

**META-ANALYSIS OF MICROPLASTICS AND ASSOCIATED MICROBIOMES  
IN WINAM GULF OF LAKE VICTORIA, KENYA**

**Sigei Sharon**

**A Thesis Submitted in Partial Fulfilment for the Award of the Degree in Master of  
Science in Microbiology of Masinde Muliro University of Science and Technology**

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**Sign.....Date.....**

**Dr. OKOTH Patrick, PhD.**

Department of Biological Sciences

Masinde Muliro University of Science and Technology

**Sign.....Date.....**

**Dr. KOLLENBERG Mario, PhD.**

Department of Biological Sciences

Masinde Muliro University of Science and Technology

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SMB/G/01-70159/2021

**SUPERVISORS**

**Sign.....Date.....**

**Dr. OKOTH Patrick, PhD.**

Department of Biological Sciences

Masinde Muliro University of Science and Technology

**Sign.....Date.....**

**Dr. KOLLENBERG Mario, PhD.**

Department of Biological Sciences

Masinde Muliro University of Science and Technology

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## **DEDICATION**

This work is dedicated to my parents, Sigei John and Agnes Sigei, for giving me the opportunity I needed to advance my academics.

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I want to start off by extending my sincere appreciation to the omnipotent God for providing me the strength to finish my research. Secondly, I am very grateful for the help, direction, and moral support I have received from Dr. Patrick Okoth and Dr. Mario Kollenberg, without whom this research would not have been feasible. I am grateful to Masinde Muliro University of Science and Technology, SONAS, Department of biological sciences for their assistance in developing my research project via corrections and providing lab space to carry out this work plus the Jomo Kenyatta University of Agriculture and technology for the lab space. Credit also goes to laboratory personnel, Josphat and Joyline (Jkuat) Microplastics identification, Oscar (Warma) sampling for microplastics, Sandra Khatiebi (MMUST) and Dr. Clabe Wekesa (PHD) for all their support for making this research a reality. Finally, I want to thank my family, friends, and coworkers for their advice, help, and emotional support throughout the entire study time.

## ABSTRACT

In recent years, an imperative discussion has been built around microplastics across different landscapes pointing to the threat of microplastic toxicity, including complexes with their environmental interactions regarding it as a fundamental concern on a global scale. While studies have explored this burgeoning problem, there is a paucity of data regarding their interaction with the environment in resource-limited settings of Western Kenya. Winam Gulf of Lake Victoria is an outstanding and delicate ecosystem that supports a wide range of livelihood including humans to the microorganisms. The aim of the study was to meta-analyze microplastic profiles and microbiomes in Winam Gulf with the aim of determining polymers, microbial diversity, degrading microbes and functions and the gaps in knowledge regarding microplastic-microbe interactions and enzymatic activities to inform bioremediation strategies. The study used a random but purposive sampling strategy by picking a number of samples, 70 water and 40 sediments samples in total across the Winam Gulf region based on hotspots affected by industrial, domestic and agricultural activities. Fourier Transform Infrared Spectroscopy analyses of water, sediments and fish samples revealed microplastic pollution of diverse origin with polymer types including polyethylene (PE), polypropylene (PP), polyacrylamide (PAM), ethylene copolymer (EC), polybutene (PB), nylon, vinyl chloride (VC). Shotgun metagenomics was employed to characterize microbial communities in water and sediment samples. The quantified libraries were sequenced using the Illumina platform. Taxonomical abundance was determined by comparing metagenomic reads to a database of taxonomically informative gene families (MicroNR database). Species annotation was done using DIAMOND software (V0.9.9.110) extracted from NCBI's NR database. Functional annotation was inferred based on its similarity to the sequence in the databases (KEGG) while functional category was annotated using MG-RAST Subsystems. Shotgun metagenomics analyses of taxonomic phyla revealed Firmicutes at [88%] abundance. The bacilli were most abundant in the class taxonomy at [78%] and *Bacillus* was the most abundant in the genome taxonomy. The highest microplastic degrading bacteria were *Paracoccus denitrificans* with *Streptomyces sp.* ranking second with the enzymes present including *3HV dehydrogenase*, *proteases*, *PHB depolymerase*, *nylonases*, *PET-hydrolase* as well as *polyesterases* among others. These results suggest the presence of important microplastics-microbiomes interactions and the possibility of leveraging the functional capabilities of the microbiomes through bioremediation. The paper offers important information regarding microplastic contamination in Winam Gulf, the microbial communities linked to degradation and their possible enzymatical pathways. Short-term goals could involve the use of specific pollution reduction strategies such as community education and enforcements of policy, but long-term should be aimed at studying the prospects of utilizing the microbial and enzymatic activities of the naturally occurring microbial communities in order to develop a bioremediation strategy to eliminate microplastics in Winam Gulf of Lake Victoria in Kenya. Hence, future studies should develop controlled models to validate microbial degradation, explore genetic modifications to enhance biodegradation of Microplastics pollution.



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## ABBREVIATIONS & ACRONYMS

<b>AMR:</b>	Antimicrobial resistance
<b>FTIR:</b>	Fourier transform infrared spectroscopy
<b>gDNA</b>	genomic DNA
<b>HDPE</b>	High Density Polyethylene
<b>KEGG:</b>	Kyoto Encyclopedia Genes and Genomes
<b>LDPE</b>	Low Density Polyethylene
<b>MPs:</b>	Microplastics
<b>PE:</b>	Polyethylene
<b>PET:</b>	Polyethylene terephthalate
<b>PP:</b>	Polypropylene
<b>PPE:</b>	Personal protective equipment
<b>PVC:</b>	Polyvinyl chloride
<b>SDS</b>	Sodium Dodecyl Sulfate
<b>WHO:</b>	World Health Organization
<b>WWTPs:</b>	Wastewater treatment plants

## CHAPTER ONE

### INTRODUCTION

#### 1.0 Background Information

Microplastics are small plastic particles, typically less than 5 millimeters in size, that arise either from the breakdown of larger plastic items (secondary microplastics) or are intentionally manufactured as small particles for use in cosmetics and cleaning agents (primary microplastics) (Duis & Coors, 2016). Microplastic (MPs) pollution in aquatic environments is becoming a growing global public health burden. Following concerns expressed by the World Health Organization (WHO), a call to action by governments, industry, researchers and the public to address the issue has become imperative. WHO notes that microplastic particles are capable of entering the food chain, and warns that the health risks associated with exposure to microplastics are still largely unknown (WHO, 2020). Because microplastic polymers are durable and widely used, there is a global problem with plastic trash in the environment. In recent years, microplastics (MPs) have gained attention as a serious water contaminant that may have detrimental impacts on human and animal health (Elgarahy *et al.* 2021). The most common types of microplastic particles are nylon, polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (Sun *et al.*, 2022). These particles are rampant in marine, freshwater, and terrestrial environments, posing potential risks to ecosystems and human health (Browne *et al.*, 2017). Aquatic environments have as well been a center of concern as they remain susceptible to microplastics pollution, as a result of many human and environmental forces. Studies have reported microplastics persistence in the aquatic environments in recent years; associating their sources to be anthropogenic pollutants,

hence posing a threat to aquatic flora and fauna (Andrady & Zhu, 2021). MPs exist in different forms, including microbeads, microfibers, and microplastic films (Nguyen *et al.*, 2017). It is notable that most studies have concentrated on microplastics, while others focus on the microorganisms present in aquatic environments (Borrel *et al.*, 2020). Nevertheless, not much research have studied aquatic microbiomes using an integrative approach that considers both the free-existing and microplastic-associated bacteria and other aquatic life within an ecosystem. (Grossart *et al.*, 2020). The microbiomes in water are impacted by depth, temperature, and geographic location, among other factors. The microbial populations in water are mostly determined by their geographic location. Different environmental conditions, like temperature, sunlight, and nutrition availability, might cause different types of microorganisms to inhabit different places.

Holding a case study on Lake Victoria, which is the largest lake in Africa and second largest fresh water lake in the world (Awange, 2020) after Lake Superior in North America, provides an extensive insight into the microscopic interaction between specific microplastics and microbes. Narrowing down to Winam Gulf of Lake Victoria, which is the Kenyan part of the Lake Victoria, previous studies have shown evidence of extensive microplastic particles in Lake Victoria (2,834 - 329,167 particles per square kilometers) comprising of mainly fibers and fragments of plastic (Egessa *et al.*, 2020). Most of the particles are less than 5 mm in size.

Primary production, the nitrogen cycle, and nutrient metabolism are examples of essential microbial processes that are commonly maintained in aquatic environments (Zettler *et al.*, 2013). However, the presence of microplastics largely interferes with such processes, hence interfering with the ecosystem. This study seeks to investigate microplastics

association with microbial processes example metabolism, microbiomes and how they account for major human health implications. Microplastic have been associated with complex human health issues including cancer, immune response and inflammations however, this intrinsic relationship has not been adequately explored (Smith *et al.*, 2018, Gruber *et al.* 2023). The four main groups of organisms found in aquatic environments are decomposers such as bacteria, free-floating, minuscule creatures, and benthos that live on the bottom. Additionally, pipes transport large densities of microorganisms to waterways, leaving their urban imprint on the natural microbial community of rivers and urban coastal waters (Hoover *et al.*, 2022). There could be several detrimental consequences if non-native bacteria are released in large quantities into natural streams (Cantonati *et al.*, 2020). This includes altered nutrient fluxes into aquatic food webs, greater genetic transfer between two distinct genetic features, and the formation of reservoirs for new human viruses, including their carcinogenic potential.

According to Delgado-Baquerizo *et al.* (2020), biomes, which are made up of biological communities made up of various species and organisms adapted to a specific climate and habitat, have the capacity to affect one another's behavior. Nearly all of the microplastics under study showed weathering and the formation of a plastisphere, according to an analysis of PE, PP, PS, Polyvinyl Chloride, and Nylon (Forero-López *et al.*, 2022). Thorough examinations of the plastisphere populations revealed that the most prevalent microbes were those that form biofilms and break down plastic (Nguyen *et al.*, 2022). It has been observed that biofilm formers exploit this characteristic to evade phagocytosis. Most bacterial communities on all forms of plastic are composed of orders such Rhodobacterales, Cytophagales, Rickettsiales, Alteromonadales, Chitinophagales, and

Oceanospirillales (Stabnikova *et al.*, 2021). Furthermore, distinct microbial communities reside in distinct polymer types, and there are notable differences in the microbiome population composition on Polyethylene (PE) in comparison to Polypropylene (PP) and Polystyrene (PS) (Sun *et al.*, 2022). Because they can have harmful consequences and act as platforms for microbial colonization and diffusion, plastics have a direct impact on microbiomes (Yuhang *et al.*, 2022). According to Kirstein *et al.*, 2016, certain *Vibrio* bacterial species can cling to floating microplastics. Pathogens like *Vibrio* bacteria are known to affect both animals and humans by causing infections, gastroenteritis, wound infections, and septicemia (Visick *et al.*, 2021). Plastic debris and microplastics can linger in the environment for a very long time and act as vectors for infections since they are not or rarely biodegradable. Through the formation of biofilms, plastic may propagate pathogen into air and water (Freire *et al.*, 2013). Several species of potentially pathogenic *Vibrio* spp., Gram-negative bacteria, facultative anaerobes that typically live in saline environments, and potentially harmful *Vibrio* spp. have been identified on polypropylene debris from the North Atlantic (Zettler *et al.*, 2013) and the floating microplastic particles (polyethylene, polypropylene, and polystyrene) in the North and Baltic Sea (Kirstein *et al.*, 2016). It was found that bacteria belonging to the Burkholderiaceae family were present in the biofilm-covered polyethylene terephthalate that had been disposed of in the river (Stabnikova *et al.*, 2021). The study also claims that a lot of aquatic species feed on microplastics because they think they are food. When humans eat these aquatic species, the MPs can either obstruct their internal food routes or cause death, or they can be consumed by humans (Sharma *et al.*, 2022). The lowest levels of microplastic that impair aquatic life are far higher than those seen in marine environments (Sharma *et al.*, 2022). Insufficient

investigation into the consequences of microplastics (MPs) has created a knowledge gap that needs to be filled in order to thoroughly assess any potential environmental risks associated with microplastics. Notably, microplastics have distinct properties that make them a danger to the environment. Most plastics are known to be non-biodegradable. This makes them more dangerous to aquatic life as they stay in the environment for long periods of time, potentially accumulating in water and sediment (William *et al.*, 2022). This means that the toxins and other pollutants that may be attached to the microplastics could be more easily ingested by aquatic life, which can lead to serious health problems. Microplastics are different from other biodegradable effluents to aquatic environments because they do not decompose over time as other biodegradable effluents do, and can accumulate in aquatic habitats and bioaccumulate in the food chain. This can cause long-term damage to aquatic habitats and to the organisms living in them. For example, after nine months, almost a year, of incubation in mangrove soil, only 4.2% of the polythene bags (MPs) had biodegraded (Almohana *et al.*, 2022).

*Aspergillus* species and representatives of the bacterial genera *Streptococcus*, *Staphylococcus*, *Micrococcus*, *Moraxella*, and *Pseudomonas* are among the microbial species that are involved in the biodegradation process (Kathiresan, 2003). Sequences from bacterial genera such as *Pseudomonas*, *Sphingomonas*, and *Klebsiella sp* were found to be more common in biofilm on MPs in wastewater treatment plant (WWTP) effluent than in sewage, indicating that the microorganisms' ability to use microplastics as growth substrates may be the cause of this degradation (Kelly *et al.*, 2021). Vaksmaa, *et al.* 2021 a study on Microbial communities on polymers in the Mediterranean Sea found that Flavobacteriales, Rickettsiales, Rhodobacterales, Oceanospirillales, Alteromonadales,

Chitinophagales, and Cytophagales orders were common on all plastic types. *Sphingomonas paucimobilis*, a strain that was isolated from Lake Nakuru, Kenya, breaks down plastics by 17.5% after 90 days; polythene, in particular, degrades by 37.5% (Wanjohi *et al.*, 2013). In addition, several recent studies have shown that certain fungi and bacteria are capable of decomposing particular types of plastic (Zhang *et al.*, 2019). It is reported that the *Rhodococcus ruber* C208 bacterial strain has the potential to degrade polyethylene film to a level of up to 8 percent and has a high cell-surface hydrophobicity; the percentage cell-surface hydrophobicity was enhanced approximately 50 percent in the presence of mineral oil (Gilan *et al.*, 2004). Zhou *et al.* (2019) revealed that the use of polyethylene (PE) could be degraded by species of *Alcanivorax* and *Acinetobacter* by using it as a carbon source, which decomposes it into smaller fragments in marine environments. On the same note, Mor *et al.* (2019) also indicated that fungus *Aspergillus tubingensis* exhibited a possibility of degrading polystyrene (PS) plastic in the laboratory. Harb *et al.* (2020) identified that bacteria found on soil and water samples were capable of biodegrading polyurethane (PU) through the production of enzymes that degraded the plastic into smaller and less harmful molecules. Although these results emphasize potential mechanisms of biodegradation, there are still challenges impeding the processes including slow degradation rates and the inability to scale up such processes. Therefore, the link between MPs and microorganisms (naturally occurring) needs to be assessed and come up with a bioremediation strategy to combat microplastics in the aquatic ecosystem.

### **1.1 Statement of the Problem**

Globally, it is estimated that 5.25 trillion plastic particles are littering the oceans, and 92% are microplastics (Sooriyakumar *et al.*, 2022). Around the world, more than 400 million

tons of plastic are generated year, of which 19 to 23 million tons are thought to find their way into lakes, rivers, and oceans. Less than 10% of the yearly global plastic manufacturing gets recycled, nevertheless. Growing urbanization and population are causing a rise in single-use plastic in Africa. Africa produces 5% of global plastic and utilizes 4% raising environmental pollution and health risks (UNEP, 2021; AWF, 2020). In Kenya, there is widespread MPs pollution in surface waters (WIO- Western Indian Ocean, 2020). Microplastics are likely to result from plastic items used by local communities, such as plastic packaging and fishing nets, and since these are daily activities in the Winam Gulf, there is likelihood that the microplastic population has accumulated to great levels after a long period of using plastic-based items in Lake Victoria. As from 2019, Kenya has had Covid 19 that has contributed a lot of MPs via masks, personal protective equipment (PPE); gloves used only once, tissues among other things (Linda Rusinque-Quintero *et al.*, 2022) Also, there was a ban of plastic bags in Kenya, but even after that plastics from the old bags and single use plastics are still lying around. There is still a dearth of information regarding the ultimate location of effluents, or the direct disposal of human and animal sewage, runoff from farms, hospital runoffs, and other anthropogenic variables in the aquatic environment. MP-related environmental pollution is a developing problem that requires attention and eradication. Given the increased usage of plastics, a bioremediation strategy against microplastics in the aquatic environment is required, with consideration given to the microorganisms present in the aquatic environment that are involved in bioremediation. Most studies on microplastic-degrading microbes are from marine or terrestrial environments. Freshwater ecosystems, especially tropical lakes like Lake Victoria, are severely understudied. Very few studies have identified or characterized

indigenous microbial taxa in freshwater bodies capable of breaking down plastics, and there is a gap in knowing whether natural occurring microorganisms in Lake Victoria or similar systems have unique biodegradation potential.

