ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CENTER FOR ENVIRONMENTAL SCIENCE

ASSESSMENT OF MICRO-PLASTIC POLLUTION IN SURFACE WATER AND SEDIMENT OF LAKE ABA SAMUEL, ADDIS ABABA, ETHIOPIA

MASTER THESIS

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Assessment of micro-plastic in surface water and sediment of Lake Aba Samuel, Addis Ababa, Ethiopia

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A thesis submitted to Center for Environmental Science in partial fulfillment of the requirement for the degree of master science in environmental science

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This is certified that the thesis prepared by *Michael Girimay Gebremedhine* entitled Assessment of micro-plastic in surface water and Sediment of Lake Aba Samuel, Addis Ababa, Ethiopia. Submitted to the Center for Environmental Science in partial fulfillment of the requirement for the degree of master's science in Environmental Science comply with the regulations of the university and meet the accepted standards concerning originality and quality.

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Declaration

I declare that the research reported in this MSc thesis is original and has been completed independently by myself (Michael Girimay Gebremedhine), under the supervision of Dr. Tadesse Alemu (Associate Professor) and Selamawit Gebremedhin (Ph.D. Candidate). This MSc thesis has not been submitted for the award of any other degree or professional qualification. Where other sources are quoted and full references are given.

Michael Girimay Gebremedhine

Signature ----------Date----------

January 2024

Abstract

Assessment of micro-plastic pollution in surface water and sediment of Lake Aba Samuel, Addis Ababa, Ethiopia.

Michael Girimay

Addis Ababa University, 2024

Microplastic is a growing global concern with significant environmental implications in Ethiopia, there is limited study to identify the level of microplastic pollution in all environmental compartments. Therefore, the objective of this study is to assess the composition and the extent of microplastic pollution in surface water and sediment of Lake Aba Samuel located in Addis Ababa, Ethiopia. Samples were collected from three sampling points, for water samples using a stainless-steel sieve and a pump-assisted filtration device while the sediment sample was collected using a grab sampler. Sodium Iodide (NaI) was employed for density separation, and Sodium Dodecyl Sulfate (SDS), Hydrogen Peroxide (H_2O_2), and Potassium hydroxide (KOH) were utilized to remove organic matter. microplastic particles were quantified and photographed using a stereomicroscope equipped with a digital camera. Additionally, SEM captured the morphology of microplastic, and the polymer composition was determined through FTIR analysis. The abundance of microplastics was recorded in mean values ranging from 24 to 52.33 particles/L and 39.67 to 73.69 particles/Kg in water and sediment samples, respectively. The sampling site and abundance of microplastics in water and sediment samples were statistically significant at $P<0.05$. The size of microplastics <100µm was abundant in both sample types. Fragments and fiber with transparent and blue colors of MPs were present in a large number. Polyethylene (35%), polypropylene (25%), polyethylene terephthalate (14%), polystyrene (18%), and other (8%) were contained in the water sample. In the sediment, sample polyethylene terephthalate (35%), Polyethylene (25%), Polyvinyl chloride (20%) polypropylene (10%), polystyrene (5%), and other (5%) were contended. The NPI value of the lake was 0.55, which indicates light pollution, and the PHI value of the lake was 32.87, which is below the threshold value and suggests minimum risk. The findings of the study emphasize the need for prompt action. To prevent more contamination and protect the ecological integrity of urban water bodies such as Lake Aba Samuel and other Ethiopian lakes, it is crucial to implement policy interventions, effective waste management techniques, regulations on plastic usage, and public awareness programs.

Keywords*:* *Lake Aba Samuel, Microplastic Pollution, Polyethylene, polyethylene terephthalate, polystyrene*

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List of Abbreviations and Acronyms

ATR—μFT-IR	Attenuated total reflection micro-Fourier transform infrared spectrometer
CA	Cellulose Acetate
EDS	Energy Dispersive X-ray Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy
PS	Polystyrene
MPs	Microplastics
NPI	Nemerow Pollution Index
OPAs	Organic Plastic Additives
PA	Polyamide
PC	Polycarbonate
PE	Polyethylene
PET	Polyethylene terephthalate
PFE	Pressurized Fluid Extraction
PHI	Polymer Hazard Index
PP	Polypropylene
POPs	Persistent Organic Pollutants
Pyr-GC/MS	Sequential pyrolysis-gas Chromatography Mass Spectrometry
PVC	Poly (vinyl chloride)
TGA	Thermos-Gravimetric Analysis
SDS	Sodium Dodecyl Sulfate
SEM	Scanning Electron Microscopy
WWT	Wastewater Treatment
UV	Ultraviolet

Chapter One

1. Introduction

1.1. Background of the study

Plastics are synthetic or semi-synthetic materials made from organic compounds, chains of molecules made up of monomers derived from petroleum and natural gas (Shah *et al*., 2008). Plastic has significantly enhanced our quality of life due to its affordability, adaptability, and hygienic properties it can maintain products in a state free from contamination.

These adaptable materials find extensive use across diverse sectors such as construction, household appliances, medical equipment, and food packaging industries (Fogh *et al.*, 2021). Hence, the rate of global plastic production has expanded dramatically over the past seven decades. In 1950, the world produced just two million tons of plastics; today it produces over 450 million tons as a direct result of the halt of the COVID-19 epidemic, mostly driven by rising demand for plastic in medical supplies, personal protective equipment, and packaging materials (Hannah Ritchie, 2023; Janssens, 2022; Plastics Europe, 2019).

The issue of plastic pollution has become a significant global environmental concern due to inappropriate usage, improper management, and the absence of legislation on industrial and single-use plastic waste (Edo *et al*., 2020; SAPEA, 2019). The issue of plastic pollution is seeing rapid growth with the environment impacted by the introduction of plastic waste

at an exponential rate. According to (Hale *et al*., 2020), the equivalent of one garbage truck's worth of plastic is being deposited into the environment every minute.

Plastic items in natural environments without serving their intended purpose exhibit persistence, mobility, and ubiquity in aquatic and terrestrial ecosystems, encompassing urban, rural, and remote areas (Auta *et al*., 2017). Over time, plastics in the environment can break down into smaller microscopic plastic particles; when plastic particles are less than 5 mm in size, they are called microplastics (Frias & Nash, 2019).

Microplastic particles found in the aquatic environment are categorized as primary and secondary microplastics (Auta *et al.*, 2017). Primary microplastics are produced intentionally and used in industrial chemicals, cosmetics, or pellets used to produce plastic materials (Auta *et al*., 2017; Sharma & Chatterjee, 2017). Secondary microplastics are generated due to the degradation of larger plastic items due to the natural weathering mechanism after their introduction into the environment, from wastewater treatment plants, and also enter the aquatic environment through direct release (Browne *et al*., 2011).

Microplastics may contain chemical additives such as stabilizers, plasticizers, flame retardants, and pigments added during their manufacturing process and these toxic chemicals could leach from plastic particles leading to possible negative impacts on an organism (Alimi *et al*., 2018; Hermabessiere *et al*., 2017). The widespread presence of microplastics in the environment gives rise to significant concern over their impact on animals and ecosystems (SAPEA, 2019). Ingestion of microplastics can block the digestive tract, reduce growth rates, block enzyme production, lower steroid hormone levels, affect reproduction, and cause the adsorption of toxicants (Wright & Kelly, 2017). One potential

environmental danger connected with microplastics is their ability to be taken up and distributed throughout the food web, hence posing a potential threat to many organisms within the ecosystem (Karbalaei *et al*., 2018).

Many microplastic studies have been conducted in all environmental matrices across the world and Africa; in freshwater environments, marine water environments, salt, sediment, fish, and other organisms, and, food and beverage (Di & Wang, 2018; Kosuth *et al*., 2018; Zheng *et al*., 2019; Wang *et al*., 2020; Merga *et al*., 2020).

In Ethiopia, only a few studies have been conducted on the occurrence and abundance of microplastics. However, microplastic has been found in fish and sediment in Lake Zeway, Bahir Dar City reported microplastic in sediment, and sewage water, Lake Hawassa reported microplastic in sediment (Jeevanandam *et al*., 2022 : Merga *et al*., 2020 : Gela & Aragaw, 2022). However, there is limited information on the microplastic study in Ethiopia across in all water resources like rivers, lakes, reservoirs and other water bodies. Hence, this study was carried out to identify and analyze the occurrence of microplastics in surface water and sediment for a better understanding of microplastics' implications for plastic pollution from Lake Aba Samuel Addis Ababa, Ethiopia.

1.2. Statement of the problem

The global plastic distribution rate will reach 348 billion tons as of 2030, which is several manifold times higher than in the early 1950s; however, abundant production has contributed to the accumulation of plastic debris in the sea (Plastics Europe 2018). Most of the studies were conducted in the marine environment and there is currently a large abundance of microplastic pollution in Africa especially in terrestrial environments. For example, across all studies, 55 % of all sampled aquatic organisms contained microplastics, and aquatic ecology can be polluted and damaged; High levels of microplastics were reported in fish Egypt from another African country (Kalčíková *et al*, 2020).

Addis Ababa has the fastest population growth, uncontrolled urbanization, industrialization, and poor waste management practices (Yohannes and Elias, 2017). No legislation requests people not to use or collect plastic waste; yet, metropolitan areas are increasingly using and producing a greater variety of plastic-based products. There are many sources of microplastics in the city single-use- plastic bottles, from food packaging, aged plastic materials, and road marking are the biggest sources of microplastics, followed by dust and particles from plastic-based paint, facial cleansers, cosmetics (Sundt *et al.*, 2014).

Microplastics are easily accessible to organisms at all levels of the food chain due to their microscopic size. Due to their composition and significant surface area, they are susceptible to sticking to organic contaminants in water and to the release of harmful plasticizers. Hence, the ingestion of microplastics could potentially transport harmful

substances to the lower levels of the food web, where they have the potential to accumulate. (Teuten *et al*., 2009)

Microplastics can enter water bodies through terrestrial routes since they are carried by the flow of surface water and river channels, as well as by human activities including aquaculture, shipping, and recreation. Both primary and secondary microplastics have an equally harmful impact on the aquatic ecosystem because they can enter the food chain through biomagnification and bioaccumulation in aquatic species (Pan *et al*., 2022).

In Ethiopia, only a few studies reported micro-plastic presence in fish and sediment *(Merga et al*., 2020), Abundance and Characterization of Micro-plastics (Gela and Aragaw, 2022). However, this analysis infused in different countries throughout the world but in our country Ethiopia, it is limited to identifying the level of micro-plastic pollution in surface water and sediments. Therefore, this study Assesses the level, to identify the occurrence, distribution, and type of microplastics in Aba Samual Lake Addis Ababa, Ethiopia, microplastic pollution in surface water, sediment, and the effects of microplastics on humans.

1.3. Research question

- What is the level of microplastics in surface water, and sediment?
- What types of microplastic can we find in surface water and sediment?
- What is the implication of health impact on the Environment?

1.4. Objective of the study

1.4.1. General Objectives

To assess the composition and the extent of microplastic pollution in surface water and sediment of Lake Aba Samuel Addis Ababa, Ethiopia.

1.4.2. Specific objective

- To identify the occurrence of microplastic in surface water and sediment
- To characterize major morphology types and their polymeric compositions of microplastic
- To assess the Environmental risks associated with microplastic pollution

1.5. Significance of the study

The present study will generate baseline scientific evidence on the status and distribution of microplastics in water and sediment. The data collection method and research report will contribute to the scientific information and evidence for academia and governments concerning the contamination levels of microplastics from land-based samples. Additionally, it will provide insights into the contaminants that enter surrounding water bodies, specifically Lake Aba Samuel. It will also serve as a valuable resource for policymakers in their efforts to effectively manage and mitigate plastic pollution. In conclusion, there is a need to assist government and non-governmental organizations in their endeavors to shift public and industrial attitudes towards single-use plastics, ultimately leading to the implementation of regulatory measures that prohibit their usage.

1.6. Limitation of study

The process of identifying and analyzing microplastics is a complex and laborious undertaking and there are certain constraints to this research. During microscope examination of microplastic samples, utilizing a dissecting microscope as a standard method. However, this stage is the most susceptible to human error. Knowing the color, and shape of microplastic was challenging. Synthetic fibers were confused with other materials; pieces can be lost, and melted by "the hot needle test" challenging to handle using forceps when placing them into a vial for counting. Finally, due to the unavailability of sophisticated analytical instruments like micro-FTIR spectroscopy and SEM/EDX in Ethiopia could not be determined in terms of their polymeric and elemental composition.

Chapter Two

2. Literature Review

2.1. Plastics

Plastics have changed our daily lives because they are multipurpose, versatile, low-cost, lightweight, robust, durable, corrosion-resistant, and insulation. Versatile materials employed in the production of several consumer goods that are utilized daily, encompassing a wide range of products such as automobile exteriors, kitchen utensils, sanitary fixtures, and toothbrushes.

The process of polymerization, wherein tiny molecules undergo a chemical reaction to form a large, chain-like polymer molecule, produces plastics. Polymers possess the ability to undergo numerous manufacturing processes such as molding, extrusion, and casting into diverse shapes and films, as well as being pulled into filaments for utilization as textile fibers. A wide range of plastic materials exists and each has diverse applications. It predominantly consists of synthetic materials that are derived from polymers.

The manufacture of plastics on a wide scale can be traced back to around 1950. Since then, global plastic production has significantly expanded from 2 million metric tons (MT) in 1950 to 380 million MT in 2015 (Geyer *et al*., 2017). Thermoplastics and thermosets are the two primary categories of plastics (Plastics Europe, 2018). Thermoplastics, which include frequently used materials including, polypropylene, and polyamides, are solid at normal ambient temperature and may be repeatedly liquefied, stronger, and molded (Plastics Europe, 2018). Thermosets can be either solids or liquids at room temperature.

