest percentage of recovery of 100% from zinc chloride, sodium sulphate and sodium carbonate and 95% of recovery from sucrose solution then the lowest percentage of recovery is coconut oil which is 65%. Furthermore, PP has the highest percentage of recovery among the density separation solution, which is from zinc chloride, sucrose, sodium sulphate, sodium carbonate by 100% of recovery and then followed by 90% of recovery from coconut oil. Finally, PS has the highest recovery percentage of microplastics on sucrose and sodium carbonate which is 100% compared to the others solution while the lowest recovery of percentage of PS is in coconut oil with only 15%. In average, we can see that the highest average percentage recovery is sucrose by 88.30%, followed by zinc chloride by 84.10%, sodium carbonate by 76.67%, sodium sulphate by 71.67% and lastly coconut oil with only 47.50% average percentage recovery. Based on the average percentage recovery, we can see that sucrose shows the best microplastics extracting solution by having the highest percentage, however, based on overall different microplastics that able to be extracted, we can see that sodium carbonate shows the best result where it manages to recover four types of microplastics by 100% efficiency and Na2CO3 also have better economical characteristics which is cheap and recyclable.

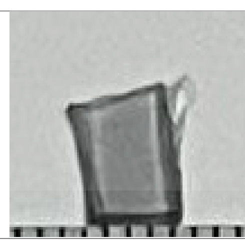
Table 4.2 : Percentage of different type of plastic polymer recovered from different type of density separation solution and the average percentage recovery

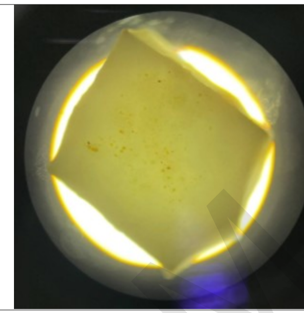
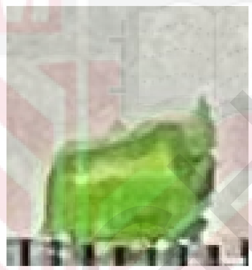
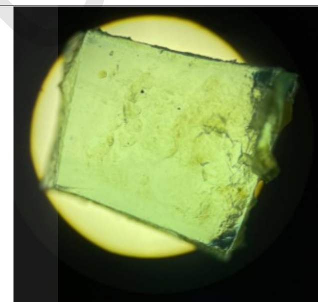
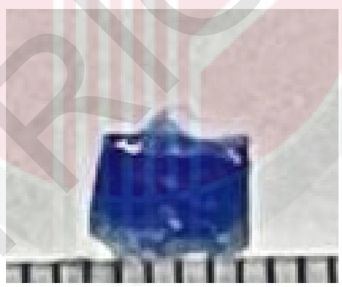
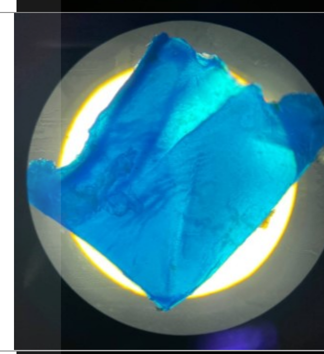
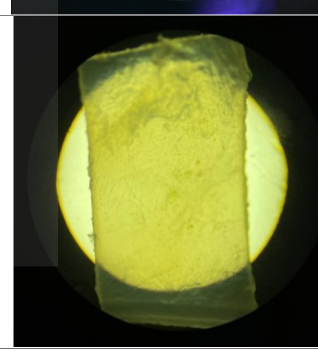
	Zinc Chloride	**Sucrose**	**Sodium Sulphate**	**Sodium Carbonate**	**Coconut Oil**
PET	75%	85%	30%	40%	25%
HDPE	95%	100%	95%	100%	90%
PVC	60%	50%	25%	20%	0%
LDPE	100%	95%	100%	100%	65%
PP	100%	100%	100%	100%	90%
PS	75%	100%	80%	100%	15%
Average Percentage Recovery	84.10%	88.30%	71.67%	76.67%	47.50%