## **1.2 Justification of the Study**

The study of microplastics and their potential health consequences is of particular importance. The lake supports the lives of many individuals. However, a research by Egessa *et al.*, 2020 revealed that the lake is polluted with microplastics and other pollutants. Microplastics (MPs) research in Kenyan marine environment has been limited. As pointed out by Okuku (2020), plastic pollution has been witnessed in several towns such as Mombasa, Kwale, Lamu, and Kilifi. Kosore *et al.* (2018) examined MPs in surface water and zooplankton at Gazi Bay and oceanic waters within the Exclusive Economic Zone (EEZ). Awour *et al.* (2020) investigated MPs presence in benthic invertebrates, while Okuku *et al.* (2020) analyzed marine macro-litter composition and distribution. Furthermore, Okuku *et al.* (2021) explored temporal trends in marine litter at Mukomani beach and the impact of the Covid-19 pandemic on marine litter pollution along the Kenyan coast. Kerubo *et al.* (2020) concentrated on the occurrence and concentration of microplastic in surface water in coastal creeks and Kerubo *et al.* (2021) investigated microplastic in the Kenya coast marine sediments among others. Thus, this will be the first research to examine the presence and type of microplastics in the Winam Gulf of Lake Victoria Kenya and explore Microbiomes responsible for degradation of Microplastics found in the Gulf. Knowing and monitoring the microbiomes and microplastics at the Winam Gulf of Lake Victoria will enable us to manage the resources of the lake better and safeguard the aquatic organisms that rely on the lake. Although an increasing amount of

research on microplastics pollution has been conducted, the gap areas currently include the lack of knowledge regarding the ecological effects of microplastics, entry routes, and the best mitigation strategy for the lake ecosystem. The rapid rate at which microplastics are accumulating and their potential to persist in the environment calls for immediate and targeted action to prevent irreversible damage and potential human health risks. There is hence a dire necessity to look into sustainable, low-cost and environment friendly options like bioremediation. The effects of microplastics on aquatic life, water quality, and human health are extensive and thus require immediate management and control measures. Microplastics may be the byproduct of plastic products that are utilized by the locals, including plastic bags to package their products and nets to fish, and as these are everyday activities in the Gulf, These pieces of plastic provide a substrate niche to colonize, creating a microbiome (Hao *et al.*, 2021). The release of toxins, a change in the structure and functioning of ecosystems and other various damaging effects are some of the possible outcomes of this microbiome. Being exposed to microplastics may predispose one to several health related complications such as cancer and endocrine disturbance (Smith, 2021). This research will not only address the big knowledge gaps concerning the occurrence and diversity of microplastic-degrading microbes in freshwater ecosystems but also will form the scientific basis of coming up with nature-based solutions to address plastic pollution. Policies and practices can be informed by this knowledge to mitigate the effects of microplastics on human health and environment. Also, the research may be used to contribute to the global activities to prevent plastic pollution as well as mitigate its effects on the environment and suggest a microplastic pollution bioremediation approach making use of native microbiota.

## **1.3 Objectives**

### **1.3.1 General Objective**

To perform meta-analysis of microplastic and associated microbiomes in Winam Gulf of Lake Victoria, Kenya, and come up with a potential bioremediation strategy based on microbial activity.

### **1.3.2 Specific Objectives**

1. To identify the types of Microplastic polymers present in Winam Gulf of Lake Victoria, Kenya.
2. To identify the microbiome profiles that exists in Winam Gulf of Lake Victoria, Kenya.
3. To determine microbial species engaged in microplastic degradation and associated enzymes and functional processes.

### **1.3.3 Research Questions**

1. What are the microplastics polymers in the Winam Gulf of Lake Victoria?
2. What are the microorganisms in the winam Gulf?
3. What microorganisms in the Winam Gulf of Lake Victoria can degrade microplastics and what are their functional categories and processes?

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.0 Background Information

This chapter is an in-depth literature review of the literature dealing with microplastic pollution and microbial interactions in particular as applied to MPs degraders. It is designed according to the objectives of the study that involve characterizing types of microplastic polymers found in Winam Gulf, characterizing microbiome profiles in the Gulf and to identify microbiological species that have a potential to degrade microplastic as well as related enzymes and functional activities.

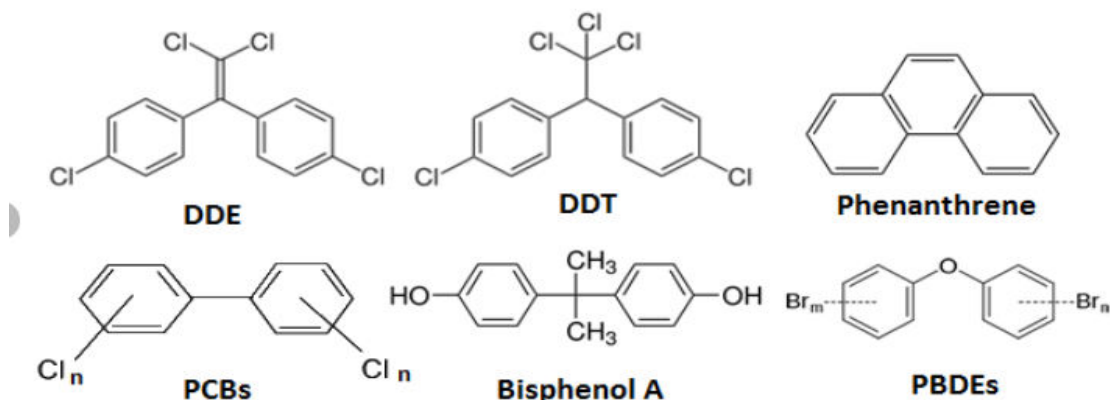
The growing rate of microplastics pollution in Lake Victoria has become a topical matter and this fact indicates the growing gravity of this environmental concern. These tiny-molecular plastic particles that do not exceed 5 millimeters in size are inflicting severe damage to the ecosystem of the lake and individuals relying on it (Coyle *et al.*, 2020). Lake Victoria is fresh water lake being among the biggest in the world and a resource that plays a crucial role to the communities residing around the lake. Nonetheless, its waters are becoming more and more polluted with microplastics that come about from so many different sources including industrial waste, domestic waste, and plastic items utilized in agriculture, fishing and tourism. The consequences of microplastics on the ecosystem of the lake are yet to be completely comprehended, but researchers have discovered that they may be consumed by fish, birds and other wild animals, leading to physical harm and even death (Clere *et al.*, 2022). Moreover, microplastics have the capacity to absorb and carry toxic chemicals as well, which can trickle down to the food chain and thus the health of the human population.

Synthetic materials are typically made using polymers, long chains of molecules (Mitrano *et al.*, 2021). Plastics have been used in most applications due to their flexibility and their ability to be shaped in various shapes. Plastics are widely utilized in both industrial and domestic settings for a variety of purposes, including toys, construction materials, textiles, and packaging. 92% of the 5.25 trillion plastic particles thought to be floating in the waters worldwide are microplastics (Coyle *et al.*, 2020). If nothing is done, it is anticipated that this number would increase by 2050. According to Kubíková & Rudý 2024, China is the greatest plastics manufacturer, accounting for 32% of global production in 2020, with plastics production predicted to reach 367 million metric tons (Hossain *et al.*, 2022). Plastic waste that is smaller than five millimeters is known as microplastics (Islam *et al.*, 2022). They either originate from the breakdown of bigger plastic items or are purposefully created here, like the microbeads used in personal care products. All significant aquatic habitats, such as rivers, lakes, oceans, and even the atmosphere, have been discovered to contain microplastics (Coyle *et al.*, 2020).

## **2.1 Microplastics Types**

According to Coyle *et al.* (2020) and Arienzo *et al.*, (2021) there is evidence that microplastics can both absorb and emit several types of pollutants, including bacteria, heavy metals, and persistent organic pollutants. These pollutants can have diverse effects on aquatic animals and their habitat. Islam *et al.* (2022) mentioned polyethylene (PE), polyvinyl chloride (PVC), polypropylene (PP), and polyethylene terephthalate (PET) as the most common microplastic polymers in water environment. Particles in the topsoil of Brahmapuram India were between 2.36 and 4.75 mm in size (Luckins *et al.* 2022). The level of microplastic pollution is not equal in various continents, regions, and countries,

which is mainly determined by economic organization and level of development (Garcez-Ordóñez *et al.*, 2022). Figure 2.1 indicates the chemical structures of microplastics polymer.



**Figure 2. 1: Chemical compositions of the materials used to make plastic products. (Bogusz *et al.*, 2017)**

Similar to Luckins *et al.* (2022) found, microplastic pollution was visible in a river, which is surrounded by an open dumping area. It means that the river serves as the major source of pollution and a real ecological hazard. The type of plastic and the concentration of salt of the surrounding conditions also predetermine the nature and characteristics of the biofilm that helps to form a specific microbial community (Garcez-Ordóñez *et al.*, 2022).

Biofilms can be formed in low salinity but it may not occur in high salinity. High salt level can inhibit bacteria, reduce nutrients, and reduce the production of extracellular polymeric materials (EPS) which is essential in the growth of bacterial biofilms (Prasanthi *et al.*, 2022). Low salinity on the other hand can be favourable to the growth of bacteria and can increase nutrient availability that increases the generation of biofilms. In fresh water lakes lying in low salinity like Lake Victoria, biofilms multiply rapidly in lakes. The COVID-19

pandemic has affected the quantity of plastics manufactured and the many found in the environment (Celis *et al.*, 2021). The single-use plastic wastes (gloves, masks, personal protective equipment, PPE, tissues, and others) are more likely to be poorly disposed of, leading to the disposal of the plastic wastes in the water systems. These wastes degrade mechanically and naturally, which has detrimental consequences on human and aquatic health. Because of the intoxication of aquatic life, the distortion of the carbon cycle, and the general health of aquatic life, humans, and ecosystem changes, it poses a serious threat to food safety, climatic changes, and changes in natural biomes.

The body of knowledge about microplastics and nanoplastics is constantly expanding, particularly with regard to their effects on aquatic life, which mostly involve mechanisms such as particle ingestion, diseases, algal photosynthesis, and the way in which they transfer harmful substances (Mitrano *et al.*, 2021). On the other hand, not much is understood about the interactions between microplastics and microbes and how they might affect human health. According to a paper (Koelmans *et al.*, 2019) released by the World Health Organization (WHO) and the European Commission's Science Advice for Policy Organisations (SAPEA), there is a dearth of published information on the toxicity of nanoplastics and microplastics in people as well as their exposure. There is evidence that microplastics and nanoplastics can cross microorganisms' cell membranes and bioaccumulate in the tissues and organs of larger creatures (Kokilathasan & Dittrich, 2022). According to the study by Goodman *et al.*, 2022 ingesting MP causes toxicological issues with cell metabolism and intercellular communication. This was linked to exposure to (Microplastics) MP, which caused cellular stress as well as morphological, metabolic, and proliferative alterations (Goodman *et al.*, 2022). Drinking water (DW) has been shown to contain plastic particles

and this becomes another channel for humans to consume the microplastics (Eerkes-Medrano *et al* 2019).

A study conducted on fish in Turkey revealed that the gastrointestinal tracts of all three fish samples contained 232 microplastics. ‘The most prevalent color was black making up (39-58%), the most shared shape was fiber making up (88%), fragment (6%), and pellet (6%); MPs in the range of maximum 1001-2000 mm were detected in size’ (Atamanalp *et al.*, 2022). Ingestion of MP can cause acute and chronic inflammation and irritation (Rahman *et al.*, 2021).

## **2.2 Microplastics: Fluorescence and FTIR Techniques**

Microplastics in water and sediment samples can be characterized with the Fourier Transform Infrared Spectroscopy (FTIR) and Fluorescence. It is possible to know the precise chemical compositions of the microplastic particles, including what type of polymer was used to manufacture them, with the use of the FTIR (Veerasingam *et al.*, 2020). The FTIR spectroscopy can be applied to identify the surface area, composition, size and structure of the microplastics. FTIR allows all the aspects to be attained including deep knowledge of the molecular structure of the sample and chemical composition. It applies infrared radiation in detecting any chemical bond in the sample. The analysis of the absorption spectrum might be used to determine the nature of the polymers and other chemical compounds. Further, the hydrocarbons, oxygen containing functional groups as well as the nitrogen containing functional groups are all functional groups which can be observed under FTIR. In a number of studies, the FTIR technique has been capable of implementing microplastic researches. The fact that the microplastic polymers were detected in the beach sediments was discovered by Song *et al.* (2015) and the authors

proceeded to identify the most widespread polyethylene (PE) and polypropylene (PP) by the FTIR technique. In addition to 17 other materials such as polyethylene (PE, 48%), polyamide (PA, 1.65%), the most prevalent polymers on the Rhine River were polystyrene (29.7) and polypropylene (16.9) (Mani et al., 2015). Similarly, MPs occur in arctic sea ice, and PE, PP, and polyurethanes varnish-coatings (PE, PP, and varnish) are the most common types of the polymers (Peiken et al., 2018).

Since the fluorescence produced by some of the polymers and additives can be used to determine the different kinds of particles, one can get to know more about the composition of the microplastic using fluorescence. Fluorescence spectroscopy is based on the light that is emitted when a sample is exposed to the ultraviolet light. It is a method that studies the optical characteristics of polymers that include the light wavelength and the intensity of the light emitted through fluorescence. The size and shape of the microplastics in the sample, their type, can be ascertained using fluorescence spectroscopy. In addition, when a sample exposes to fluorescence, one can identify whether it is made entirely of plastic or it contains any biological material (Costa et al., 2021).

### **2.3 Microbiomes on Microplastics (MPs)**

The collection of collective genetic content of microbes present in a particular environment is referred to as the microbiome. Whenever consumed by animals, the plastics have been linked to various adverse mechanical, chemical, and biological effects on the animals, directly or through trophic transfer. Great quantities of microplastics can lead to a blockage of the digestive system, killing birds, turtles, and cetaceans (Wilcox et al., 2018). More so, the filter feeders hematocytes and hemolymph may pass the microplastics to the body organs including kidneys and liver (Deng et al., 2017). It implies that in case of

consumption, microplastics can enter the intestinal mucosa and contaminate the blood (Avio et al., 2015).

Additionally, microplastics act as substrates for microbial communities, creating microbiomes that may include both harmful microbes that produce toxins and helpful microbes that can break down plastics. Because plastic additives like flame retardants (like polychlorinated biphenyls and naphthalenes), plasticizers (like bisphenol A), and UV stabilizers (like benzotriazoles) give some microorganisms organic carbon sources, plastics have an effect on both gut and environmental microbiomes (Andrary *et al.*, 2021). Although these additives alter the structure and function of microbial communities, pure plastic polymers are typically chemically inert (Bauer *et al.*, 1966). *Prochlorococcus* species, for example, exhibit decreased population density and cell growth in a dose-dependent manner when exposed to plastic leachates from high-density polyethylene (HDPE) and polyvinyl chloride (PVC) (Tetu *et al.*, 2019). Aquatic ecosystems depend on microbiomes for a variety of ecological functions, such as nutrient cycling, organic matter breakdown, and water quality maintenance. The "plastisphere," or microbial communities linked to microplastics may change the functions of natural microorganisms, encourage the growth of pathogenic species, or even release genes that confer resistance to antibiotics into the environment (Zhu *et al.*, 2022, Amaral-Zettler *et al.*, 2020). Besides giving spaces to microbial settlement, plastics have the potential to augment the dissemination of pathogens and microbes around the globe, affecting the gut microbiota of host organisms and potentially altering microbial communities (Tetu et al., 2019). Microplastics can be found in human stool and colon, which is probably because they were found in sea food, fruits, vegetables, drinking water, and commercial salt. This implies that

human beings could be exposed to microbial communities that are linked to microplastics (Luqman et al., 2021). The World Health Organization (WHO) has requested the assessment of the effects of microplastics on the environment and human health (Borrel et al., 2020). This microbial adhesion to plastic particles that forms biofilm not only protects microorganisms against environmental stressors but also increases their survival and dispersal to enable horizontal transmission of beneficial properties (Cantonati et al., 2020). A number of studies have indicated that the thick biofilm forming microbes communities supported by micro plastics in aquatic environments are quite dissimilar in relation to the adjacent waters. Zettler et al. (2013) originally described the specific microbial communities, consisting of both autotrophs and heterotrophs, on plastic trash in the ocean as the plastisphere. Oberbeckmann et al. (2016) have also concluded that bacteria communities in polyethylene and polypropylene in the North Sea are enriched in Flavobacteriaceae and Rhodobacteraceae, which were not in the surrounding water, and that microplastics host unique microbial communities, including potentially pathogenic taxa such as *Pseudomonas* and *vibrio* in comparison to the surrounding water.

In African freshwater systems, though research is limited, studies in the Nile River have revealed microplastic presence in the Nile River, including in fish (Khan *et al.*, 2020) also in Lake Naivasha, Kenya (Migwi, 2020). MPs colonizing communities composed predominantly of Proteobacteria and Bacteroidetes, with significant variation depending on plastic type and age ((Kim et al., 2022, Chen *et al.*, 2021, Dey *et al.*, 2022). Marine plastic-associated biofilm communities, such as those on plastics in the ocean, have been found to harbor pathogens like *Vibrio parahaemolyticus*, which can cause gastroenteritis and septicemia in humans (Kirstein *et al.*, 2016). Microbial communities that colonize plastics

likely aid the dispersal of invasive marine species worldwide by supporting the settlement and colonization of organisms like bryozoans and polychaete worms (Barnes, 2002; Rech, 2018). The microbiomes associated with plastics differ from those of surrounding environments, with overrepresented bacterial phyla like Proteobacteria, Bacteroidetes, and Cyanobacteria, and fungi such as Chytridiomycota (Borrel *et al.*, 2020). Studies on biofilms growing on various plastic types (HDPE, LDPE, PP) have shown similar overall community assembly patterns across surfaces, including abundant families like Rhodobacteraceae, Phyllobacteriaceae, and Flavobacteriaceae (Lemos *et al.*, 2020). In addition, the paper has also shown that weathered plastics host various communities of microbes in comparison with glass or non-weathered plastics (Erni-Cassola *et al.*, 2020).

Nevertheless, literature about how aquatic microbial communities interact with microplastics and can specifically degrade them is very sparse. The problem of the long-term health effects also correlates with the possibility of microplastics accumulation into human tissues and toxic substances bioavailability (Li *et al.*, 2022). Microbial communities on plastics may cause persistent organic pollutants that accumulate in the human tissues and cause cancers (Kerestin *et al.*, 2022). In addition, the buildup and retention of these harmful materials in human bodies may be the outcome of the biofilm communities on microplastics. The connection between the microbial biomes and microplastics and the human cells and tissues, especially with regard to cancer development and progression, has not been thoroughly studied (Oussama *et al.*, 2022; Clere *et al.*, 2022). Microplastics are now among the priorities on the list in the WHO due to the ongoing role in the environment and carrying carcinogenic compounds (Koelmans *et al.*, 2019).

