MICROPLASTIC CONCENTRATIONS IN WATER AND FOUR COMMERCIALLY IMPORTANT FISH SPECIES IN KISUMU BAY, LAKE VICTORIA, KENYA

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Master of Science Degree in Limnology of Egerton University

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented in this university or any other for the award of a degree.

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Recommendation

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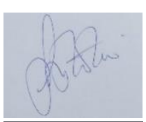
Signature:  Date:

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DEDICATION

To my family and friends for their selfless support and encouragement to see me go through my master's programme.

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ABSTRACT

Microplastics (MPs) are fragments of any type of plastic formed through processes such as abrasion and photodegradation and are considered emerging pollutants in aquatic ecosystems. They can be mistakenly ingested by aquatic organisms. Despite the high plastic pollution within Kisumu City, there is inadequate data and information on the levels of microplastic concentrations in water and fish within Kisumu Bay of Lake Victoria. This study aimed to determine levels of microplastics in water and identify polymers of microplastics in water and fish as well as evaluate the impact they have on fish. The study was conducted at four sites with different anthropogenic impacts; the Airport area (close to the shore with minimal anthropogenic activities), Kichinjio (a near shore site characterized by anthropogenic disturbance), River Nyalenda mouth (a site preceded by a wetland and a tourist beach adjacent.), and the Open water (the site was situated several kilometres off shore, representing the pelagic zone) within Kisumu Bay of Lake Victoria. Water quality parameters were measured in situ at each sampling site. Sampling was conducted monthly between March and May 2022. A plexiglass water sampler was used to collect composite water samples. Bulk water samples were filtered through a series of stacked sieves (45 - 300 µm mesh sizes) in the field. Deionized water was added to the residue collected in 250 mL glass bottles. Samples of fish species were caught using 3 and 6-inch gill nets set overnight. Microplastics concentrations in the water ranged between 0.85 ± 0.04 - 2.41 ± 0.15 particles/L at different sites. The concentration of microplastics differed significantly among the sites (Mood's median test, $\chi^2 = 18{,}22$, df=3, $p < 0.05$). Fish gastrointestinal tracts were examined, and 62 out of 95 (FO = 65.26%) contained MPs. The sampled fish had consumed MPs with different proportions among the species, 75% (*Clarias gariepinus*), 71.43% (*Lates niloticus*), 59.26% (*Oreochromis niloticus*) and 75% (*Synodontis victoriae*). Poly (perfluorobutadiene) and poly (vinylidene fluoride-co-hexafluoropropylene) were the main plastic polymers found in water. Polystyrene and poly (perfluorobutadiene) were the main plastic polymers found in fish as analyzed by ATR-FTIR spectroscopy. Condition factors for *O. niloticus*, *S. victoriae* and *L. niloticus* were > 1 and below 1 for *C. gariepinus*. Positive correlations were observed between microplastic numbers and fish length and weight. However, the low R^2 values obtained implied a weak relationship between these parameters. These findings reveal that Kisumu Bay, Lake Victoria is experiencing escalating plastic pollution, with quantitative evidence underscoring the urgent need for targeted environmental interventions and comprehensive plastic waste management strategies.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
APHA	American Public Health Association
COVID-19	Coronavirus Disease 2019
EDX	Energy Dispersive X-ray Spectrometry
FAO	Food and Agricultural Organization
ATR-FTIR	Attenuated total reflectance fourier transform infrared spectroscopy
LWM	Limnology and wetland management
KOH	Potassium hydroxide
MPs	Microplastics
NaCl	Sodium chloride
PCBs	Polychlorinated Biphenyls,
PAHs	Polyaromatic Hydrocarbons
PVC	Polymerizing vinyl chloride
SEM	Scanning Electron Microscopy
SIMPER	Similarity percentage
UN-HABITAT	United Nations Human Settlements Programme
WHO	World Health Organization
WPO	Wet Peroxide Oxidation
PCA	Principal component analysis

CHAPTER ONE

INTRODUCTION

1.1 Background information

Plastics are synthetic and semi-synthetic materials that have had a surge in utilization in recent years. Contamination of the environment with plastic debris has been gaining increasing attention from the public, environmentalists, scientists, and policy makers since the 1970s (Rochman *et al*., 2016). In 2019, China contributed 31% of the world's 368 million tonnes of plastics production, while the Middle East and Africa both contributed 7% to global production (Plastics Europe, 2020). Based on the current trends, it is estimated that the global mismanaged plastic waste will reach 155-265 million tonnes annually by 2060 (van Wijnen *et al*., 2019). Plastic waste management is a challenge for many countries, especially in developing countries. Many strategies have been formulated to resolve this problem but their implementation has faced setbacks (Guerrero *et al*., 2013). In 2017, the Government of Kenya through the Kenya Gazette Notice No. 2334 enacted a strict regulation banning the manufacture and use of plastic carrier bags entirely (NEMA, 2017). This law has been a significant accomplishment since the same initiative had already failed two times in the past decade (Bahri, 2005). Even though Kenya banned the use of plastic bags, plastics are being used both as an ingredient in the manufacturing of other products and packaging of manufactured goods (Horvath *et al*., 2018).

A considerable amount of solid waste, which includes plastic debris generated from terrestrial environments aided by runoff often finds its way into aquatic ecosystems (Horton *et al*., 2017). For example, the quantity of plastics entering a river is influenced by the size of its catchment and land use activities. Once large plastics are in an aquatic ecosystem, they are degraded as a result of abrasion and photodegradation into macro, micro and nano plastics (Barnes *et al*., 2009). Due to their small size and low density microplastics can linger in the environment for longer, creating an increasing environmental danger compared to macroplastics. Microplastics in water systems were first reported in North America as spherules in plankton tows along the coast of New England in the 1970s (Carpenter *et al*., 1972). Microplastics have been observed in marine (Barboza & Gimenez, 2015; Qiu *et al*., 2015) and freshwater (Peng *et al*., 2018; Toumi *et al*., 2019) across the globe, and their biotic interactions are widely documented.

Many microplastics are buoyant and hence are found at the surface microlayer of water bodies where hydrophobic compounds such as hexachlorobenzene, polychlorinated biphenyls (PCBs), and polyaromatic hydrocarbons (PAHs) may concentrate up to 500 times that of the underlying water column (Wurl & Obbard, 2004). Chemical additives incorporated into plastics during manufacturing can leach out as most of them are not chemically bound. Consequently, this an increasing ecotoxicological risk for aquatic organisms (Hermabessiere *et al*., 2017).

In Kenya, poor waste management is becoming a threat to ecosystem health by contributing an enormous number of pollutants into the environment, with the main sources of pollutants in aquatic systems being storm drains, sewerage effluents as well as poorly managed dumpsites. Kisumu Bay is located adjacent to Kisumu City in Kisumu County. The County has been experiencing a fast growing rural and urban settlement with a population density of 550 persons per km^2 (Simiyu *et al*., 2017). With the increasing population, Kisumu County generates about 500 tonnes of solid waste per day, which comprises 79.5 tonnes of plastics (Dianati *et al*., 2021; Oyake-Ombis *et al*., 2015). The larger part of the city's waste remains uncollected and accumulates in heaps, openly burnt, illegally dumped on vacant land, alongside roads or in drainage systems (Sibanda *et al*., 2017).

Fish have diverse feeding habits, acting as predators, herbivores, detritivores, or omnivores, therefore, many species at least in principle, have the capacity to ingest microplastics depending on their feeding strategies (Suckling, 2021). Nile tilapia (*O. niloticus*) has been documented to have an ontogenic shift in its feeding with zooplankton forming the major diet for the young tilapia whereas mature fish have a range of food items in their diet and they inhabit shallower waters closer to the surface (Munguti *et al*., 2022; Njiru *et al*., 2004; Outa *et al*., 2014) exposing them to microplastic ingestion. The North African catfish (*C. gariepinus*) consumes a wide variety of food items, which include terrestrial and aquatic insects, snails, zooplankton and several benthic organisms and fish. In some eutrophic water bodies in Africa, the North African catfish have been reported to portray a shift to ram-feeding mode and filtering large quantities of zooplankton using its long and compact gill rakers (Dadebo, 2009). *L. niloticus* are predatory and demersal fish feeding on small fish and macroinvertebrates while *S. victoriae* is an omnivorous and benthopelagic species (Natugonza *et al*., 2021).

The exposure pathways include direct consumption by organisms that cannot differentiate between MPs and prey. Predators and detritivores may also indirectly ingest MPs while consuming prey or scavenging on detrital matter containing MPs (Nelms *et al*., 2018). Hamed *et al*. (2021) reported that MPs can produce various changes, ranging from biochemical changes in single cells to lesions within the tissue, all of which can affect fish vitality and life. Fishing activities involving capture fisheries and aquaculture are also important but overlooked sources of MPs. Fibre plastic debris, from the abrasion of fishing nets and ropes, is a major source of secondary MPs in water systems (Napper *et al*., 2022). Therefore, the feeding mechanisms of these species make them susceptible to feeding on microplastics in aquatic ecosystems. The major aim of this study, therefore, determining the levels of microplastics in the water and four common fish species and the effects of MPs on their well-being is important considering the increasing environmental pollution at Kisumu Bay. The chosen fish species in this study are common and of high commercial value for Lake Victoria.

1.2 Statement of the problem

Freshwater systems having closer proximity to point sources of pollution face several pollution threats including microplastic pollution as compared to marine environments. Kisumu Bay of Lake Victoria is located adjacent to Kisumu City, the County headquarters. Like many urban and peri-urban areas of developing countries, it has been experiencing an increase in waste generation, overflowing dumpsites and pollution from uncontrolled discarding of wastes. The waste includes plastic which is the third major component of municipal waste in the city after organic waste and paper waste. Plastic waste when poorly managed may enter aquatic ecosystems especially those adjacent to urban areas, where it degrades into microplastics. Microplastics lead to pollution and destruction of the aquatic ecosystems. Ingestion of microplastic particles causes stress to the organisms including but not limited to cellular necrosis, inflammation, lacerations of tissues in gastrointestinal tracts and risks for trophic transfer and biomagnification. Most of the scientific research into microplastic pollution has continued to focus on the marine environment. Plastic pollution monitoring of freshwater environments such as Lake Victoria, is still in its infancy and is not adequately understood. Therefore, this study aims to determine the levels of microplastics in the water column and those ingested by *O. niloticus, L. niloticus, S. victoriae* and *C. gariepinus*, and their effects on the well-being of the four fish species within the Kisumu Bay of Lake Victoria.

1.3 Objectives

1.3.1 General objective

To assess levels and identify polymers of microplastics in water and fish and the effect on fish well-being within Kisumu Bay of Lake Victoria, Kenya.

1.3.2 Specific objectives

i. To determine the levels of microplastics in water and four fish species within Kisumu Bay of Lake Victoria
ii. To identify the composition of microplastics in fish and water within Kisumu Bay of Lake Victoria
iii. To determine the relationship between microplastic pollution and the well-being of fish within Kisumu Bay of Lake Victoria

1.4 Hypotheses (Ho)

i. There is no significant difference between the levels of microplastics in water and four fish species within Kisumu Bay of Lake Victoria
ii. There is no significant difference in the composition of microplastics in fish and water within Kisumu Bay of Lake Victoria
iii. Microplastic pollution has no significant influence on the well-being of fish within Kisumu Bay of Lake Victoria

1.5 Justification

Freshwater ecosystems face threats such as water pollution, flow modification, habitat degradation, invasion by exotic species and overexploitation of their resources. Microplastics are emerging pollutants in aquatic ecosystems. Therefore, it is essential to assess the stresses that microplastics cause in the aquatic environment, especially to fish. Nile tilapia (*O. niloticus*), Nile Perch (*L. niloticus*), North African catfish (*C. gariepinus*) and Lake Victoria squeaker (*S. victoriae*) are commercially important species and are filter feeders, predatory demersal fish omnivorous bottom feeders and omnivorous benthopelagic species respectively. These feeding habits expose them to accidental ingestion of microplastics. Therefore, they represent logical choices to monitor microplastic pollution in Kisumu Bay, a possible hotspot. This study will fill the knowledge gap of microplastic pollution in freshwater systems in Kenya and their effects, especially on fish well-being. The information obtained will help local authorities within Kisumu

County identify appropriate ways to regulate environmental pollution in aquatic ecosystems, particularly plastics in the achievement of Kisumu County Integrated Development Plan II, 2018-2022, and align with Kenya's Vision 2030, especially the economic pillar where agriculture is one of the six major sectors targeted and in which fisheries management and development falls and Africa's Agenda 2063. Moreover, the Sustainable Development Goal 6 (Clean water and sanitation), two of the sub goals targets improving water quality by reducing pollution, eliminating dumping and minimizing the release of hazardous chemicals and materials which aligns with the subject of this study. Sustainable Development Goal 3 (Good health and well-being) with a sub-goal of substantially: reducing the number of deaths and illnesses from hazardous chemicals and air, water and soil pollution and contamination. Sustainable Development Goal 14 (Life below water) through the sub-goal of preventing and significantly reducing pollution of all kinds, in particular from land-based activities, including plastic debris.