4.2 Impact of Spiked Plastic Polymer in Different Types of Density Separation Solution

Table 4.3 shows that there was no difference on the surface between the initial appearance of microplastics and after being extracted from the various density separation solutions. Previous study by Srivasta (2013), coconut oil has been shown to be stable when being stored in different type of plastic container that include LDPE, PET and HDPE which only shows slight changes after 12 months that being measured by peroxide value (PV), free fatty acid (FFA), thiobarbituric acid (TBA), total carbonyl (TC) and anisidine value (AV). However, no previous data available for the stability of PVC and PS in coconut oil.

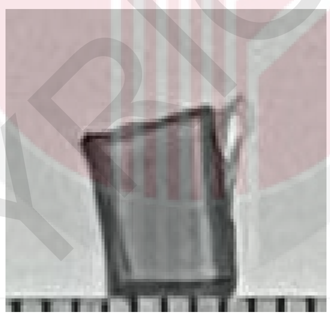
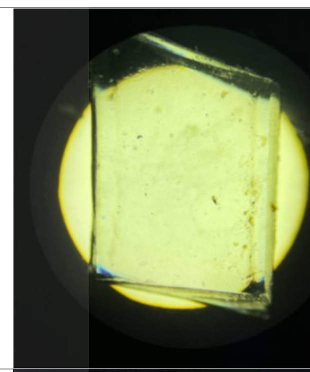
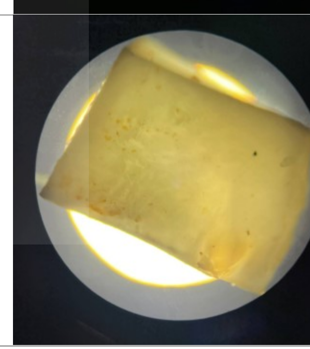
Table 4.3 : Surface and color of spiked microplastics polymer before and after being extracted by coconut oil solution

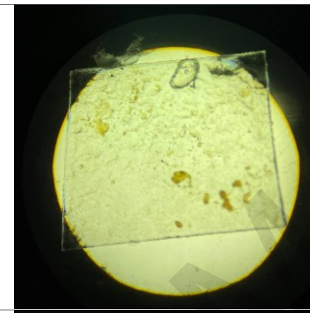
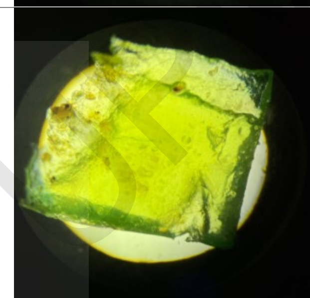
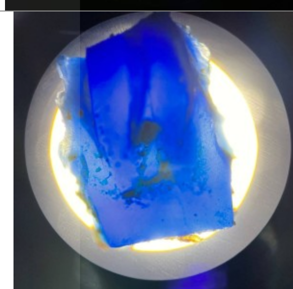
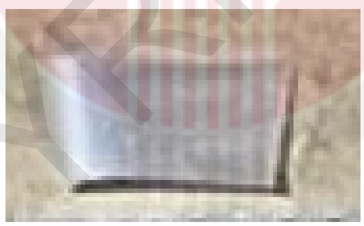
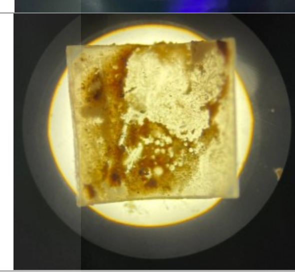
Plastic Polymer	Before	After
PET		Not extracted

HDPE		
PVC		Not extracted
LDPE		
PP		
PS		

Next, sucrose is a common sugar, a disaccharide that is composed of two monosaccharides glucose and fructose. From table 4.4, there was no significant changes in the colour and surface of the microplastic after being extracted b sucrose. However, sucrose has been known to have the ability to corrode steel by hydro erosion which varies according to the concentration of sucrose (Nekoz et al., 1968). The data availability on the effect of sucrose is currently limited. A study by Ball (2002) found that sucrose could turn polythene, polystyrene, and polypropylene into biodegradable in the soils by enhancing the action of bacteria in the soils.