## 2.4 Microplastics Degradation

The possibility of microbial degradation of microplastics is the subject of the study, but the list of unsolved problems is rather high. Currently, the lack of data to evaluate the presence of microbial communities degrading plastic with a critical eye, or recreate experimental findings is existing (Lear et al., 2021). As an example, degradation of 99.9% pure polystyrene (PS) film by *Pseudomonas* sp. has been determined using X-ray photoelectron spectrometry (XPS) and scanning electron microscopy (SEM) (Lee et al., 2020). Other studies have also shown that polystyrene can be recycled by *Rhodococcus ruber* and *Pseudomonas* sp. (Lee et al., 2020). However, the current laboratory studies are performed over relatively short periods since plastics remain in the environment, and they may not be extensive enough to comprehend the degradative capability of the plastisphere microbiomes. *Pseudomonas*, *Vibrio*, *Rhodococcus*, *Acinetobacter* and *Sphingomonas* are several of the plastic-degrading bacteria found in the North Atlantic Gyre based on Zettler et al. (2019). These bacteria all have the enzyme capacity to break down such ubiquitous polymers as polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET). The active species of *Pseudomonas* and *Bacillus* species were also observed by Su et al. (2020) on the microplastic surfaces in the South China Sea. Other studies can also observe that bacteria like *Bacillus*, *Pseudomonas* and *Acinetobacter* are also prominent in the biodegradation of other plastics like polyethylene and polystyrene in freshwater like Oberbeckmann et al. (2019) and Liu et al. (2021) do. Plastics are degraded by microorganisms under different types of functions. One of the most vital ones is the formation of biofilms, which contribute to the increase in the breakdown rate, in addition to colonization of plastic surfaces. The biofilms provide the community of microbes with a protective environment and invert the extracellular enzymes around the plastic interface,

which enhance faster degradation (Zhang et al., 2023). The polymer is adsorbed by the enzyme and adsorption is then accompanied by the enzyme phase of bond hydrolysis or hydro-peroxidation. Microorganisms of different environments have enzymes needed to decompose plastic (Mohanani et al., 2020).

In the discovery of enzymes in different aquatic conditions, the invention of metagenomics has also facilitated this. Indeed, there have been studies showing that the molecular communities in the seas contain genes that encode cutinase, lipases, PET hydrolase (PETase) and mono (2-hydroxyethyl) terephthalate hydrolase (MHETase). (Sun, 2024; Carr et al., 2020). The recent developments in metagenomics have made it possible to investigate further the functional genes and enzymes that are involved in the microplastic degradation. Indicatively, Kirstein et al. (2016) identified genes that encode the enzymes lipases and esterases that are required to break down the plastic polymers within the marine microbiomes. This genetic knowledge can be useful in the planning of certain bioremediation methods.

In a bid to address microplastic pollution in waters, the bioremediation solution has appeared as the potential solution to the issue and cost-effective (Stabnikova et al., 2021). The microplastics can be attacked by the natural bacteria in an enzymatic process. The enzymes of the species like *Pseudomonas* can be used to break plastic polymers down into small and more biodegradable molecules, and the degradation rate can be accelerated by the inclusion of nutrients or by enhancing the activity of symbiotic bacteria capable of decomposing the polymer such as photosynthetic algae. Depending on the types of polymers, types and varieties of microorganisms use the types of polymers, and the length of incubation time determines the degree of microplastics breakdown (Tang and

Hadibarata, 2022). Bioaugmentation of the secondary biological treatment systems can be carried out in WWTPs, i.e. aerobic conventional activated sludge, sequencing batch reactor, membrane bioreactor, and rotating biological contactor (Tang and Hadibarata, 2022).

Beyond natural microbial action, engineered strains offer promising biotechnological solutions. For example, Guo *et al.* (2020) developed a recombinant *Escherichia coli* strain that can enzymatically hydrolyze PET into its monomers. Yang *et al.* (2021) further identified a novel polyesterase produced by *Pseudomonas citronellolis* which is capable of degrading PS and PE polymers under laboratory conditions. In addition, Gao *et al.* (2021) revealed the promise of these types of consortia in wastewater treatment facilities through their ability to isolate a microbial consortium of activated sludge that could degrade PE and PP. All these works show that these types of consortia have a potential to be used in the reduction of microplastic pollution in water bodies using both naturally occurring and introduced microbial communities. The bioremediation methods in the future should consider the unique enzymatic processes and microbial groups able to degrade the various types of plastic.

## **2.5 Metagenomics**

Metagenomics is one of the useful methods of studying the composition of microbial communities and their interactions with the environment (Zhang *et al.*, 2021). It does not require the separation and growth of the broad spectrum of organisms so that researchers can study their genetic composition. By applying the method of metagenomics, it is possible to predict variations in the microbial diversity and functioning (Fierer *et al.*, 2012). Metagenomic analyses of microplastic samples can be used to establish the

composition and abundance of the microbial communities associated with the particles, their metabolic activity, and the potential pathways of microplastic degradation (Reddy, C. N et al., 2023). Many microplastic samples, e.g. collected on the surface waters, wastewater treatment plants, and marine sediments, have undergone metagenomics analysis. Moreover, metagenomic studies have been applied to identify genes responsible of the microplastic biodegradation and to ascertain microplastic toxicity.

It is possible to identify the genetic material of a sample of microbial communities through 16s metagenomics and shot gun metagenomics (Crisafi et al., 2022). This type of analysis is done on the genetic material of multiple species of a variety of microbes in a sample. The 16s metagenomics analysis analyses the rRNA gene which is a marker gene which allows the determination of the species within a sample. The 16s rRNA gene of the sample is sequenced and aligned with a database of known microbial species to achieve this (Barbosa et al., 2020). The sequencing of the 16s rRNA gene will provide the species contained in the sample, its relative abundance as well as the genetic diversity of the species. The short gun sequencing method can appropriately sequence the whole genome of a sample allowing a more detailed analysis of the genetic material that is present. This approach enhances the understanding of the genetic variation of a sample and enables one to find more species that are rare.

Shot gun sequencing provides more specific information hence it is a preferred method to 16s sequencing in characterizing microbiomes in aquatic environments. Shotgun sequencing is able to identify more bacterial and archaeal species and provide a more precise sequencing of the microbial communities as compared to 16s sequencing. Also, shot gun sequencing provides a more comprehensive view of the microbial community

within a certain environment as it identifies microbial gene functions that cannot be identified through 16s sequencing. To discuss enzymes coded by the genes, researchers tend to focus on specific genes of interest associated with enzyme production. This can be done through methods such as genome sequencing that provides information about the entire DNA sequence of a given organism and polymerase chain reaction (PCR) which amplifies specific DNA regions. Bioinformatics tools allow the amino acid sequence of the corresponding protein to be determined when the DNA sequence of enzyme of interest is known. This is based on the genetic code which translates nucleotide triplets (codons) into specific amino acids. Moreover, numerous tools of molecular biology such as recombinant DNA technology and system of protein expression allow researchers to clone genes, express proteins in heterologous hosts, and characterize their functions. Enzyme assays and biochemical analyses are additional methods of elucidating the enzymatic properties of the encoded proteins along with their substrate specificity and catalytic mechanisms (Kumar et al., 2021).

Behera et al. (2020) have used metagenomic analysis on the Ganga and Yamuna rivers of India to demonstrate a variety of communities of bacteria and fungi that can be utilized in bioremediation of pollutants, such as pesticides. A study on the Yamuna River was conducted using whole-genome shotgun metagenomics to determine a library of genes associated with antibiotic resistance and metabolism in river water pollution. The presence of genes related to metabolism of nitrogen and sulfur, breaking down of the aromatic compounds, and various antibiotic resistance mechanisms indicated that the study was a complex response of the microbes to the pollution (Mittal et al., 2019). Metagenomic sequencing of the Hangzhou Bay coastal sediments in China revealed that the morphology

and functions of the microbial groups were influenced by microscopic microplastics ([?]100 mm). The ecological impact of microplastic pollution was implied by the results that the occurrence of microplastics was interconnected with the alterations of carbon metabolism pathways and that this was potentially dangerous to eukaryotic communities (Zheng et al., 2024). In studies conducted on Lake Michigan, it has been demonstrated that wastewater treatment plants (WWTPs) contribute to the introduction of microbes and genes, some of which are resistance to antibiotics, into aquatic sediments. Metagenomic analyses were used to show the effects of WWTPs on the microbial community composition and gene content in the receiving water bodies with a higher percentage of WWTP derived genes found in the sediments closer to points of effluent discharge (Chu et al., 2018). Based on the metagenomics analysis of mangrove sediments, the pollution altered the genes of its microorganisms enhancing methanogenesis and sulfate reduction pathways and augmenting the tally of genes that oppose both metals and antibiotics-resistance. As per the results, pollution can change the metabolic ability of microorganisms (Li et al., 2019).

## **2.6 Research Gaps**

In the case of the Winam Gulf of Lake Victoria, no concrete data is available on the types of microplastics and microorganism in connection with it, although the problem of microplastic pollution and microorganism interaction is getting more attention on a global scale. Research has described microplastics in water bodies across the board (Ricciardi et al., 2021, Andrady, 2011, Ivleva et al 2017), but little has been done to identify the exact types of polymers that occur in freshwater bodies in Africa with a particular focus on East Africa. Effective local mitigation plans and policy actions suited to the Lake Victoria

basin are hampered by this shortcoming. Although microbial communities on microplastics (plastisphere) have been studied in marine and temperate environments (Zhang *et al.*, 2022; Zhai *et al.*, 2023, Andrady, 2011), limited research exists on the microbiome profiles unique to the Winam Gulf. Understanding the composition and functional potential of these microbial communities is vital, since some microbes may act as pathogens or microplastic degraders.

There is also a growing interest in the role of microorganisms in the degradation of plastics through enzymatic action (Lear *et al.*, 2021; Bhardwaj *et al.*, 2013) but the identification of specific species and enzymes responsible for microplastic breakdown in Lake Victoria Winam Gulf remains unexplored. All of these knowledge gaps point to the pressing need for localized research that combines enzyme analysis, microbial community characterization, and polymer characterization. In addition to improving scientific knowledge, addressing these gaps will help shape public health and environmental management plans in the Winam Gulf of Lake Victoria and even throughout Kenya.

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.0 Introduction

This chapter presents the materials and methodology employed to achieve the objectives of the study. It outlines the research design, study area, sampling methods, data collection and data analysis. Given the nature of the study in that it spans microbiology, molecular, environmental science, ecology and chemistry this chapter integrates both field-based and laboratory-based methods. Microplastic types were identified and characterized using Fourier Transform Infrared Spectroscopy (FTIR) while microbiomes and microplastic degrading bacteria and enzymes present were identified using shotgun metagenomic sequencing.

#### 3.1 Research design

This study adopted a cross-sectional research design integrating both purposive and randomized sampling strategies to investigate the types of microplastic polymers and microbiome profiles present in the Winam Gulf of Lake Victoria, Kenya, as well as to identify microbial species involved in microplastic degradation and their associated enzymes. Urbanization, inadequate waste management, and riverine inflows are all potential contributors to the rise in microplastic contamination in Lake Victoria's Winam Gulf. The kinds or types of microplastic polymers that are present and the microbial populations that are associated with them especially those that are involved in the degradation of plastic are not well explored. Planning for bioremediation, ecosystem management, and microplastic pollution control all depend on an understanding of those interactions. The experiment involved the integration of the laboratory-based analysis

techniques with the field-based environment sample of Fourier Transform Infrared Spectroscopy (FTIR) to determine the existence of microplastic polymers and shotgun metagenomic sequencing to describe the microbial communities. This type of a methodological approach allowed investigating physical contaminants containing microplastics and biological components containing microbiomes. Water samples were collected using 500 mL sterile plastic and glass containers as necessary, while sediment samples were obtained using sterile glass bottles, ensuring minimal contamination. Standard protocols for environmental sampling, transport, and storage were followed to preserve sample integrity for laboratory analysis. Strict contamination control protocols were followed, including the use of non-plastic tools where applicable, protective clothing, and a sanitizer. Environmental control sites entailed offshore site in the Gulf and outlet water after treatment. I also used distilled laboratory water as a control. The study timeline spanned from sample collection in March–April, laboratory processing through May, metagenomic analysis between May and July, and data interpretation from August to October 2023.

### **3.2 Sampling Sites**

Sampling was purposive and was done in the Winam Gulf, as shown in Figure 1 below, Winam Gulf, with geographical coordinates approximate Latitude: 0° 14' 14.40" N and Longitude: 34° 34' 28.79" E. Table 1 shows the exact geographical coordinates of the sample areas.

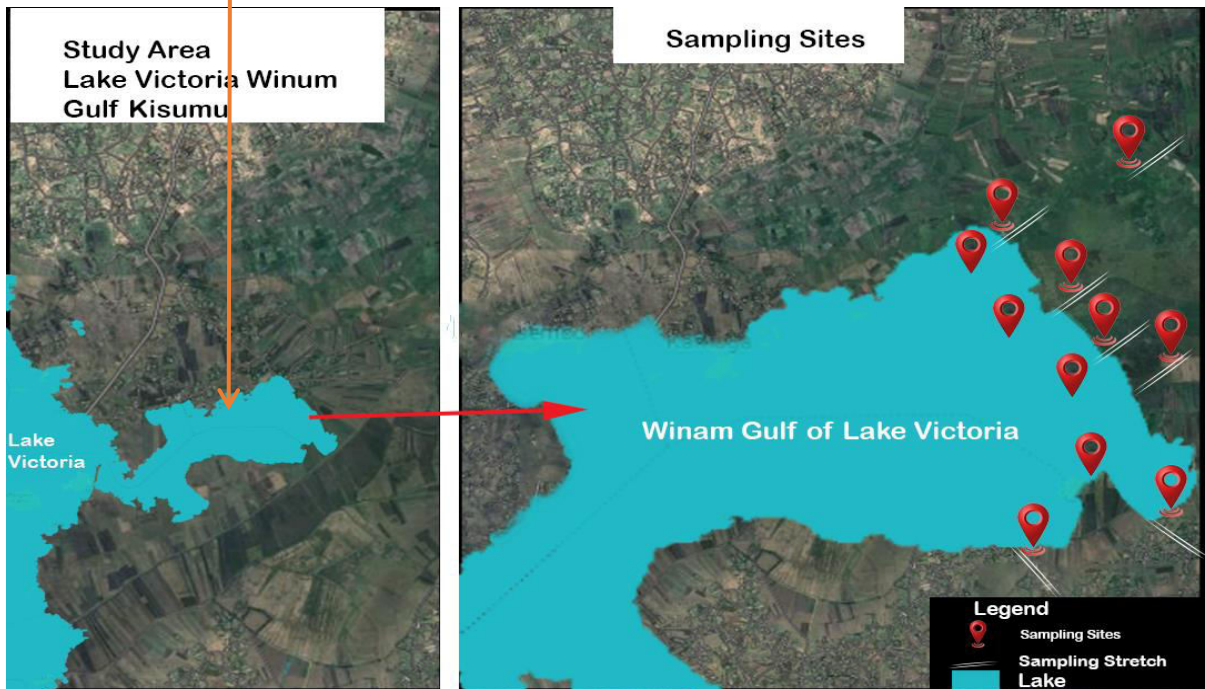


Figure 3. 1: Sampling area of Winam Gulf of Lake Victoria

*Table 3. 1: Summary of the Longitudes and latitudes values for the sampling sites.*

<i>Sampling area</i>	<i>Latitude</i>	<i>Longitude</i>
<b>Kiwasco TP</b>	-0.119826	34.774636
<b>River. Kisat</b>	-0.083049	34.748828
<b>Storm water</b>	-0.122048	34.772785
<b>Kiwasco Lagoons</b>	-0.1210017	34.7729328
<b>River. Nyamasaria</b>	-0.118596,	34.787563
<b>Ndungu Beach</b>	-0.134571	34.741412
<b>Block Beach</b>	-0.118600	34.787574
<b>River. Wigwa</b>	-0.116824	34.772435
<b>River. Uhuru</b>	-0.1210017	34.7729328
<b>Lake Location 1</b>	-0.1100	34.7700
<b>Lake Location 2</b>	-0.1250	34.7500

The potential to propagate microplastics and microbes was key to defining the sampling areas. In addition, the sampling areas were defined to establish the characteristic relationship between the microplastics and microbiomes found in these sites to the microbes established in the Winam Gulf of Lake Victoria. The exact sampling points were marked through these coordinates (1-11) from the sampling sites; multiple samples were picked, totaling 70 samples. The Gulf sustains livelihood in the area juxtaposing the lake in many ways. According to Nyamweya *et al.*, 2023 approx.42million people rely on Lake Victoria for their diverse livelihoods. The most prevalent economic activity for most families is fishing, which is done both for household use and commercially. In addition,

Winam Gulf has recently experienced a storming commercial wave, with a good number of factories located in Kisumu and its environment. Industrialization of the cities and towns within a 50 km radius of the lake potentially increases the microplastics and microbial bioavailability in the lake. The sampling points were subject to the objectives of the study considering their natural characteristics that were anticipated to hub significant Microplastics and microbiomes. The Kisumu water and sewerage system (KIWASCO) treatment plant fits the study in numerous ways. Wastewater treatment plants are known to be a significant source of microplastics, as they receive large quantities of domestic, industrial, and commercial wastewater that may contain microplastic particles. Additionally, wastewater treatment plants harbor diverse microbiomes, which play a crucial role in the degradation of organic matter and the removal of pollutants from the water. It was anticipated that microbial communities associated with microplastics were distinct from those in the surrounding water and sediment, suggesting that microplastics provide a unique habitat for microbes. Sampling in KIWASCO lagoons included areas such as the inlets to lagoons, water lagoons, the outlets to lake, and the storm water.

Rivers Kisat , Uhuru, Wigwa and Nyamasaria play a huge role in defining the population of microbiomes in Winam gulf of Lake Victoria in various ways, with some more pronounce in some rivers dependent on some factors such as the level of urbanization around the places that the river flows, the agricultural activities along the rivers, and waste treatment. River Kisat is one of the main rivers that flow through Kisumu Town in western Kenya. The river originates from the hills in Kisumu and flows through several city areas before emptying into the Winam Gulf of Lake Victoria. The factors that define the nature and amount of microplastics and microbiomes propagated by River Kisat include

population density and urbanization, land use practices, seasonal variations, the level of wastewater treatment, and the presence of industrial activities. The main reason that details the nature of microplastic and microbiomes that can be associated with the river is that, it flows near the KIWASCO treatment plant, harboring significant microplastics and microbes from the sewage despite the fact that the treatment of the plant is monitored; the levels anticipated cannot be negligible.

River Nyamasaria on the other hand, is also a potential source of microplastics and microbiomes that can end up in Winam Gulf of Lake Victoria. The river originates from the hills and wetlands in Kisumu and flows through several residential and industrial areas before emptying into Winam Gulf as well. The discharge of untreated wastewater from domestic and industrial sources into the river can introduce significant amounts of microbiomes into the river. Similarly, littering and improper waste disposal can lead to the generation of microplastics that can end up in the river. Just like Kisat River, the Nyamasaria and Uhuru Rivers hold the effluent of the second sewerage treatment plant of Kisumu Town. This gives an opportunity that it will add a significant number of microbiomes to the river. Equally, inadequate garbage disposal and littering may create microplastics, which may be deposited in the river. Practically, the slums of Nyalenga are encircled by the River Wigwa that is filled with plastic bottles, household waste, and bottles of detergents that are released to the lake. The majority of them are drained into the lake.