They change chemically when heated, making it impossible to re-melt and shape them. Polyurethane (PUR), silicone, acrylic, and epoxy mastics are examples of thermosets (Plastics Europe 2018).

Plastics' widespread use can be ascribed to their distinctive qualities, such as their lightweight durability practicality, and adaptability for a variety of uses across several industries, with the most popular plastics used today being PE, PP, PS, and PVC (Plastics Europe, 2019). A substantial rise of plastic waste, primarily thermoplastic materials such as low-density polyethylene (PE), high-density polyethylene (PE), polypropylene (PP), and polyamide (PA) fibers, has been observed along with the quick increase in plastic production, which appears to be continuing (Geyer *et al*., 2017). Because of the durability of plastics, a large portion of what has been manufactured since they began to be mass-produced is still in use today, this has resulted in the accumulation of plastic waste within seven decades (World Data, 2023).

Plastic garbage has been produced at an accelerated rate, and its improper disposal has resulted in a global catastrophe that could have detrimental effects (Plastics Europe, 2019). The widespread utilization of plastics, inadequate waste management practices, along the long-lasting nature of traditional plastic, pose a substantial peril to the ecosystem. (Geyer *et al*., 2017). Although some less obvious effects have also been documented because of digestion or uptake of smaller particles, wildlife entanglement in bigger debris such as fishing nets, plastic bags, and packaging remnants is one of the more obvious results. Plastic waste can come from a diversity of sources and can thus take on a variability of arrangements (Geyer *et al*., 2017).

Table 1 Densities and application of some common polymers (Plastics Europe, 2019)

POLYMER	DENSITY (gm^{-3})	EXAMPLES OF USE
Polypropylene	0.89	Packaging, toys, household appliances, lighting diffuser, CD case, fishing lines
Polyethylene	0.96	packaging, plastic bags, bottles bulletproof vests
Acrylonitrile-butadiene-styrene	1.05	Sports equipment, toys, car parts
Polystyrene	1.06	Packaging, household appliances, electronics, disposable medical items, building, and construction
Polyamide	1.14	Textiles, fishing lines, carpets food packaging
Polycarbonate	1.21	Bottles, CDs and DVDs, food containers, eyeglass lenses
Cellulose acetate	1.30	Eyeglass frame, toothbrush, tool handles, wrapping
Polyester	1.37	Textiles, ropes, insulation, plastic bottles
Polyvinyl chloride	1.39	Building products, piping, coating, low voltage insulation, packaging, medical, leisure products
Polyethylene terephthalate	1.39	Engineering plastics, external building parts

2.2. Occurrence of microplastic in the environment

Plastic waste may exist in many forms of plastics that are often employed in the literature, and categorized plastics into two main groups: macro-plastics, which have a size greater than 5 mm, and microplastics, which have a size less than 5 mm (Gabriel et al., 2017; Arthur *et al*., 2009). Microplastics not only vary in size but also in various shapes such as microbeads, fibers, debris, film foam pellets, and filaments (Hidalgo-Ruz *et al.*, 2012; Wright *et al*., 2013).

Microplastics are divided into primary and secondary microplastics based on their origin (Arthur *et al*., 2009). Primary microplastics are intentionally produced by industry for cosmetic purposes, while secondary microplastics are plastic residues in the environment due to wave action, biofilm growth, solar radiation, mechanical shear, and thermal oxidation. It arises from weathering/degradation of objects (Andrady, 2011; Hale *et al*., 2020). There are now more plastics in both the land-based and marine environments than ever before, with significant negative effects on ecological balance.

The majority of plastic that finds its way into the ocean is driven by rivers, which also serve as the outflow point for sewage from cities (Gela & Aragaw, 2022). We cannot easily outlaw or immediately replace plastics because of their beneficial qualities and our growing reliance on them. Indeed, both their improper management and encroachment into the natural environment are growing, as is their global utilization. These plastics will over time break down into microplastics and Nano-plastics (Hale *et al*., 2020).

Microplastics (MPs) can originate from many origins, interact with various environmental mediums (such as freshwater, marine, groundwater, sediments, and soils), as well as

undergo multiple pathways during their transportation and transformation (Tirkey & Upadhyay, 2021). Various categories of plastics are employed in packaging, including Polyethylene, Polypropylene, Polystyrene, Poly (ethylene terephthalate), and Poly (vinyl chloride).

Their extensive utilization is shown in their production statistics, which increases the probability of them, being deposited in the marine ecosystem. The future influx of plastic garbage into the oceans will be amplified by overfishing, and recreational and marine activities, along with shifting populations that encourage immigration to coastal locations (Ribic *et al*., 2010). Around eighty percent of the plastic waste comes from sources on land, such as litter seen on beaches.

All fishing vessels worldwide currently utilize plastic equipment (Watson *et al*., 2006) and it is common for some of this equipment to be lost or irresponsibly thrown at sea during its use. Polyolefin (PE and PP), along with nylons, are predominantly employed in fishing gear applications. The fishing sector is responsible for approximately one-eighth of the marine plastic trash present in the ocean ecosystem.

Aquaculture can also play a substantial role in the accumulation of plastic waste in the oceans. (Hinojosa & Thiel, 2009). The remaining portion primarily originates from terrestrial sources, predominantly beach debris. Virgin resin pellets, which are frequently found in garbage, regularly make their way into the oceans through accidental losses during transportation or by runoff from processing facilities (Doyle *et al*., 2011; Ogata *et al*., 2009).

Since the commencement of commercial manufacture around 1950, society has experienced a growing dependence on plastics. The global demand for them has been driven by their adaptability, stability, lightweight nature, and low production costs. The majority of plastics are initially utilized and subsequently disposed of on land. Nevertheless, it is projected that the quantity of microplastics in certain oceanic compartments will increase twofold by the year 2030 (Hale *et al*., 2020).

2.2.1. Primary microplastic sources

Primary microplastic particles are deliberately produced in small dimensions to serve various purposes and manufactured in several shapes for a variety of applications. The substances present in abrasive face scrubs, shampoos for the body, hand soaps, as well as dental products are the most current and public concern. Certain brands of these products began incorporating polyethylene bits, or "scrubbers," to change the ordinary that manufacturers previously used (Fendall & Sewell, 2009; Gregory, 1996).

The production of primary microplastics is expected to see growth due to their utilization in electronic devices, pharmaceuticals, automobiles, and aircraft (Roex *et al*., 2013). These plastic fragments are flushed down the drain together with the product after being used and ultimately end up in municipal wastewater systems. Additional sources of primary microplastics are relatively unfamiliar to the general population; however, they have existed for an extended period.

Microplastics employed in air-blasting technologies facilitate the elimination of corrosion and pigment from equipment ship vessels, and motors Sharma & Chatterjee, 2017). Additionally, virgin resin pellets used to make a variety of plastic products could be

classified as microplastic pollution, but they may be larger than the permitted size limit. Pellets enter the oceans regularly.

Due to losses incurred during transportation across the ocean and the discharge from processing facilities (Alam *et al*., 2023). Common primary microplastic particles are likely to be retained in their original form when collected from industrial and domestic sewage. Subsequently, these particles are subjected to processing at sewage treatment (WWT) facilities before their discharge into the aquatic ecosystem. (Roex *et al*., 2013).

2.2.2. Secondary microplastic sources

Secondary microplastic particles are formed because of the degradation of larger plastic fragments caused by exposure to UV radiation and mechanical forces (Cole *et al*., 2011). The majority (80%) of plastic litter that enters the marine ecosystem originates from terrestrial sources, particularly densely inhabited or industrialized regions, as a result of littering and improper solid waste management. (Derraik, 2002; Li *et al*., 2016). Known sources of pollution include coastal recreational activities, raw industrial wastes, sewage, and refuse site drainage (Browne et al., 2011; Li *et al*., 2016).

Plastics are transported to the ocean through river systems, wastewater treatment plants, and even during extreme weather events (Barnes et al., 2009; Browne *et al*., 2011; Cole *et al*., 2011). Secondary microplastic pollution can originate from several types of plastics derived from terrestrial sources, primarily plastic packaging (including disposable single-use items) and waste from the fishing sector (Andrady, 2011).

Approximately one-third of plastic resin manufacturing is attributed to consumer packaging and disposable items, making them highly susceptible to becoming ocean debris.

"Several broad kinds of plastics are utilized in packaging," says Andrady, "including polyethylene, polypropylene, polystyrene, poly (ethylene terephthalate), and poly (vinyl chloride). Other kinds of plastics that are regularly encountered in the maritime environment are Foamed Polystyrene, Nylon, and Cellulose Acetate (Andrady, 2011).

Much of this waste winds up on beaches as litter and is vulnerable to natural weathering. Beaches, once in the ocean or on a beach, the fragmentation process of macro-plastic litter begins with chemical or mechanical weathering, or photo-degradation caused by sunshine, all of which leads the plastic to become weak and brittle. Tiny synthetic fibers, which most people encounter daily, are another form of microplastic pollution that scientists are increasingly paying more attention. According to Habib, and Locke, the textile industry initially used synthetic fibers over 50 years ago to supplement natural fibers such as wool, cotton, and linen. As clothing is washed and dried repeatedly, worn and torn fibers break free due to mechanical action and become suspended in gray (waste) water. They are subsequently treated as wastewater and frequently remain as part of the effluent or sludge, which is eventually absorbed into the natural environment in various ways.

2.3. MPs in the environment

Microplastics (MPs) are little fragments of plastic that pollute the environment and are present in various mediums such as air, sediments, soils, freshwater, ocean, and organisms, including human beings (Priya *et al*., 2022). The assessment and investigation of the abundance and distribution of MPs (microplastics) in water, sediment, and biota have been conducted in many regions across the globe. MPs possess unique attributes about their

physical structure, consistency, concentration, coloration, and prevalence, all of which contribute to their dispersion within the environment (Prabhu *et al*., 2022).

MPs can have negative impacts on the environment because of their notable characteristics such as high durability, stability, small size, and lightweight nature. The small size of MPs makes them susceptible to ingested by aquatic animals, which can subsequently lead to the accumulation of MPs within their bodies and the subsequent multiplication of this accumulation along the food chain.

2.4. Microplastic studies at the Global level

Microplastic abundance in sediments and water varies greatly around the world. Many studies have reported the concentration of microplastics in surface water and sediments, with the Mediterranean Sea sediments in Spain having the greatest concentration of microplastics. The Algarve coastal sediments in Portugal (Fries *et al*., 2015) and the Baltic Sea near Russia have relatively low quantities (Zobkov & Esiukova, 2017).

Polypropylene, polystyrene, polyvinyl chloride, polyester, poly (ethylene-propylene), poly (vinyl acetate), polyethylene terephthalate, polyamide, and rayon were among the components discovered, with polypropylene and polyethylene being the most frequent (Zobkov & Esiukova, 2017).

China has The principal components of microplastics are dominated by polypropylene and polyethylene, as in Europe, and were studied using a stereomicroscope and an attenuated total reflection micro-Fourier transform infrared spectrometer (ATR—μFT-IR) (Zheng *et al*., 2019). Furthermore, the abundance of microplastics in surface water ranged from 1597

to 12,611 items/m3 and in sediments from 25 to 300 items/kg wet weight (Di & Wang, 2018).

Other countries and locations with microplastics The concentrations of microplastics in Lake Ontario's nearshore sediments reached 27,830 items/kg dry sediment, the highest documented value among nearshore sediments worldwide (Ballent *et al*., 2016). Sediments from the Complex Lagoon-Channel of Bizerte (Tunisia) and the Atoyac Rivers basin (Mexico) have high amounts of microplastics, with more than 3000 items/kg dry sediment Microplastic concentrations were lower in the studied areas of Iran (Naji *et al*., 2017) and the United States than in the Yellow Sea ((J. Wang *et al*., 2019).

Microplastics have been found in isolated areas (Peng *et al*., 2018). Microplastic concentrations in the Mariana Trench sediments ranged from 200 to 2,200 particles per liter, while microplastics were observed in Polar Regions (Mu *et al*., 2019).

A few microplastic studies conducted in Ethiopia reveal microplastic quantity and analysis. The initial research Fish and sediment were discovered in Lake Zeway. Using attenuated total reflection (ATR) - Fourier-transform infrared (FTIR) spectroscopy, polypropylene, polyethylene, and alkyd-varnish were shown to be the most prevalent polymers in fish and sediment (Merga *et al*., 2020). The second research conducted in Bahir Dar city's urban dishes was sediment and agglomerated sewage water analyses in two class sizes (> 0.5 and 0.5 mm). The mean abundances of 0.5 mm particles in sediment were 5 1.00 items/50 g and 3.00 1.00 items/ml in agglomerated sewage water, with polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), polyamide (PA), and polyvinyl chloride (PVC) being the most prevalent polymers (Gela & Aragaw, 2022).

The other study was conducted in Hawassa entitled Evidences of Microplastics in Hawassa Lake, Ethiopia, twenty-five surface sediments from the shoreline were isolated by the utilization of a $ZnCl_2$ solution. These sediments were then subjected to microphotography using a scanning electron microscope (SEM), and the specific type of microplastic (MP) present was determined by analyzing the Fourier-transform infrared (FTIR) spectra. The concentration of microplastics (MPs) ranged from 11 to 74 pieces per cubic meter in the vicinity of the lake's catchment region on the eastern side (Jeevanandam *et al*., 2022). Therefore, the objective of the present studies is to identify and analyze the occurrence of microplastics in surface water and sediment from Lake Aba Samuel Addis Ababa, Ethiopia.