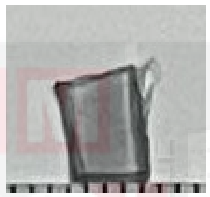
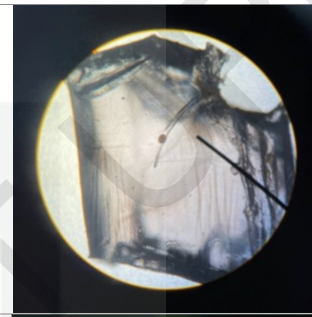
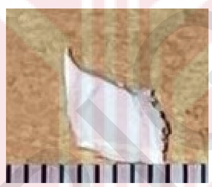
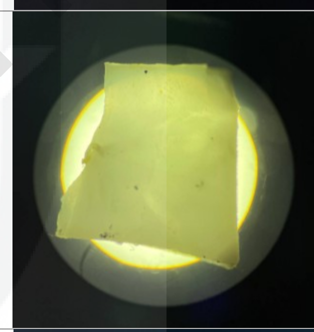
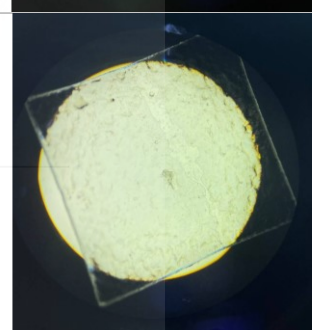
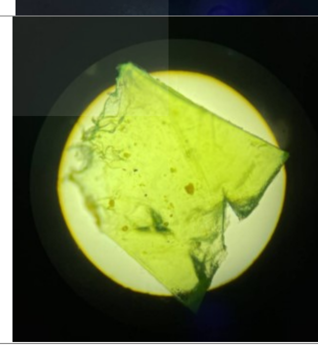
Table 4.4 : Surface and color of spiked microplastics polymer before and after being extracted by sucrose solution.

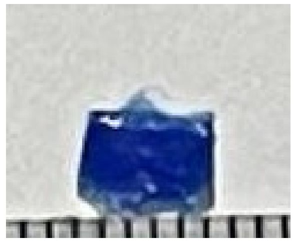
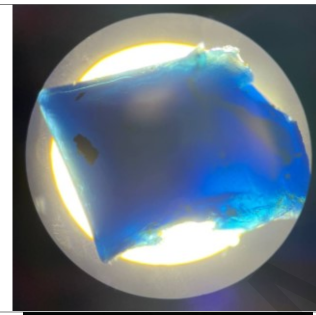
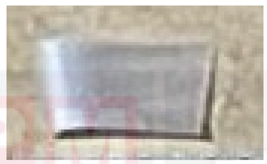
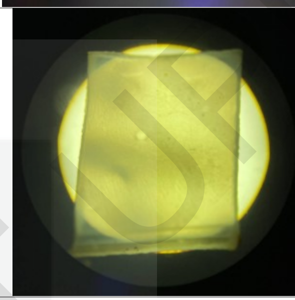
Plastic Polymer	Before	After
PET		
HDPE		

PVC		
LDPE		
PP		
PS		

Meanwhile, zinc chloride has been known to have corrosive properties however only limited data on metal and no study shows that this solution has the ability to corrode plastics. Hannes et al. (2012) mentioned that the corrosive nature of the zinc chloride aids the detachment of plastic particles from sediment particles and/or organic material that makes this solution ideal to be used in separating microplastics. This supports the result from this study that shows the high average percentage recovery of microplastics in zinc chloride solution.