Lastly, samples need to be gathered on the Dunga and Block of the Winam Gulf of Lake Victoria to know the nature and quantity of microplastics and microbiomes in the Gulf. Whereas Block beach is a new and recent leisure centre that has become popular over the

recent years, Dunga beach has been a preferred option amongst the locals, tourists and fishermen. Taking into account all the number of people and activities on the beaches and nearby both of the beaches could be the sources of the microplastics and microbiomes. An example is the microplastics that can be discharged into the water through the use of plastic fishing nets, use of other fishing gears. Due to fish washing and beach drying some microbiomes may be released into the water and sand, which later may be transported by the down stream. The leisure activities like swimming, tanning and picnicking at the Block Beach will be bringing on board micro plastics and microbiomes in the gulf. As an example, poor waste and litter management can result in the production of microplastics, which will enter the gulf through water and wind. In addition, human and animal activities on the beach such as defecation can contaminate the sand and water with microbiomes. Therefore, surveying of the Dunga and Block beaches can provide data on the source of microplastics and microbiomes and their abundance in the Gulf.

### **3.3 Sampling Methods**

The sampling procedure was purposive and randomized to collect a wide variety of pollutants. The samples were collected in March and April 2023. Collecting each sample involved the use of sample collection containers for water samples, 500ml sterile plastic for shotgun and glass containers for microplastics were used and for sediments, sterile glass bottles were used. For MP's the water samples were filtered through a stainless steel filter net with a small pore size 5 mm. They were then sealed and transported to the laboratory in cooler boxes in less than 12 hours of sampling and stored at  $-80^{\circ}\text{C}$  awaiting further processing. The sediment samples (0-2cm layer) were done the same after being placed in a sterile glass bottle. Fish were captured using gillnets and angling. Fishing was

conducted in compliance with local fisheries regulations, and ethical considerations were observed to ensure humane handling. A total of 16 specimens were collected. Each fish specimen was immediately rinsed with distilled water to remove external contaminants and then wrapped in aluminum foil and placed in sterile glass containers that had concentrated 96% Ethanol. The samples were stored on ice and transported to the laboratory within 24 hours. DNA extraction and metagenomics sequencing were then done (Sequencing were done at Novogene (UK) Company Ltd) and sequences banked in the NCBI database with accession number PRJNA992979.

### **3.4 Physico-chemical Properties**

The pH levels, temperature, conductivity, salinity, turbidity, total dissolved solids (TDS), and Chemical Oxygen Demand (CoD) were determined. A thermometer Hanna EN13485 was used to determine the temperature of the water before collecting the sample. Secondly, to determine the PH levels, a pH meter Hanna HI98107 was first calibrated using standard buffer 4.0, standard buffer, 7.0, and finally, standard buffer 10.0, to verify the integrity of the pH meter. For each 4ml taken from the sample collected, individual pH readings were taken and recorded. The rod was rinsed before taking successive pH readings. Thirdly, conductivity, which indicates the ability of a solution to conduct electricity, was measured using a conductivity meter. The meter was calibrated using a standard solution, and the conductivity probe was immersed directly into the water sample. The meter then measured the electrical conductivity, and the result was recorded. Fourthly, salinity was determined using a salinometer or a refractometer. A small volume of the water sample was placed on the prism of the refractometer, and the refractive index was determined which was then converted to salinity using an established conversion table or equation. Turbidity, on the

other hand, was measured using a turbidimeter or a nephelometer. The water sample was placed in a cuvette, and a light beam was passed through it. The apparatus recorded the turbidity value and measured the amount of light scattered by the suspended particles in the sample. Additionally, 5 ml of the water samples were evaporated in a pre-weighed container before the remaining solids were weighed in order to calculate TDS. To determine the TDS concentration, the container was reweighed after the sample was heated to evaporate the water. The weight of the dried solids was divided by the volume of the original sample to determine TDS, which was then expressed in milligrams per liter (mg/L). Finally, the Chemical Oxygen Demand levels were measured using a Chemical Oxygen Demand meter. The probe was immersed directly into the water samples, and the instrument measured the partial pressure of oxygen dissolved in the solution. The reading was recorded as the Chemical Oxygen Demand concentration.

### **3.5 Microplastics Identification**

70 water samples were filtered through a filter membrane (Stainless steel) with a small pore size below 5 mm to capture the microplastics. To get rid of any last contaminants, distilled water was used to rinse the filter membrane. The water was then put in a sample holder for liquid samples in the FTIR (Shimadzu Corp., 03191). The presence of microplastics was then verified by irradiating the samples and comparing the resulting spectra with known microplastic polymers in the instrument's library. To stop microplastics from sticking to the soil particles, the sediment samples were first dried at a low temperature (40–50°C). To concentrate the microplastics and eliminate larger particles, the soil was subsequently run through a 5 mm mesh sieve. Each specimen of fish was set on a sterile dissection tray and using sterile dissection scissors, an incision was

made along the opercular cavity to reach the fish gills. Extracted gill samples were rinsed with sterile saline solution to remove surface contaminants and then preserved in 70% ethanol in glass sterile labeled containers for further analysis. Sediment samples and gills were ground with potassium bromide in a ratio of 1:10 (sample:KBr) then placed in a sample holder and attenuated with infrared radiation (IR). The FTIR spectra obtained were compared with the FTIR spectra of known microplastics to identify any microplastics present.

### **3.6 Shotgun Metagenomics**

The water and sediment samples were pooled together and mixed thoroughly then filtered through grade one filter papers (Whatman TM) to remove any suspended particles, including pieces of wood, larger plastics, and animal and clothe debris. 10 mL of 20-liter sample were taken and centrifuged followed by decantation of the supernatant. The experiment was repeated for the entire homogenous sample. The sediment cell debris were vortex and placed into 2ml Eppendorf tubes and further centrifuged for 10minutes at 5000×g for 10 minutes to obtain the pellets for DNA extraction and metagenomics sequencing. The sample was placed in 2 mL Eppendorf tubes followed by addition of 2% of 0.7 mL of extraction CTAB buffer (20 mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, 2 % CTAB. 0.4 % Beta-mercaptoethanol was added right away and Incubated at 65 °C for 45 min while gently mixing by inversion after every 15 min. The mixture was added 0.6 mL chloroform-isoamyl in a ratio (24:1) and gently mixed for 1 min followed by centrifugation for 10 min at 16000 ×g and procedure repeated twice. 0.7 mL of cold isopropanol (-20 °C) was added to the mixture and the mixture was gently mixed by inversion. The solution was then centrifugation at 16000×g for 10 min. The extracted DNA

was washed twice with 1 mL of 70 % ethanol to eliminate salt residues and set to dry for overnight with the tubes inverted over a filter paper at room temperature. Pellet was then re-suspended in 100 mL TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0) and stored at -20 °C for shotgun metagenomics. DNA was quantified with a Qubit 2.0 fluorometer (Thermo Fisher Scientific) and integrity verified by agarose gel electrophoresis. The DNA was then used for library construction. DNA sequencing was done at Novogene (UK) Company Ltd.

### **3.7 Data Analysis**

The quantified libraries were pooled and sequenced using the Illumina platform. Taxonomical abundance was determined by comparing metagenomic reads to a database of taxonomically informative gene families (MicroNR database). Species annotation was done using DIAMOND software (V0.9.9.110) for alignment of Unigenes sequences with those of bacteria, fungi, archaea, and viruses extracted from NCBI's NR database. Functional annotation was inferred based on its similarity to the sequence in the databases (KEGG) while functional category hits distribution was annotated using MG-RAST Subsystems classification. For microplastics, Fourier Transform Infrared (FTIR) spectroscopy was used. The equipment was configured to do a total of 30 scans with a spectral resolution of 4 cm<sup>-1</sup> for both background and sample spectra recorded swiftly in the 4500-500 cm<sup>-1</sup> range. The spectra were analyzed using lab solutions software and compared to a library of known spectra to identify the type of plastic.

## CHAPTER FOUR

### RESULTS

#### 4.0. Physico-chemical Parameters

In this study, various physical and chemical parameters were examined. The parameters that were taken into account included pH levels, temperature, conductivity, salinity, turbidity, total dissolved solids (TDS), Chemical Oxygen Demand (COD) levels, total nitrogen (TN), and total phosphorus (TP) for all the samples taken from the sampling sites shown in figure 4.1 and 4.2

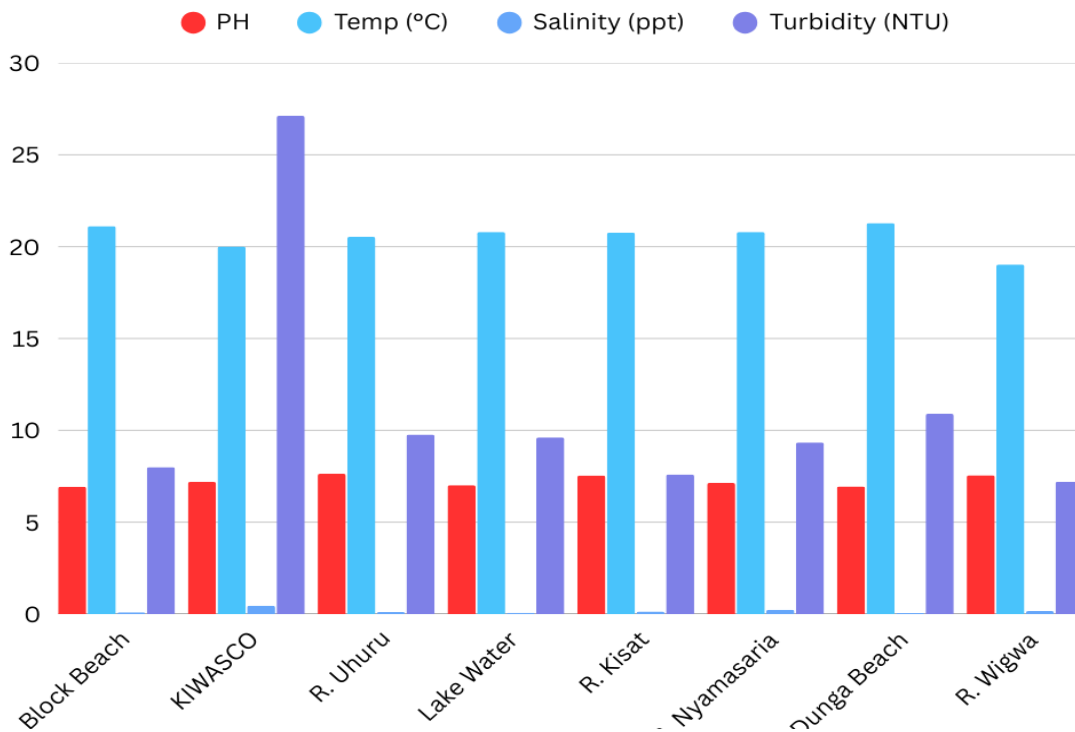
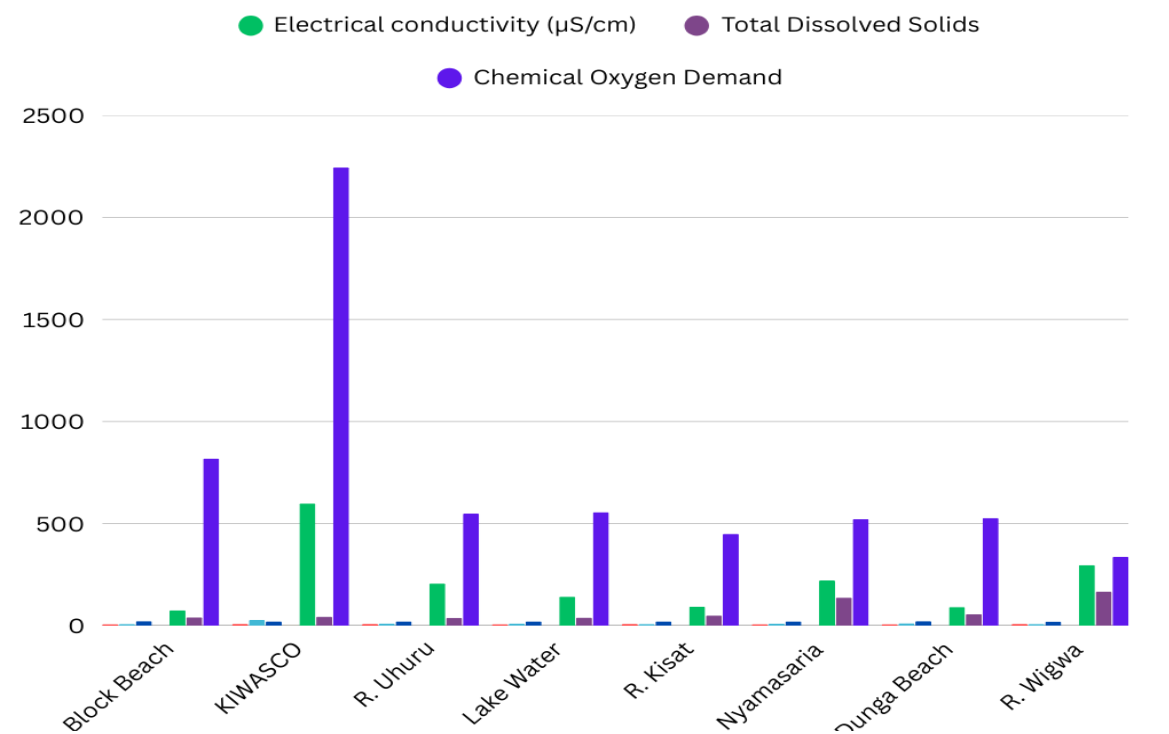


Figure 4. 1: Mean Values of Physicochemical Parameters of Water



**Figure 4. 2: Mean Values of Physicochemical Parameters of Water**

Evaluation of the data revealed that most of the water sources were within the WHO standards. These standards include, pH of 6.5-8.5, salinity of >0.5ppt, turbidity of >5NTU, TDS of >500, electron conductivity of >1000S/m, and COD <5mg/O<sub>2</sub>l.

**Table 4. 1: Parameter Ranges, Standard Deviation and Observations of Physicochemical Parameters of Water**

Parameter	Mean Range	Standard Deviation	Notable Observations
<b>Ph</b>	6.93 – 7.63	Block Beach (0.12), KIWASCO (0.49), R. Uhuru (0.67), Lake Water (0.26), R. Kisat (0.89), R. Nyamasaria (0.27), Dunga Beach (0.16), R. Wigwa (0.64)	All sites are within neutral to slightly basic pH. Highest at R. Uhuru (7.63).
<b>Temperature (°C)</b>	19.03 – 21.28	Block Beach (0.82), KIWASCO (0.83), R. Uhuru (0.32), Lake Water (0.42), R. Kisat (0.59), R. Nyamasaria (0.30), Dunga Beach (1.01), R. Wigwa (0.69)	Relatively stable across sites; lowest at R. Wigwa.
<b>Salinity (ppt)</b>	0.052 –	Block Beach (0.18), KIWASCO	Highest at KIWASCO,

	0.45	(0.14), R. Uhuru (0.04), Lake Water (0.05), R. Kisat (0.04), R. Nyamasaria (0.13), Dunga Beach (0.06), R. Wigwa (0.07)	lowest at Dunga Beach.
<b>Turbidity (NTU)</b>	7.2 – 27.12	Block Beach (0.94), KIWASCO (5.81), R. Uhuru (3.63), Lake Water (3.70), R. Kisat (1.26), R. Nyamasaria (2.11), Dunga Beach (2.69), R. Wigwa (2.10)	Highest turbidity at KIWASCO (27.12 NTU); lowest at R. Wigwa.
<b>Electrical Conductivity (µS/cm)</b>	74.68 – 598.43	Block Beach (9.74), KIWASCO (243.23), R. Uhuru (89.18), Lake Water (54.53), R. Kisat (35.27), R. Nyamasaria (78.34), Dunga Beach (13.01), R. Wigwa (88.47)	Significantly higher at KIWASCO
<b>Total Dissolved Solids (TDS)</b>	37.3 – 165.69	Block Beach (11.25), KIWASCO (13.65), R. Uhuru (14.06), Lake Water (16.58), R. Kisat (26.17), R. Nyamasaria (85.35), Dunga Beach (17.45), R. Wigwa (35.29)	R. Wigwa has the highest TDS
<b>Chemical Oxygen Demand (COD)</b>	336.5 – 2245.5 mg/L	Block Beach (48.76), KIWASCO (317.73), R. Uhuru (164.68), Lake Water (100.87), R. Kisat (97.48), R. Nyamasaria (112.09), Dunga Beach (65.82), R. Wigwa (61.22)	High at KIWASCO (2245.5 mg/L)

#### 4.1 Microplastic Characterization

The scores obtained from the analysis were used to rank the samples based on their similarity to the reference spectra, with an FTIR score of between 500-1000. The FTIR analysis and qualitative comparison with the reference spectra provided a total of 90 types of polymers in the surface water and the sediments of which 32 of them are plastic polymers and were consistent in all the sampling points. From analysis we have natural biodegradable polymers which consists of two categories from the table below that is proteins and cellulosic and there are synthetic polymers and are categorized as Polyethylene (PE), Polypropylene (PP), Polyacrylamides, Polybutene, Nylons and Vinyl Polymers. The FTIR data qualitatively identified the microplastics. The microplastics

identified were tabulated in the table 4.2. The microplastics have been grouped based on their chemical composition and properties.