CHAPTER TWO

LITERATURE REVIEW

2.1 Threats to freshwater ecosystems

Freshwater ecosystems which include streams, rivers, and lakes are vital for human well-being as they provide vital goods and services (Carpenter *et al*., 2011). Anthropogenic activities pose threats that have consequences to freshwater ecosystems. Ecological responses are the result of interactions among multiple threats and their associated ecological alterations have been experienced in different catchments (Craig *et al*., 2017). The threats include water pollution, over exploitation, flow modification, habitat degradation and invasion by exotic species (Dudgeon *et al*., 2006). Other emerging threats to these ecosystems include climate change, harmful algal blooms, freshwater salinization, expanding hydropower, infectious diseases and recent concerns about microplastics pollution (Reid *et al*., 2019).

2.2 Microplastics pollution

While plastics are considered an achievement in modern society and the apex of technological innovation, they are increasingly becoming a renowned environmental threat that humans have ever known (Crawford & Quinn, 2016). Plastic debris enters natural ecosystems in all shapes and sizes (Figure 2.1). It was not until recently that larger plastic items gained media attention and caused serious public concern due to their entanglement on organisms such as turtles and sea birds (Kühn *et al*., 2015; Wilcox *et al*., 2016). Jambeck *et al*. (2015) estimated that between 4.4 and 12.7 million tonnes of plastic enter the marine environment annually. Lebreton *et al*. (2017) estimated that the global riverine system release between 1.15 and 2.41 million tonnes of plastic into the oceans every year. According to Hoffman and Hittinger (2017), the Great Lakes alone receive about 10,000 tonnes of plastic annually, making the issue of plastic pollution in freshwater systems just as critical as that in marine settings.

The plastics can be classified according to their size; macroplastics (>5 mm), microplastics (5 and 0.05 mm), and nanoplastics (<50 µm) (Andrady, 2011; Hidalgo-Ruz *et al*., 2012; Wright *et al*., 2013). Microplastics are likely to be the most abundant items of plastic debris in water bodies, and their quantities are projected to increase because large, single plastic items ultimately degrade into millions of microplastic pieces (Isobe *et al*., 2019). Microplastics are of environmental concern because of their small size, rendering them bioavailable to aquatic organisms even small zooplankton, with potential for physical and toxicological harm (Migwi *et al*., 2020).

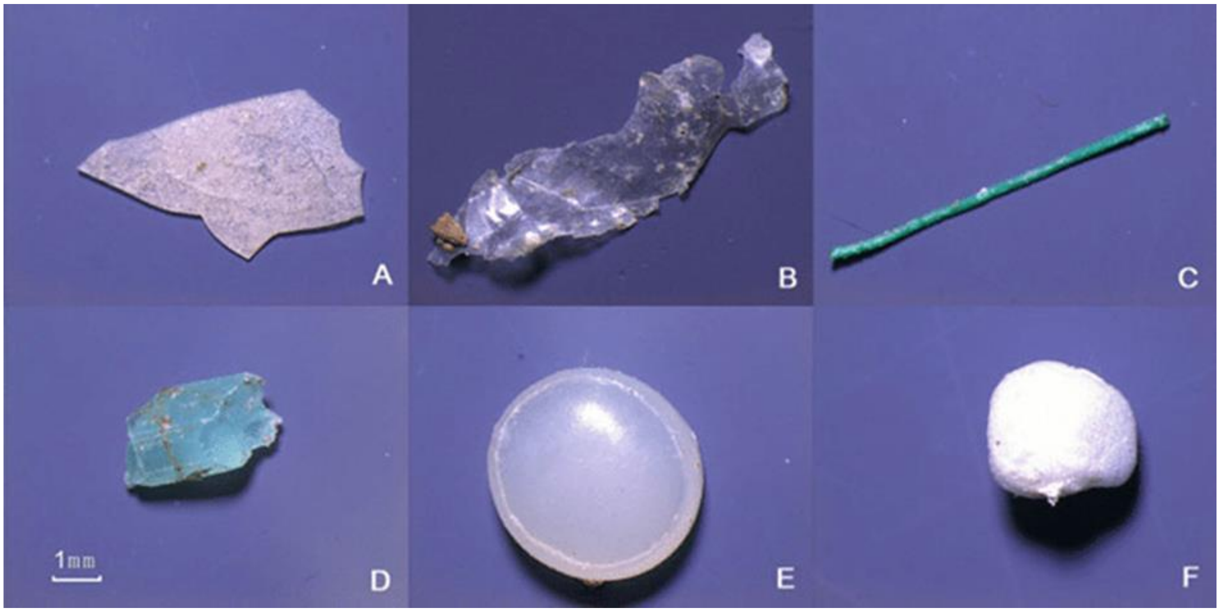

Figure 2.1: Common shapes of typical microplastics (a, sheet; b, film; c, line/fibre; d, fragment; e, pellet/granule; f, foam) (Wu *et al*., 2018).

Plastics have the ability to change their chemical structure or formulation, hence their final properties allow them to be used in various applications (Millet *et al*., 2019). Additives in plastics enhance their functionality, longevity and aesthetics. These include flame retardants, ultraviolet inhibitors and colourants among others. Plastics are used in many industries since they are inexpensive. However, their wide usability and mismanagement lead to their introduction into the environment. Plastics are emerging pollutants that pose a great threat to the environment, especially aquatic ecosystems (Avio *et al*., 2017).

Microplastics are characterized by their small size, weight and relative density which ranges between 0.9 and 1.5 g/cm^3 (Andrady, 2011). Therefore, once they get into an aquatic ecosystem, they are able to float on the water surface or sink to the base of the thermocline in the case of marine waters, while others sink to the bottom. Thus, many studies preferably sample the water surface and where possible, along the water column (Dai *et al*., 2018). However, processes such as wind action, hydrodynamic alterations, degradation, sedimentation, adsorption, aggregation, biofouling, resuspension, burial, ingestion, and excretion of microplastics by aquatic organisms influence their transport and accumulation, resulting in a complex and dynamic equilibrium. These processes bring about the horizontal and vertical movement of particles, along with sedimentation in deposition areas (Chubarenko *et al*., 2016; Kukulka *et al*., 2012; Zhang, 2017).

In addition, factors such as flow velocity, water depth, storms, floods or anthropogenic activities might also influence their physical and temporal detection (Kessarkar *et al*., 2010; Rocha-Santos & Duarte, 2015). Lenaker *et al*. (2019) reported an exponential decrease in microplastics with an increase in water depth in freshwater systems, although no statistically significant difference was observed. The study of Rodrigues *et al*. (2018) in Antuã River, Portugal provided new insights into microplastic abundances and distribution. The study emphasized the importance of rivers as carriage systems of microplastics and recommended further investigations to be performed to identify rivers as point sources, to mitigate the microplastic contamination in aquatic systems. Freshwater microplastics pollution can influence marine environments since rivers are major pathways of plastics. Indeed, studies on the Austrian Danube River show that more than 1,500 tonnes of plastics smaller than 5 mm but bigger than 500 µm were estimated to enter the Black Sea annually (Lechner *et al*., 2014).

The density of microplastics is affected by several factors that influence their distribution in the water column. Different microplastic polymers have different densities, and thus their vertical movement will vary according to that density (Eo *et al*., 2019). Most plastic debris is originally buoyant such as polystyrene, polyethylene, and polypropylene, while denser plastic such as polyvinylchloride and nylon readily sink in seawater (Miller *et al*., 2017). A number of factors, including those linked to anthropogenic activities and physical watershed/stream characteristics, may have an impact on the spatial distributions of microplastics in freshwater systems (Talbot & Chang, 2022). For instance, increased riparian zone slope can result in higher levels of microplastic abundance in surface water samples (Grbić *et al*., 2020). There is evidence that water velocity affects the buildup of microplastics since lower flow rates and weaker hydrodynamics may encourage this process (Barrows *et al*., 2018). For instance, river channel centres have been found to have lower microplastic concentrations than river banks, according to research by Dris *et al*. (2018). Van Melkebeke *et al*. (2020) created conditions that allowed the formation of adequate biofilm on microplastics with a surface coverage of at least 90 %, as a result, there was an observed change in microplastics density. They reported that a spherical microplastic particle with a density of 925 kg m^{-3} and diameter of 20 μm requires a biofilm thickness of at least 18 μm to induce settling in seawater as a result of density alteration, while a similar particle with a diameter of 2 mm would require a biofilm thickness of at least 1.8 mm. However, this depends on the physicochemical parameters of water and the microbial strains (Lehaitre & Compère, 2008).

2.3 Sources of microplastics

Microplastics are divided into two types, primary and secondary. Primary microplastics such as microbeads are used in personal care products such as exfoliates in face scrubs, peelings and shower gels because of their rough-edged particles (Drohmann, 2018) as well as microfibres shed from clothing and other textiles. On the other hand, secondary microplastics are particles resulting from the breakdown of larger plastic items such as water bottles among others. However, the cycle and movement of these microplastics in the environment are not fully known, but there is a lot of research interest currently to investigate this phenomenon.

Anthropogenic activities have led to oceans being dustbins, and macro and microplastics are some of the components which are not only polluting shorelines but also freshwater bodies globally (Sharma & Chatterjee, 2017). Microplastics enter freshwater environments in several ways: primarily from surface run-off and wastewater effluent (both treated and untreated), but also from combined sewer overflows, industrial effluent, degraded plastic waste and atmospheric deposition (World Health Organization, 2019). Secondary microplastics are the most common type and are discharged through point (e.g., effluent) and diffuse (non-point, e.g., sludge) sources (Meng *et al*., 2020).

Prior to the occurrence of the COVID-19 pandemic plastic waste management was a problem. Moreover, according to Klemeš *et al*. (2020), the COVID-19 pandemic increased the production of several plastic products, mainly those needed for personal protection and healthcare, which stimulated changes in plastic waste management practices throughout the world. Personal protective equipment such as masks and gloves have been reported to be disposed of haphazardly in different environments (Fadare & Okoffo, 2020). Restriction of movement by different governments in order to control the pandemic resulted in a rise in e-commerce activities which are associated with plastic packaging likely to worsen microplastic pollution (Han *et al*., 2021).

Fibres have been documented as microplastics present in the environment with textiles being their origin (Sait *et al*., 2021). Synthetic clothing have gained popularity in recent decades. They are made from fabrics such as nylon, acrylic, rayon and polyester which have different knit gauges and techniques. Clothes made from synthetic fabric are cheap to produce and hence abundant (Samanta *et al*., 2015). Napper and Thompson, (2016) indicated that the number of fibres released from washing six (6) kg of laundry could reach more than 700,000 fibres through shedding in the washing process. Also, wastewater from a washing machine could contain 100–300 fibres per litre (Browne *et al*., 2011). Carney *et al*. (2018) measured the quantity of microfibres that were shed from synthetic textiles where acrylic, nylon and polyester materials with different knit gauges were used. All textiles shed microfibres, but polyester fleece fabrics shed the greatest amounts, averaging 7360 fibres/m^2/L in one wash, compared with polyester fabrics which shed 87 fibres/m^2/L. They found that loose textile constructions shed more, as did worn fabrics, while high twist yarns had shed reduction.

Several studies have documented the role that wastewater treatment plants play in the release of microplastics into the environment (Prata, 2018; Talvitie *et al*., 2017; Ziajahromi *et al*., 2017). The reason being microbeads are added in the manufacturing of personal care products such as shampoos, facial cleansers, and toothpaste which are discharged directly into wastewater through wastewater effluents (Kelkar, 2017; Tang *et al*., 2020). The removal of microplastics in a wastewater treatment plant depends on the treatment units that are used. However, it has been reported that microplastics could bypass the wastewater treatment plants, entering into the aquatic water bodies and finally accumulating in the environment (Sun *et al*., 2019). Michielssen *et al*. (2016) documented that wastewater treatment plants with tertiary treatment such as granular sand filtration and the membrane bioreactor exhibited high overall removal of small anthropogenic litter, with fibres being the most abundant of the small anthropogenic litter in the effluents from these plants with a percentage of (79 and 83%, respectively) than the plant with activated sludge (secondary treatment) as a final step (44% fibres).

Fishing activities, involving capture fisheries and aquaculture, are also important sources of microplastics that haven’t received a lot of attention. A lot of plastics are used in making fishing gear such as nets and ropes. Fibre plastic debris in water systems may result from mechanical abrasions while using fishing nets and ropes (Lusher *et al*., 2017).

2.4 Microplastics identification and composition

Over the last decade, different protocols have been used in the identification and determining polymer composition and to describe the particle properties, such as size, shape or colour of microplastics (Prata *et al*., 2019). However, the choice of protocol depends on the sensitivity of the microplastic polymers to the oxidizing agent (Bianchi *et al*., 2020; Pfeiffer & Fischer, 2020). The best approach therefore is trying out relevant protocols on test samples similar to the real sample. This ensures that the selected approach works for the specific sample. The analysis method should be fast, reliable and easy to handle to facilitate routine analysis of environmental samples (Käppler *et al*., 2016). The United States National Oceanic and Atmospheric Administration provides procedural advice on the laboratory methods for the analysis of microplastics in the marine environment, with recommendations on how to quantify synthetic particles in both waters and sediments (Masura *et al*., 2015).