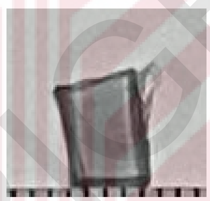
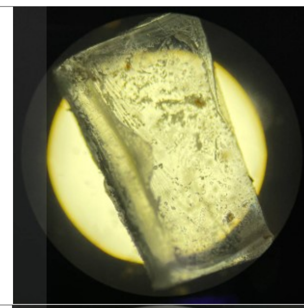
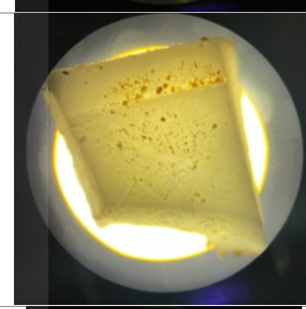
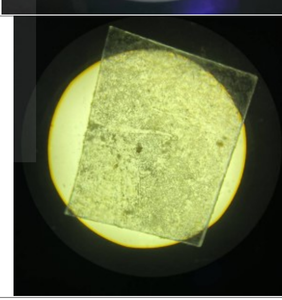
Table 4.5 : Surface and color of spiked microplastics polymer before and after being extracted by zinc chloride solution

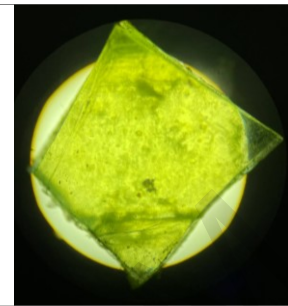
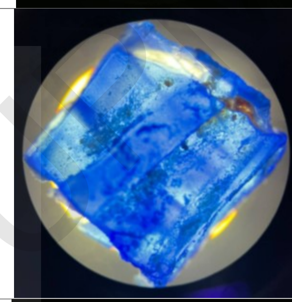
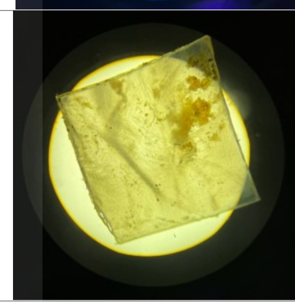
Plastic Polymer	Before	After
PET		
HDPE		
PVC		
LDPE		

PP		
PS		

Sodium sulphate being known to have the ability to recover microplastic and being used widely in previous studies (Cutroneo et al. 2020). In addition, Corrosionpedia (2018) stated that sodium sulphate (Na_2SO_4) is a chemical compound that may be found in nature as a mineral or as a byproduct of certain industrial operations. It has a wide range of commercial uses and is categorized as a non-toxic chemical when handled properly. Soaps and detergents, especially powdered soaps, are typically made using sodium sulphate (Garzena, 2004). Textiles, paper and paper pulp manufacture, glass manufacture, and a range of other uses are all made using it. Sodium sulphate is the sodium salt of sulfuric acid. When anhydrous, it forms a white crystalline solid of formula Na_2SO_4 known as the mineral thenardite. Sodium sulphate is one of the most essential minerals in the chemicals sector. Sodium sulphate is chemically highly stable, being non-reactive toward most oxidizing or reducing chemicals at normal temperatures. At high temperatures, it may be transformed to sodium sulfide through carbothermal reduction. It produces hot corrosion, which is the breakdown of materials caused by the presence of a deposit or ash, usually sodium sulphate Corrosionpedia, 2018). However, this research only dissolved the sodium sulphate into the distilled water at room temperature. Therefore, from table 4.6 we observe that the sodium sulphate is in a stable state and does not damage the plastic polymer throughout the extraction process.

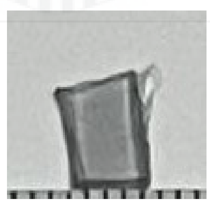
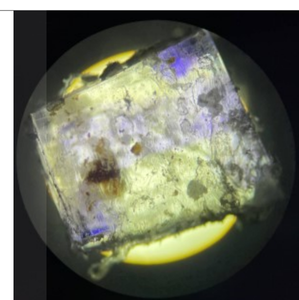
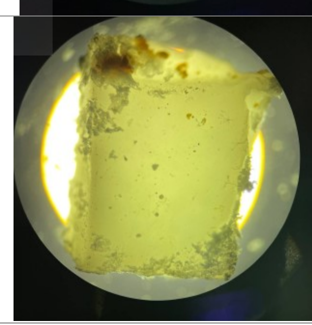
Table 4.6 : Surface and color of spiked microplastics polymer before and after being extracted by sodium sulphate solution