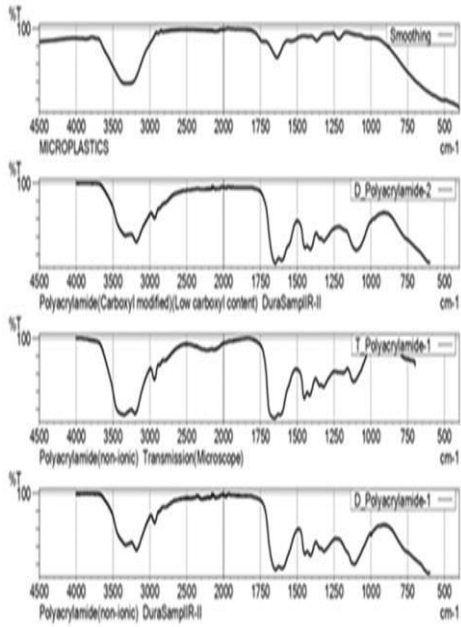
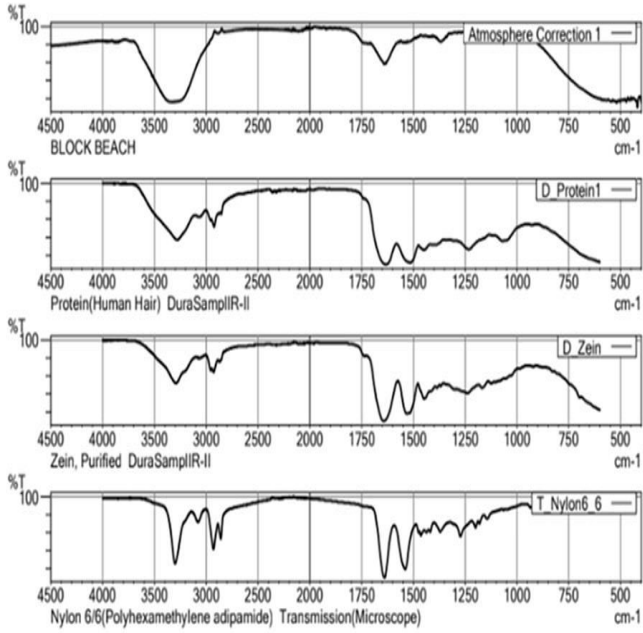
**Table 4. 2: Categories of identified microplastics in Winam Gulf of Lake Victoria.**

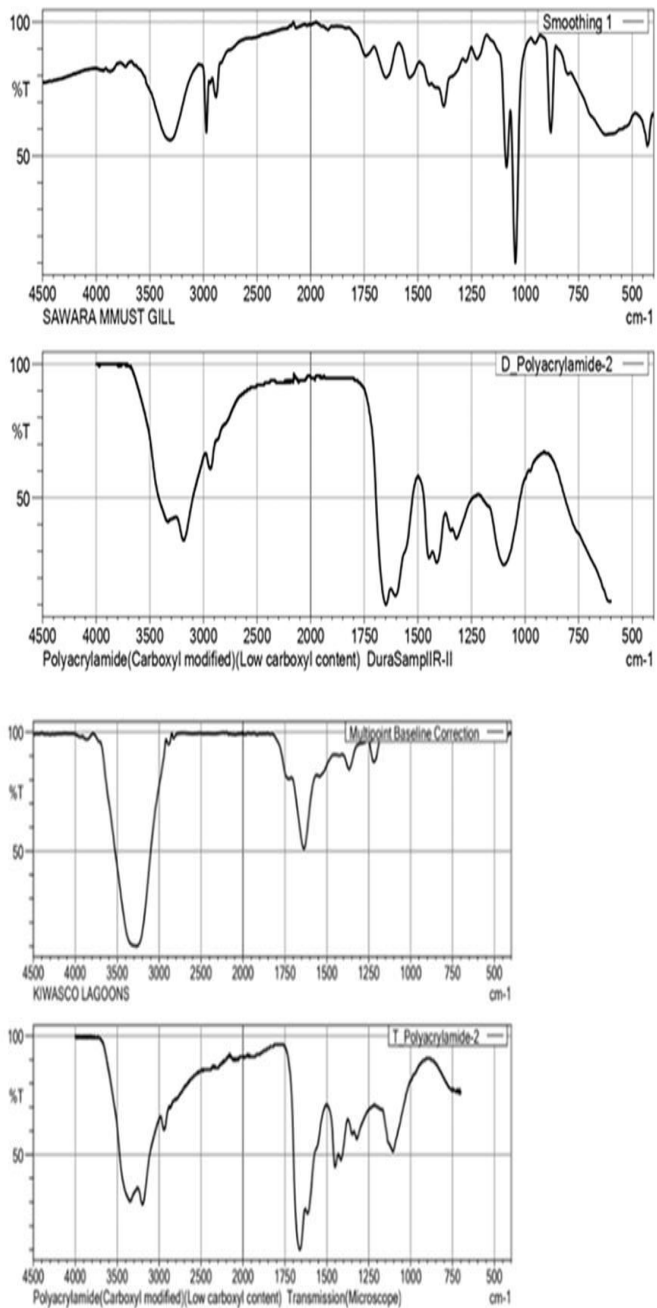
<b>Category</b>	<b>Polymer Names</b>	<b>Score</b>
<b>Proteins</b>	Soy Bean Powder Protein, Human Hair Protein, Protein (Human Hair), Zein (Purified)	687-733
<b>Polyethylene</b>	Polyethylene (PE), Low-Density Polyethylene (LDPE), High-Density Polyethylene (HDPE)	624-628
<b>Polypropylene</b>	Polypropylene (PP)	624-626
<b>Polyacrylamide</b>	Polyacrylamide-1, Polyacrylamide-2	667-692
<b>Ethylene Copolymer</b>	Ethylene/Acrylic Acid Copolymer (20% Acrylic Acid content), Ethylene/Propylene Copolymer (60% Ethylene content), Ethylene/Ethyl Acrylate Copolymer (18% Ethyl Acrylate content), Ethylene/Vinyl Acetate Copolymer (14% Vinyl Acetate content), Ethylene/Vinyl Acetate Copolymer (18% Vinyl Acetate	594-631

	content)	
<b>Styrene-Ethylene-Butylene</b>	Styrene-Ethylene-Butylene	594-603
<b>Nylon</b>	<p>Nylon 6 (Polycaprolactam)</p> <p>Nylon 6/6 (Polyhexamethylene adipamide)</p> <p>Amorphous Nylon, Nylon 6 (Polyamide), Nylon 6/12 (Polyamide), Nylon MXD6 (Polyamide), Nylon 6/10 (Polyhexamethylene sebacamide)</p>	629-645
<b>Vinyl Polymers</b>	<p>Vinyl Chloride/Vinyl Acetate Copolymer (81% Vinyl Chloride, 17% Vinyl Acetate, 2% Maleic Acid), Vinyl Chloride/Vinyl Acetate Copolymer (87% Vinyl Chloride, 13% Vinyl Acetate), Vinyl Chloride/Vinyl Acetate Copolymer, Carboxylated (86% Vinyl Chloride, 13% Vinyl Acetate, 1%</p>	626-638

	Carboxyl), Poly(Vinyl Acetate) (PVAc), Vinylpyrrolidone/Vinyl Acetate 60/40 Copolymer, Polyvinylchloride with Acethyl Tributyl Citrate	
<b>Cellulosics</b>	Cellulose Triacetate (43.6% Acetyl content), Cellulose Acetate (39.8% Acetyl content), Cellulose Acetate Butyrate (17% Butyryl, 29.5% Acetyl, 1.5% Hydroxyl)	625-639
<b>Other</b>	Ionomer (Na Type), Ionomer (Zn Type), Polyacetylene, Poly(Vinyl Pyrrolidone), Poly(Trimethyl Hexamethylene Terephthalamide), Polyethylene, Chlorinated (Chlorine content 25%, 36%) with Talc, Polyethylene Oxidized, Cellulose Triacetate (43.6% acetyl content), Cellulose Acetate (Acetyl content 39.8%), Poly(Vinyl Acetate)(PVAc), Poly(Vinyl Pyrrolidone), Poly(Trimethyl Hexamethylene	580-639

Terephthalamide)





**Figure 4. 3: Some of the Spectra yielded from FT-IR for microplastics chemical characterization**

#### 4.1.1 Polymers Present in Fish Gills of Fish Samples Obtained from Winam Gulf.

The specific microplastics polymers identified in the fish gills included; Polyacrylamide, Polyurethane, Tencel, Polyvinyl Alcohol (PVAL), Cellulose, Bemberg (Cupra) Fiber, Nylon. The FTIR scores ranged between 615 and 716. Polymers were identified through FTIR analyses, which revealed the presence of several microplastics shown in table 4.3.

**Table 4. 3: Polymers present in Fish Gills obtained from Winam Gulf**

<b>Chemical</b>	<b>Structure</b>	<b>Polymer Descriptions</b>
<b>Zein (Protein)</b>	D_Zein	Zein, Purified
<b>Proteins</b>	D_Protein2	Protein(Soy Bean Powder), D_Protein1 Protein(Human Hair), Human Hair Protein(Human Hair)
<b>Animal-Based Fibers</b>	WOOL	Wool FiberATR/diamond, Human skinATR/Diamond
<b>Polyacrylamides</b>	D_Polyacrylamide-2	Polyacrylamide (Carboxyl modified) (Low carboxyl content)
<b>Polyurethane</b>	RETHAN	Polyurethane Form ATR/ZnSe
<b>Polyvinyl Compounds</b>	D_PVAL	Polyvinyl Alcohol (PVAL)
<b>Cellulose and Related Compounds</b>	D_Cellulose2	Paper, D_Cellulose4 Bemberg (Cupra), D_PVAL-1 Poly(Vinyl Alcohol)(PVAL)(100% hydrolyzed), D_PVAL-2 Poly(Vinyl Alcohol)(PVAL)(98% hydrolyzed)

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<b>Cellulose and Cellulose Derivatives</b>	BEMBERG Bemberg (Cupra) Fiber ATR/diamond, NYLON-A Amorphous Nylon (Selar PA) Film, RAYON Rayon FiberATR/diamond, CELLOPHA Cellulose, ARABIC Arabic gum Film
<b>Nylon and Related Polymers</b>	D_Nylon6_6 Nylon 6/6(Polyhexamethylene adipamide), D_Nylon6 Nylon 6(Polycaprolactam), D_Polyamide8 Polyamide(Nylon 66), D_Polyamide4 Polyamide(Nylon6/12), D_Polyamide7 Polyamide(Nylon 66), NYLON6 Nylon 6 ATR/diamond, Nylone66 Nylone66ATR/DIAMOND, D_Polyamide1 Polyamide(Nylon6)

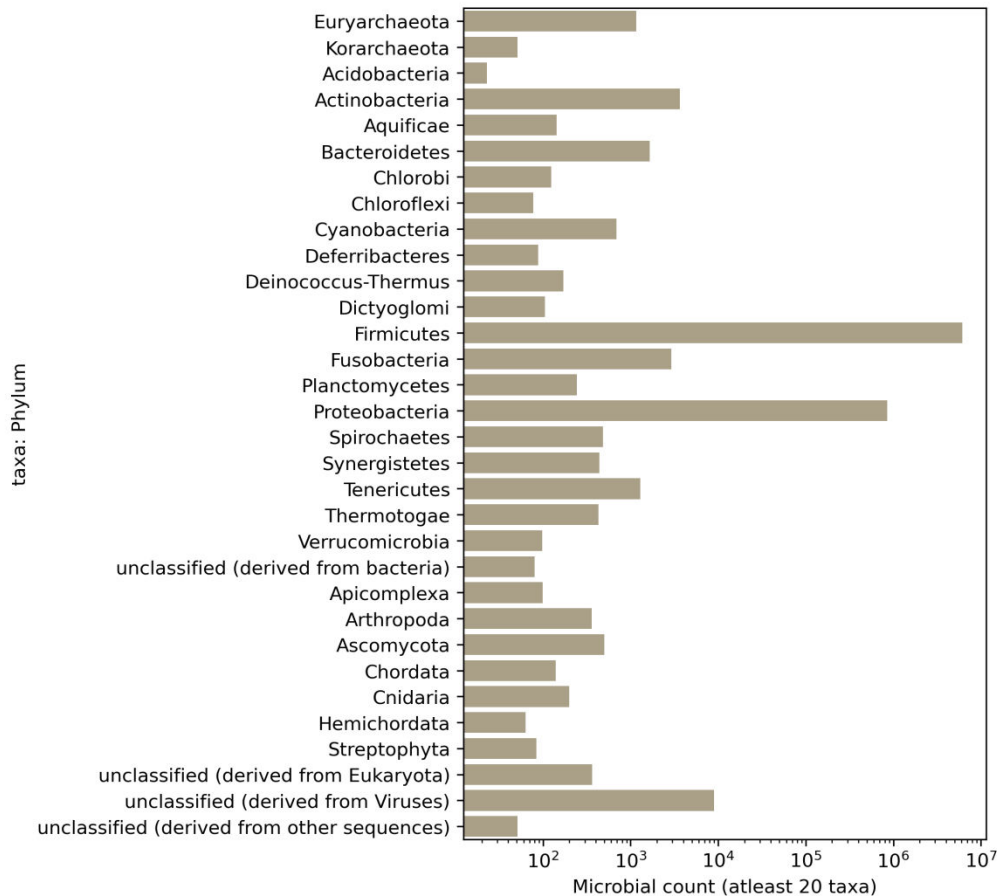
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#### 4.2 Microbiome Profile

The short gun metagenomics analysis of the composite sample from all the sampling points in Winam Gulf of Lake Victoria revealed a significantly rich microbial community. The microbial analysis exhibited a comparison of various taxa including the class, the genus, and the phylum distribution and their abundance. The results of the detected phylum, the classes, and the genus, showed the presence of Bacteria, Eukarya and Archaea, with each taxon singly detected with its abundance. Approximately, 95% of the identified microbial community in all taxa identified above were made of bacteria, while Eukarya and Archaea contributed to about 5% of the total microbial assemblage in Winam Gulf.

The microbial phyla constituted highly diverse compositions of the taxa, with a distinct group of microorganisms with unique traits and genetic diversity. Phylum *Firmicutes* was

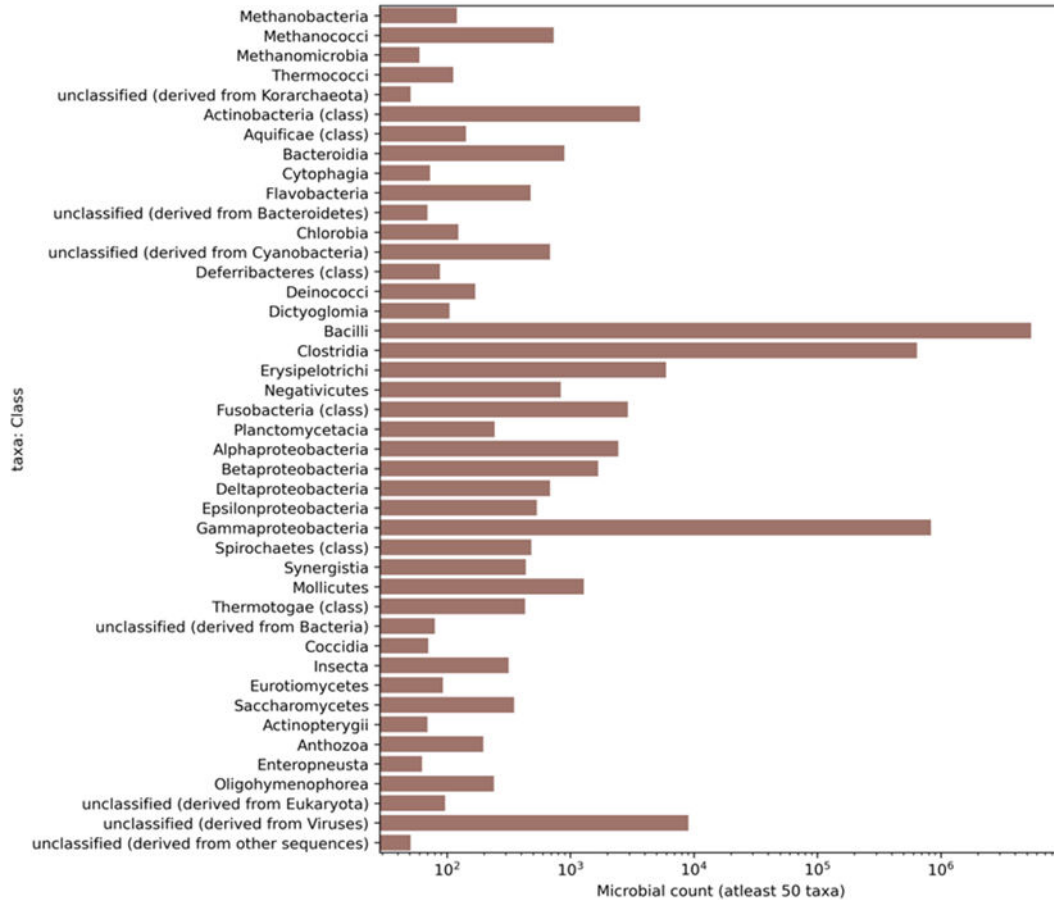
found to be the most abundant with *88% composition* in the sample as shown in figure 4 below. Phylum *Proteobacteria* on the other hand, was the second abundant phylum in the sample accruing a 12% relative abundance. The phylum taxonomic information also evidenced other microbial phyla with a relative abundance <1%, however, they had a specific microbial count of between 200-10000 counts, which provides a significant information about the taxonomic distribution; these phyla include; Actinobacteria, Fusobacteria, Bacteroidetes, Tenericutes, Euryarchaeota, Cyanobacteria, Ascomycota, Spirochaetes, Synergistetes, Thermotogae, unclassified (derived from Eukaryota), Arthropoda, Planctomycetes, Cnidaria, Deinococcus-Thermus, Aquificae, Chordata, Chlorobi, and Dictyoglomi. For the percentage abundance please refer to the figures attached in the appendix.