Analytical techniques can be used to accurately determine microplastic morphology and composition, including harmonizing combinations of manual or automated Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM) or Raman micro-spectroscopy (Löder & Gerdts, 2015). For example, through vibrational spectroscopy, molecule vibrations of a microplastic sample are excited and detected, which leads to characteristic spectral fingerprints with a unique signature profile in the FTIR. Therefore, FTIR spectroscopy provides a distinct spectrum for a definite functional group. Different materials have different specific bonds, making it possible to identify an unknown material by comparing its spectrum with the spectra of known materials (Kumar *et al*., 2021; Primpke *et al*., 2018; Shim *et al*., 2017; Xu *et al*., 2019). When coupled with focal plane array detectors it provides images (Huang *et al*., 2021). It also has the ability to give information on the physiochemical weathering of microplastics by analyzing their oxidation intensity (Corcoran *et al*., 2009). Thus, making it one of the most commonly used techniques in the chemical characterization of microplastics recovered from environmental samples (Konechnaya *et al*., 2020). However, a factor such as cost limits the adoption of FTIR (Corcoran *et al*., 2009).

Raman spectroscopy is a vibrational spectroscopy technique that uses the inelastic scattering of light to give information on the molecular vibrations of a sample in the form of a vibrational spectrum. The Raman spectrum output is related to a fingerprint of the chemical structure allowing identification of the components present in the sample (Araujo *et al*., 2018). The non-destructive advantage of Raman spectroscopy enables it to be coupled with confocal laser scanning. This allows for the detection and location of (polymer) particles in organic tissues and an understanding of microplastic distribution and effects on the organisms (Zitouni *et al*., 2020). Other advantages of Raman spectroscopy include better spatial resolution, low sample size requirement, high output screening and environmental friendliness, wider spectral coverage, higher sensitivity to non-polar functional groups, lower water interference and narrower spectral bands (Anger *et al*., 2018; Huppertsberg & Knepper, 2018). However, it has disadvantages which include fluorescence interference, requires extensive sample preparation and low signal to noise ratio. Also, the use of a laser as a light source might cause sample heating, leading to background emission occasionally followed by polymer degradation (Birch *et al*., 2021).

Scanning Electron Microscopy (SEM) when used in the identification of microplastics provides extremely clear and high-magnification images of plastic particles, enabling the distinction of microplastics from organic particles (Cooper & Corcoran, 2010). Energy Dispersive X-ray Spectrometry (EDX) can be used to characterize inorganic additives in microplastic particles, this includes those that have been sorbed to the plastics and those added to plastics during manufacturing (Fries *et al*., 2013). However, when SEM is coupled with EDX, it is expensive with laborious sample preparation steps. It is also time-consuming in cases where many samples are to be analyzed (Dekiff *et al*., 2014). This study adopted ATR-FTIR spectroscopy since it is efficient in the analysis of samples obtained from the environment and it provides distinct spectral fingerprints that can be compared to a polymer reference library.

2.5 Ingestion of microplastics by fish

Many studies in different parts of the world have documented the ingestion of microplastics by fish from both marine (Naidoo *et al.*, 2016; Vendel *et al*., 2017) and freshwater ecosystems (Biginagwa *et al*., 2016; Khan *et al*., 2020; Peters & Bratton, 2016). Exposure pathways to microplastics include direct consumption by organisms such as zooplankton (Cole *et al*., 2015; Migwi *et al*., 2020), fish (Jovanović, 2017) and mussels (Digka *et al*., 2018), owing to their inability to differentiate between microplastics and prey. Active ingestion of microplastics can either be through accidental consumption, while passive ingestion can occur through ventilation such as uptake through the gills. The microplastic encounter rate can increase when a predator comes into contact with its prey (Setälä *et al*., 2018). Predators (*L. niloticus*) and detritivores (*O. niloticus*) may indirectly ingest microplastics while consuming prey or through scavenging on detrital matter that contains microplastics (Santillo *et al*., 2017). Indiscriminate omnivorous bottom feeders such as *C. gariepinus* are the most prone to microplastic ingestion (Murray & Cowie, 2011). Thus, trophic transfer occurs when predators and detritivores ingest microplastics while consuming prey or scavenging detrital matter containing microplastics (Nelms *et al*., 2018). An increase in concentrations of microplastics inevitably increase the exposure of organisms at the base of the food webs, which may also be the case at higher trophic levels (Egbeocha *et al*., 2018).

The level of microplastic uptake by an organism is influenced by several factors, such as foraging location, feeding strategies, life stage, and type of plastic in the environment. The location of foraging plays an important role in what is ingested (Schuyler *et al*., 2014). Feeding mode has been recorded to correlate to the amount of plastic ingested by fish (Battaglia *et al*., 2016; Romeo *et al*., 2015). Early life stages of fish are reported to have increased exposure to microplastics, as they dwell close to the water surface where floating microplastics concentrate or in the water column where the particles are masked by microbial communities (Le Bihanic *et al*., 2020; Prinz & Korez, 2020). Cousin *et al*. (2020) reported very small microplastic particles transferred to zebrafish and marine medaka larvae through experimental exposure to prey that had microplastics from the onset of feeding. Unicellular and plankton organisms ingest microplastics and their associated contaminants and effectively transfer these to sensitive early life stages of vertebrates, hence presenting a whole-life exposure. Ory *et al*. (2017) reported that the Amberstripe scads (*Decapterus muroadsi*) captured along the coast of Rapa Nui (Easter Island) ingested microplastics resembling their natural prey. The fish confuse blue microplastics with blue-pigmented copepods. In the Mediterranean Sea, stomach analyses from large pelagic predators of swordfish and tuna revealed that 18.5 % of the fish examined contained microplastics (Setälä *et al*., 2018). Some species commonly consumed by humans such as tuna and swordfish contain notable levels of microplastics (Rochman *et al*., 2015). Microplastics in terrestrial ecosystems are, astoundingly, less studied than marine microplastics (He *et al*., 2020). This study focused on determining microplastic levels in four common freshwater fish species, mainly *C. gariepinus*, *L. niloticus*, *O. niloticus* and *S. victoriae* due to their different feeding behaviours.

2.6 Effects of microplastics on fish

The effects of microplastics on aquatic organisms are yet to be fully understood (Santana *et al*., 2017; Shen *et al*., 2020). A review of plastic impact on fish has shown that microplastic particles cause damage leading to cellular necrosis, inflammation, and lacerations of tissues in gastrointestinal tracts according to Rochman *et al*. (2016). Microplastics could also inflict damage on organisms and cause inflammation due to their minor size and sharp ends by damaging the stomach lining. It has been observed that ingestion of tiny microplastics could cause malnutrition, blockage of the digestive tract and reduce feeding, all leading to starvation, hence affecting the well-being, especially in fish. Similarly, microplastics could cause alterations in reproduction for some organisms (Besseling *et al*., 2014; Gall & Thompson, 2015). Mattsson *et al*. (2017) reported changes in behaviour and an appetite reduction in crucian carp (*Carassius carassius*) that fed on *Daphnia magna* that had ingested nanoparticles. Additionally, the fish had morphology changes in the brain and cellular disorders. Other effects of microplastics across a range of organisms include enhanced susceptibility to oxidative stress, reduced growth, reduced ability to remove pathogenic bacteria, reduced energy reserves and balance, and decreased lysome stability (Taylor *et al*., 2016). Condition factor was used to assess the well-being of fish as an indicator of the impact of microplastic pollution.

2.7 Lake Victoria Fisheries

In the past Lake Victoria fishery was dominated by diverse haplochromine cichlids. However, multispecies fishery has undergone a decline over the past four decades, evolving into the current commercial fishery consisting mainly of Nile perch (*L. niloticus*), North African catfish (*C. gariepinus*) Nile tilapia (*O. niloticus*) and Cyprinid (*Rastrineobola argentea*) species (Njiru *et al*., 2018; Outa *et al*., 2020).

Nile tilapia (*O. niloticus*) is a commercially important fish in African lakes and is most preferred by the fishing folks (Britton & Harper, 2008). Different aspects of the biology of the species have been investigated by scientists. According to Njiru *et al*. (2006), the length-weight relationships for *O. niloticus* from different depth zones and life stages within Nyanza Gulf significantly differed. Weight increases as the length of fish increases however this varies in different species and may also be affected by changes in seasons within the year (Fulton, 1904) and water quality. Njiru *et al*. (2004) reported that Nile tilapia was initially known to be herbivorous feeding mostly on algae, however, it was later reported to have a diversified diet which includes insects, fish, algae and plant materials. Nile tilapia has been documented to have ontogenic shift in its feeding with zooplankton forming the major diet for the young tilapia whereas bigger fish have a range of food items in their diet (Outa *et al*., 2014). *O. niloticus* has a diel feeding regime indicating that it is a diurnal feeder. Diet shifts may be a result of ecological and environmental changes.

The North African catfish (*C. gariepinus*) is an omnivorous bottom feeder that consumes a wide variety of food items such as algae, macrophytes, zooplankton, insects, fish, detritus, amphibians and sand grains (Tesfaye *et al*., 2020). This species has qualities which make it suitable to adapt to different environmental conditions. These include its rapid growth, hardiness, high disease resistance, high yield potential, high fecundity, air-breathing characteristics and good market potential (Ayo-Olalusi, 2014).

Nile perch (*L. niloticus*) was introduced in Lake Victoria in 1963. However, its population increased significantly between 1980 and 1985 (Taabu-Munyaho *et al*., 2016). Nile perch are predatory fish feeding on small fish in the genera *Barbus*, *Clarias*, *Haplochromis* and macroinvertebrates (Goudswaard *et al*., 2008). A major ecological change, where it was thought that some 200 endemic haplochromine species (which previously comprised about 90% of the fish biomass) had become extinct from the lake as a consequence of Nile perch introduction (Matsuishi *et al*., 2006). Agembe *et al*. (2019) reported shifts in the Nile perch diet stating that the diet comprised *Caridina nilotica* with little contributions from haplochromines, *Rastrineobola argentea* and other fish prey.

The species Lake Victoria squeaker (*S. victoriae*) belongs to the Mochokidae family of the order Siluriformes (Catfish). They are also known as squeakers due to their ability to make stridulatory sounds through their pectoral fin spines when handled or disturbed (Friel & Vigliotta, 2006). They are omnivorous feeding on a wide spectrum of different foods. The diet of *S. victoriae* mainly comprises molluscs, crustaceans, insects, detritus and fish scales (Yongo & Wairimu, 2018).

2.8 Fish well-being

The well-being of fish is influenced mainly by abiotic and biotic factors in aquatic ecosystems which is particularly observed in physiological mechanisms and responses of organisms. Environmentally acceptable physico-chemical and microbiological characteristics in aquatic environments create favourable conditions for fish to thrive (Dragun *et al*., 2013). Compromise in the ecological status of aquatic ecosystems results in fish experiencing changes in growth, metabolism, reproduction, immune capacity and behaviour (Barton *et al*., 2002). Different approaches such as gonadosomatic index, length-weight relationship and condition factor are used to determine fish well-being (Rocha *et al*., 2021).

Condition factor measures the deviation of fish from the average weight in a given sample in order to assess suitability of biotic and abiotic interactions in a given water environment for growth of fish (Yılmaz *et al*., 2012). Juveniles of a planktivorous fish (*Acanthochromis polyacanthus*) that were exposed to microplastics the same size as their natural food particles (mean 2 mm diameter), showed no significant effect on fish growth, body condition or behaviour. While those exposed to microplastics of a reduced particle size approximately one quarter the size of the food particles (< 300µm diameter), the frequency of occurrence of microplastic particles in the gastro-intestinal tract increased. Also, when the food was replaced with microplastics, there was a negative effect on the growth and fish condition (Critchell & Hoogenboom, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was undertaken within Kisumu Bay at the Nyanza Gulf of Lake Victoria, which is a transboundary freshwater lake in East Africa shared between Tanzania, Kenya and Uganda. The Nyanza Gulf in the western region constitutes the major portion of the Kenyan part of Lake Victoria, with an area of 1920 km^2, a length of 60 km and a width varying from 6 to 30 km. The Gulf is shallow with a mean depth of 6 m and lies at an altitude of 1134 m above sea level (Njiru *et al*., 2007), Kisumu Bay is within Nyanza Gulf of Lake Victoria in Kenya. The Bay is located at latitudes 0° 05'S and 0° 09'S and longitudes 34° 42'E and 34° 45'E (Figure 3.1).