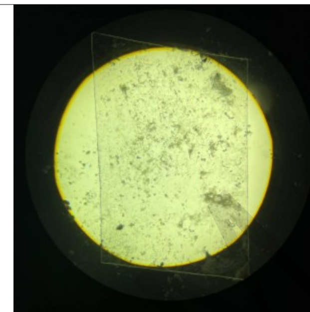
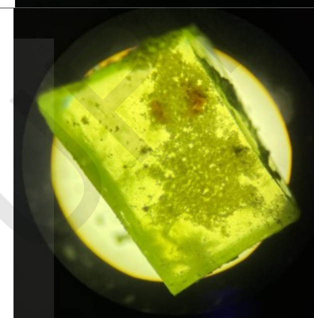
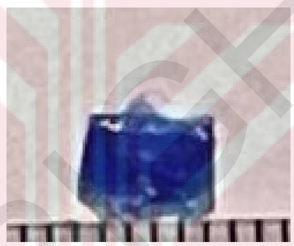
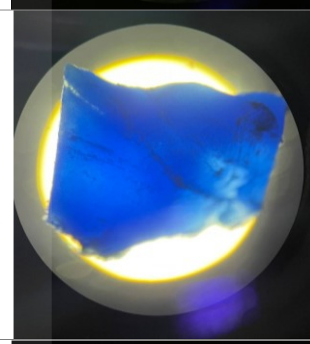
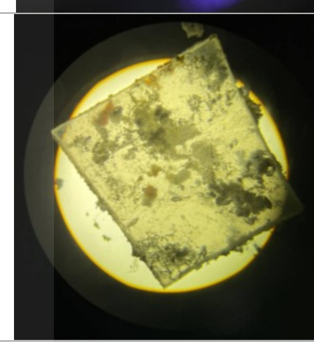
Plastic Polymer	Before	After
PET		
HDPE		
PVC		

LDPE		
PP		
PS		

The color of the spiked microplastics polymer that was extracted in this study has shown no difference in most of the density separation solutions that are being used except for sodium carbonate (Table 4.7). This is because the solution other than sodium carbonate does not have a bleaching or cleaning agent properties. Peltier (2020) stated that sodium carbonate is an inorganic substance containing the sodium salt of carbonic acid and is an alkali with a high pH, when concentrated. Corrosionpedia (2018) stated that the most common use of sodium carbonate is as a cleaning agent. It is also used as a common additive, bonding agent, and electrolyte in the glass and brick industries. Plastics and certain metals, such as aluminium, lead, zinc, and zinc brasses, are corroded by sodium carbonate. Soda ash and washing soda are two more names for sodium carbonate. The sodium salt of carbonic acid, sodium carbonate, has an alkaline characteristic. In water, sodium carbonate produces carbonic acid and sodium hydroxide. It forms sodium hydroxide and carbon dioxide when it combines with water vapor at temperatures over 400 °C (752 °F). It forms sodium bicarbonate by absorbing moisture and carbon dioxide from the air. Bleaching agents, solids separation agents, and finishing agents are just a few of the applications for sodium carbonate. Some polymers, such as polyacrylates and polysulfides, are also attacked by sodium carbonate solutions. Nylon, polyethylene, polypropylene, polyvinyl chloride (PVC), Teflon, various fluorocarbons, and certain elastomers are not affected by its solutions. Therefore, table 4.7 has shown reduction of color in LDPE and PP due to the characteristics of the solution.