2.5. Method for detecting and identifying microplastic

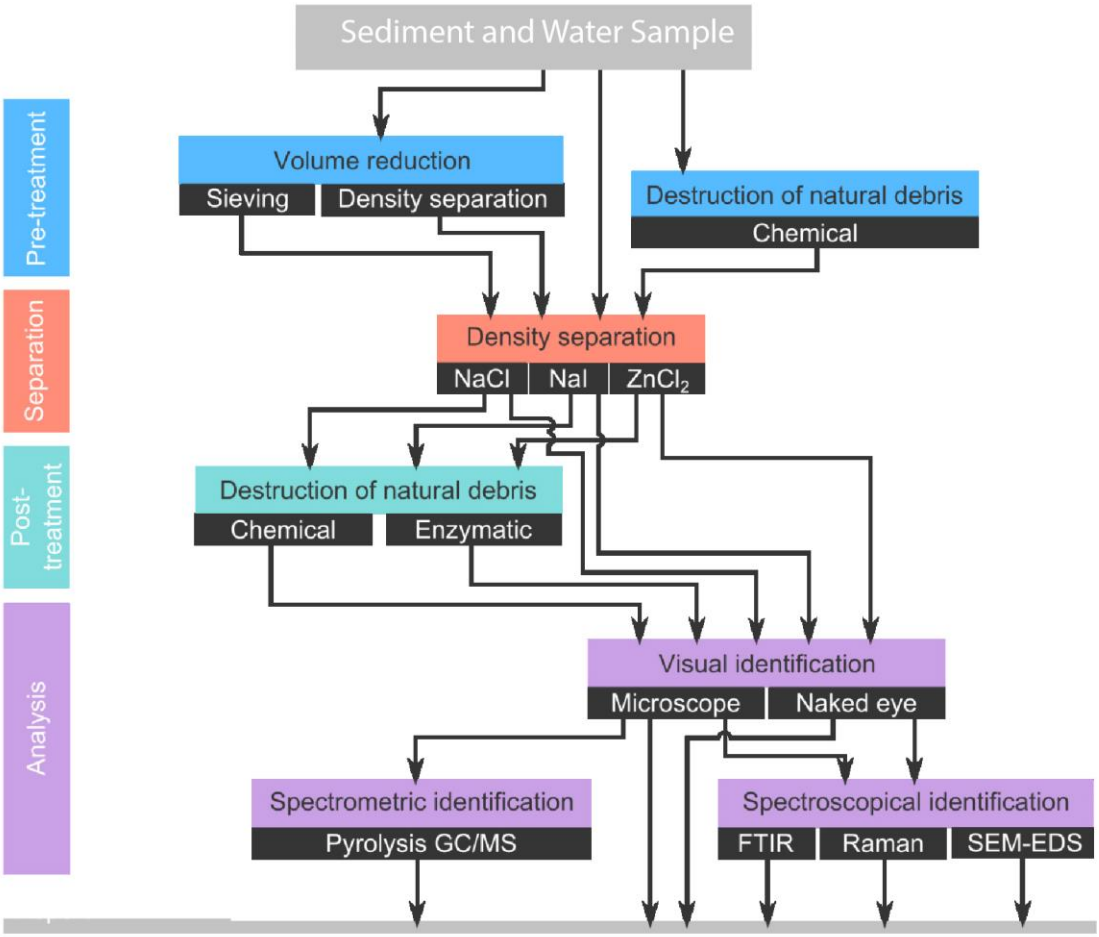


Figure 1 Graphical representation of a method for detecting and identifying microplastics (Klein, 2015)

Wide ranges of analytical techniques have been utilized in diverse microplastic studies to detect and identify microplastics in different environmental samples, including surface water, sediment samples, and marine debris. Within the realm of scholarly literature, a multitude of non-instrumental methodologies has been proposed to differentiate plastics from non-plastics. These include techniques such as the hot needle method and Nile Red staining. The hot needle technique, as an illustration, encompasses a reasonably straightforward configuration wherein a stainless-steel needle is heated to a temperature at

which it emits a red light. (Leads & Weinstein, 2019). When a hot needle approaches a particle, it is said to be plastic if the particle melts or curls as a result. Nile Red staining is also being used to identify microplastics (Maes *et al*., 2017; Shim *et al*., 2016; Tamminga, 2017).

Because plastics are hydrophobic, they can be fluorescently labeled when a lipophilic dye, Nile Red, is added and studied under blue light (Shim *et al*., 2016). When compared to instrumental approaches (FTIR, Raman spectroscopy, or Pyrolysis), Nile Red staining is comparatively simple and inexpensive. it has relatively limited accuracy in detecting the presence of various plastic polymers in samples, such as PVC and PET (Nel *et al*., 2021).

In terms of plastic identification, the FTIR analytical approach offers numerous advantages. FTIR spectroscopy is an appropriate measure of polymer type within sediment samples based on the high concentration of studies. One advantage of FTIR reflectance spectroscopy is that it is non-invasive, allowing samples to be studied without harming them. Raman spectroscopy is a spectroscopic technique that uses the interaction of a sample and the resulting changes in photons in monochromatic light to offer structural information about plastics, which is studied to determine the polymer type. *(Allen et al*., 1999).

Raman spectroscopy is less impacted by particle morphology, such as shape or thickness, and is ideal for wet materials, whereas FTIR is affected by the presence of water because it is infrared active, However, Raman spectroscopy is subject to interference from fluorescent chemicals such as some biological materials and colorants (Araujo *et al*., 2018). Because of this potential interference, Raman spectroscopy necessitates more stringent

organic matter clearance to reduce erroneous signals (Mai *et al*., 2018). Raman takes significantly longer to process than FTIR (Käppler *et al*., 2015).

SEM provides detailed information on a particle's size and shape. SEM can help identify inorganic plastic additives by providing a clear image of the particle's size and topography. SEM was used in different studies to identify plastic particles in soil samples (Gniadek & Dąbrowska, 2019). Combining SEM with other identification techniques, such as FTIR, can be beneficial when certain plastic kinds have certain forms.

According to one study, most of their polyethylene samples were spherical, while the majority of their polypropylene samples were fragmented. However, SEM/EDS is time-consuming and needs expensive apparatus and facilities, thus it may not be appropriate for all applications. SEM/EDS, on the other hand, is time-intensive and requires expensive instruments and infrastructure, making it unsuitable for routine monitoring applications (Gniadek & Dąbrowska, 2019).

Another new analytical tool for identifying microplastics is pyrolysis gas chromatography/mass spectrometry (GC/MS), used to determine the polymer present in environmental samples. Pyrolysis is a technique for (TGA) thermos-gravimetric analysis in which a sample which burned in the absence of oxygen.

The temperature-related polymer decomposition provides a distinctive signature relevant to individual polymers. If combustion is supplied into a gas chromatography for the separation of chemical ingredients, the chemical identification can be determined using mass spectrometry (MS). When paired with Pry-GC, this yields structural information

about macromolecules via GC/MS analysis and is a significant method for polymer identification.

The main disadvantage is that you just get the mass of polymer per sample and no information on the amount, kind, and morphology of the polymers in the sample. Using pressurized fluid extraction (PFE), a new technique for determining the amount of plastics in solid materials has been reported (Stephen Fuller & Gatutam, 2017).

Whole polymers (melting, destructive) are appropriate for measuring the total amount of plastics contained in a sample, but they do not catch the morphological properties, which are currently only obtained through labor-intensive visual counting (Cabrera, 2018).

2.6. Health and environmental impacts of microplastic

2.6.1. Health impacts of microplastic

Plastics have been recognized as an important component of marine litter for several decades, their ecological and biological impacts have only been acknowledged and understood recently (Derraik, 2002). Numerous scientific investigations have been conducted to explore the potential health implications associated with exposure to microplastics, hence raising concerns regarding their detrimental consequences on human well-being (Campanale *et al*., 2020).

Based on epidemiological studies, it has been shown that microplastics have the potential to penetrate the human body via many routes, including ingestion, inhalation, and skin contact. Upon entering the human body, microplastics have been observed to accumulate within various organs and tissues. MPs are detectable in numerous aquatic organisms and

human samples, therefore requiring a risk assessment of MPs for human health (Brachner *et al*., 2020).

The presence of microplastics has been linked to a range of health hazards, including inflammation, oxidative stress, and the possibility of genotoxic effects (Thornton Hampton *et al*., 2022). In addition, microplastics have the potential to serve as vehicles for the transportation of harmful substances, including persistent organic pollutants (POPs) and heavy metals, which can adhere to their exteriors.

Subsequently, these chemical substances can be discharged into the human body, potentially resulting in adverse toxicological consequences (Senathirajah *et al*., 2021). The exposure of humans to microplastic particles (MPs) is an issue recognized as a potential health hazard by scientists, authorities, politicians, non-governmental organizations, and the public.

2.6.2. Environmental impacts of microplastics

The presence of microplastics has become widespread in both aquatic and terrestrial habitats, hence presenting substantial ecological hazards. These microplastics have the potential to be consumed by a diverse array of organisms, spanning from planktonic organisms and fish to avian and mammalian species, resulting in detrimental effects such as harm to the body, obstructions within the gastrointestinal tract, diminished eating efficacy, and compromised reproductive capabilities (Hasan Anik *et al*., 2021).

Additionally, these alterations can facilitate the transmission of pollutants up the food chain. Moreover, microplastics can adsorb various contaminants that exist in the surrounding environment, resulting in their extensive distribution over long distances and

the possibility of accumulating at higher levels of the food chain. Eco-toxicity assays should be performed more often on the types and sizes of particles that are most frequently observed in the environment. Yet, the hazard dataset shows good coverage of species required by regulation, and the exposure dataset relies on measurements performed all over the globe (Wyl & Nowack, 2021).

2.6.3. Environmental risk of microplastic pollution

One of the primary environmental risks of microplastics is their bioavailability for aquatic organisms. Bivalves are of particular interest because their extensive filter-feeding activity exposes them directly to microplastics present in the water column (Li *et al*., 2016). The Nemerow Pollution Index (NPI) and Polymer Hazard Index (PHI) have been observed in several research activities to evaluate the magnitude of pollution and the associated risk posed by microplastics. An investigation carried out in Bangladesh employed the NPI and PHI methods to assess the dispersion patterns of microplastics in the water and soil along the shores of the Ganges River Basin up to the Meghna Estuary (Alam *et al.*, 2023).

The research findings revealed that the NPI had a remarkably elevated value, suggesting a substantial degree of contamination. A separate investigation carried out in China employed the NPI and PHI as a means of assessing the extent of pollution and the associated risk of microplastics on bathing beaches (Wu *et al*., 2023). The study determined that both indexes served as effective instruments for conducting a comprehensive assessment of both the magnitude and potential hazards associated with microplastic pollution.

The NPI and PHI were employed in a research investigation aimed at assessing the environmental hazard posed by microplastics in maritime bays (Liu *et al*., 2022). The research presented a novel approach for assessing the environmental danger associated with microplastics, employing a two-dimensional system. In general, the Nemerow Pollution Index (NPI) and Polymer Hazard Index (PHI) have been employed in numerous microplastic investigations to evaluate the degree of pollution and potential risks associated with microplastics in diverse environmental settings.

2.6.4. Health and ecological implication of microplastic pollution on water bodies

This study provides valuable information on to significant consequences of plastic contamination of aquatic ecosystems. Lake Aba Samuel, an important freshwater reservoir, exemplifies the effects of plastic contamination on urban water systems. The study results indicate the abundance of microplastics in sediment and water, giving rise to various concerns regarding, the environment, and human health. An important issue is the environmental consequences of plastic contamination on aquatic habitats, because their size readily accumulates in sediment, presenting danger to the habitat and a variety of aquatic species. This pollution poses a high risk to aquatic life, ranging from microscopic organisms to bigger species by disrupting food systems as well as causing damage. Furthermore, the deterioration of water quality emerges as a critical issue. MPs can absorb and release harmful pollutants, potentially contaminating the water column. This not only impacts aquatic life but also poses a risk to human health, especially for communities that depend on these water sources for consumption and irrigation.

Chapter Three

3. Materials and Methods

3.1. Description of the study area

The research was carried out in Lake Aba Samuel. Aba Samuel is a man-made body of water, specifically classified as an artificial lake or reservoir, situated around 37 kilometers to the south of Addis Ababa. The establishment of the reservoir in 1939 was primarily aimed at generating electricity for the city of Addis Ababa. Notably, this reservoir holds historical significance as it served as the location for Ethiopia's inaugural hydroelectric power plant.

The reservoir, which may be described as an artificial lake, is an integral component of the Upper Awash River Basin (Solomon, 2007). The Akaki-Aba-Samuel wetland system is formed by the flooding of the reservoir and its surroundings for a significant portion of the year. The estimated storage capacity of the primary reservoir, which is obstructed by a concrete masonry dam with a height of 22 meters, is around 35 million cubic meters.

Due to the accumulation of sediment, the current storage capacity of the reservoir is lower than its initial capacity. The reservoir's initial storage capacity experienced a reduction of around 30% due to the process of siltation (Solomon, 2007).

The explanation of urbanization in Addis Ababa has led to the establishment of various industries, including those involved in food and beverage production, chemical manufacturing, tannery operations, garment manufacturing, urban agriculture, wastewater treatment, and transportation. These industries discharge their waste products into the two

river systems known as the Little Akaki and Great Akaki Rivers. The Aba Samuel reservoir acts as a sink for pollutants originating from the Great Akaki and Little Akaki Rivers, along with their tributaries. These rivers serve as a natural conduit for the disposal of domestic, agricultural, chemical, and industrial waste generated within the city of Addis Ababa.