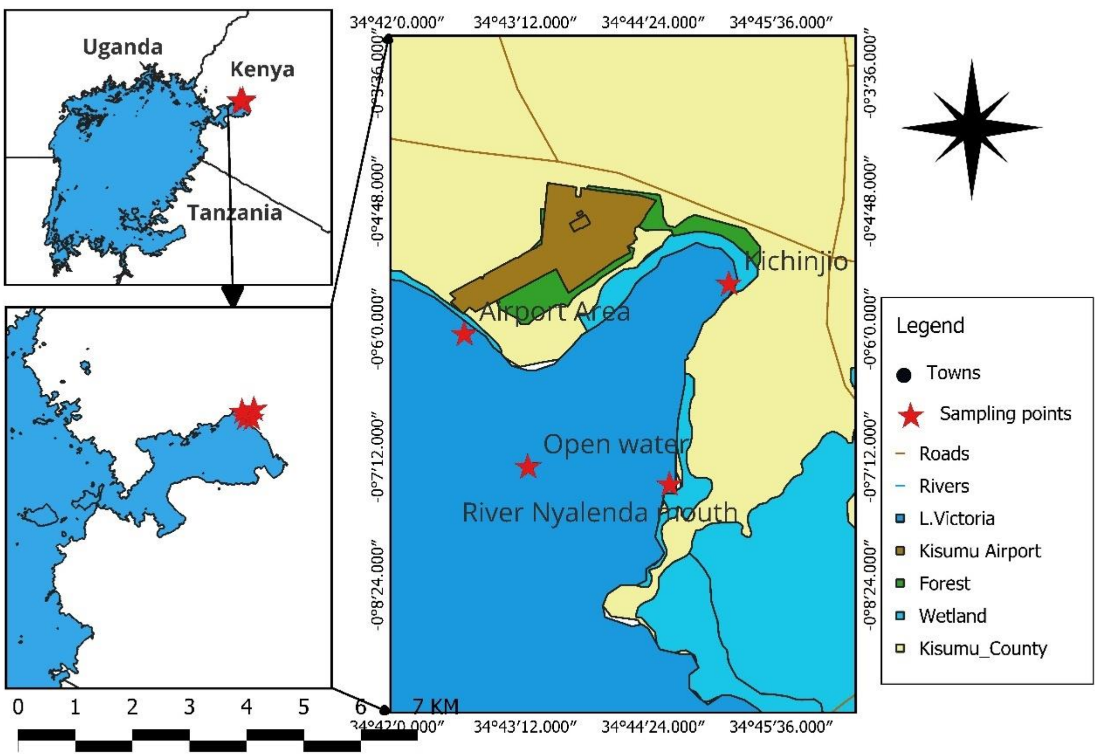

Figure 3.1: A map of Kisumu Bay showing sampling sites (Source: Adopted from the Topographical map of Kenya scale 1:50,000) (Survey of Kenya).

The average temperature in Kisumu is 23.1 °C per annum and an areal annual rainfall of up to 1966 mm (Climate-data, 2020). There are two rainfall seasons; March to June (long rain season), September to November (short rain season), followed by a relatively dry season in December to February and July to August (Misigo & Suzuki, 2018). The catchment area of the Bay is characterized by agricultural activities including rice farms, sugarcane plantations, informal settlements and industries within and around Kisumu City, including slaughterhouses, textile mills, feed mills and many small-scale industries (Adhiambo, 2017).

Description of the sampling sites

a) Inshore sites

(i) Kichinjio

This site was located at S 00° 05' 34.66" (longitude) and E 34° 45' 06.68" (latitude) near the city’s industrial area. This site was more disturbed compared to other sites through the washing and mending of fishing nets by the fishermen. The site had a lower secchi depth and exhibited increased amounts of algae growth implying high nutrient concentration at the site (Plate 3.1).

Plate 3.1: Photographs showing (a) fishermen washing and mending nets and (b) eutrophic water at Kichinjio Site

(ii) River Nyalenda Mouth

This site was located at S 00° 07' 27.55" (longitude) and E 34°44' 33.27" (latitude). The site was just after River Nyalenda drained into Lake Victoria. There was a wetland at the river’s mouth and a tourist beach adjacent to it. There was a lot of solid waste in the river before the wetland and Kisumu Water and Sanitation Company Limited discharges its treated wastewater (Plate 3.2).

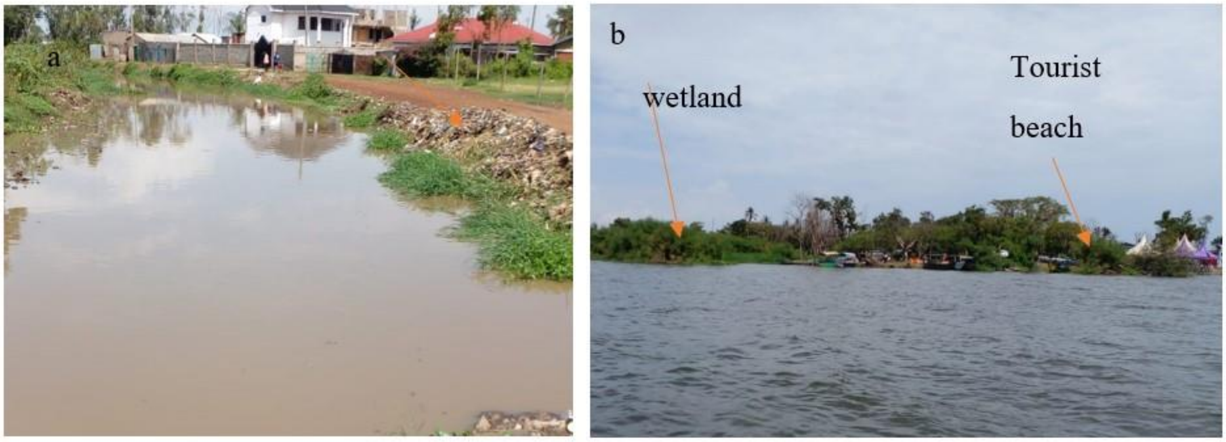

Plate 3.2: Photographs of (a) River Nyalenda before the wetland, littered with waste and (b) A tourist beach adjacent to River Nyalenda Mouth as it discharges into Lake Victoria

(iii) Airport Area

This site was located at S 00° 06' 2.93" (longitude) and E 34°43' 37.84" (latitude). The site was used to represent an area that had minimal anthropogenic activities (Plate 3.3).

Plate 3.3: Photograph showing the Airport Area site, with minimal human activities along the shoreline

b) Offshore Site

(i) Open Water

This site was located at S 00° 07' 17.65" (longitude) and E 34°43' 13.63" (latitude). The site was several kilometres from the shore representing the pelagic zone of Kisumu Bay (Plate 3.4).

Plate 3.4: A photograph from the Open Water site away from anthropogenic activities

3.2 Study design

The study adopted a purposive sampling design based on the likely entry points of microplastics into the lake. Sampling focused on locations that are adjacent to anthropogenic activities as likely entry points of microplastics into the lake and one site away from the anthropogenic activities to act as the control. Specifically, water samples were collected from four sampling sites; Kichinjio, River Nyalenda mouth, Airport area and Open water within Kisumu Bay. Fish samples were obtained within the sampling sites. Sampling was conducted three times monthly, between March and May, 2022.

3.3 Sampling

3.3.1 Measurement of water quality parameters

At each site, water quality parameters were determined at every sampling session. Electrical Conductivity (units), pH and temperature were measured using a HACH HQ 40d meter. Dissolved Oxygen was measured using a HACH HQ 30d meter while, turbidity was measured using a HACH HQ 11d meter at every sampling session. Measurements were taken in triplicate at the water surface and at one metre intervals beneath the water surface. The probes were rinsed with distilled water after use at every sampling site (APHA, 2012). A typical Secchi disk (20 cm in diameter) with two black and two white quadrants was used to estimate the Secchi depth *in situ* It was determined as the average depth at which the Secchi disk first disappeared and then reappeared in the water column by measuring in the shadowed part of the boat (Sitoki *et al*., 2010).

3.3.2 Water samples for microplastics analysis

A total of 36 water samples were collected during the three sampling sessions by taking triplicate samples at each of the four sampling sites. At each site bulk water samples were collected; 6 replicate samples were taken using a 3.7 litre plexiglass water sampler from the water surface to the bottom at sampling depth intervals of 1 metre to make a composite sample. Depth at the four sampling sites varied (Figure 3.2). The composite water samples were filtered through a series of stacked metal sieves with the following mesh sizes 45 µm, 150 µm, and 300 µm in the field (volume-reduction approach) (Plate 3.5). The sieves were then washed with deionized water. The residue and the deionized water were collected in 250 mL glass bottles and fixed with 5% formalin (Ding *et al*., 2019). The water samples collected were transported in ice-cool boxes to the Egerton University, Limnology and Wetland Management Programme laboratory for microplastics identification and ATR - FTIR analysis of microplastics at Chemistry Department Laboratory at the United States International University Africa.

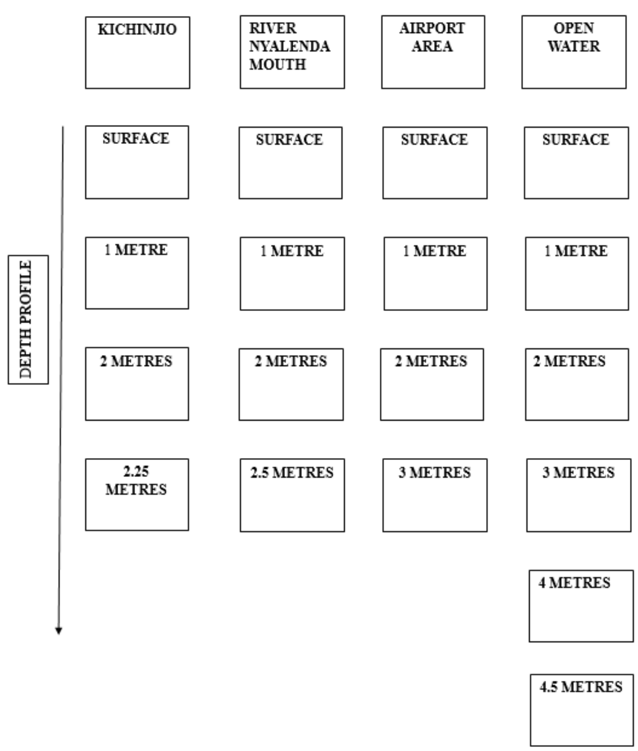

Figure 2.2: A Schematic diagram showing the depth profile at different sites indicating depths at which samples were collected in replicates

Plate 3.5: A photograph showing (a) water sampling with a plexiglass water sampler and (b) water filtration through stacked metal sieves

3.3.3 Fish sampling and determination of fish well-being

A total number of 95 fish samples were collected during three sampling sessions at the four sampling sites. Samples of the four fish species were caught using 3 and 6 inch gill nets set overnight at each sampling site with assistance from local fishermen. The fish were killed humanely as per stunning (Brijs *et al*., 2021). Total Lengths (TL) in cm were measured using a measuring board and the weights were measured in grams using a weighing scale at the beach site. Length-weight data was used to calculate the length-weight relationship (LWR) using the following equation as described by Froese (2006);

$$W = aL^b \tag{1}$$

Where:

W is weight in grams

a is a constant

b is the allometry coefficient

L is the total length in centimetres

Condition factor (K) was determined as described by (Eberhardt & Ricker, 1977) using the following equation;

$$K = W/L^3 \quad (2)$$

Where;

W is the weight

L is the length

The value of exponent b in equation 1 provides information on growth dimensions or an interpretation of individual well-being. When b = 3, increase in weight is isometric and if the value of b $\neq$3, weight increase is allometric. When b is less than 3, fish become slimmer with increasing length, and growth will be negatively allometric. When b is greater than 3.0, fish become heavier showing a positive allometric growth and reflecting optimum conditions for growth. This relationship is a useful tool that provides important information regarding the structure and function of fish populations (Connor *et al*., 2017; Guissé & Niass, 2021). While for the condition factor an overall fitness for fish species is assumed when K values are equal or close to 1 in equation 2.

The fish samples were then injected with 10 mL of 4% formalin in the abdominal region in order to neutralize the digestive action at the beach. Each whole fish was wrapped in aluminium foil and put in a cool box as per Silva-Cavalcanti *et al*. (2017) and transported to the Egerton University Biological Sciences Department Limnology and Wetland Management (LWM) laboratory for analysis.

3.4 Determination of microplastics in water samples

At the laboratory, water samples were refrigerated at 4°C prior to analysis. The water samples were sieved through a stacked arrangement of sieves in the following order of mesh sizes; 45 µm, 150 µm and 300 µm and rinsed thoroughly using distilled water to rinse the formalin. A dry and acid washed 500 mL beaker was weighed to the nearest 0.1 mg and the solids collected in the sieves were transferred into the beaker using a spatula which was rinsed using a squirt bottle containing distilled water for each sample. The beaker was placed in the oven at 40°C for 24 hours or longer until the sample dried up. To determine the mass of the total solids, the beaker with dried solids was weighed using an analytical balance to the nearest 0.1 mg. A sample of distilled water was similarly subjected to all analysis tests to as a control and assisted in monitoring the presence of any plastic contaminants from laboratory clothing, laboratory air, improper cleaning, squeeze bottles, or gloves.