Table 4.7 : Surface and color of spiked microplastics polymer before and after being extracted by sodium carbonate solution

Plastic Polymer	Before	After
PET		
HDPE		

PVC		
LDPE		
PP		
PS		

4.3 Field sample testing using the best density separation solution

Table 4.8 shows that the field sample has 4.79% of organic matter content. Due to the low content of organic matter in the field sample, it does make the microplastics polymer that has been spiked into the samples easily extracted from the chosen solution (sodium sulfate and sodium carbonate). Moreover, Table 4.9 shows that the soil texture class for field samples is in loam form according to USDA classification. The percentage of clay, silt and sand in the field sample is 16.95%, 34.25% and 48.61% respectively.

Table 4.10 shows the percentage recovery of microplastics extracted from sodium carbonate for field samples which is 84.29%. This result is slightly higher compared to the recovery percentage of microplastics from sodium carbonate in experimental soil sample in table 4.2. This shows that sodium carbonate has the ability to extract the microplastics even though the microplastics have been left in the soils for a longer time. Economically, sodium carbonate is cheap and can be easily recovered and recycled (Yang et al., 2012). These properties make sodium carbonate an ideal solution to be used as a density separation solution and economically wise.

Table 4.8 : Organic Matter in the Field Sample Soil

Percentage of Organic Matter
4.79%

Table 4.9 : Particle Size Analysis of Field Sample Soil

Total Particle Size (%)			**Soil Texture Class**
Clay	Silt	Sand	USDA
16.95	34.25	48.61	Loam

Table 4.10 : Average percentage of recovery of six types of microplastic in sodium carbonate solution in field sample soil

Percentage of Recovery (Sodium Carbonate)
84.29 %

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In conclusion, there are numerous density separation solutions that are potentially suitable for the density separation of microplastics from soil samples. From this study, the performance was evaluated by calculating the percentage recovery of microplastics in selected density separation solutions ($ZnCl_2$, sucrose, Na_2SO_4, Na_2CO_3 and coconut oil). The highest percentage recovery of microplastics in tropical soil was sucrose 88.30%, followed by zinc chloride 84.10%, sodium carbonate 76.67%, sodium sulfate 71.67% and lastly coconut oil 47.50%. However, sodium carbonate is also capable of extracting most of the type of microplastics efficiently except PVC and PET and the average number of total microplastics recovered including floated and non-floated was also the highest. Thus, sucrose. zinc chloride and sodium carbonate have shown efficiency in extracting microplastics involving tropical soil compared to other solutions. Among the best three solutions, sodium carbonate has been chosen to be the solution to be used for microplastic extraction in field sample testing due to its capability and economical value. The percentage of recovery of microplastics in sodium carbonate was higher when being tested in field samples compared to experimental samples. Hence, this solution is the best separation solution.

Future research in these areas may be beneficial to better understanding of the extraction of microplastics in tropical soil using these tested density separation solutions. Besides, given the broad variety of methods and density separation solutions presently used to extract microplastics from soil, comparing the findings of different studies conducted across the globe to obtain a truly representative picture of the extent of microplastic contamination is becoming more challenging and limited, especially involving tropical soil. This study can only determine the most appropriate techniques and obtain a realistic picture of the microplastics recovery involving tropical soil by using standardized, verified processes.

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APPENDIX I

(PROCEDURE ORGANIC CARBON ANALYSIS)

Procedure

Day 1: Weigh the crucibles and fill the LOI data table with the empty weight. Place a small amount of sample in a crucible (1-5 cc), weigh the crucible and sample, and write the weight in the "100ºC weight" column of the LOI table. Dry samples for at least 12 hours in a drying oven set to 100°C.

Day 2: Turn off the oven and leave the samples in there to cool. Remove from the oven and weigh each item, noting the weight in the "dry weight" column. Place the samples in the oven. Turn on the furnace and set the temperature to 550 degrees Fahrenheit. (It takes about an hour to get the desired temperature.) Allow for a 4-hour burn time at 550°F. Allow crucibles to cool overnight after turning off furnace and unplugging it.

Day 3: Weigh each crucible and write the weight in the column labelled "550ºC weight." Place the crucibles in the furnace, plug it in, and set the temperature to 1000 degrees Celsius (takes about 2 hours to reach target temperature). Allow for a 2-hour burn time at 1000°C. Allow crucibles to cool overnight after turning off furnace and unplugging it.