The river systems in question serve as dumpsites for both solid and liquid waste, posing a significant hazard to the city's ecology due to the presence of toxic substances (Kassegne *et al*., 2018). The Little Akaki River exhibits a higher degree of pollution compared to the Great Akaki River system due to its close association with numerous industrial establishments and drainage systems, which are directly linked to the river system.

Table 2 Sampling site with GPS coordinates

No	**Sampling Site**	**Latitude**	**Longitude**
1	Site 1	8 ^{0}48’55.26”	38.42’30.16”
2	Site 2	8 ^{0}47’50.42”	38 ^{0}42’15.73”
3	Site 3	8 ^{0}47’22.37”	38 ^{0}43’11.52”

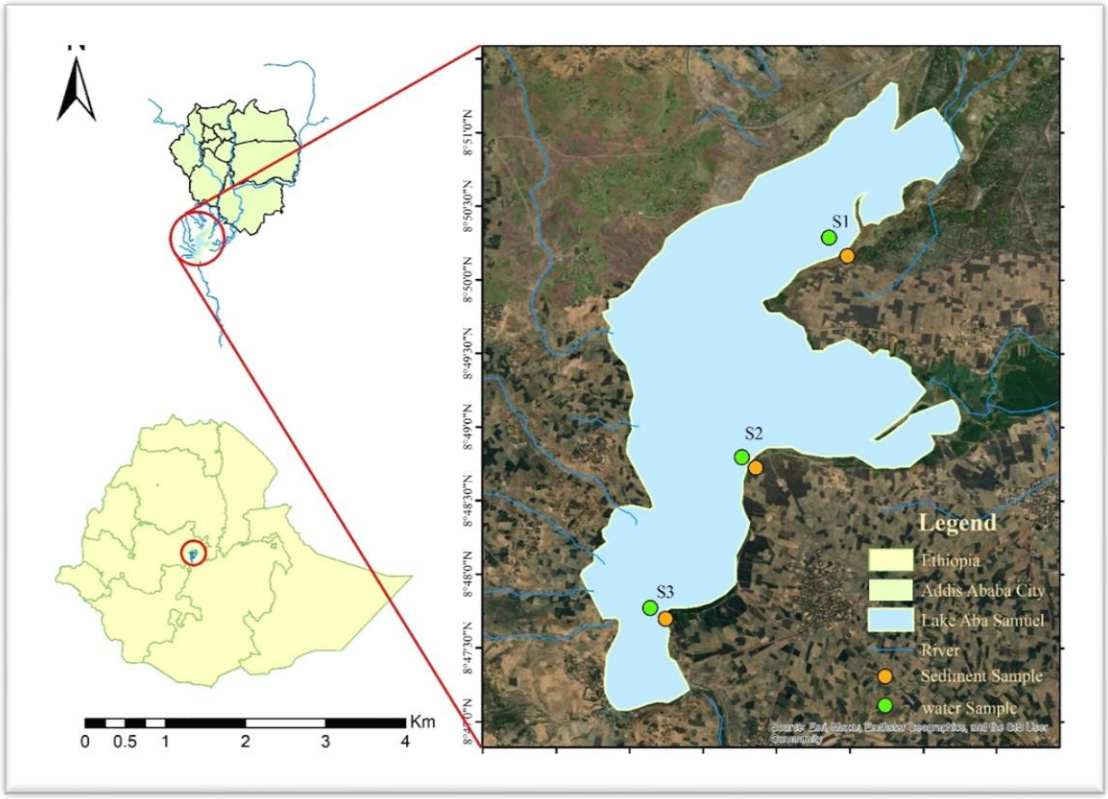


Figure 2 Study area map

3.2. Sampling procedure

3.2.1. Water Sampling Technique

A sampling device for the extraction of MPs at each sampling point in a freshwater body was manually constructed for this research. The device was made up of a centrifugal pump (Leo 4xcm 120C, China). The inlet of the pump was connected to the 5 m long flexible hose. The front of the hose was also connected to a cylindrical stainless-steel cage with 5 mm pore sizes, which prevented large debris from entering and damaging the pump device. Next to that, it also is the theoretical upper limit of microplastics according to the definition for microplastics that was adopted in this thesis.

The cage was weighted down with two 1 kg weights so that the hose would go straight into the water. The pump had two outlets, one of which was connected to another hose of 3 meters in length and used to redirect some of the water back to the river to lower the flow over the filters and prevent leakage in the system due to the high pressure. The other hose was used to connect the pump to a filtration system for MPs, which was vertically installed on the back of the cart.

The filtration system consisted of two branches containing two filter holders each. The two branches were used to alternate during the sampling to ensure continuous operation of the device. One branch was then used for sampling water, whereas the used filters from the other one, that just had been sampled, were removed, and replaced with new ones. The alternation between the branches was possible thanks to two valves, which were incorporated into the installation at the top of each branch.

The first filter holder of each branch held a 150 µm pore size stainless-steel filter whereas the second one held a 51 µm (Spectra labs). The device filtered the water until 50 L of water had been reached or the filters clogged. Then, the branches of the filters were alternated. Finally, a little bit of water remained in the filter holders after sampling. If opened, the water would've flooded out with partial or total loss of the MP sample as a result.

Therefore, between each filter, another valve with an air filter was installed to let air in when removing the water with a vacuum pump afterward. Afterward, the filters were removed, replaced, and stored in glass beakers, shielded through aluminum foil. They were

then transported back to the lab and were stored under the fume hood until further processing.

Figure 3 water sample collection

3.2.2. Sediment Sampling Technique

Samples of sediment were collected from three different zones of the lake, which were the inlet, middle, and outlet areas. Within each of these zones, there were three sampling sites. To collect the samples a wooden square frame was used with a side length of 1 meter and the top 2.5 cm of the sediment from within each $1m^2$ quadrat of the frame was collected using a grab sampler stainless steel shovel.

The quadrat line was set parallel to the shoreline at each sampling location. This process resulted in a total of approximately 1.5 kg for each sampling site with 3 replications. The sediment samples were then placed in wide-mouthed bottles that had been cleaned, sealed with aluminum foil, and transported to the laboratory. The sample was stored in the laboratory at room temperature until it could be processed.

Sample processing

The overall pretreatment, extraction, digestion, and identification procedure was carried out as per the National Oceanic and Atmospheric Administration protocols NOAA (Masura *et al*., 2015), and Laboratory guidelines for the detection and quantification of plastic particles from freshwater environmental samples, and water research with some modifications.

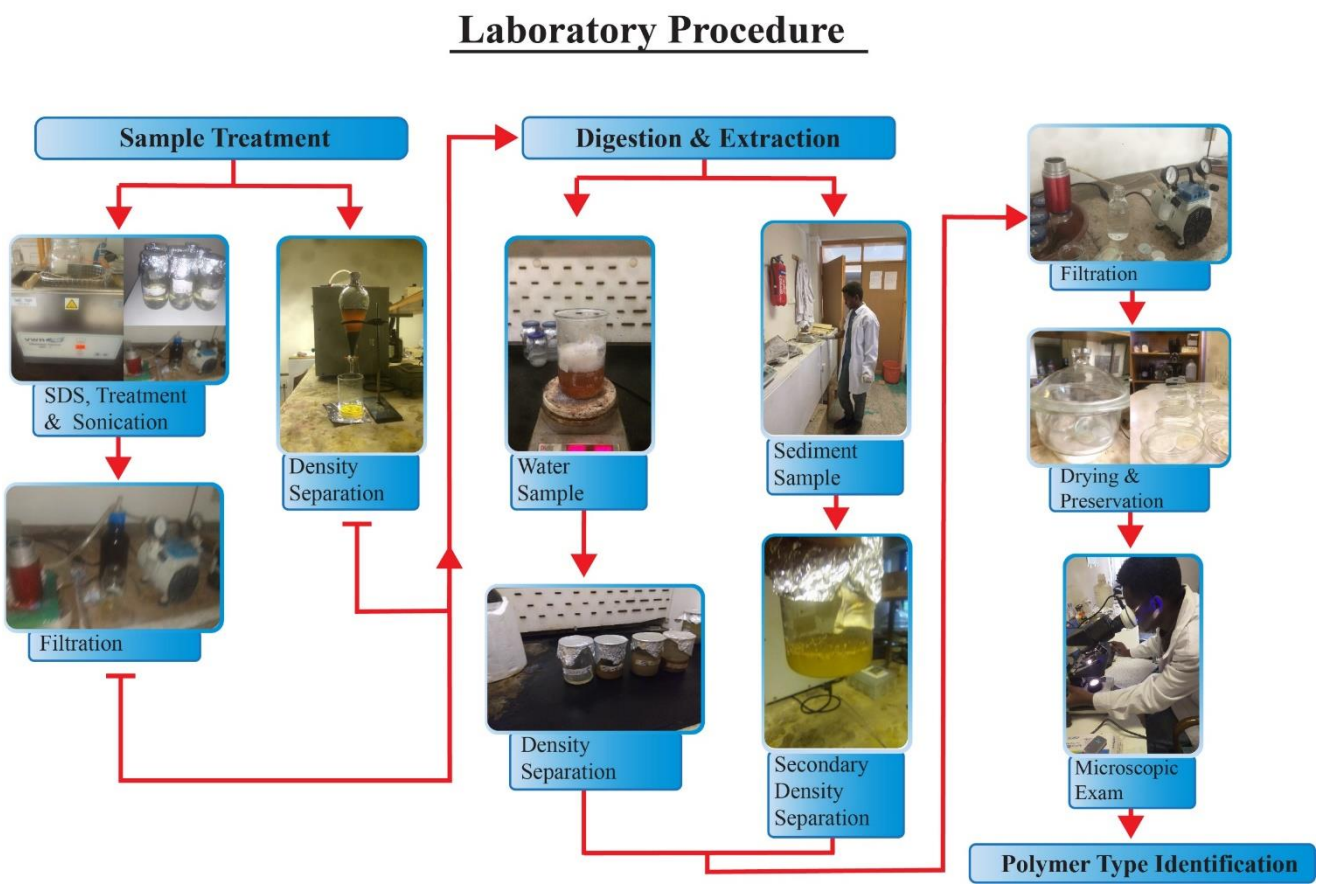


Figure 4 Schematic presentation of microplastic identification

3.3. Sample pre-treatment

3.3.1. SDS - Treatment and sonication for water sample

A 5% SDS solution was prepared by dissolving 100 g of SDS in 2 liters of filtered distilled water. The solution was stored in glass bottles; similarly, ethanol was filtered on 1.2 µm GF/C filter paper using the glass filtration system, and the filtrate was stored in a glass bottle. The prepared SDS solution was poured into the filter sieve for each filtrate of 100 ml of 5% SDS solution. The filters were placed in a separate pre-cleaned beaker and filled with 100 ml of 5% SDS solution per filter.

The beaker was sealed with aluminum foil and a small hole was made in the aluminum foil to avoid any pressure. The filtrate was incubated on a heating plate at 40 °C for 24 hours. After the heating step, each beaker was placed in the ultrasonic bath and sonicated for 5 minutes for each filter, After the 5 minutes of sonication; the filter was taken from the beaker thoroughly with clean tweezers and flushed with filtered ethanol thoroughly to remove SDS. Then the solution was transferred to the rinsed beaker with ethanol, then the mixture (SDS solution, Ethanol, Particles from the Filters) was filtered on 8-µm cellulose nitrate using a vacuum pump (VWR, USA) and a glass filtration system (Thermo Fisher, USA) and to remove the remaining chemicals on the filters were rinsed with distilled water; While filtering, the solution produces a significant amount of foam to safeguard the vacuum device.

To counter this issue, butanol was employed as a defoaming agent between the glass filtration system and the vacuum device (Ren *et al*., 2023). After filtration, the filtrate was

removed from the glass filtration system using the stainless-steel tweezers and placed in the labeled glass vial by folding it 2 times without disturbing the particles on the filter.

3.3.2. Pretreatment, density separation, and extraction of sediment sample

The sediments were placed in the large glass Petri dishes and were dried at 60 °C in the oven for 72 hours to remove the sediment moisture based on the heat deflection temperature (HDT) of plastic samples oven dried at less than 60°C can also be a good technique (Osswald, 2006). The dried sediments were transferred in a sieving apparatus for size fractionation. Each dried sample was sieved to retain 500µm. The sieve equipment was positioned on an orbital shaker and subjected to agitation for 30 minutes at a speed of 150 revolutions per minute.

A 250 ml saturated sodium iodide NaI solution of (1.8 g cm^{-3} BDH, England) was added to a 1000 ml beaker after 75 g of dried sediment samples were weighed using an analytical balance and transferred there (Li *et al*., 2018). In addition, sodium iodide solution was added to the beaker until it held 750 ml, stirred for 15 minutes at 150 RPM, and then transferred to a glass separation funnel to settle overnight.

The higher-density material settled to the bottom of the beaker and the supernatant was separated by using a stainless steel still metal sieve having a mesh size of 500 µm particles from the solution. After having properly rinsed with distilled water to remove the salt solution, the separated particles on the metal sieve were allowed to dry for a suitable amount of time.

3.4. Digestion, density separation, and filtration

3.4.1. Water sample

The filtrate was transferred to cleaned glass beakers after undergoing SDS and sonication steps. To the filtrate in the beaker, 75 ml of potassium hydroxide (KOH) with a concentration of (12.5% BDH, England,) was added, and it was then incubated on a heating plate for 5 days at 35°C. After 5 days of digestion, 50 ml of hydrogen peroxide (H_2O_2, 30% BDH, England), was added, and it was incubated at 35°C for 24 hours. Inorganic particles were separated by performing a density separation process using a hypersaline solution of sodium iodide (NaI 1.8 g/cm^3 from BDH, England).