Immediately the dried solids were subjected to Wet Peroxide Oxidation (WPO) by adding 20 mL of aqueous 0.05 M Fe (II) solution in the beaker containing solids, followed by 20 mL of 30% hydrogen peroxide. The mixture was left to stand on a laboratory bench at room temperature for five minutes prior to the next step. A stir bar was put in the beaker and covered with a watch glass. The mixture was heated to 75°C on a hotplate. If natural organic material were still visible, another 20 mL of 30% hydrogen peroxide was added. This was done until there was no natural organic material visible. Six (6) g of sodium chloride (NaCl) per 20 mL of sample was added to increase the density of the aqueous solution. The mixture was further heated to 75°C until the NaCl had dissolved. After cooling the solution was transferred to a separating funnel (Plate 3.6). The beaker which contained the WPO solution was rinsed with distilled water and all remaining solids were transferred to the separating funnel. The separating funnel was covered loosely with aluminium foil. The solids were allowed to settle overnight and the settled solids drained from the separating funnel. The floating solids were collected in a Whatman GF/C filter paper (0.45 µm) through vacuum filtration. The separating funnel was rinsed several times with distilled water during filtration. The Whatman GF/C filter paper (0.45 µm) with the solids was transferred to an acid washed glass petri dish, covered with aluminium foil and oven-dried at 40 °C for 24 hours and stored in a desiccator awaiting microscopic identification (Migwi *et al*., 2020).

An empty 4mL glass vial was acid washed, dried and labelled. A dissecting microscope at x40 magnification was used to identify microplastics from the Whatman GF/C filter paper. Using forceps, the identifiable microplastics were transferred to the glass vial. The number of microplastic particles were counted and quantified as the number of particles per litre of water sampled (Masura *et al*., 2015).

Plate 3.6: A photograph of a separating funnel used to separate microplastics through density separation

3.5 Determination of microplastics in fish samples

At the laboratory, the fish species were refrigerated at 4°C and the gastrointestinal tracts of each fish removed within 24 hours from the sampling time. The dissection involved the removal of the entire gastrointestinal tract which includes the oesophagus, stomach and intestines (Lusher *et al*., 2017). This procedure was done in lamina flow hood to reduce the risk of contamination from the working environment and was completed as soon as possible. The dissecting utensils were thoroughly cleaned with 70% ethanol and lint free paper used between dissections to avoid sample contamination (Khan *et al*., 2020). Gastrointestinal tracts and their contents were then rinsed with distilled water through a 50 µm mesh sieve, to drain the formalin solution before processing.

Dissected tissues were placed in 250 or 500 mL conical flasks, and the gut weight was measured using a digital weighing balance (Silva-Cavalcanti *et al*., 2017). Potassium hydroxide (KOH, 10%) was added to the dissected tissues placed in 250 or 500 mL conical flasks, in a 5:1 ratio (500 mL acid to 100 g tissue). The fish tissues were then digested by incubating in 10% KOH in an oven at 40°C overnight (Lusher *et al*., 2017).

Post digestion, the samples underwent density separation using a separating funnel as described in section 3.4, where lighter microplastics were expected to float on the surface of the saline solution. The floating plastic debris was collected from the separating funnel using 0.45µm Whatman GF/C filters, with the walls of the filtering device washed multiple times with pre-filtered distilled water. The filter papers were transferred into clean petri dishes, covered with aluminium foil and oven-dried at 40 °C for 24 hours and stored in a desiccator awaiting microscopic identification (Migwi *et al*., 2020).

An empty 4mL glass vial was acid washed, dried and labelled. Microplastic particles were identified under a light dissection microscope at x40 magnification. Using an ocular micrometre attached to the microscope's eyepiece, the length of the identified plastic particles was determined from their largest cross-section (Merga *et al*., 2020). Forceps were used to collect identifiable microplastics from the Whatman GF/C filter paper to the glass vial. The number of microplastic particles were counted and quantified as the number of particles in each gastrointestinal tract. Images were taken using a MotiCam digital camera mounted on the microscope.

The percentage frequency of occurrence of microplastics within digestive tracts was calculated, following the equation by Pegado *et al*. (2018).

$$FO\,\% = (Ni\,/\,N) \times 100 \tag{4}$$

Where:

FO % = frequency of occurrence of microplastic particles

Ni = number of gastrointestinal tracts that contained microplastic particles

N = total number of gastrointestinal tracts examined

3.6 Determination of polymer composition

The chemical composition of all suspected plastics was identified non-destructively using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy. The analysis was done on an FT/IR-4700 type A Spectrometer (equipped with a diamond crystal ATR accessory unit (ATR PRO ONE)) (Plate 3.7). Background measurements were performed on the blank sample carrier before each sample measurement. The MP particles were placed onto the ATR crystal using forceps. The analyses were carried out in % transmittance mode and the spectrums were collected in 50 scans (4 cm^{-1} resolution, 549.613 – 4000 cm^{-1} wavenumber range) (Scopetani *et al*., 2020). The spectra readings were corrected by subtracting the background measurements of a blank sample which greatly reduces inaccuracies in the identification of polymers (Su *et al*., 2020). The measurements were processed using the Know It All software. The spectra obtained from the unknown microplastic samples were compared against spectra from the Know It All polymer reference library to determine their polymer origin.

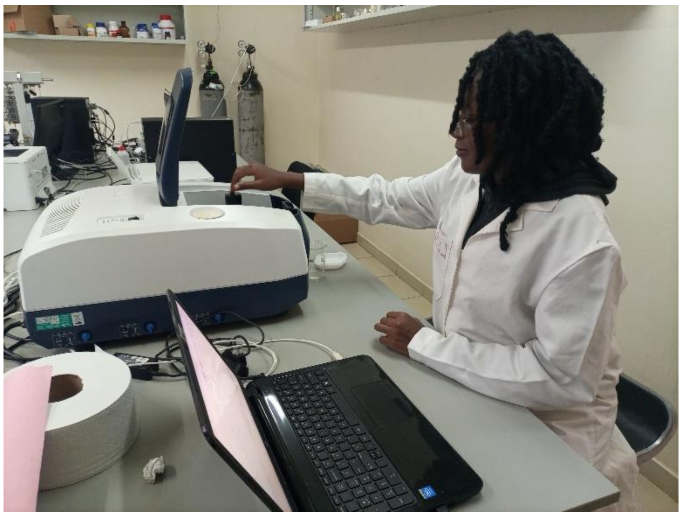

Plate 3.7: A photograph showing ATR-FTIR analysis in the laboratory

3.7 Quality control

To avoid contamination, cotton laboratory coats were worn during laboratory procedures. A lint roll was used to remove excess fibres from the laboratory coats. Laboratory benches were thoroughly cleaned with methylated spirit prior to procedures and lined with fresh clean brown paper. Glass beakers were used, rinsed with distilled water and covered with aluminium foil to prevent contamination prior to use. Blank samples containing only filtered distilled water were processed alongside test samples, which were treated identically. The samples were immediately covered with aluminium foil when they were not being processed. All the experimental procedures were done in the lamina flow hood to prevent environmental contamination and the process was completed as soon as possible. The blanks were analyzed by ATR-FTIR. Chips of all plastic equipment used were subjected to ATR-FTIR analysis to determine their polymer composition. These were compared with the results obtained to identify the presence of contamination.

3.8 Data analysis

All data collected was stored in Microsoft Office Excel. Statistical tests were done using R statistical software version 3.6.3 and SPSS version 25. The normality of the data was tested using the Shapiro-Wilk test and the homogeneity of variance using Levene's test, wherever the data was normally distributed, a parametric test was conducted. Descriptive statistics were used to present the average length and weight of the four fish species and the water quality parameters. Principal component analysis was used to determine the relationship between microplastics and physico-chemical parameters. Mood's median test was used to test differences in the concentration of microplastics in water at different sites. Binary logistic regression was used to determine the effects of fish species and sites on the occurrence of microplastics. A Kruskal-Wallis test, was employed to determine the variability in microplastic abundance in fish guts across different sampling sites and species. Polymer identification was performed by comparing the measured spectra (549.613 – 4000 cm^{-1}) with the reference spectra. A renowned Sadtler™ libraries reference database and Know It All software (https://sciencesolutions.wiley.com) were used for comparison. Correlation analysis and bivariate plots were used to show the relationship between fish length and fish weight, fish length and microplastic numbers and fish weight and microplastic numbers. All statistical tests were done at a significance level (α) of 0.05.

CHAPTER FOUR

RESULTS

4.1 Water quality parameters

The site's water quality parameters are presented in (Table 4.1). The levels of dissolved oxygen varied from (5.43 ± 0.33 mg/L) at the Kichinjio site to (5.85 ± 0.26 mg/L) at the River Nyalenda Mouth. The Open water site had the lowest temperature (27.30 ± 0.51°C), whereas the Kichinjio site had the highest (27.97 ± 0.70°C). There were significant differences in mean temperature levels across the different sampling sites $p < 0.05$ (One-way ANOVA, Temp ($F_{3,32} = 2.965$, $p = 0.047$). The Tukey test indicated that the temperature at the Open water site were significantly lower than those at the Kichijio and Airport Area sites ($p < 0.05$). Electrical conductivity showed different trends across all the sampling sites with a slight decrease towards the Open water site (103.80 µS/cm). The Kichinjio site recorded the greatest mean electrical conductivity (175.13 ± 2.39 µs/cm). Mean water electrical conductivity differed significantly among the sites (Kruskal-Wallis, H= 30.113, df=3, $p < 0.001$). A post hoc Dunn's test revealed a statistically significant difference between three pairs: Open water and River Nyalenda Mouth, Open water and Kichinjio, and Airport Area and Kichinjio ($p < 0.05$). Airport Area point and Kichinjio site had the lowest and highest pH readings, respectively, and the pH ranged from 7.09 to 7.99. Water pH was not significant among the sites (Kruskal-Wallis, H=4.525, df=3, p=0.210). The mean dissolved oxygen in water had significant differences among the sites (One-way ANOVA, DO ($F_{3,32} = 3.114$, $p = 0.04$). The Tukey test indicated that the DO levels at the Airport Area site were significantly higher than those at the Kichijio site ($p < 0.05$). Significant differences were observed in mean secchi depth levels across the different sampling sites (Kruskal-Wallis, H=20.939, df=3, $p < 0.005$). A post hoc Dunn's test revealed a statistically significant difference between two pairs: Kichinjio and River Nyalenda Mouth, and Open water and Kichinjio ($p < 0.05$).

Table 4.1: Mean ± SD of water quality parameters values at the different sites within Kisumu Bay

Parameter		Airport Area	Open Water	R. Nyalenda Mouth	Kichinjio
Total Depth (m)		3.00	4.50	2.25	2.50
Dissolved Oxygen (mg/L)	Mean	5.72 ± 0.36	5.72 ± 0.17	5.85 ± 0.26	5.43 ± 0.33
	Range	5.39 - 5.89	5.38 - 5.90	5.08 - 5.99	5.06 - 5.88
Temperature (°C)	Mean	27.88 ± 0. 52	27.30 ± 0.51	27.41± 0.35	27.97± 0.70
	Range	27.3 - 28.60	26.8 - 28.1	26.9 - 28.1	26.8 - 28.6
Electrical Conductivity (µS/cm)	Mean	113.67 ± 2.70	106.04± 1.99	173.40 ± 3.86	175.13 ± 2.39
	Range	110.30 - 115.70	103.80 - 109.10	170.10 - 179.5	171.70 - 178.20
pH	Range	7.12 - 7.35	7.09 -7.99	7.10 - 7.83	7.09 - 7.94
Secchi Depth (m)	Mean	0.60 ± 0.12	0.79 ± 0.13	0.66 ± 0.07	0.51 ± 0.03
	Range	0.49 - 0.71	0.65 - 0.96	0.57 - 0.77	0.47 - 0.58

4.2 Levels of microplastics in water and different fish species

4.2.1 Levels of microplastics in water

The sites had different concentrations of microplastics ranging between 0.85 - 2.41 particles/litre. The highest concentration of MPs (particles/litre) was recorded at the Kichinjio site with the lowest at the Open water site (Figure 4.1). However, the concentration of microplastics differed significantly among the sites (Mood's median test, χ^2 = 18,22, df=3, p < 0.05) (Appendix A). Kichinjio site had the highest count of MPs, whereas the Open water site had the lowest count of MPs (Table 4.2).