Day 4: Weigh each crucible and write the weight in the column labelled "1000 oC weight." Remove any leftovers from the crucibles. Until clean, wash in warm soapy water (Some discoloration may remain). Rinse with fresh water. Place in furnace and bake for two hours at 1000°C. Allow crucibles to cool overnight after turning off furnace and unplugging it.

Day 5: Remove the crucibles from the furnace; they are now ready to be used for the next round of samples.

APPENDIX II

(PROCEDURE SOIL PARTICLE ANALYSIS)

Procedure

1. Weigh 20 g of dry soil (about 2 mm) on a weighing scale; pour 1000 ml of water into a tall.

2. Add 100 mL H202 (30%) hydrogen peroxide and let sit overnight.

3. Place the beaker on a hot pan and gradually add additional alcohol until there is no more foaming.

4. Pour in 100 mL 0.2 N HC1 and 400 mL distilled H2O.

5. Cook for 15 minutes on a hot plate, then remove and cool.

6. Remove the supernatant liquid and add 50 mL of Calgon solution, followed by three washes with 400 mL distilled water.

7. Fill the stirrer with the contents of the beaker and weigh the empty crucible.

8. Using a mechanical stirrer, stir for 5 minutes.

9. Strain the contents through a 50 µm filter into a 1000 ml cylinder after stirring. Clay and silt particles are found in the cylinder's contents. The sand fraction is comprised of particles that remain on the 50 µm filter (see step 17 for the sand separation).

10. Rinse the mechanical stirrer blades with distilled water until no trace of dirt remains, then pour the wash water into the cylinder via the sieve.

11. Fill the cylinder halfway with 1000 mL of water.

12. The cylinder is placed in a water bath that is kept at a constant temperature of 23 degrees Celsius.

13. The materials are fully mixed for 1 minute using the plunger.

14. Allow 6 hours and 39 minutes for the suspension to settle at 230°C, then pipet an aliquot at a depth of 10 cm.

15. Pour the contents of the pipette onto an aluminium dish that has already been weighed, and bake for 24 hours at 105°C. The weight of clay particles (A) is equal to this.

16. Allow the aluminium dishes to cool in a desiccator after they have been oven-dried before weighing.

17. The sand fraction on the 50 µm sieve is oven-dried (105 °C) and then moved to a nest of sieves in the following order: 1000-, 500-, 250-, 100-, and 50-µm.

18. Using a mechanical shaker, agitate the sieve nest for two minutes.

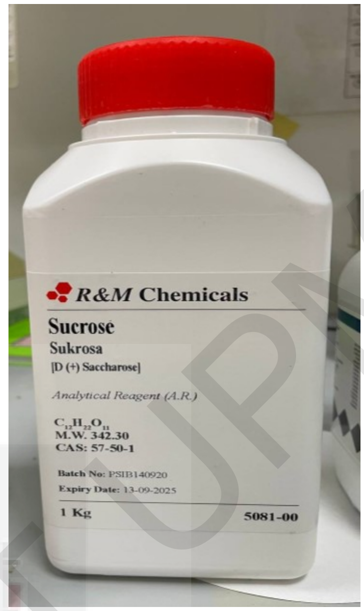
19. In a nest of sieves, weigh each sand fraction. All sand fractions have a total weight of B.

20. There must be an adjustment for the weight clay (A) calculated in step 15 since 50 mL of Calgon solution was added (step 7). 50 mL Calgon, pipetted into three dishes Place them in the oven until they are completely dry, then cool in the desiccator before weighing. Calculate the three duplicates' average weight (C).