The solution was left to settle overnight in a glass separation funnel. The materials with a density above 1.8 g/cm^3 settled at the bottom and were removed through the outlet valve. The supernatant-containing particles were filtered using a cellulose nitrate membrane filter placed on a glass filtration system (Thermo Fisher, USA). The filtered particles were collected in a pre-cleaned glass Petri dish in a closed desiccator using silica gel to enhance the drying process for further analysis.

3.4.2. Sediment sample

Afterward, moist peroxide oxidation was employed to eliminate the organic matter that had accumulated in the samples. By mixing 7.5g of Fe_2SO_4 with 500 ml (SD Fine Chine limited, China) of distilled water and 3 ml of concentrated sulfuric acid (H_2SO_4), a 0.05M Iron (Fe (II)) solution was produced. Subsequently, 20 ml of an aqueous solution containing 0.05 M of Fe (II) and 20 ml of 30% hydrogen peroxide solution (H_2O_2 BDH, England) were added to the glassware containing the dried sample.

The mixture was left for 5 minutes at room temperature before the heating process started. After 5 minutes, a stir bar was added and the mixture was heated to 75º C on a hotplate. When the gas bubbles were observed at the surface of the beakers the beaker from the hotplate then added distilled water and the mixtures were heated to 75º C again for additional minutes. To increase the density of the aqueous solution 6g of sodium chloride (NaCl Sigma, Aldrich).

The process of digestion was carried out until the digestion liquid was clear. (Masura *et al*., 2015). After the digestion process, the mixtures were removed from the hot plate covered with aluminum foil cool it down, and retained for filtration. After removing organic matter by the wet oxidation process, the supernatant was filtered using vacuum filtration with a fiberglass filter Whatman ™ membrane (cellulose nitrate The cellulose nitrate filter paper used has a 47 mm diameter and a 0.45 µm pore size).

The filtered, particles were rinsed with distilled water to remove the remaining chemicals, moved to a labeled petri dish, and dried in a closed desiccator using silica gel to enhance the drying process for further analysis.

3.5. Microplastic identification and analysis

3.5.1. Microscopic identification

The filtered samples were identified by using a dissecting microscope (OMAX, China) with 40X magnification and attached with a 10 MP USB 3.0 digital camera. The identification procedure was done according to (Hidalgo-Ruz *et al*., 2012; Masura *et al*., 2015) Particles were considered plastic-like because the structure was elastic and durable, with no breaking when handling tweezers.

Additionally, the hot needle test was applied to distinguish between plastic and non–plastic particles (De Witte *et al*., 2014). The identifiable particles were removed by using tweezers and transferred to the cleaned Petri dishes to characterize the quantities, shapes, and colors of the plastics.

The size of the microplastic was determined by using image j software. Additionally, to examine the morphology of microplastic particles SEM JCM-6000Plus with High-vac. SED PC-std. 15kV with magnification x100 (44mm) was applied. Finally, the identified microplastic particles were moved to a small glass vial for FTIR characterization.

3.5.2. Spectroscopic identification characterization of microplastic

A total of 23 microplastic particles were selected from both sediment (13) and water (10) samples and were analyzed Spectrum 65 FT-IR (PerkinElmer) The polymer compositions of MPs were identified using Fourier Transform Infrared Spectroscopy (FTIR). The FTIR absorption spectra were recorded by averaging 4 scans in the mid-infrared band from 4000 to 400/cm, with a resolution of 4/cm.

The identification of the polymer type was determined by analyzing the absorption frequencies of specific chemical bond types found in the relevant polymer samples. To make comparisons standard plastic polymers Such as PP, PE, PVC, PET, and PS were collected from the Ethiopian Plastic Factory Share Company.

3.6. Quality assurance and control

In the field and laboratory in both settings, blanks were used to avoid the possibility of cross-contamination between samples that come from filter sieves, bottles, and hoses. All measurements were conducted to prevent possible background contamination. Before the examinations, all solutions utilized in the study underwent filtration using a 1.2 μm membrane filter. During the experimental period, wore gloves and lab coats made of cotton.

3.7. Environmental risk of microplastic pollution

To assess the level of microplastic pollution in the lake the Nemerow Pollution Index (NPI) was used based on the bioavailability and toxicity of microplastics calculated by the following equation(Wu *et al*., 2023).

$$\sqrt{\frac{\frac{Qi^2}{s_{i\ max}}+\frac{Qi^2}{s_{i\ ave}}}{2}} \qquad (1)$$

Where: - Qi is the microplastic abundance of samples collected from each site i, Si is the reference standard value of microplastic abundance Si is the reference standard value of microplastic abundance, and the Si value was taken as 540 particles/kg dry weight, (Everaert *et al*., 2018). Based on the NPI value, if NPI is less than 2, it shows that the lake microplastic pollution is light, and if NPI is greater than 2, it shows that the lake microplastic pollution is serious.

The quantification of microplastic toxicity was conducted by the utilization of a mathematical calculation known as the polymer Hazard Index (PHI). The equation for the PHI variable is as follows:

$$PHI=\sum Pn \times Sn \quad (2)$$

Where:- Pn refers to the average proportion of each microplastic polymer observed in all samples, while Sn represents the risk score associated with each polymer (Liu *et al*., 2022; Wu *et al*., 2023) that has provided the risk rating for various types of plastics namely PP, PET, PE, PS Nylon PVC, and others. The risk ratings assigned to these plastics are 1, 10, 11, 30, 150, 11, and 100. If the value of Pn is below 100 it indicates a low risk of microplastic pollution, and the value of Pn is above 100, it indicates a high risk of microplastic pollution.

3.8. Data analysis

The abundance and size (mean ± standard deviation) were graphed using the Origin 2022 program (Version 9.65). The statistical data analysis was conducted using the SPSS program (Version 26), which is a tool specifically designed for social sciences. To evaluate the disparity in concentrations among the three sampling locations, namely site 1, site 2, and site 3, a one-way analysis of variance (ANOVA) was utilized. The factors employed in the analysis encompassed plastic concentration and site location. The alpha value was established to be ($p \leq 0.05$).

Chapter Four

4. Result and Discussion

4.1. Abundance of microplastics in water and sediment

The abundance of microplastic particles was identified at three different sampling sites. The mean of the three triplicates was found to be 109 ± 5.5 particles per liter for water samples and 155 ± 7.6 particles per kilogram for sediment samples. The figure presents the abundance of microplastics in water and sediment samples across the three sampling locations.

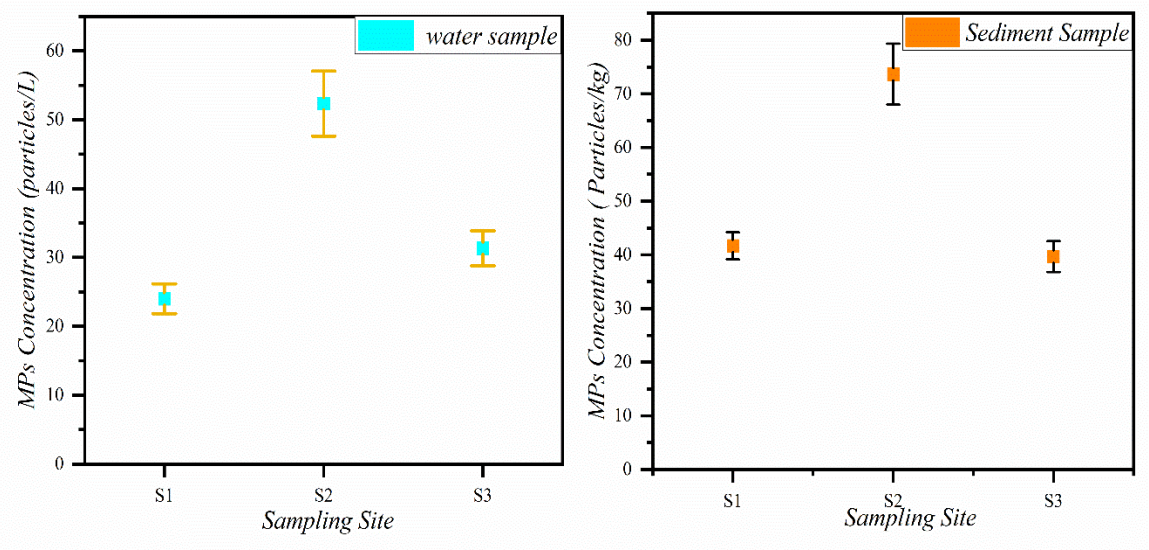


Figure 5 Abundance (Mean ± SE) comparison of microplastics in the water particles/L and sediment particles/kg samples at three sampling locations of the lake

The mean values of microplastic levels in water samples for each sampling site were 24 ± 2, 52.33 ± 4.72, and 31.33 ± 2.56 particles per liter for S1, S2, and S3 respectively. On the other hand, the mean values of microplastic levels in sediment samples for each sampling site were 41.67 ±2.55, 73.67 ±5.69, and 39.67 ± 2.87 particles per kilogram for S1, S2, and S3 respectively.

The highest concentration of microplastics (52.33±4.72 particles/L) was detected in S2. The average concentration of microplastics decreased, as follows: S2 > S3 > S1. On the other hand, the sediment samples displayed a range of mean values from 39.67 to 73.69 items/kg, with the highest concentration (73.67±5.69 particles/kg) identified in S2.

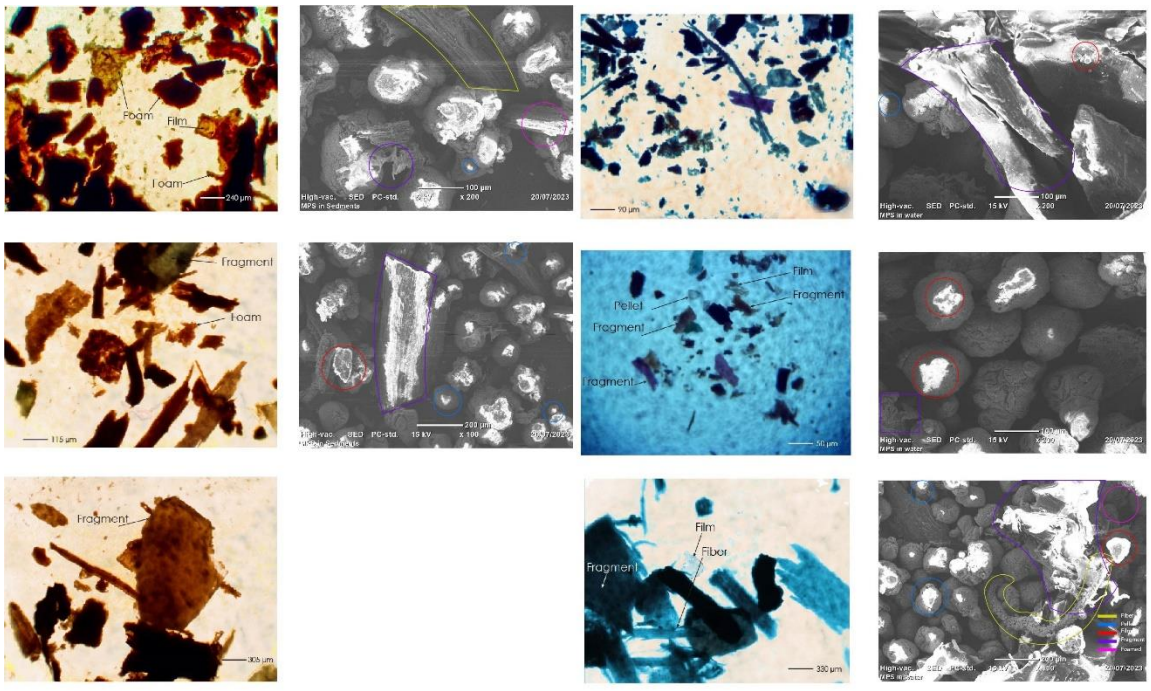
Microplastic sediment concentrations generally decreased from S2 through S3 to S1 on average. For both water and sediment samples, the highest microplastic concentration was observed in the middle zone of the lake. This might be the area, potentially attributed to the direction of water flow, and topographic factors (Hossain *et al*., 2023). Due to the lake's large area in site two and prolonged hydraulic retention time, microplastic has the potential to accumulate in the area.

In general, the occurrence of microplastic particles in the lake tributaries was polluted from point and non-point sources. like residential areas, urban runoff from transportation, commercial wastes, industrial effluent connecting the septic tank without proper treatment, improper waste disposal around the river area, and agricultural runoff usage of different agrochemicals (Peng *et al*., 2017; Gela & Aragaw, 2022; Jeevanandam *et al*., 2022).

4.2. Physical characteristics of microplastics

4.2.1. Shape of microplastics

During microscope identification, five unique morphologies of microplastics were identified: fragment, fiber, film, foamed plastic, and pellets, from all sampling sites, and both water and sediment samples. Scanning electron microscopy (SEM) scans revealed distinct variations in surface roughness across microplastics, indicating the presence of detailed surface topographic features such as rough, porous, fractured, or severely damaged. The presence of fragmented microplastics causes morphological changes in both water and sediment samples. These microplastics had rough or uneven surfaces, and there were clear traces of wear at the ends. The detail picture of the morphology types of microplastics captured from compound microscope and scanning electron microscope presented below.