**Figure 4. 4: Distribution of microbial taxa phyla illustrating counts of different microbes present in the Winam Gulf.**

The class taxonomic information gathered on the other hand showed the following; the bacterial class *Baccilli* was the most abundant with 78% abundance, *Gammaproteobacterial* was the second abundant bacteria with 12% abundance while *Clostridia* was the third highest class with 9% abundance. Other microbial classes that were found to have a significant count included; collective unclassified class from viruses. Additionally, microbial community with <1% abundance included; *Erysipelotrichi*, *Actinobacteria* (class), *Fusobacteria* (class), *Alphaproteobacteria*, *Betaproteobacteria*, *Mollicutes*, *Bacteroidia*, *Negativicutes*, *Methanococci*, *Deltaproteobacteria*, *unclassified* (derived from *Cyanobacteria*), *Epsilonproteobacteria*, *Spirochaetes* (class), *Flavobacteria*,

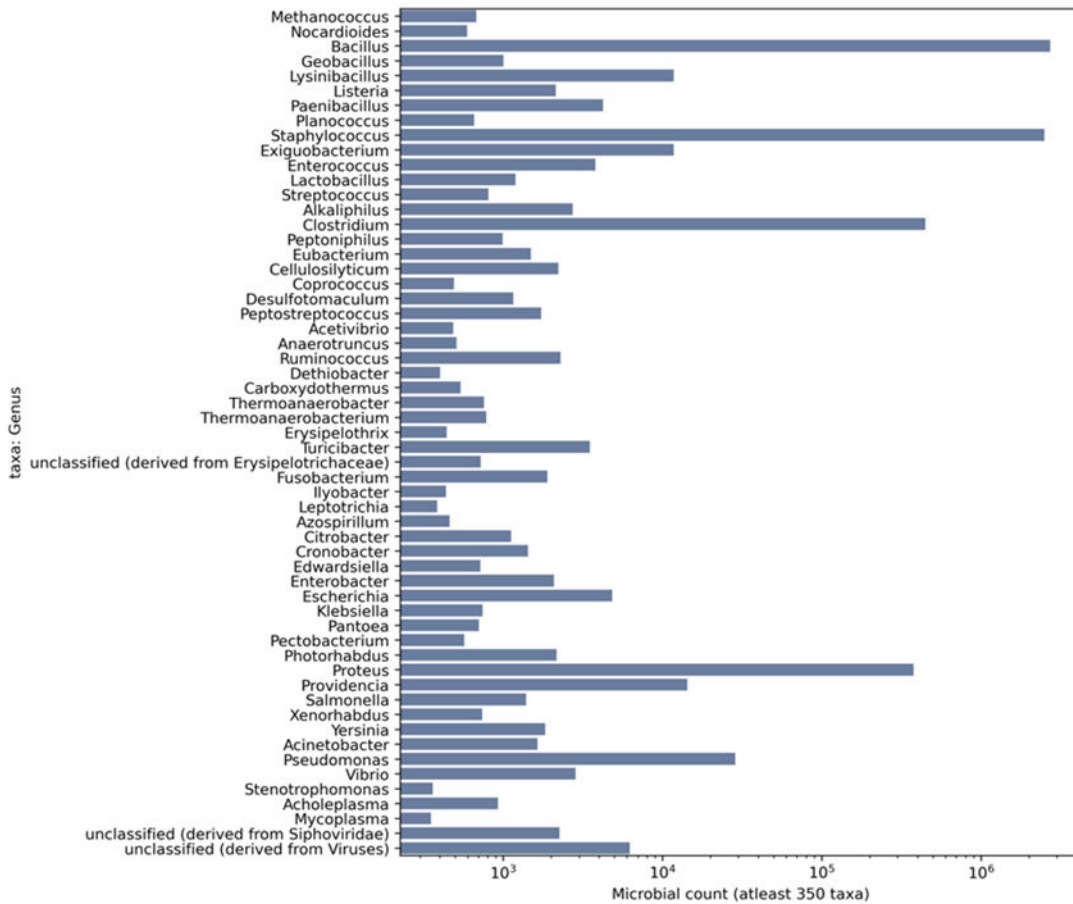
*Synergistia*, *Thermotogae* (class), *Saccharomycetes*, *Insecta*, *Planctomycetacia*, *Oligohymenophorea*, *Anthozoa*, *Deinococci*, *Aquificae* (class), *Chlorobia*, *Methanobacteria*, *Thermococci*, *Dictyoglomia*, *unclassified* (derived from *Eukaryota*), *Eurotiomycetes*, *Deferribacteres* (class), *unclassified* (derived from *Bacteria*), *Cytophagia*, *Coccidia*, *unclassified* (derived from *Bacteroidetes*), *Actinopterygii*, *Enteropneusta*, *Methanomicrobia*, *unclassified* (derived from *Korarchaeota*), *unclassified* (derived from other sequences), *Verrucomicrobiae*, *Spartobacteria*, *Chloroflexi* (class), *unclassified* (derived from *Streptophyta*), *Mammalia*, *Ktedonobacteria*, *Aconoidasida*, *Sphingobacteria*, *Halobacteria*, *Sordariomycetes*, *Bryopsida*, *Branchiopoda*, *Amphibia*, *Solibacteres*, *Chlamydiae* (class), *Liliopsida*, *unclassified* (derived from *Euryarchaeota*), *Fibrobacteres* (class), *Dothideomycetes* among other classes with less than 50 counts in the sample obtained from Winam Gulf as shown in figure 4.5. For the percentage abundance please refer to the figures attached in the appendix.



**Figure 4. 5: Distribution of microbial taxa classes illustrates the counts of different microbes present in the Winam Gulf.**

The genus composition was found to have to be diverse comprehensively with the phylum and respective class of the microbial population of specific bacteria. *Bacillus*, which lies in phylum *Firmicutes*, and class *Bacilli*, was found to have 44% relative abundance in Winam Gulf of Lake Victoria, closely trailed in relative abundance by the genus, *Staphylococcus* at 41% relative abundance. In addition, the genus *Clostridium* and genus *Proteus* were among the significantly higher relative abundance at 7% and 6% respectively. Among other genera that were present included, *Yersinia*, *Peptostreptococcus*, *Acinetobacter*, *Eubacterium*, *Cronobacter*, *Salmonella*, *Lactobacillus*, *Desulfotomaculum*, *Citrobacter*, *Geobacillus*, *Peptoniphilus*, *Acholeplasma*, *Streptococcus* *Thermoanaerobacterium*,

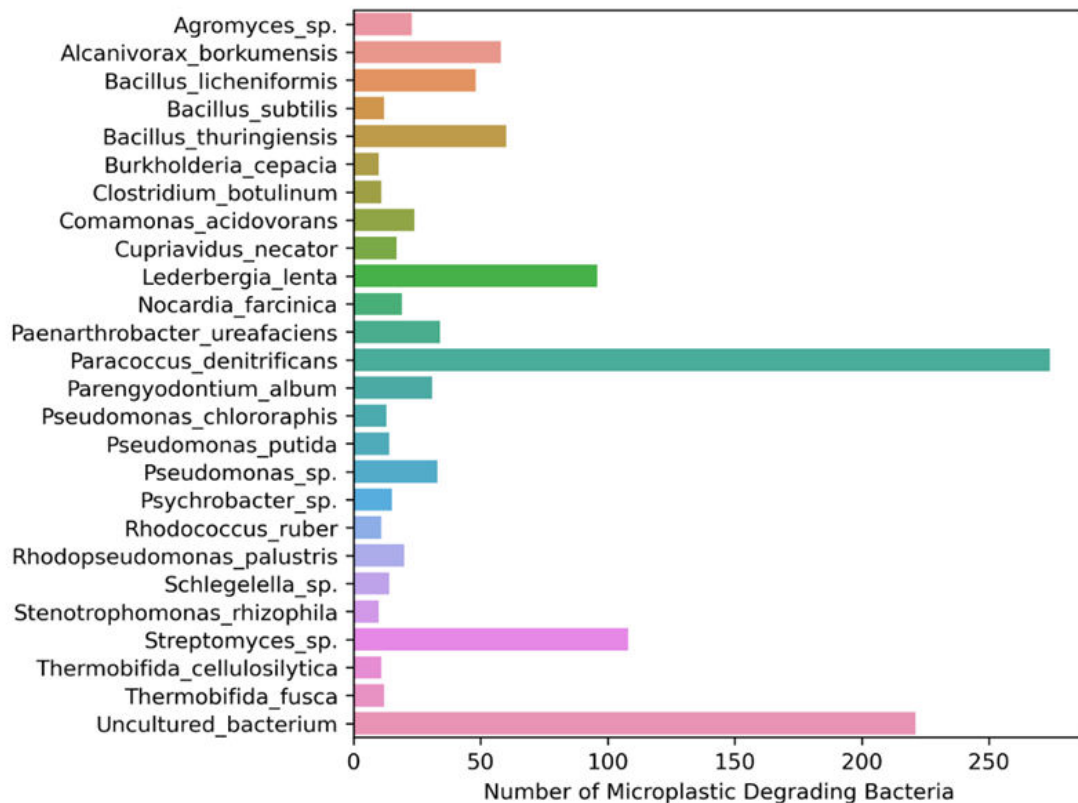
*Thermoanaerobacter*, *Klebsiella*, *Xenorhabdus*, *unclassified* (derived from *Erysipelotrichaceae*), *Edwardsiella*, *Pantoea*, *Methanococcus*, *Planococcus*, *Nocardioides*, *Pectobacterium*, *Carboxydotherrmus*, among others, each with a relative abundance of <1%; however, their individual count relatively differ depending on the class and phylum they lie into as evident in figure 4.6. For the percentage abundance please refer to the figures attached in the appendix.



**Figure 4. 6: Distribution of microbial taxa genus illustrates the counts of different microbes present in the Winam Gulf**

### 4.3 Microplastic Degrading Microbes

The short-gun metagenomics also evidenced the presence of microplastics degrading bacteria which sought to provide a basis for a biodegradation and remediation strategy and showed us the relationship microplastics have with bacteria. The analysis of microplastic degrading bacteria provided the following as outlined in figure 4.7

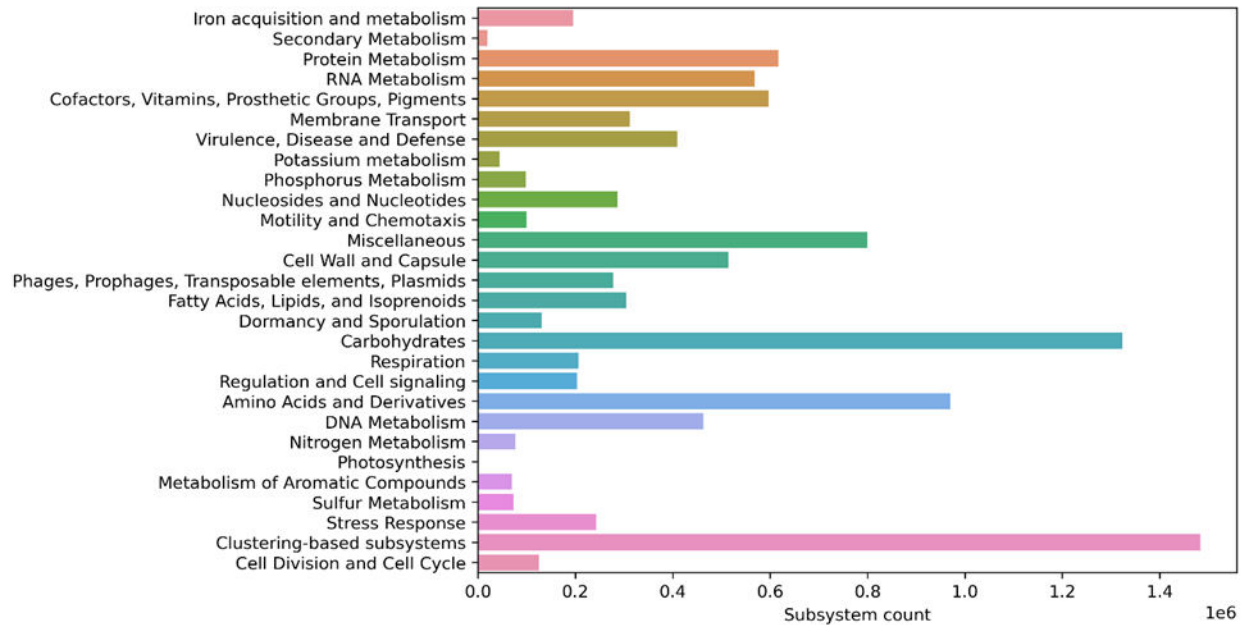


**Figure 4. 7: Counts of Microplastic Degrading Bacteria Present in Winam Gulf of Lake Victoria.**

*Paracoccus denitrificans* was the highest microplastic degrading bacteria in the Winam Gulf followed by *Streptomyces sp.* and the least being *Streptomyces sp.* The other microplastic degrading bacteria found in the Winam Gulf were: *Agromyces sp.*, *Alcanivorax borkumensis*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Burkholderia cepacia*, *Clostridium botulinum*, *Comamonas acidovorans*, *Cupriavidus*

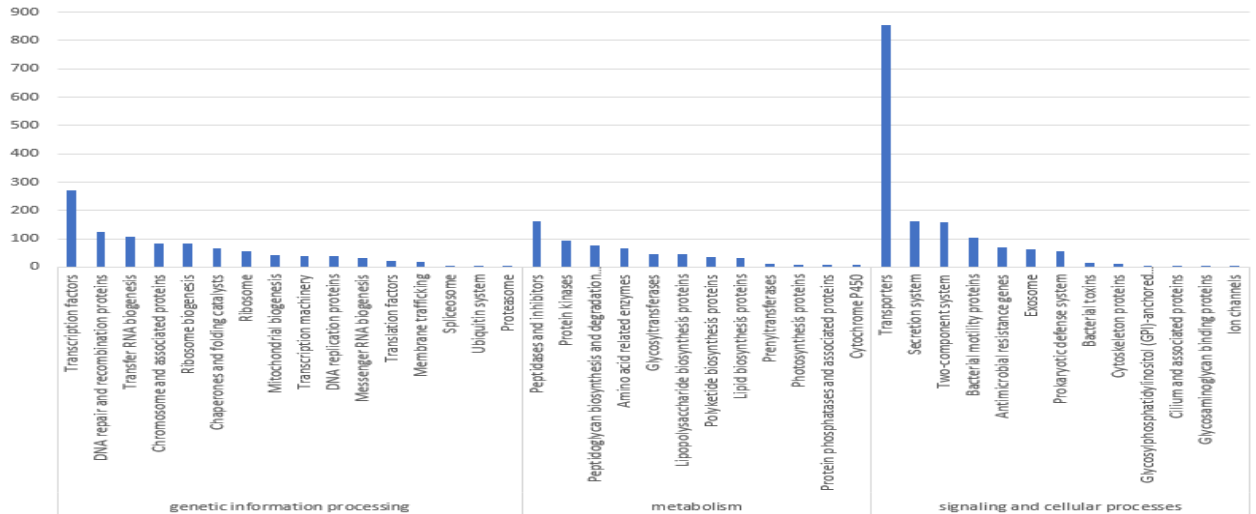
*necator, Lederbergia lenta Nocardia farcinica Paenarthrobacter ureafaciens, Paracoccus denitrificans Parengyodontium album, Pseudomonas chlororaphis, Pseudomonas putida, Pseudomonas sp. Psychrobacter sp., Rhodococcus ruber, Rhodopseudomonas palustris, Schlegelella sp. ,Stenotrophomonas rhizophila, Streptomyces sp., Thermobifida cellulosilytica Thermobifida fusca and Uncultured bacterium.*

The study also evaluated the nature of the functions of the microbial communities that enhance the degradation of various compounds found in Winam Gulf of Lake Victoria. These functions were observed through shotgun metagenomics analysis of the subsystems. The analysis of subsystems in the sample obtained from Winam Gulf of Lake Victoria provides insights into the functional potential of the microbial community in this environment. Subsystems represent specific functional categories of genes and pathways within the microbial community. Different functional categories are represented, indicating the adaptability and versatility of microorganisms in this environment. The subsystems analysis indicates significant roles played by the clustering-based subsystem with the highest abundance in the sample at 14%. The data reveal the relative abundances of the subsystems as follows: *Carbohydrates (13%), Amino Acids and Derivatives (9%), Miscellaneous (8%), Protein Metabolism (6%), Cofactors, Vitamins, Prosthetic Groups, Pigments (6%), RNA Metabolism (5%), Cell Wall and Capsule (5%), DNA Met Sulfur Metabolism, Metabolism of Aromatic Compounds, Potassium metabolism, Secondary Metabolism, and Photosynthesis have a relative abundance of less than 1% and were detected as other systems with the least number of microbes.* The following graph shows the number of results of the subsystems as determined by the microbial communities evident in Winam Gulf of Lake Victoria.

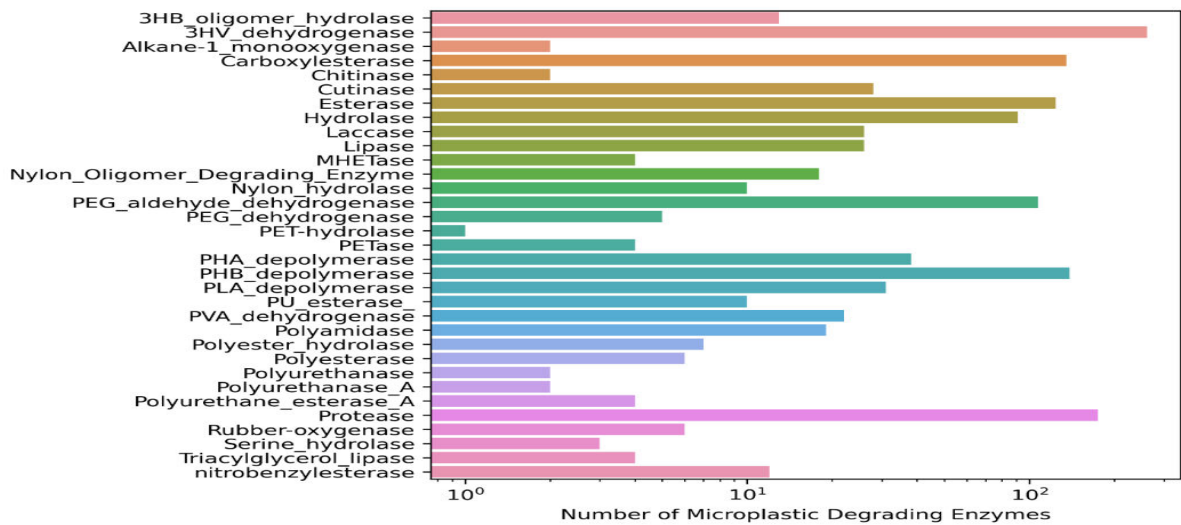


**Figure 4. 8: Subsystems counts from Winam Gulf of Lake Victoria.**

In addition to shotgun metagenomics analysis, the KEGG analysis provided a comprehensive insight of the various categories of microbial characteristics of the Winam Gulf. These categories included; genetic information processing microbes, Metabolism and signaling and cellular processes. Each category consists of significantly varying abundances of the processes. Overly transporters under signaling and cellular processes were the most abundant microbial process with  $>800$  counts; higher than the other systems and processes in the other categories. The KEGG pathways also showed transcription factors with a count of 287 which is under genetic information processing. Under Metabolism factors the highest was peptidases and inhibitors followed by protein kinases with counts  $>100$ . See Fig. 4.9.



**Figure 4. 9: KEGG pathways result showing categorical microbial process in the Winam Gulf of Lake Victoria.**



**Figure 4. 10: Graph Showing the Number of Microplastics Degrading Enzymes.**

Figure 4.10 shows that 3HV\_dehydrogenase enzyme was the highest in number and it's produced by *Paracoccus denitrificans* bacteria (Lu *et al.*, 2014). The second was protease, then PHB depolymerase and the others were 3HB oligomer hydrolase, Alkane-1 monooxygenase Carboxylesterase Chitinase Cutinase Esterase Hydrolase Laccase Lipase MHETase Nylon Oligomer Degrading Enzyme, Nylon hydrolase, PEG aldehyde dehydrogenase PEG\_dehydrogenase, PET hydrolase, PETase PHA depolymerase, PHB

depolymerase, PLA depolymerase, PU esterase PVA dehydrogenase, Polyamidase,  
Polyester hydrolase, Polyesterase, Polyurethanase, Polyurethanase A Polyurethane esterase  
A Protease Rubber-oxygenase Serine hydrolase Triacylglycerol lipase nitrobenzylesterase  
not in any specific order and the least was PET-hydrolase.