APPENDIX III

(CHEMICALS)

Zinc Chloride (R&M Chemicals)



Sucrose (R&M Chemicals)

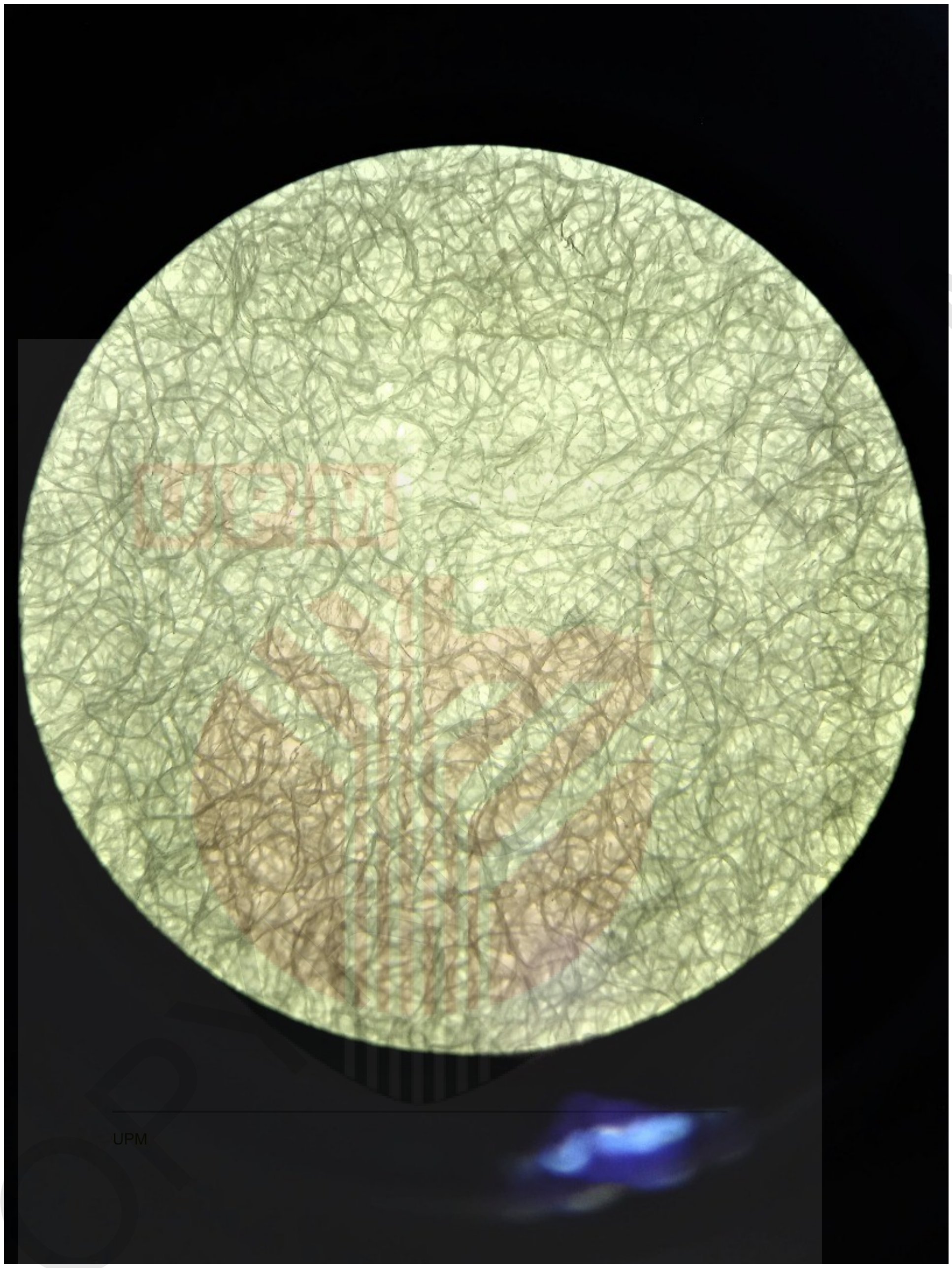
Sodium Chloride (Bendosen) Sodium Carbonate (R&M Chemicals)

Sodium Sulphate (R&M Chemicals)

Virgin Coconut Oil (Country Farm Organics)

APPENDIX IV

(BLANK DISTILLED WATER)



Microscope imaging of distilled water