Sediment sample water sample

Figure 6 microscopic and SEM image of microplastics sediment and water sample

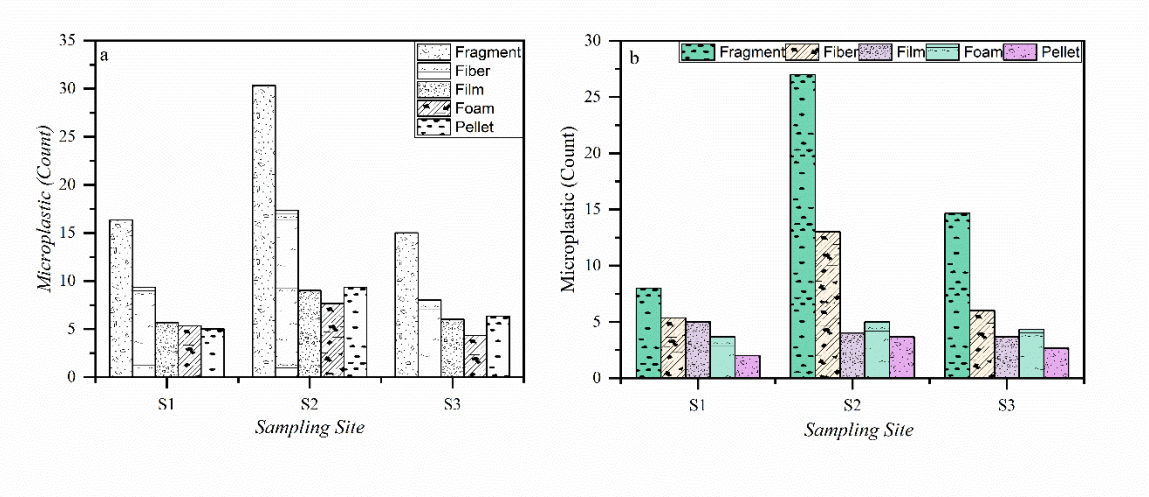


Figure 7 the proportion microplastic morphology from a water (a) and sediment sample (b)

The proportion of fragmented microplastics was 46% in water and 40% in sediment samples, respectively. The fragments obtained from the samples primarily originate from the degradation of large plastic items like jugs, plastic plates, aged plastic tubs, bags, and food containers. Due to the action of different environmental factors such as wind and ultraviolet rays (UV) which leads to the formation of small microplastic fragmented particles in the water bodies as described by Priscilla *et al*., (2019); Hengstmann *et al*., (2021).

The prevalence of fiber microplastics (MPs) in water sediment samples was found to be the second highest, accounting for 23% and 22% of the total MPs detected. It might be from synthetic fabrics and clothing is the primary source of the fibers, the cleaning process of synthetic materials can cause a significant release of fibers into the water body (Andrady, 2011; Z. Wang et al., 2020). The third most prevalent microplastic particle was documented as a film-type particle present in both water and sediment samples. The percentage of fiber content in water and sediment was 12% and 13%, respectively. The occurrence of pellets

is a small amount compared to other shapes in the water sample. Additionally, the presence of foamed microplastic particles in the sediment sample was low quantities.

Films and foams are mostly composed of crushed remnants of polythene bags, plastic wrappings, and other materials used in the packaging industry (Tanaka & Takada, 2016). These materials were fragmented to produce films and foams. The major sources of pellets are cosmetics, personal care items facial cleanser scrubs, microbeads, and the plastic industry are the major contributors of pellets to the water body (Amrutha *et al*., 2022). This indicates that there are many people dependent on these goods and that these industries are located near the river.

4.2.2. Color of microplastics

The identified MPs were sorted by color, which accounts for transparent, black, blue, red, green, and yellow. In water, the proportion of transparent MPs was recorded as 48% of the total no of MPS. The other colored MPs for water sample types in general, were given in order: Green>Blue>Black>Yellow>Red. In addition, in sediment, the transparent MPs 45 % of the total were recorded significantly with the other colors MPs. Green > Blue > Red > Black > Yellow.

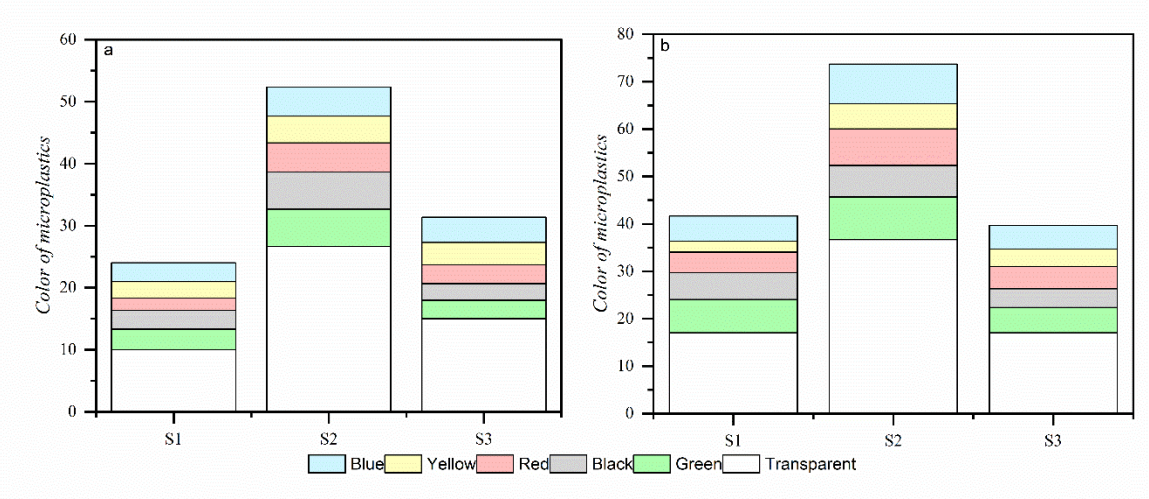


Figure 8 The Color proportion of microplastic from water (a) and sediment sample (b)

This suggested that the majority of transparent microplastics are typically used for single-use and disposable plastic bags plastic cups, and plastic bottles for water and soft drinks, and some colorful microplastics may have originated from food and beverage products (Gela & Aragaw, 2022).

Additionally, certain colorful plastics such as blue, green yellow red may lose their original hue upon entering the water bodies due to exposure to sunlight the primary cause of plastic aging which induces polymer chain reaction, is changes in physical properties like yellowing and fading, and chemical degradation (Sutkar *et al.*, 2023; Zhao *et al.*, 2022; Corcoran, 2021).

Another significant factor in knowing the color of microplastics is their potential to increase the probability of ingestion as visual predators on small zooplankton, some commercially important fish species and their larvae may consume MPs that closely resemble their natural prey this might lead to bioaccumulation, and biomagnification through the food chain with the plastics additives (Zhao *et al*., 2022).

4.2.3. Size of microplastics

The size of microplastic particles was measured using the Image J software rather than MPs with particle three size categories: > 500 µm, 500–100 µm, and < 100 µm for both water and sediment samples. The size distribution of microplastic in this study, the smallest size of microplastic particles was measured at 9.33 µm and almost half of the microplastic particles were below 100 µm in both water (56%) and sediment (54%). In water, 26.6% of MPs were categorized in a size range between 500 to 100 µm the remaining 16.5 % was within the size range > 500 µm. In addition, 29% of sediment MPs were categorized in a size range between 500-100 µm, and 16.7% were categorized in > 500 µm size categories.

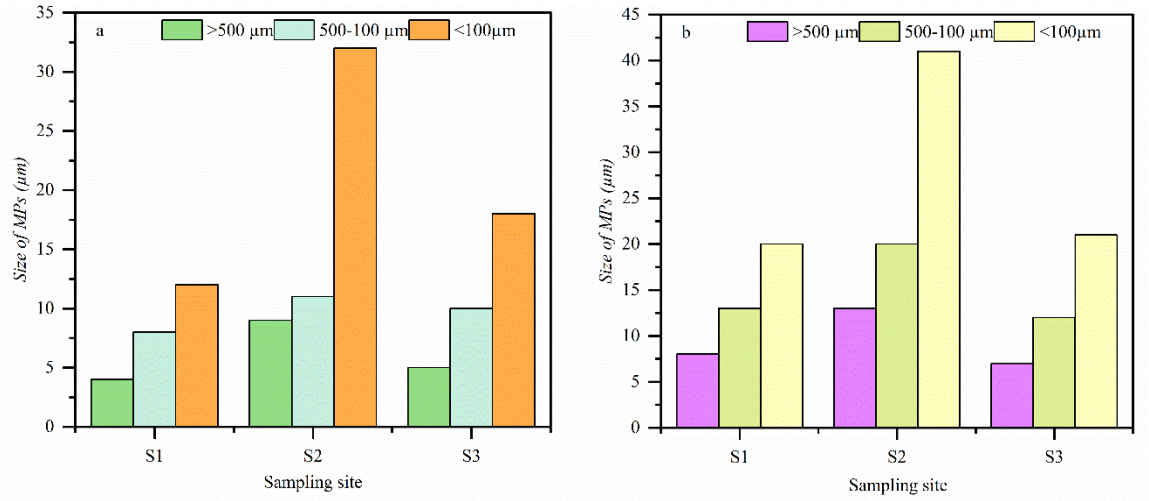


Figure 9 the size classification of microplastics measured by using Image J software from a water (a) and sediment sample (b)

The abundant particle sizes are shown in both water and sediment samples particularly at the middle sampling location sampling locations compared to > 500 µm particle size MPs. All the sampling sites exhibit the presence of MPs of 500–100 µm, and < 100 µm, which falls under the size defined for MPs.

Larger plastic items may be breaking down into smaller microplastic particles due to various factors such as sunlight, wind, and waves causing photo-degradation and mechanical degradation respectively.

Microorganisms can also break down larger plastics into smaller particles through microbial activity, while the polymer types of plastics may also affect the degradation process (Webb *et al*., 2012; Bajt, 2021; Liu *et al*., 2022).

Small-sized microplastics (MPs) are more likely to be ingested by aquatic organisms due to their small size and ability to form biofilms, causing adverse health effects for both low and high-trophic organisms (Selvam *et al*., 2021).

4.3. Polymer type identification

4.3.1. Polymer spectral analysis

The analysis of the selected MP particles, together with their matching standard reference plastic polymer spectra, was conducted using Fourier transform infrared spectroscopy (FTIR). Polyethylene $(H_2C=CH_2)_n$ displays a wide and intense stretching band in the range of 2850 cm^{-1} to 2900 cm^{-1}, primarily associated with CH bonds. Additionally, a less pronounced bending band attributed to CH_2 bonds may be observed between 1470 cm^{-1} and 1360 cm^{-1}, Furthermore, a rather weak rocking band related to CH_2 bonds is discernible at approximately 720 cm^{-1} (Amelia *et al*., 2016).

These features include a prominent CH_2 stretching band observed at approximately 2850 cm^{-1} and 2920 cm^{-1}, a CH_3 symmetric stretching band at roughly 2950 cm^{-1}, and a CH_2 bending band at approximately 1460 cm^{-1} (Fang *et al*., 2012). The spectral characteristics suggest that the polymer is polypropylene $(CH_3H_6)_n$.

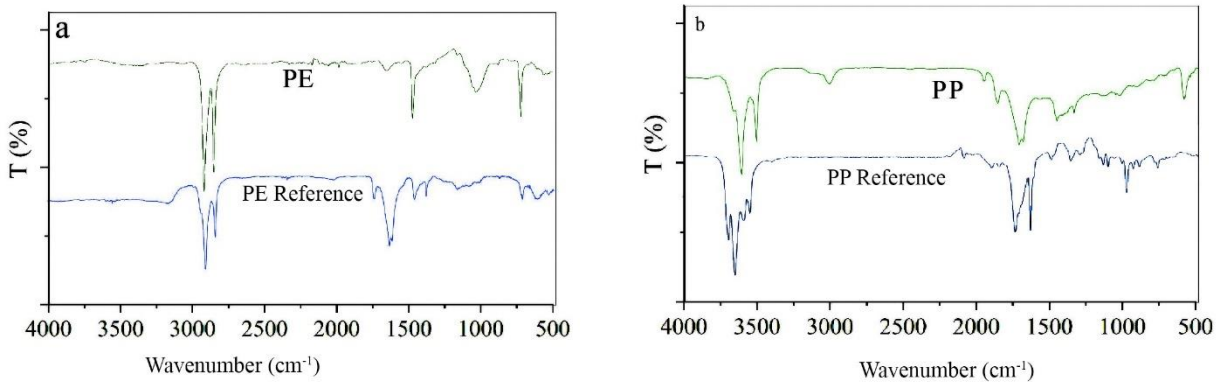


Figure 10 Identification of microplastics using FTIR spectra identified polymer and reference spectra (a) polyethylene and (b) polypropylene.

The prominent absorption peaks encompass a robust carbonyl C=O stretch occurring at approximately 1700 cm^{-1}, an aromatic C=C stretch taking place at around 1600 cm^{-1}, and a C-O-C stretch manifesting at approximately 1250 cm^{-1} (Garton *et al*., 1981). All of the aforementioned selections have provided evidence of the existence of PET $(C_{10}H_8O_4)_n$ particles. The absorption spectrum of PS $(C_8H_8)_n$ is distinguished by the presence of several prominent peaks. Notably, a robust aromatic C-H stretching vibration is observed at approximately 3050 cm^{-1}. Additionally, a distinct phenyl C=C stretching vibration is observed at around 1600 cm^{-1}. Furthermore, a characteristic benzene ring deformation vibration is observed at approximately 1000 cm^{-1}, providing a further description of the PS molecule (Thinh *et al*., 2012).