## CHAPTER FIVE

### DISCUSSION

#### **5.0 Types of Microplastic Polymers Present in Winam Gulf of Lake Victoria, Kenya**

The results presented here bring forth the characteristics of microplastic and microbiomes and their diversity within the Winam Gulf of Lake Victoria. Microplastic abundance in the Winam Gulf of Lake Victoria was diverse, in terms of the microplastic. The FTIR data presented qualitative results (types) rather than quantitative analysis (concentrations) of the various microplastics present in Winam Gulf of Lake Victoria, identifying a total of 90 types of polymers, 32 of them being plastic polymers with an FTIR score of 580-733. The identified microplastics were grouped into different categories based on their polymer composition, i.e. polypropylenes, Polyacrylamides, polyethylene, vinyl chloride, Nylon Polymers, and other polymers. Studies in Lake Taihu, China (Huang *et al.*, 2022), and the Northern European Lake (Uurasjärvi *et al.*, 2020), microplastics polymers in urban freshwater lakes Songshan Lake of Dongguan, China (Tang *et al* 2022 ) reported presence of polyethylene, polypropylene and nylon polymers as some of the common polymers consistent with findings in Winam Gulf .The agricultural, industrial, and household wastes largely account for the high presence of these polymers as the most imminent sources of these microplastics are; plastic products, coatings, or additives (Gupta *et al.*, 2021). While proteins (D\_Protein1, D\_Protein-2, D\_Zein) themselves are biodegradable and not considered traditional plastics, they can be part of biodegradable plastics and composite materials. In cases where these materials are not disposed of properly, they can break down into microplastic particles. Polyacrylamides are synthetic polymers used in various

applications, including water treatment and agriculture. Improper disposal, runoff from treated fields, or wastewater discharge can introduce polyacrylamide particles into lake water (Sojka *et al.*, 2007). Polyvinyl compounds, these polymers can be used in various consumer products and industrial applications (Grosu, 2021). Nylon polymers are commonly used in textiles, fishing nets, and other applications (Charter & Carruthers 2022). Polyethylene is a widely used plastic in packaging and various products (Chen & Lin 2021). Polyethylene products are also plastic wastes that can break down over time to smaller microplastic wastes. Improper disposal and littering are the two causes of this pollution. Many other artificial polymers and plastics also could add to the microplastic pollution since they also degrade due to environmental factors, or malpractice in waste disposal.

Besides this, the study also investigated the presence of microplastics in the gills of the fish to establish the potential of microplastics to enter the food chain. Several microplastics identified in the previous analysis, including Zein, Polyacrylamide, polyvinyl, Amorphous Nylon, and various types of Nylon, were found in the fish gills samples. Based on a study by Zhang *et al.*, (2019), the majority of the microplastics in fish that were discovered was blue, fiber-shaped, and composed of polyester polymer. In a study in Beibu Gulf, South China Sea fish gills detected presence of polyester and nylon polymers (Koongolla *et al.*, 2020). In a study in Anzali Wetland, Iran, it was discovered that a sizable percentage (>80% of the fish specimens) had ingested microplastics, suggesting that the wetland ecosystem was heavily contaminated. Most of the MPs that were consumed were found to be fibers (Saemi-Komsari *et al.*, 2023). The presence of these polymers in fish gills which represent an aspect of the human food chain relates to the biochemical movement of

microplastics from their anticipated agricultural, industrial, and household sources. This is because most of the microplastics identified in the fish gills like Nylon, polyacrylamides and polyvinyl were also evident in the water and sediment samples obtained from the Winam Gulf. This evidences the possibility of ingestion of these microplastics as humans consume the fish from the Winam Gulf.

The observed microplastics can be related to causing cancer in two major ways. First, through the accumulation of microplastics in body tissues and secondly, through harboring microbial communities that are associated with cancer; such as Class Gammaproteobacteria as earlier mentioned. The physical presence of microplastics in the body can induce chronic inflammation and tissue damage, which are known risk factors for cancer (Rahman *et al.*, 2021). The small size and irregular shape of microplastics enable them to penetrate biological barriers and accumulate in organs and tissues. This physical contact can trigger an immune response and lead to chronic inflammation and production of reactive oxygen species (ROS) (Saifried *et al.*, 2007). DNA damage and cancer cell formation may be the long-term outcomes of the oxidative stress and inflammatory changes on the human body. Alternatively, microplastics that get accumulated in the body tissues may lead to exposure to potentially harmful substances related to the microplastics (Osman *et al.*, 2023). Microplastics have a high surface area, and it implies that the products have the potential to absorb and concentrate a diverse array of harmful materials found in the environment, including heavy metals and persistent organic pollutants (POPs) (Okoye *et al.*, 2022). These chemicals adsorbed may leak out of the microplastics and enter the tissues that surround them and cause damage to the cells, and facilitate the growth of cancerous cells.

Second, the microplastics in the environment may provide a good environment in the colonization and growth of microorganisms some of which have been linked to cancer development. On the microplastics that can be found in the Winam Gulf, it has been made known that some of them contain bacteria that cause the cause of cancer. To provide an example, Bacteria of such types as Firmicutes, Gammaproteobacteria and Clostridium have been discovered to be attached to a microplastic e.g. nylon and polyethylene based polymers (Abed et al., 2024). Some of these bacteria have in one way or the other been associated with the carcinogenic processes (Rajilic-Stojanovic et al., 2020). One of them is the firmicutes, which has been linked to the high risk of colorectal cancer due to their production of toxic metabolites like secondary bile acids (Louis et al., 2014). The number of pathogenic strains of Gammaproteobacteria that are known to cause persistent infections is great, and they can further contribute to the further development of inflammation and the formation of the environment, in which the appearance of cancer may be stimulated. (Rahman et al., 2021).

### **5.1 The Microbiome Profiles Present in Winam Gulf of Lake Victoria**

The microbial analysis comparison indicated distribution of various taxa and their abundance in terms of the class, the genus, and the phylum. The detected phyla, classes, and genera show the presence of Bacteria, Eukaryotes, and Archaea, with each taxon singly detected with its abundance. The shotgun metagenomics results reveal high abundances of the phylum Firmicutes, with a notably highest abundance than the rest of the microbial phyla. According to a study by Doytchinov & Dimov, 2022, Firmicutes was one of the most commonly found Phyla in Antarctic lake waters. Kurilkina *et al.*, 2016 also found Phylum Firmicutes and Proteobacteria as some major Phyla found in freshwater

Lake Baikal. From water and sediments samples from eutrophication impacted artificial lakes in South Africa, Phylum Firmicutes was the second most abundant after Proteobacteria phyla (Ijoma *et al.*, 2024). This depicts that firmicutes play a key role in defining the microbial ecology of Winam Gulf. Among the key factors that explain the high abundance of phylum Firmicutes, one can single out the fact that Firmicutes bacteria, including some sporogenic ones, are characterized by their resistance to hostile environmental factors (Galperin *et al.*, 2022). The physicochemical parameters of the sites sampled in Winam showed that there were relatively stable pH conditions, which were neutral to slightly basic. Despite the lack of any overall variation among the sites, River Uhuru had the highest value of pH (7.63), with River Wigwa having the lowest. There were great site-specific differences in conductivity and turbidity with the highest values recorded in KIWASCO. On the same note, KIWASCO, the lowest Dunga Beach, recorded a lot more total dissolved solids (TDS) (2245.5 mg/L). They are also able to live under low oxygen levels, broad temperature variations and high salinity which makes them flexible to different types of water habitat environments (Galperin, 2016).

To gain more insight into the microbial taxonomic richness, a more subjective shotgun metagenomics analysis of microbial classes and genera in Winam Gulf was performed. This demonstrated that the phyla data, the classes, as well as the genus had a significant relationship; since the most rich classes and genera were observed to be located in the phylum Firmicutes. Bacilli and genus *Bacillus* was the richest bacterial class and genus present in the composite sample, and 78% of the microbial community comprised of this bacterial group. A test conducted to determine Bacillota distribution in Water and Sediments in Rio de Janeiro, Brazil aquatic systems revealed that the top Bacillota

genera were found in *Bacillus* (Argentino et al., 2025). Wang et al. (2022) carried out a study of the microbial communities in 15 shallow lakes located in the Hubei Province (China) where *Bacillus* and *Pseudomonas* were the most common genera. Among the numerous metabolic processes, Bacilli are famous due to the breakdown of organic matter and nutrient cycling (Jiao et al., 2019). They are probably very important to the ecosystem and contribute to decomposing complex organic substances and re-using nutrients because of their high abundance. This community of microbes is the probable reason why the Gulf environment is well-stable and functional in general. Some of the *Bacillus* species are also known to have a negative health outcome on human health besides being extremely important to the eco system. Such species can also generate toxins and virulence factors that may cause infection and diseases, an example of this is that *Bacillus anthracis* is the cause of anthrax and moreover, *B. anthracis*, other pathogenic *Bacillus* species include; *Bacillus cereus* and *Bacillus thuringiensis*. *B. cereus* is associated with foodborne illnesses, causing symptoms such as diarrhea, nausea, and vomiting. *B. thuringiensis*, while primarily known for its use as a biological insecticide, can also cause infections in immunocompromised individuals (Baldwin, 2020).

The aim of microbial data aims at evaluating the potential of these microbes to cause potential health effects to humans that entail cancer. Despite the limited research on this aspect; some Firmicutes species have been related to cancer to a greater extent. Some Clostridia species have been found to produce toxins that can promote the development of colorectal cancer by affecting cellular signaling pathways (Bennedsen *et al.*, 2022). In addition, while there is limited direct evidence linking Gammaproteobacteria to cancer, alterations in the abundance or composition of Gammaproteobacteria have been observed

in certain cancer types. For example, an increased abundance of Gammaproteobacteria has been associated with colorectal cancer, suggesting a potential association (Xu *et al.*, 2022). Moreover, Clostridia, as mentioned earlier, is a class within the Firmicutes phylum. Some species within this class, such as *Clostridium difficile*, are well-known pathogens associated with gastrointestinal infections, and they can be a complicating factor in individuals with cancer who are immunocompromised (Sartelli *et al.*, 2015).

## **5.2 Microbial Species Involved in Microplastic Degradation, Along with Their Associated Enzymes and Functional Processes**

From the results above, *Bacilli*, which was identified to be the largest class and genus, was also found to be a microplastic degrading microorganism. *Bacillus thuringiensis* and *Bacillus licheniformis* (some of the most abundant) and identified as widespread in the environment based on a study by Yao *et al.*, (2022), were identified as microplastics degraders found in the Gulf and produce PHB\_depolymerase and protease enzymes respectively. *Streptomyces sp* which was also identified (the 2nd most abundant after *Paracoccus denitrificans*) and produces PEG aldehyde dehydrogenase enzyme. Class *Gammaproteobacteria* was identified as the second most abundant *Alcanivorax borkumensis* and *Pseudomonas sp* as some of the most abundant microplastics degraders in the Gulf. Bacterial degraders from *Pseudomonas* and *Bacillus* species have potential for degrading plastics such as PE, PET, and PS (Cai *et al.*, 2023). A study by Ali *et al.* 2023 found the strains of *Pseudomonas sp.* SH5B and *Pseudomonas aeruginosa* SH6B to be responsible for degradation of plastic after 120 days. According to a study by Azizi *et al.*, 2024, in Jakarta Bay, Indonesia he identified some plastic degradationers and the constituents entailed *Pseudomonas sp.*, and *Bacillus sp.* A study in Chilean Coast identified

two strains of *Streptomyces* (González *et al.*, 2020). It was also evident that microplastics degrading microorganisms and the enzymes they produce tally in abundance. And to fully understand microbes in the Winam Gulf and its interactions with microplastics, it was vital to understand their microbial processes and functional categories and that entailed the subsystems categories and KEGG pathways.

From the subsystems, the high abundance of Carbohydrates (13%) suggests that microorganisms in this ecosystem are actively involved in carbohydrate metabolism. Carbohydrates serve as a vital energy source for microbes and are involved in various metabolic processes. Amino acids (9%) play a crucial role in protein synthesis and various metabolic pathways. The rich concentration of the microbial community is an indication of the active protein metabolism of the microbial community. Vitamins, prosthetic groups, cofactors, and pigments (6%): These substances are required in a number of the metabolic processes as well as enzyme activity. Their presence shows that the metabolism of vitamins and cofactors is very important in this ecosystem. RNA metabolism (5%) involves processes such as transcription and translation. The abundance of this subsystem suggests active gene expression in the microbial community among other functions that are prevalent in this microbial community. A study in the Himalayan Artificial Lake revealed dominant functions related to carbohydrate metabolism (10.17%), amino acid derivatives (9.95%), and cofactors/vitamins (9.57%) (Kaushal *et al* 2022). Another in the Equatorial Eastern Indian Ocean showed carbohydrate metabolism was the most prominent function with relative abundances ranging from 16.19% to 17.41% across different sampling sites. Amino acid metabolism was also among most abundant with abundances between 14.92% and 15.89% (Ding *et al* 2021). According to Bekele *et al* 2025 genes linked to amino acid

metabolism, carbohydrate metabolism, and energy metabolism accounted for 4–5% of the total genes in Chitu and Shala Soda Lakes, Ethiopia.

Under the KEGG pathways, transporters under signaling and cellular processes are the highest and they are crucial for microbial survival and adaptation. They facilitate the movement of molecules in and out of microbial cells. This may mean microbes in Winam Gulf are actively engaged in nutrient acquisition, osmoregulation, and other processes that require the movement of molecules across their cell membranes. Hence, the microbial community has an ability to efficiently utilize available resources in the Gulf environment. The transcription factors under genetic information processing entail proteins that regulate gene expression in microbes. They play a key role in controlling various cellular processes and may mean the microbes in Winam Gulf are actively regulating their gene expression to respond to environmental changes and optimize their metabolic activities hence adapting to fluctuations in environmental conditions easily. Metabolism came in as the third which may be due to the microbes being in a state of quiescence or reduced metabolic activity or pollutant stressors. Under Metabolism factors the highest was peptidases and inhibitors followed by protein kinases. In Chitu and Shala Lakes the KEGG database analysis showed that the majority were associated with metabolism accounts of 19% followed by 5% of genetic information processes and environmental information process accounts (89,412 and 86,112 genes) (Bekele *et al.*, 2025). Purine metabolism, ABC transporters, and pyrimidine metabolism were the most prevalent pathways, according to Wang *et al.*, 2020. The abundance of genes associated with nitrogen metabolism, the TCA cycle, and the two-component system was higher in sediments than in seawaters (Wang *et al* 2020).

Finally, the focus is to find a solution by creating a viable bioremediation strategy to combat microplastics in Winam Gulf of Lake Victoria to significantly lower levels that are eco-friendly. The determination of enzymes, which are most frequently linked to the degradation of microplastic, the microorganisms that are involved, and the microplastic degraded gave a favorable direction to the purpose. One of the extremely prevalent enzymes involved in the study is 3-hydroxyvalerate (3HV), which is a fragment of polyhydroxyalkanoates (PHAs) that are found in certain types of microplastics and is degraded by 3HV of the *Paracoccus denitrificans* bacteria (Lin et al., 2023). PHA is a biodegradable polymer and the existence of 3HV dehydrogenase implies that the bacteria *Paracoccus denitrificans* can degrade PHAs which would help in breaking down microplastics composed of these materials. Protease is another enzyme that was discovered in fairly large amounts, and it is renowned due to its de-proteolytic abilities (Zhou et al., 2023). It is produced by many bacteria and has been associated with the degradation of microplastics which have protein based materials such as silk and zein. The fact that this enzyme is present shows that there is a chance that some bacteria can decompose proteinaceous microplastics. This was in addition to the discovery of PHB depolymerase, an enzyme that disintegrates polyhydroxybutyrate (PHB). PHB depolymerase implies that bacteria can degrade and metabolize microplastics in the form of PHB, which is frequently found in microplastic. Nylonases ( Nylon hydrolase and Nylon Oligomer Degrading Enzyme ), PET-hydrolase ( Carr et al., 2020) and PETase (abundant in Zhang et al., 2024) were found to be other enzymes that influence polyethylene terephthalate (PET) plastic. A study of healthcare waste conducted by Siew et al. in the year 2025 revealed the presence of enzymes such as esterase, depolymerase, and oxidoreductase. The study of the

mangrove sediments revealed the presence of multiple enzymes, such as alcohol dehydrogenase, aldehyde dehydrogenase, and alkane hydroxylase (Pawano et al., 2024). These enzymes suggest that certain types of microplastics such as nylon, polyethylene terephthalate (PET), polylactic acid (PLA), polyurethane (PU), and polyvinyl alcohol (PVA) can be broken down by certain types of bacteria. Their presence indicates that these microplastics can be degraded by enzyme degradation. There were also the enzymes such as laccase, lipase, esterase, chitinase, and cutinase. These enzymes can contribute to the decomposition of various types of microplastics composed of polyesters, esters, and complex organic substances, and so on due to their broad spectrum of substrate specificity (Liu et al., 2022). The number of enzymes present and the related bacteria demonstrate that there are numerous types of microorganisms which are able to digest different types of microplastics. This finding explains why it is feasible to employ microbial communities and their enzymatic functions to bio-remediate microplastic contamination using bioremediation methods. In this case, the enzymes, which can break amide bonds in polyacrylamides, include hydrolases and esterases. One study identified esterases as the new enzymes in the alpine soils (Frey et al., 2024). Polyvinyl compounds have ester, amide and other bonds. This degradation of polymer can be caused by the targeting of these bonds by the enzymes like hydrolases, esterases and polyurethanases. Enzymes like polyamidase, nylon hydrolase and nylon oligomer degrading enzyme can possibly break nylon polymers down by hydrolyzing their amide bonds in structure. There are two types of enzymes, polyethylene oxidase and polyurethanase, which might have the capability of dismantling polyethylene in terms of its carbon-carbon backbone (Zhang et al 2022, Choi, 2025).