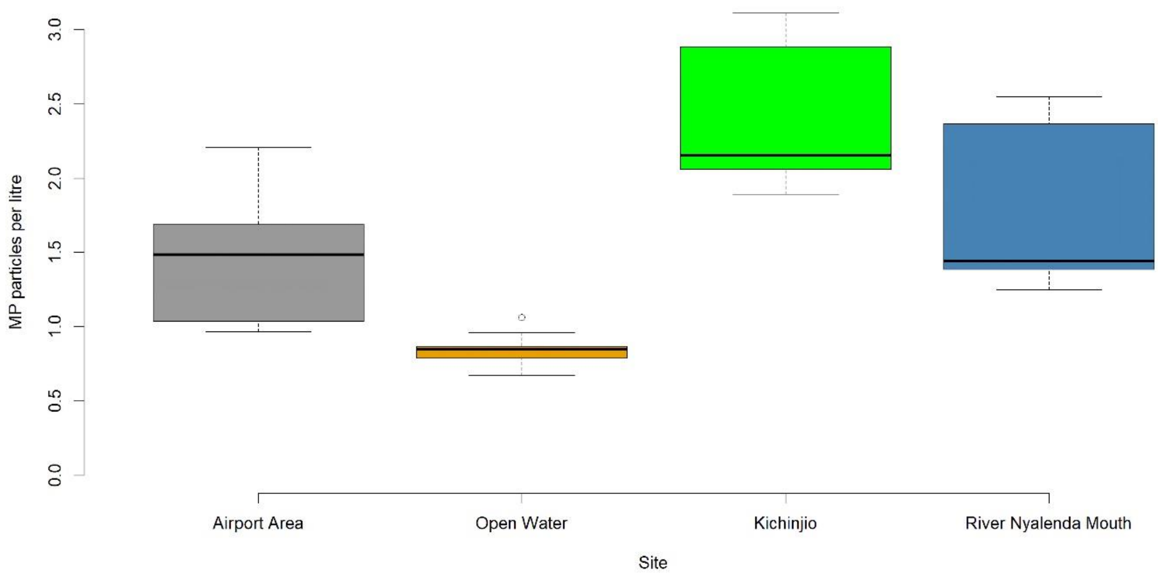

Figure 43.1: Box-whisker plots showing the concentration of MPs particles ± SD at each site. The outlier is represented by a circle, the median is represented by the horizontal line inside each box, and the maximum and minimum values are indicated by the whiskers

Table 4.2: Depth and concentration (particles/litre) of microplastic particles at the different sites within Kisumu Bay

Parameter	Airport Area	Open Water	R. Nyalenda Mouth	Kichinjio
Depth (m)	3.00	4.50	2.50	2.25
Mean MPs ± SD	129.89 ± 36.91	113.22 ± 15.02	154.33 ± 47.22	214.00 ± 41.07
Water filtered (Litres)	88.80	133.20	88.80	88.80
MPs particles per litre ± SD	1.46 ± 0.41	0.85 ± 0.11	1.74 ± 0.53	2.41± 0.46

4.2.2 Levels of microplastics in fish

Out of the 95 fish sampled 62 had MPs occurring in their gastrointestinal tracts, translating to a 65.26% frequency of occurrence (FO). A total of 421 suspected MPs were detected in all fish samples. The average abundance of MPs in the gastrointestinal tract of fish sampled was 3.28 ± 4.11 particles/fish for *C. gariepinus* (FO = 75.00%), 2.00 ± 1.70 particles/fish for *S. victoriae* (FO = 75.00%), 3.33 ± 3.81 particles/fish for L. niloticus (FO = 71.43%) and 5.57 ± 8.87 particles/fish for *O. niloticus* (FO = 59.26%). The most MPs retrieved from a single fish were 36 particles in *O. niloticus*. The detection of MPs in all four fish species shows they were susceptible to microplastic ingestion. The mean number of MPs varied for each fish species at different sampling sites (Table 4.3). The MP counts between the different species at River Nyalenda Mouth had no statistically significant differences (Kruskal-Wallis, H= 1.96, df=3, $p > 0.05$). There was no significant difference in the MP counts between the different species at the Open Water site (Kruskal-Wallis, H= 0.91, df=3, $p > 0.05$). The MP counts between the different species at the Kichinjio Site had no statistically significant differences (Kruskal-Wallis, H= 1.80, df=1, $p > 0.05$). There was no significant difference in the MP counts between the different species at the Airport Area (Kruskal-Wallis, H= 3.82, df=3, $p > 0.05$). The MP counts between sites for *O. niloticus* had no statistically significant differences (Kruskal-Wallis, H= 7.28, df=3, $p > 0.05$). The MP counts between sites for *L. niloticus* had statistically significant differences (Kruskal-Wallis, H= 188 6.03, df=2, $p < 0.05$). A post hoc Bonferroni test revealed a statistically significant difference between Open Water and River Nyalenda Mouth sites (Test Statistic = -8.800, Adj. Sig. = 0.043). The MP counts between sites for *C. gariepinus* had no statistically significant differences (Kruskal-Wallis, H= 2.64, df=3, $p > 0.05$). The MPs between sites for *S. victoriae* had no statistically significant differences (Kruskal-Wallis, H= 2.74, df=2, $p > 0.05$).

Table 4.3: Microplastics frequency in relations/and fish characteristics at different sites within Kisumu Bay

Species	**Sites**	**Sample Size (n)**	**Mean Length (cm) ± SD**	**Mean Weight (g) ± SD**	**Condition Factor (K)**	**Total Number of MPs Found**	**Frequency of Occurrence (FO%)**
O. niloticus	River Nyalenda Mouth	15	21.02 ± 2.64	177.35 ± 76.61	1.82 ± 0.17	**128**	73.33
	Kichinjio	14	18.58 ± 3.14	126.80 ± 81.13	1.78 ± 0.16	65	50
	Open Water	5	17.26 ± 2.06	103.29 ± 38.50	1.94 ± 0.05	**44**	60
	Airport Area	20	17.96 ± 1.88	104.42 ± 33.19	1.76 ± 0.18	64	55
L. niloticus	River Nyalenda Mouth	5	25.68 ± 2.20	191.74 ± 47.17	1.12 ± 0.10	**10**	80
	Kichinjio	7	26.87 ± 3.51	218.68 ± 77.50	1.09 ± 0.12	27	71.43
	Open Water	9	23.63 ± 4.14	169.40 ± 90.22	1.23 ± 0.27	**33**	77.78
C. gariepinus	River Nyalenda Mouth	4	26.45 ± 6.88	142.13 ± 105.24	0.67 ± 0.04	17	75

Species	Sites	Sample Size (n)	Mean Length (cm) ± SD	Mean Weight (g) ± SD	Condition Factor (K)	Total Number of MPs Found	Frequency of Occurrence (FO%)
	Kichinjio	1	54.5	1090.11	0.67	**4**	100
	Open Water	1	43.5	524	0.63	3	100
	Airport Area	2	46.63 ± 18.98	875.92 ± 880.94	0.69 ± 0.0005	**2**	50
S. victoriae	River Nyalenda Mouth	5	17.36 ± 0.89	51.31 ± 5.22	0.98 ± 0.05	**13**	100
	Open Water	3	16.77 ± 0.60	46.81 ± 11.13	0.98 ± 0.14	7	100
	Airport Area	4	16.50 ± 0.95	47.25 ± 7.72	1.05 ± 0.07	**4**	25

4.2.3 Characterization of Microplastics in fish and water

The recovered microplastics were classified into four main categories; fibres, films, fragments and pellets. The fibres and fragments were present in all four sampling sites while pellets and films were not identified. The number of microplastic fibres differed significantly among the sites (Mood's median test, $\chi^2 = 14.67$, df=3, $p = 0.002$). However, there were no significant differences in the mean number of fragments identified in the four sites (One-way ANOVA, Fragments ($F_{3,32} = 1.282$, $p = 0.297$) (Figure 4.2). The mean number of fibres differed significantly among the sites (Kruskal-Wallis, H= 16.24, df=3, $p = 0.001$). A post hoc Bonferroni test revealed a statistically significant difference between two pairs: Open water and Kichinjio sites (Test Statistic = 18.77, Adj. Sig. = 0.001) and Airport Area and Kichinjio sites (Test Statistic = -15.33, Adj. Sig. = 0.012).

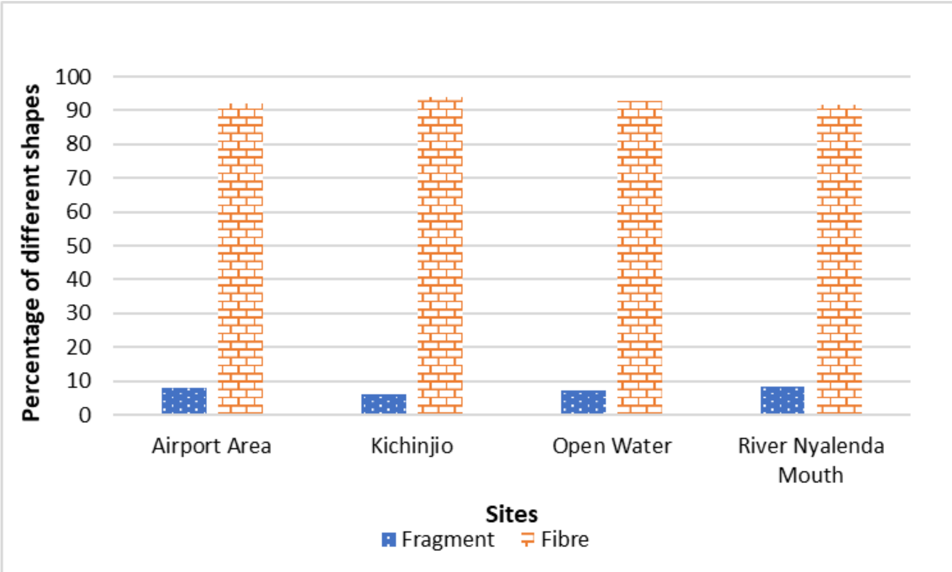

Figure 4.2: The percentage of different shapes of microplastics in water from four sites within Kisumu Bay, Lake Victoria

The most common colours of microplastics in water were clear with Open water having the highest percentage (66.63%). Green and black colours had the least percentage at Kichinjio (4.04%) and Open water (4.51%) sites respectively (Figure 4.3).

The shapes of the recovered MPs were only fibres and fragments. The Mann-Whitney U test was used for each species to compare the ingestion rates of fibrous and fragmented MPs. The results consistently showed a statistically significant difference between the ingestion rates of the two MP shapes, with fibrous MPs exhibiting a higher mean rank, indicating a greater affinity for ingestion than fragmented MPs. Specifically, the U values and associated p-values were: (8.00, $p < 0.05$) *C. gariepinus*, (101.00, $p < 0.05$) *L. niloticus*, (644.00, $p < 0.05$) *O. niloticus* and (18.00, $p < 0.05$) *S. victoriae*. Fibres were the most dominant shapes identified in fish (Figure 4.4).

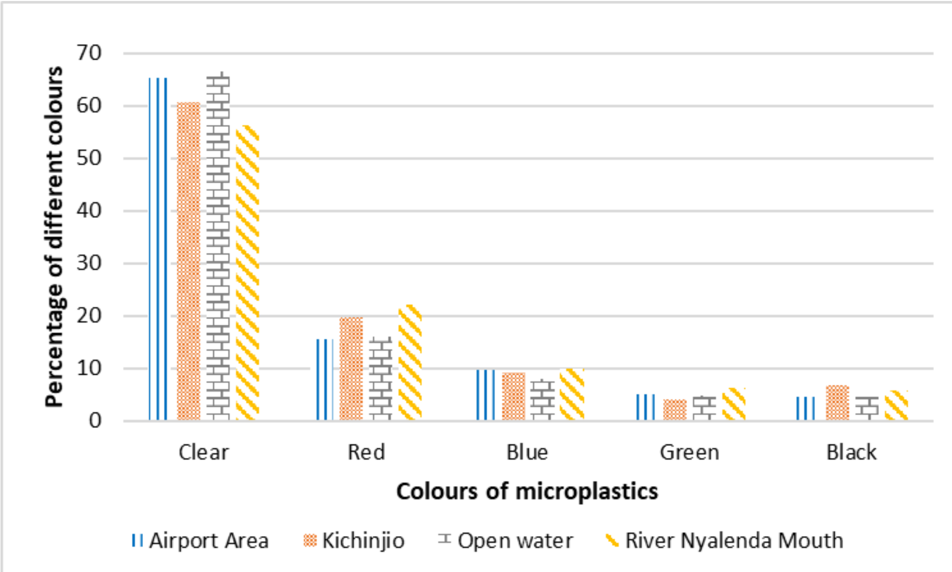

Figure 4.3: The percentage of different colours of microplastics in water from four sites within Kisumu Bay, Lake Victoria