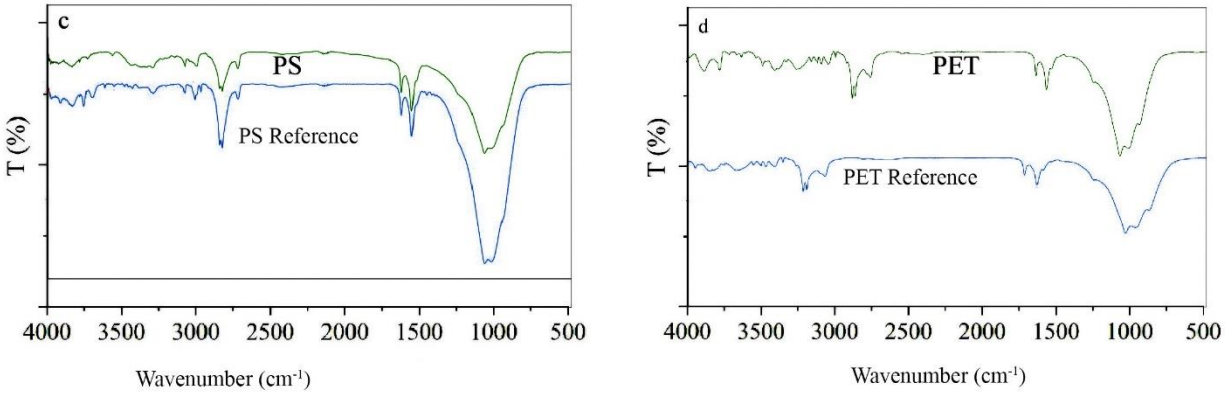


Figure 11 Identification of microplastics using FTIR spectra identified polymer and reference spectra (c) polystyrene and (d) polyethylene terephthalate.

Polyvinyl chloride $(C_2H_3Cl)_n$ exhibits discernible peaks in the Fourier-transform infrared (FTIR) spectrum, including a prominent C=C stretching band at approximately 1735 cm^{-1}, a C-Cl stretching band at roughly 650 cm^{-1}, and a C-H stretching band at approximately 2940 cm^{-1} (Pandey *et al*., 2016). All of these features have been verified, indicating the presence of PVC in the sample.

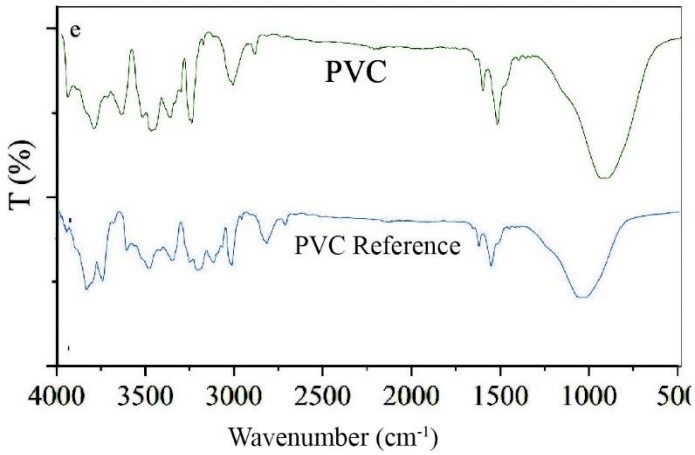


Figure 12 Identification of microplastics using FTIR spectra identified polymer and reference spectra for PVC

By conducting absorption band studies, various vibrations related to distinct functional groups were detected, enabling the identification of the predominant polymer type present in the extracted microplastics.

4.3.2. Polymer composition

The results of this study were obtained from the analysis of microplastic polymer composition in water and sediment samples collected from Lake Aba Samuel. Out of all the water samples, polyethylene (PE) was identified as the predominant polymer, making up 35% of the total polymers measured. Polypropylene (PP) ranked second, accounting for 25% of the sample. Polyethylene terephthalate (PET) made up 14% of the total, with Polystyrene (PS) and other polymers accounting for 18% and 8%, respectively. The sediment samples exhibited a marginally distinct distribution of polymers. Polyethylene terephthalate (PET) accounted for the largest proportion, representing 35%, while polyethylene (PE) constituted 25%. Polypropylene (PP) accounted for 10% of the total, while Polystyrene (PS) and Polyvinylchloride (PVC) made up 5% and 20% respectively. The remaining 5% consisted of other unidentifiable particles.

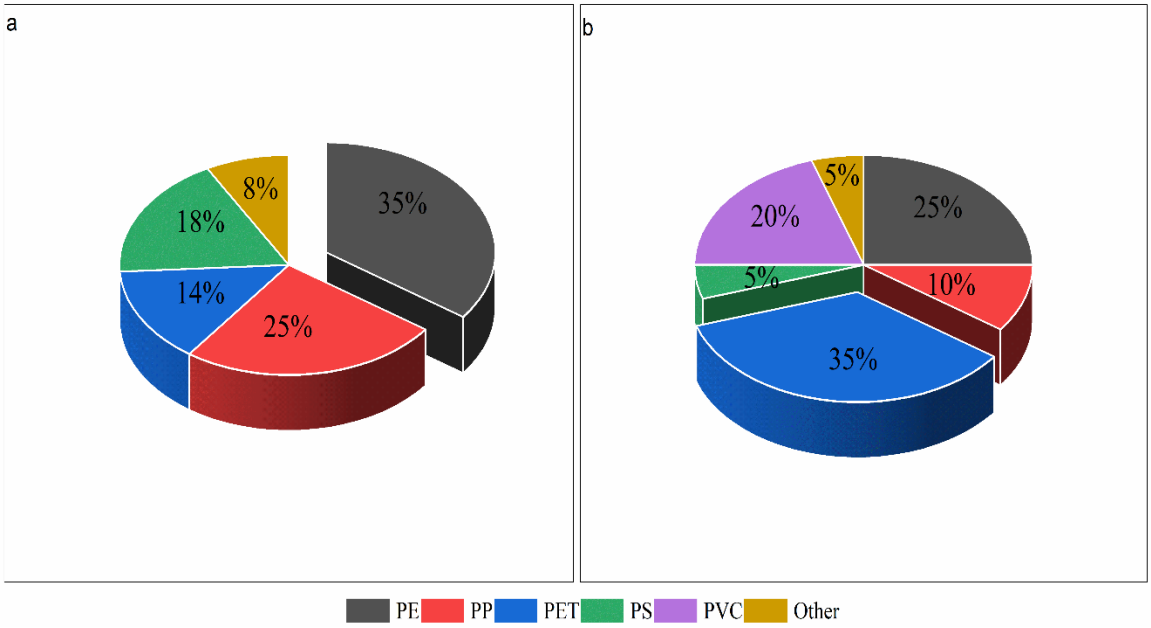


Figure 13 Polymer composition from a water (a) and sediment sample (b)

The FTIR spectra from extracted microplastics (MPs) for the polymer with compositions of 92% and 95% in both water and sediment samples respectively were confirmed by FTIR spectroscopy the rest could not be identified as common polymeric materials and thus are not considered to be plastic.

In the present study, it was observed that polyethylene (PE) and polypropylene (PP), polyethylene (PET), and polyethylene (PE) were the predominant polymers of microplastics (MPs) in waters and sediment samples respectively. In addition, Polyvinyl chloride (PVC) was found only in sediment samples it might be based on its physical characteristics like density and its additives have heavier density and sink into sediment. Polystyrene (PS) was a small amount observed from both sample types within the lake. PP (polypropylene) and PE (polyethylene), the predominant polymers, are typically found in consumer items like plastic bags, bottles, caps, films, containers, and automotive bumpers (Naji *et al*., 2017). These polymers are commonly derived from the fragmentation of bigger plastics. PE and PP are more prone to invading the environment because of their resilient and adaptable characteristics, which contribute to their extensive utilization. Polyethylene terephthalate (PET) is a widely used polymer in the manufacturing of beverage containers and packaging materials. Moreover, its fibrous forms are utilized in the textile sector for the manufacturing of garments. Another possible concern is that PET exhibits a strong attraction to oily pollutants present in seawater and can act as a conduit for carrying poisons into the food chain when organisms ingest the fibers (Mu *et al*., 2019).

4.4. Environmental risk of microplastic pollution

In this research, we applied the NPI to quantify microplastic pollution. Microplastic bioavailability in the lake was calculated as shown in Equation 1. According to the findings, Lake Aba Samuel has a value of NPI 0.55, below the threshold level 2 which indicates light pollution because according to *Liu et al., (2022)* the value of NPI less than or equal to 2 indicating light pollution of microplastic in the lake. The health effects of microplastics were calculated using the PHI, which, based on Equation (2). The PHI value of the Lake was 32.87, which is below the threshold value of 100, suggesting a minimal risk. Lake Aba Samuel has a low microplastic contamination level and health risk score *(Liu et al., 2022; Wu et al*., 2023). Nevertheless, it is expected to rise with the increasing human population and rising standards of living that accompany economic development. Therefore, even lakes in low pollution and low danger may become potential places of high microplastic pollution risk in the future, if plastic waste management is low, policy implemented, and people's awareness is not created and improved. At any point in their lifecycle from manufacturing to disposal these polymers could break down, releasing or even creating potentially harmful byproducts.

Chapter Five

5. Conclusion and Recommendation

5.1. Conclusion

The result of the study exhibited a range of mean values for microplastic levels, ranging from 24 to 52.33 particles/L and 39.67 to 73.69 particles/kg for water and sediment samples respectively. The highest concentration of microplastics was observed in site 2 for both sample types or in the middle zone of the lake. Furthermore, a significant difference was observed at ($p \leq 0.05$) location with an abundance of microplastics (MPs) in water and sediment. Abundant fragments and fibers, which were transparent and blue, were observed in both water and sediment samples. The microplastics exhibit a variety of sizes. The microplastic particles were found to have a minimum size of 9.33 µm. The majority of microplastic particles, accounting for 56% in water and 54% in sediment, were below 100 µm in size. Polymer characterization of microplastics polyethylene terephthalate (PET) and polyethylene (PE) were the major polymers of microplastics (MPs) and Polystyrene (PS) was a small amount observed from both sample types within the lake. The value of the bioavailability and toxicity of microplastics NPI and PHI was 0.55 and 32. 87 respectively. Therefore, the NPI and PHI values are below the threshold level, indicating a light level of microplastic contamination. The findings of the study emphasize the need for prompt action. To prevent more contamination and protect the ecological integrity of urban water bodies such as Lake Aba Samuel, and other Ethiopian lakes, it is crucial to implement policy interventions, effective waste management techniques, regulations on plastic usage, and public awareness programs.

5.2. Recommendation

Additional research is necessary to determine the minimum size of microplastic (MP) fractions that can be identified based on their polymeric composition using advanced analytical tools like μ-FTIR spectroscopy, SEM/EDS, Raman spectroscopy, and Pyrolysis GC-MS. These methods will provide detailed information about the surface morphology and elemental composition of the detected MP particles.

Further research is required to investigate the potential health and toxicological impacts of the predominant kind of plastic particles that are important to human risk assessment. Additionally, more studies are needed to examine the impact of water treatment facilities on these particles both before and after treatment.

Promote paper-based packaging made from recycled materials and encouraging industries and importers to shift from plastic packaging to biodegradable and recyclable paper-based alternatives can significantly decrease plastic waste sustainable way.

Advocate activities that promote the use of recycled plastics from consumer or industrial waste in production operations which involves integrating recycled plastics into new products, decreases the need for fresh plastic manufacturing, and mitigates environmental consequences.

Implement policies and strategies intended for reducing the production and usage of disposable plastics, and integrated solid waste management, this goal can be achieved by the implementation of legislative measures and the voluntary participation of industries, stakeholders, and public awareness initiatives.

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Appendixes

Appendixes I: - way ANOVA among locations with an abundance of microplastics for both sediment sample

ANOVA: Single Factor for Water Sample

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	102	34	12
Column 2	3	115	38.33333333	44.33333333
Column 3	3	109	36.33333333	49.33333333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	28.22222222	2	14.11111111	0.400630915	0.4764 707	5.1432528
Within Groups	211.3333333	6	35.22222222			
Total	239.5555556	8				

ANOVA: Single Factor for Sediment Sample

SUMMARY

Groups	***Count***	***Sum***	***Average***	***Variance***
S1	**3**	**155**	**51.666667**	**8.33333333**
S2	**3**	**176**	**58.666667**	**57.3333333**
S3	**3**	**134**	**44.666667**	**17.3333333**

ANOVA

Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	**294**	**2**	**147**	**5.31325301**	**0.046994904**	**5.14325285**
Within Groups	**166**	**6**	**27.666667**			
Total	**460**	**8**				