## CHAPTER SIX

### CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 Conclusion

The microplastics were qualitatively determined by the chemical characterization by FTIR. Due to their small size of plastic, microplastics can be stored in organisms and persist in the environment. They may accumulate in the tissues of the aquatic animals including fish and biomagnify their way up the food chain. It would mean that consumption of seafood in the Winam Gulf may expose individuals to microplastics which may be harmful. This study has highlighted the role of enzymes associated with bacteria in the degradation of various types of microplastics. The short gun metagenomics analysis of all sampling sites of Winam Gulf revealed a remarkably rich microbial community. The high bacteria and enzyme count of this study indicates the significant role they can play in the microplastic degradation process in the Winam Gulf of Lake Victoria. These bacteria and enzymes show a natural way to degrade and lessen the environmental persistence of microplastics. Their presence suggests that there is a potential for natural remediation of microplastic pollution through microbial degradation processes. Understanding the abundance and diversity of these enzymes and bacteria provides valuable insights into the potential for bioremediation strategies and the development of targeted approaches to mitigate microplastic pollution. Hence need for a viable remediation strategy to combat microplastics pollution.

#### 6.2 Recommendations

The findings in this study takes into account samples taken on March/April of 2023 hence long term monitoring is recommended that would take into account seasonal variations in

the findings. This study only acts as a first step that identifies and evaluates microplastic pollution and microbiome profile in the Winam Gulf in the surface waters, fish gills and sediments. The presence of microplastics sets it as a stepping stone for the need to analyse effects of microplastics to Winam Gulf aquatic life and humans making sure to identify the microbiome upto the species level in order identify if the lake has pathogenic microorganisms there. Despite the presence of enzymes, microplastics still persist in the Winam Gulf. This persistence can be attributed to several factors, including the continuous release of microplastics from various sources, the accumulation of microplastics in sediments, and the slow degradation rate of certain types of microplastics, low numbers of mdb compared to mps. Several suggestions can be taken into consideration in order to successfully lessen the Winam Gulf's microplastics' presence and the possible health risks they pose. To begin with, more stringent policies and regulations should be implemented to reduce the level of plastic waste that gets into the atmosphere. This involves the promotion of the use of less dangerous and more degradable materials, implementation of recycling mechanisms, and the advocacy of proper waste management methods. Second, to teach the population about the impact of microplastics on the environment and human health, it is necessary to conduct the public awareness and education campaign. By raising awareness about the consequences of harmful consumer habits and promoting responsible consumption, people can make informed decisions that will result in reduced usage of plastics and decrease the overall pollution of the microplastic. To implement effective strategies and monitoring programs to identify the concentrations of microplastics in the Winam Gulf, the collaboration of the different stakeholders such as governmental organizations, business, and scientific communities is required. Besides the further

expansion of research to more deeply comprehend the causes, flows, and consequences of microplastics in the ecosystem, this collaboration can contribute to the creation of the advanced technologies of microplastic detection and elimination. Thirdly, bioaugmentation is one of the promising strategies that could be used to solve the problem of microplastics in the lake. It has been found that, there are bacteria which can dissolve these contaminants. A great example of the use of bioaugmentation to eliminate microplastics is a study performed by Japanese researchers. They found out that *Ideonella sakaiensis* is a bacterium that has the capacity to degrade polyethylene terephthalate (PET) which is a popular type of plastic in bottles of beverages. The enzymes that are produced by this bacterium are capable of breaking down the components of PET and subsequently they can be broken down further by other microorganisms or they can be absorbed by the environment. Breakdown of microplastics could be accelerated and their concentration progressively decreased, potentially, through bioaugmentation, adding or multiplying the number of PET degrading bacteria, e.g. *Ideonella sakaiensis* in the lake habitat. The approach is one of the possible solutions to address the problem of microplastic pollution in aquatic ecosystems (Yoshida et al., 2016).

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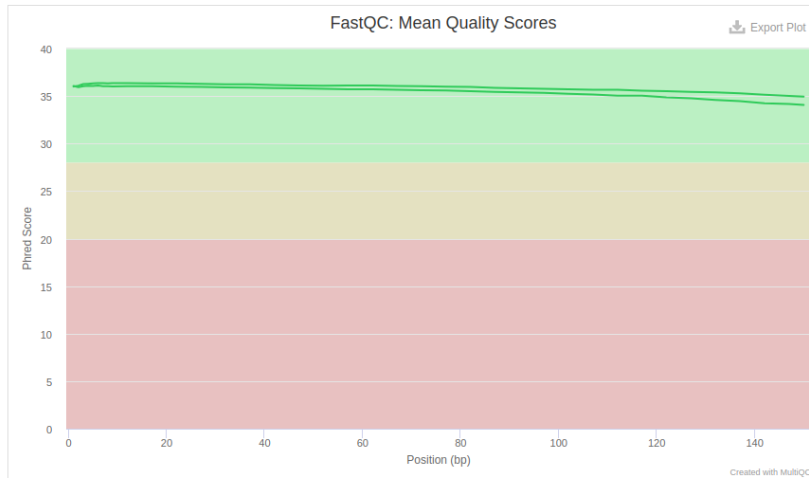
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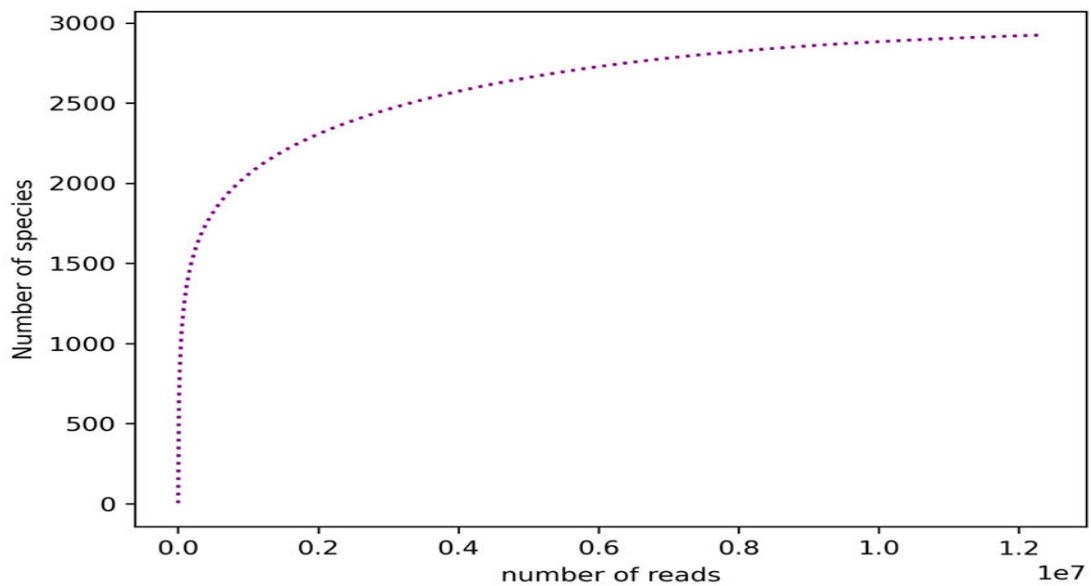
## APPENDICES

### Appendix I: Sequencing data

Sequencing data Quality control graph indicating the quality of the shotgun metagenomics results in the sample reads

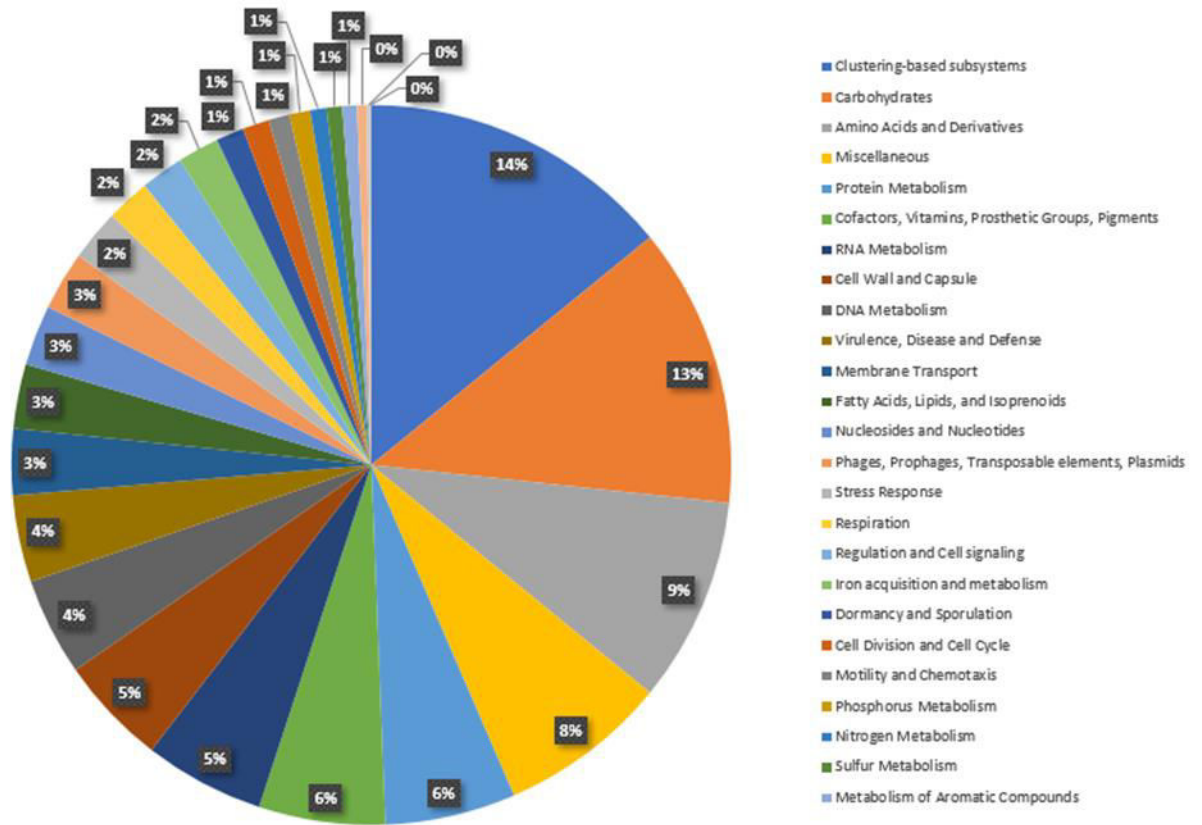


Showing the rarefaction graph obtained that verifies the quality of the reads made from the shotgun metagenomics analysis

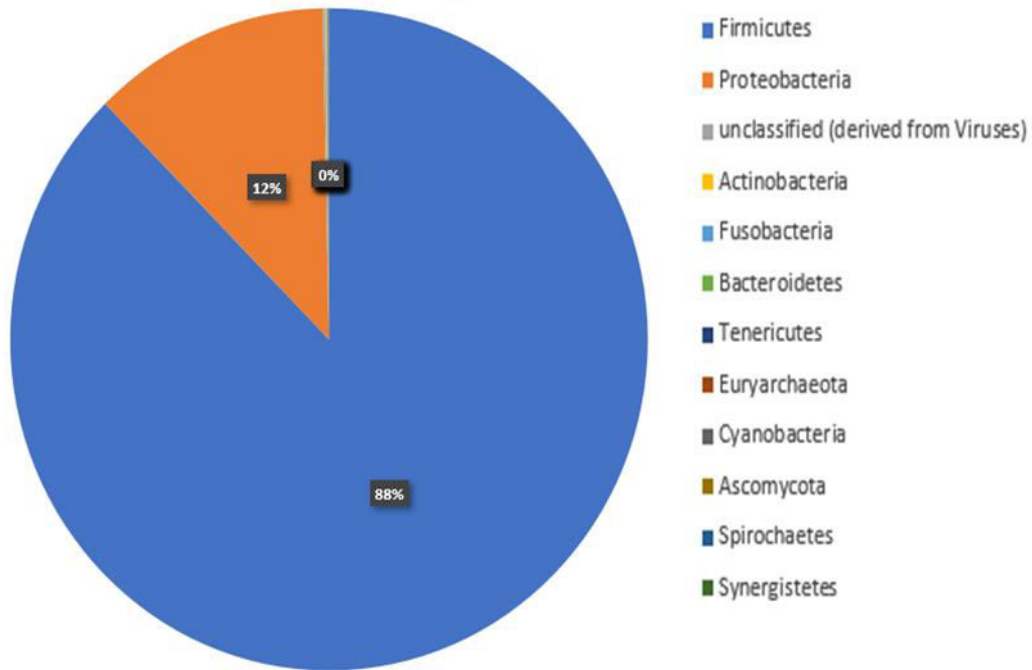


## AppendixII: Subsystems

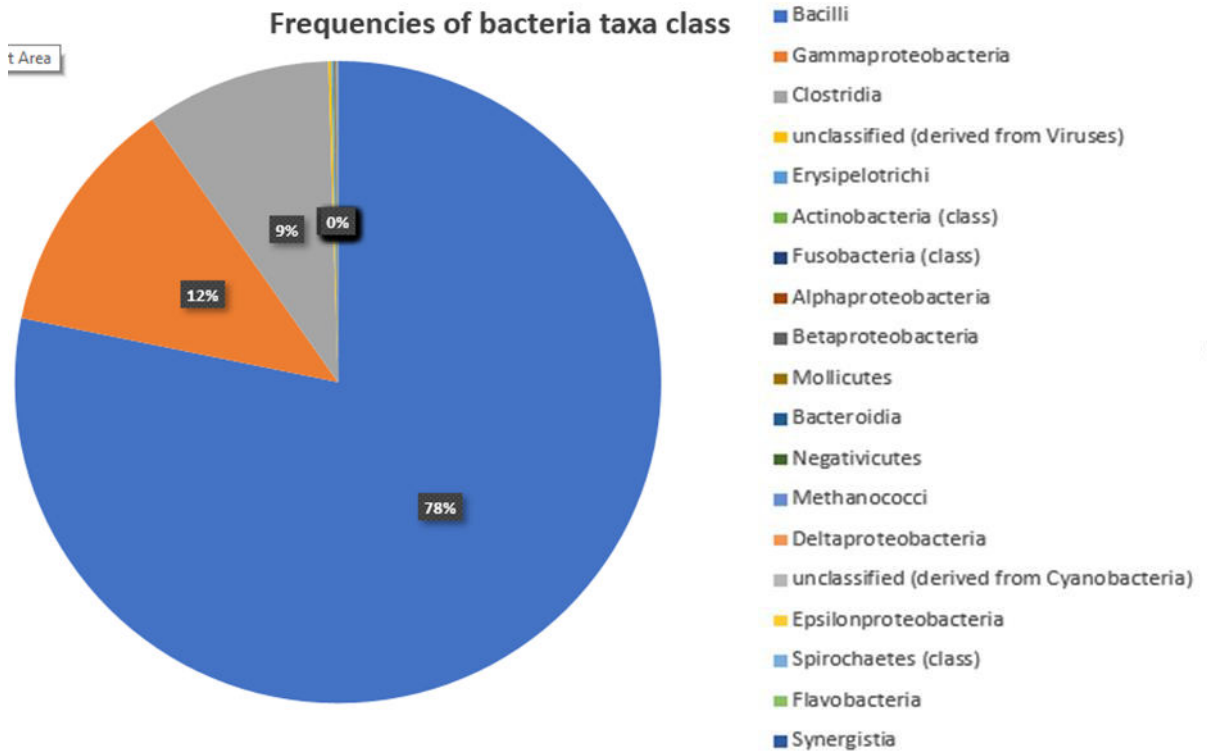
Shows Subsystem categories in percentage from clustering based subsystem at 14 percent to the last.



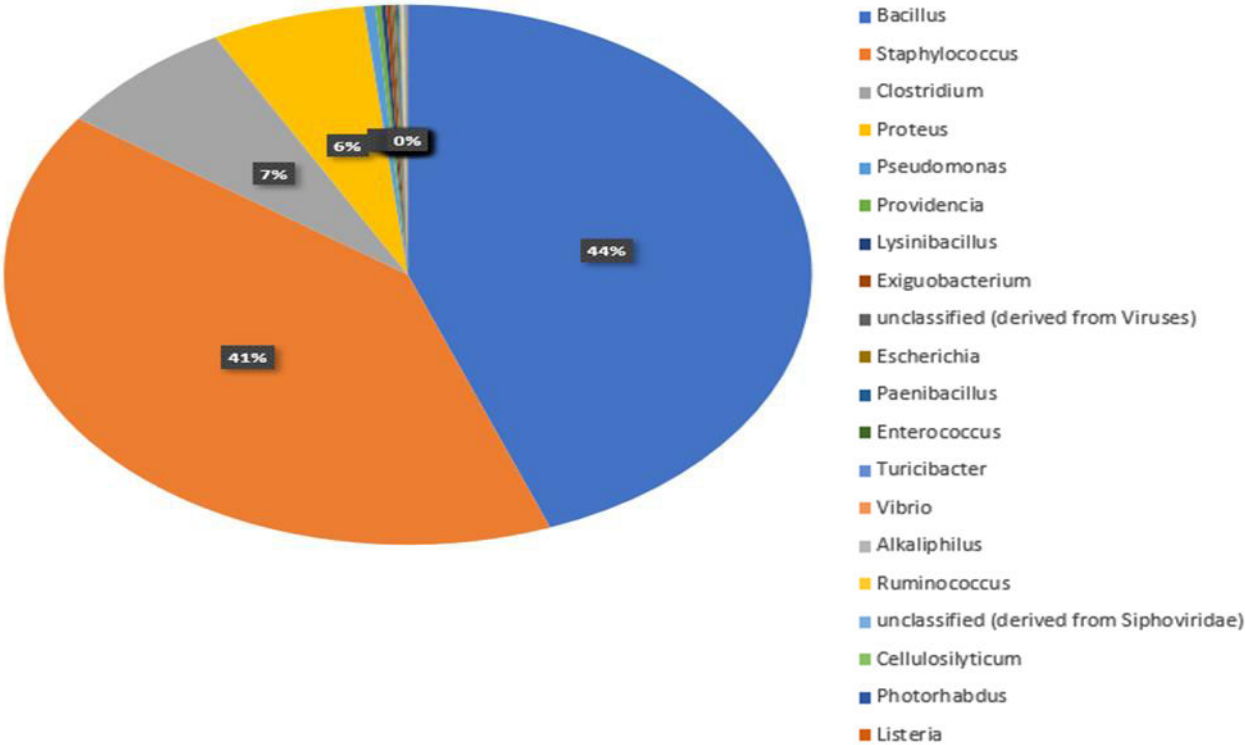
## Phylum Bacterial Count in percentage



### Class Bacterial Count



**Genus Microbial Count (Percentage)**



### Appendix III: Spectra yielded from FT-IR for microplastics chemical characterization

This are just representatives of FTIR spectra yielded from River Wigwa samples, Block beach, Gill and Dunga beach.