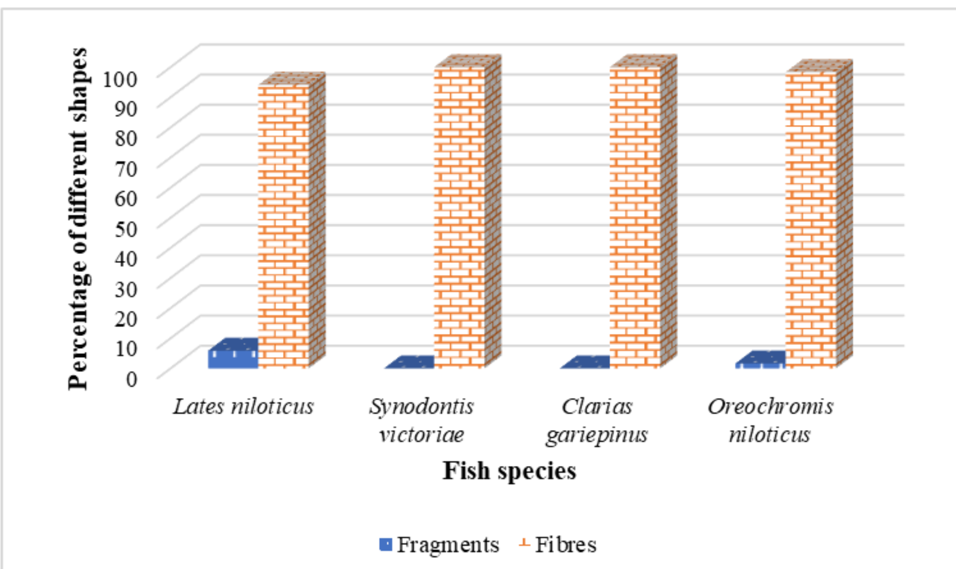

Figure 4.4: The percentage of different shapes of microplastics in the four fish species of Lake Victoria

Clear and coloured MPs were identified in fish, however, the majority of the particles were clear with small fractions of red, black and green coloured particles (Figure 4.5). There were no statistically significant differences in MP counts between colours of *C. gariepinus* (Kruskal-Wallis, H=12.78, df=4, $p > 0.05$). The MP counts in *L. niloticus* varied significantly by colour

(Kruskal-Wallis, H= 14.43, df=4, $p < 0.05$). A post hoc Bonferroni test revealed a statistically significant difference between one pair: green and clear (Test Statistic = 25.07, Adj. Sig. = 0.005). There was a statistically significant difference in the number of MPs across different colours for *O. niloticus* (Kruskal-Wallis, H= 19.61, df=4, $p < 0.05$). A post hoc Bonferroni test revealed a statistically significant difference between two pairs: green and clear (Test Statistic = 51.96, Adj. Sig. = 0.0001) and blue and clear (Test Statistic = 12.22, Adj. Sig. = 0.015). The MP counts between colours for *S. victoriae* had statistically significant differences (Kruskal-Wallis, H= 17.39, df=4, $p < 0.05$). A post hoc Bonferroni test revealed a statistically significant difference between three pairs: blue and clear (Test Statistic = -19.37, Adj. Sig. = 0.004), green and clear (Test Statistic = 19.37, Adj. Sig. = 0.004) and red and clear (Test Statistic = 17.08, Adj. Sig. = 0.02). Most of the MP particles identified from the gastrointestinal tracts were clear. A proportion of 16% of the MPs recovered from the fish were measured, and the size ranged between 70-1500 µm. Fish fed on a smaller size range than what was identified in the surface water.

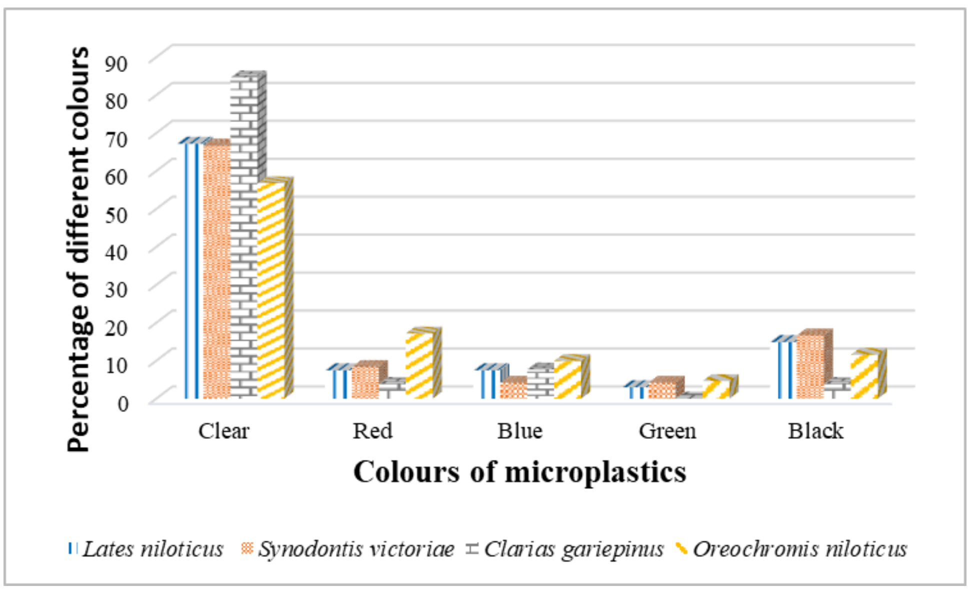

Figure 4.5: The percentage of different colours of microplastics found in the four fish species

The size of microplastics in fish ranged between 70-1500 µm. Microplastic particles in water ranged between 60 -3000µm (Plate 4.1).

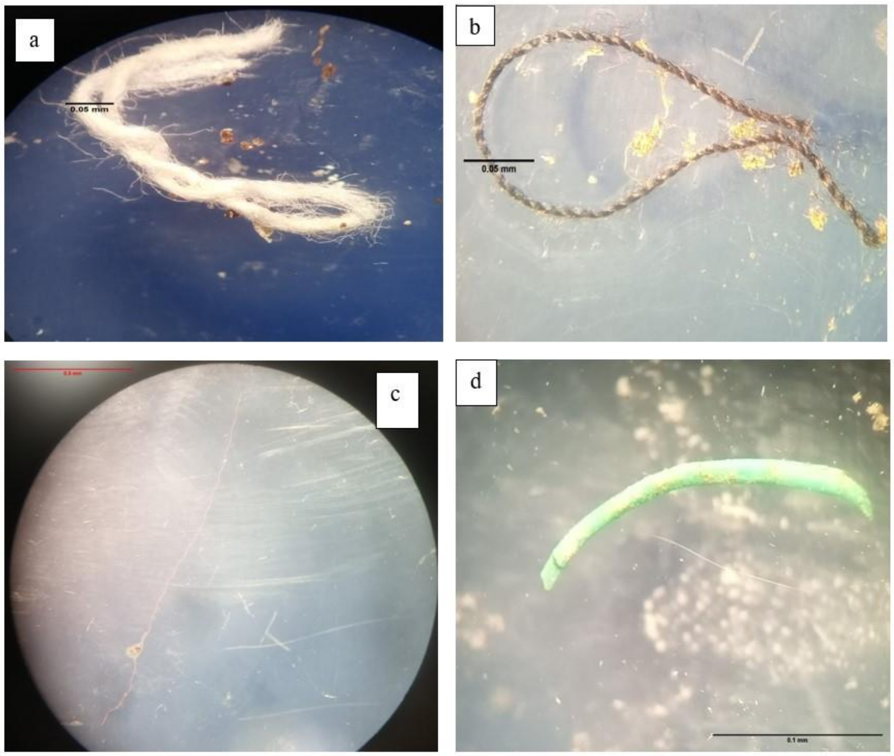

Plate 4.1: Selected microplastic particles recovered from (a, b & d) water (c) *L. niloticus* (Leica Zoom 200 compound microscope)

4.2.4 Relationship between environmental variables and microplastics abundance

Significant water quality parameters were indicated by the greatest score of the loading variable on the component axis in the principal component analysis plot. The plot displayed two principal components (PC), Component 1 (66.83%) and Component 2 (27.66%), on the x-axis and y-axis respectively. The environmental parameters included in the analysis were pH, Electrical Conductivity (EC), Temperature (Temp), Dissolved Oxygen (DO), Secchi depth, and the number of microplastics. Sampling locations were presented as points on the plot, including Open water, River Nyalenda Mouth, Airport Area, and Kichinjio (Figure 4.6).

The biplot illustrates the relationships between the environmental parameters and the sampling locations. The vectors indicate the direction and magnitude of each parameter's contribution to the principal components. For instance, pH (0.21) and EC (0.42), had a strong positive correlation with Component 1, while DO (-0.47) and Secchi depth (-0.46) showed a negative correlation with Component 1. Temperature (-0.61) had a strong negative correlation with Component 2. This suggested that PC1 primarily represented a gradient of water quality influenced by microplastic contamination and conductivity.

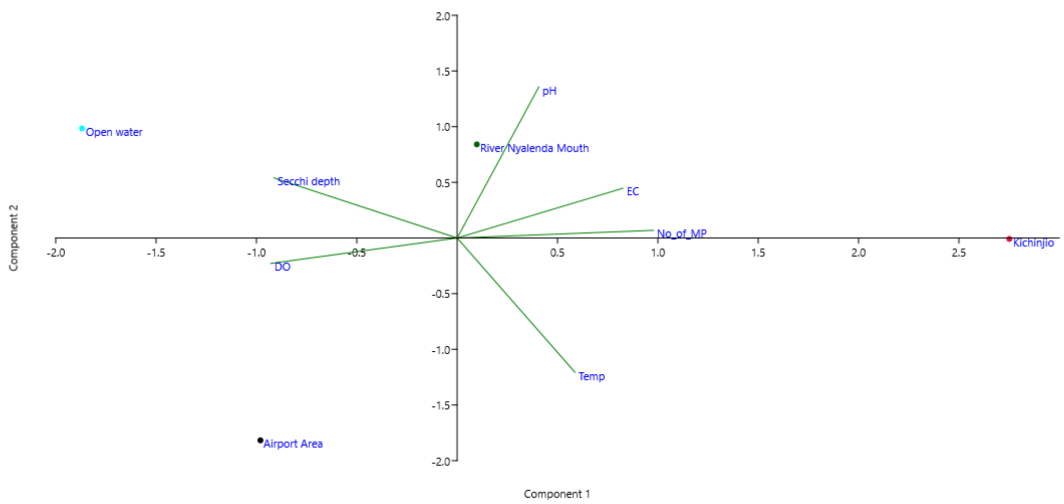

Figure 4.6: A principal component analysis (PCA) biplot showing the influence of environmental variables on microplastics abundance in the four study sites. The plot's x-axis displays Component 1 (PC1) and y-axis displays Component 2 (PC2).

4.3 Identification of the chemical composition of microplastics in fish and water

ATR-FTIR spectroscopy was used to determine the proportions of the recovered microplastics. In fish, where a total of 421 particles were recovered, 25% were analyzed and yielded Polystyrene(n=79) and Poly (perfluorobutadiene) (n=26) (Figure 4.7).

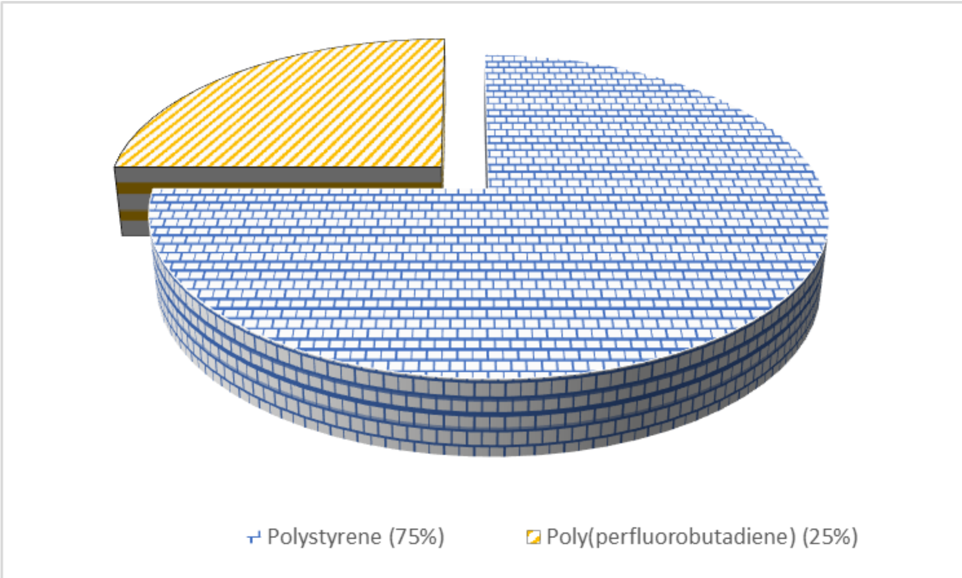

Figure 4.7: Percentage proportions of microplastic polymers recovered from fish in Kisumu Bay, Lake Victoria

The spectra obtained from the samples were linked with reference spectra in the Sadtler™ libraries reference database and yielded PS (Figure 4.8) and Poly (perfluorobutadiene) (Figure 4.9). There are two lines on the graph: (a) a red line representing the polymer from the reference library while (b) a black line represents the sample that was analyzed. Figures 4.8, 4.9, 4.11 and 4.12 show how the transmittance varies with the wavenumber for both the reference polymer and the samples. The peaks and troughs in the graph indicate the absorption of specific wavelengths of infrared light, which correspond to different molecular vibrations (fingerprints) within the samples.