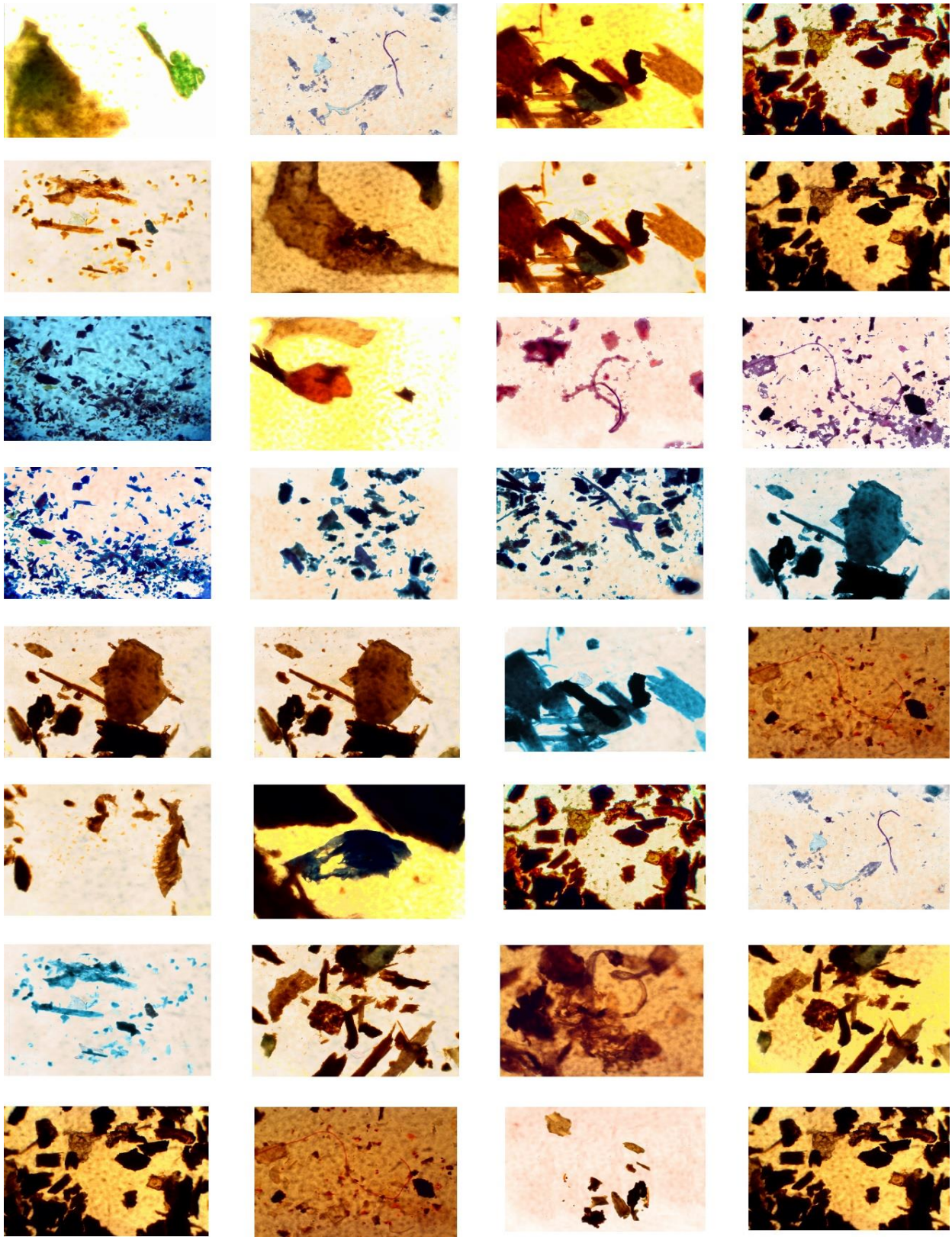
Appendix II: - Materials (chemicals, apparatus, and instruments)

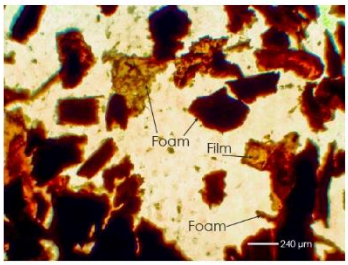
No.	Chemicals	Usage
1.	Sodium Iodide (NaI)	Density separation
2.	Sodium Chloride (NaCl	
3.	Sodium Dodecyl Sulfate (SDS)	Pretreatment and sonication
4.	Ethanol	
5.	Iron (II) sulfate ($Fe_2SO_4.7H_2O$)	Used to prepare a solution with a concentration of 0.05M of Iron (Fe (II)
6.	sulfuric acid (H_2SO_4)	
7.	Hydrogen peroxide (H_2O_2)	Digestion process
8.	and potassium hydroxide (KOH)	
9.	- Nitric acid (HNO_3)	Cleaning the materials before and after their utilization
10.	Distilled water	
11.	Butanol	Defoaming agent
12.	silica gel	Drying process

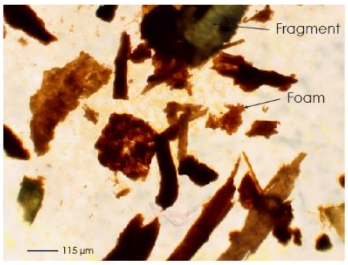
The following apparatus and instruments were employed in various stages of the sample collection, sample processing, analysis, and sample characterization.

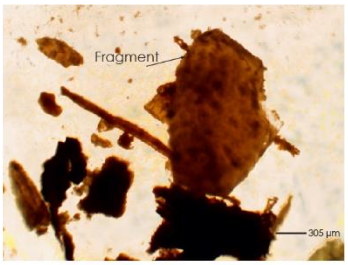
No	Materials
1.	Stainless still shovel
2.	Measuring tape and rope
3.	Wooden frame
4.	Water pump
5.	Generator
6.	Vacuum pump
7.	Inox filter holders
8.	Stainless steel filter sieves (50 and 51 µm, each with a diameter of 47mm.)
9.	5mm steel cage
10.	Flowmeters
11.	Hoses for inlet, bypass, connection, and outlet
12.	Ball valves
13.	Inox T-pieces, Inox tube connections, and Teflon tape
14.	Different-sized beakers
15.	Stainless steel tweezers
16.	GPS
17.	Fume hood
18.	Stereomicroscope with an attached digital camera
19.	Membrane filter paper
20.	Separatory funnel
21.	Petri dishes
22.	Glass vial
23.	Desiccator
24.	Scanning Electron Microscope
25.	Fourier Transform infrared spectroscopy

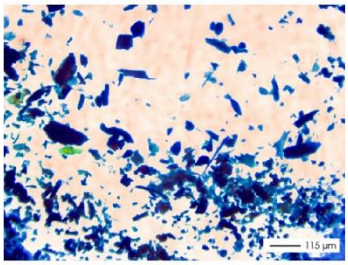
Appendixes III Photographs captured by microscope equipped with a digital camera

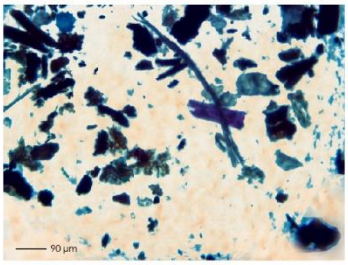


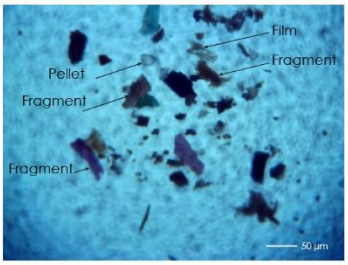

Foam
Film
Foam
240 µm

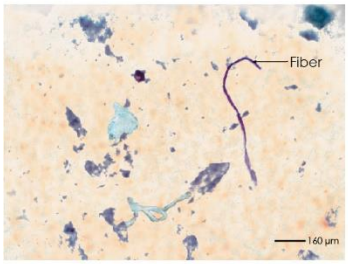

Fragment
Foam
115 µm

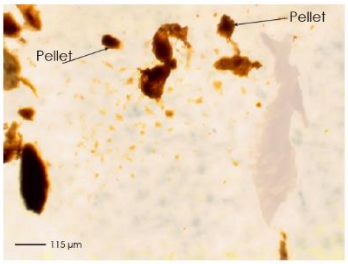

Fragment
305 µm

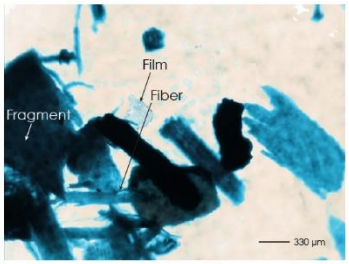

115 µm

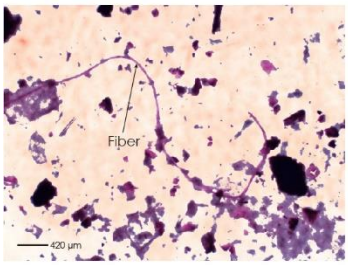

90 µm

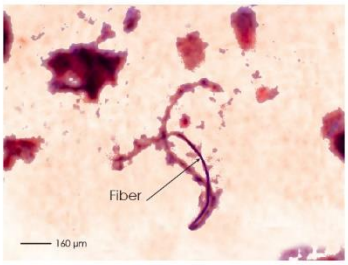

Film
Fragment
Pellet
Fragment
Fragment
50 µm

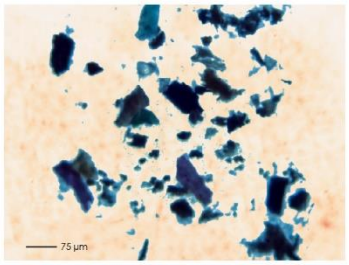

Fiber
160 µm


Pellet
Pellet
115 µm


Film
Fiber
Fragment
330 µm


Fiber
420 µm


Fiber
160 µm


75 µm

Appendixes IV. Photographs of the sampling site and sample processing and analysis

Site Visit









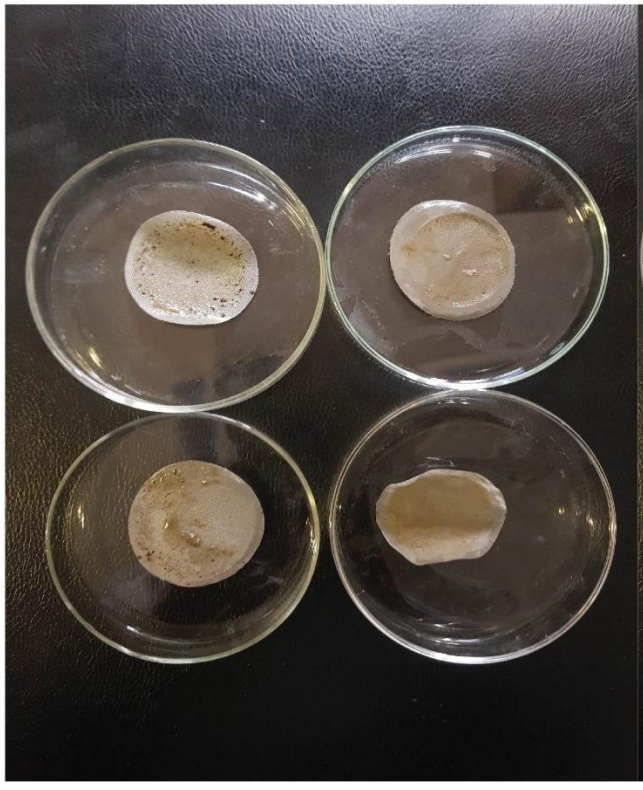
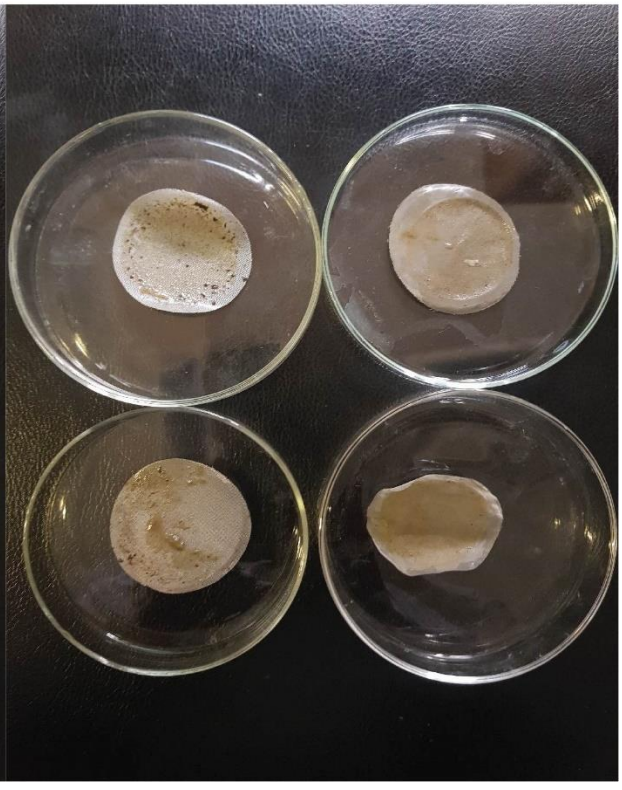


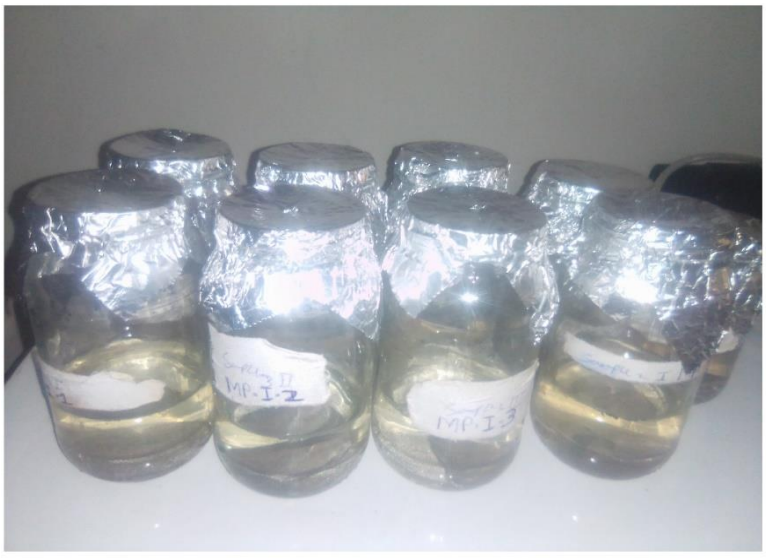

Sampling








MP-I-2
MP-I-3


VWR
Ultrasonic Cleaner


400
200
100
APPROX VOL