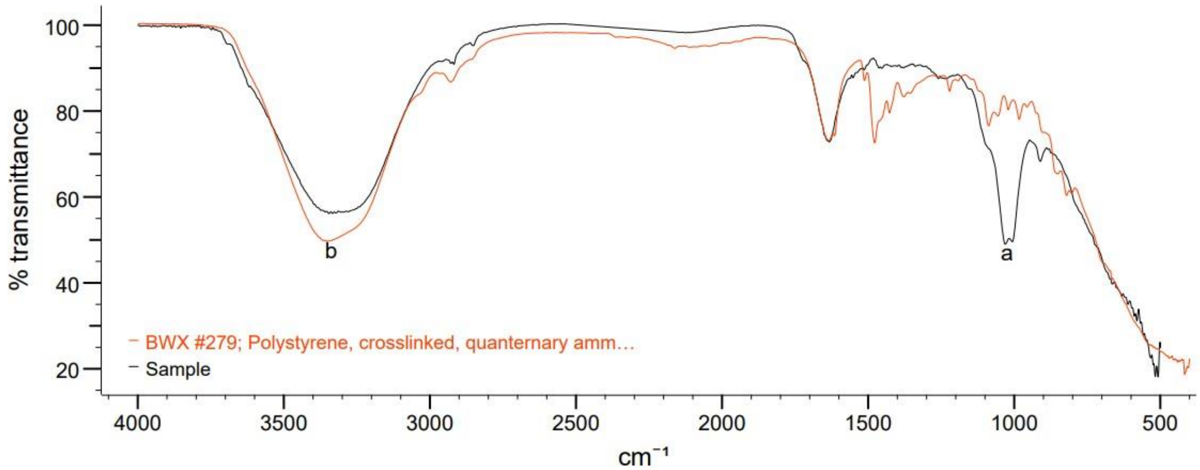

Figure 4.8: ATR-FTIR spectra for (a) sample (b) the reference Polystyrene (PS) spectra from the Sadtler™ libraries reference database spectra library. Spectral match; 84.49%

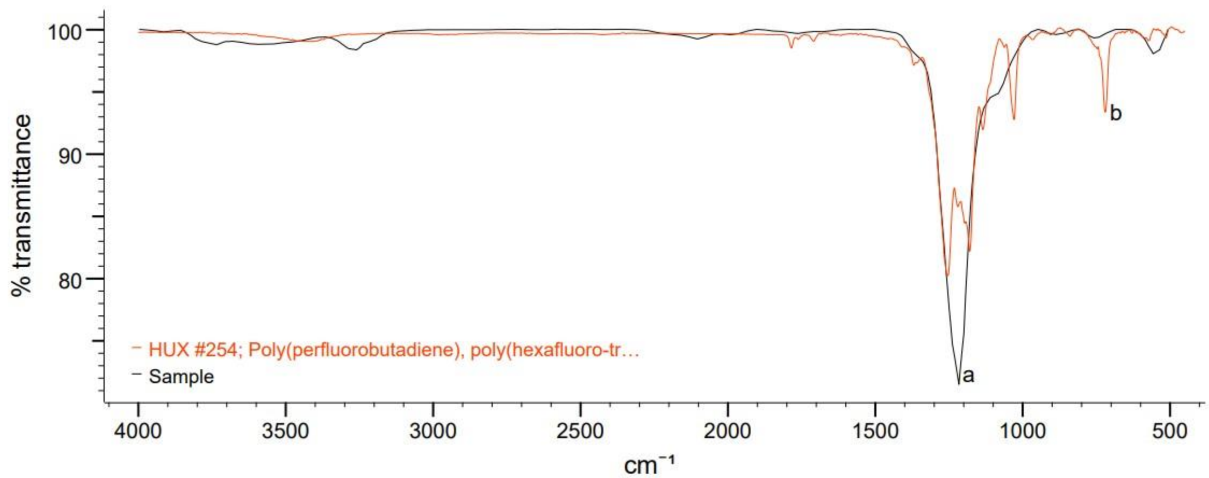

Figure 4.9: ATR-FTIR spectra for (a) sample (b) the reference Poly (perfluorobutadiene) spectra from the Sadtler™ libraries reference database spectra library. Spectral match; 81.07%

In surface waters, where a total of 5503 particles were recovered, 25% were analyzed via ATR-FTIR and yielded Poly(perfluorobutadiene) (n=1100) and Poly (vinylidene fluoride-co-hexafluoropropylene) (n=275), (Figure 4.10). The spectra obtained from the samples were linked with reference spectra in the Sadtler™ libraries reference database and yielded Poly (vinylidene fluoride-co-hexafluoropropylene) (Figure 4.11) and Poly (perfluorobutadiene) (Figure 4.12).

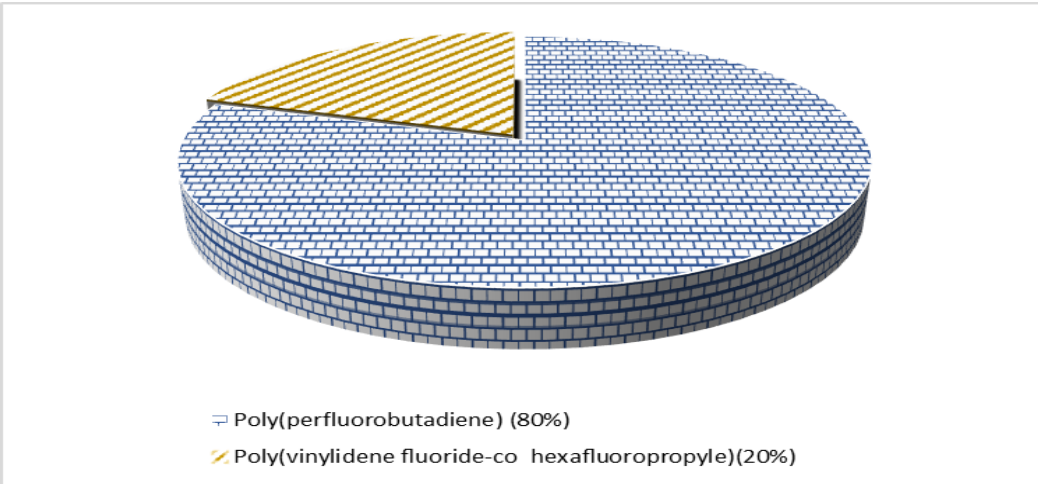

Figure 4.10: Percentage proportions of microplastic polymers recovered from Kisumu Bay, Lake Victoria surface waters

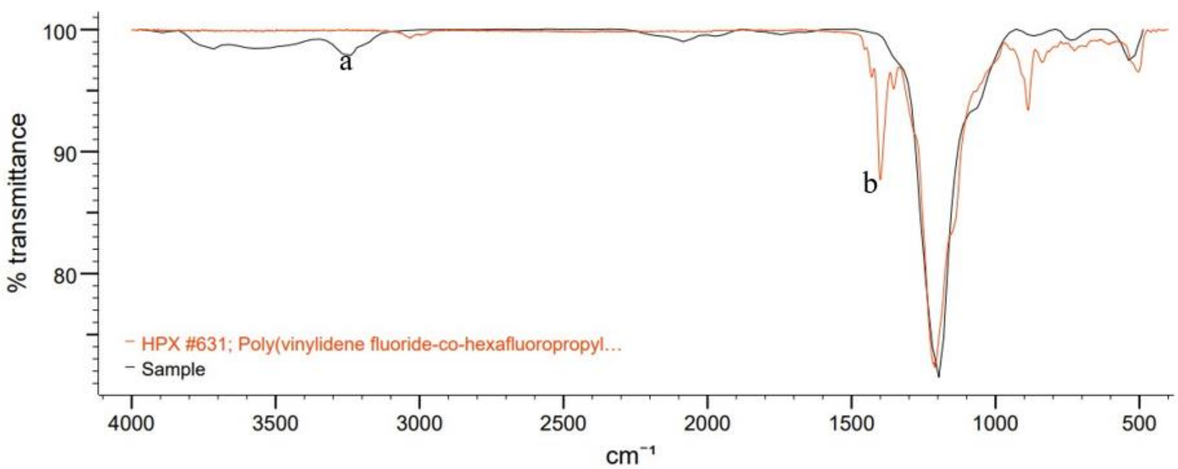

Figure 4.11: ATR-FTIR spectra for (a) sample (b) the reference Poly (vinylidene fluoride-cohexafluoropropylene) spectra from the Sadtler™ libraries reference database spectra library. Spectral match; 79.09%

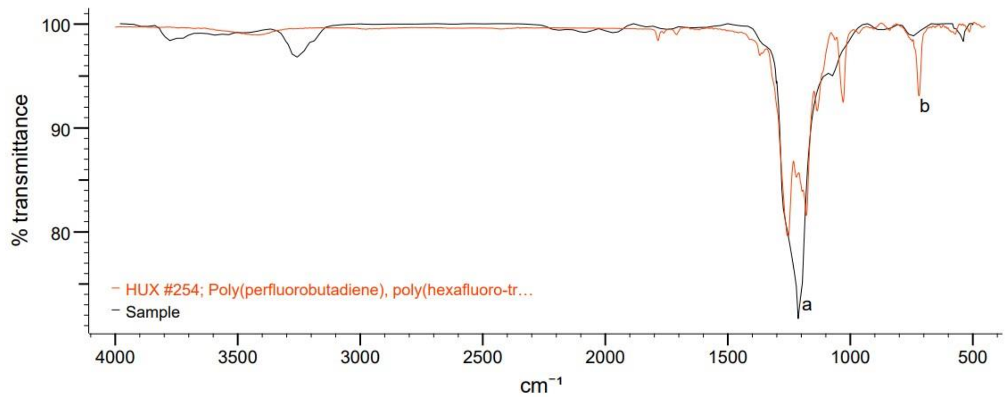

Figure 4.12: ATR-FTIR spectra for (a) sample (b) the reference Poly (perfluorobutadiene) spectra from the Sadtler™ libraries reference database spectra library. Spectral match; 81.06%

4.4 Determination of the relationship between microplastics and the well-being of fish

The total length of the different species collected within Kisumu Bay, Lake Victoria ranged between; 14.9 and 27 cm (*O. niloticus*), 18.5 and 32.2 cm (*L. niloticus*), 15.5 and 19.1 cm (*S. victoriae*) and 18.4 – 35.1 cm (*C. gariepinus)*. The weight ranged between 56.35- 446.75 g (*O. niloticus*), 81.52-390.68 g (*L. niloticus*), 39.4-61.56 g (*S. victoriae*) and 42.82 – 1498.84 g (*C. gariepinus*) (Table 4.4).

Table 4.4: Total Lengths, weights and allometric coefficients of fish species sampled within Kisumu Bay, Lake Victoria (the bolded values represent the highest and lowest values)

		Total length (cm)		**Weight (g)**		**Allometric coefficient**	
Species	n	min	max	min	max	a value	b value
O. niloticus	54	14.90	29.80	36.35	336.75	0.01	2.94
L. niloticus	21	18.50	32.20	81.52	390.68	0.01	**2.84**
S. victoriae	12	15.50	19.10	40	70.56	0.01	2.95
C. gariepinus	8	18.40	35.10	42.82	1498.84	0.006	**3**

Different values of exponent b (the slope of the regression line used in computing the condition factor of the fish species) were recorded between the sampled species. The "b" values for *O. niloticus*, *L. niloticus*, *S. victoriae* and *C. gariepinus* were 2.94, 2.84, 2.95 and 3.00 respectively (Fig. 4.13a; Fig. 4.13b; Fig. 4.13c; Fig. 4.13d). The coefficient of determination (R^2) values varied between the species, with 0.9517, 0.966, 0.9617 and 0.9984 for *O. niloticus, L. niloticus*, *S. victoriae* and *C. gariepinus* respectively. The *O. niloticus, L. niloticus*, *S. victoriae* species exhibited a negative allometric growth pattern since the allometry coefficient (b<3), whereas *C. gariepinus* exhibited normal growth with the allometry coefficient (b=3) (Table 4.4).

Positive correlations were observed between microplastic numbers and fish length (Fig. 4.14a; Fig. 4.14b; Fig. 4.14c; Fig. 4.14d). The coefficient of determination (R^2) values varied between the species, although being low in all the cases. The values of R^2 were 0.0196, 0.0081, 0.0064 and 0.0193 for *O. niloticus*, *S. victoriae*, *L. niloticus* and *C. gariepinus* respectively. While the coefficient of determination (R^2) between microplastic numbers and fish weight were 0.0006, 0.0006, 0.0008 and 0.0295 for *O. niloticus*, *S. victoriae*, *L. niloticus* and *C. gariepinus* respectively (Fig. 4.14e; Fig. 4.14f; Fig. 4.14g; Fig. 4.14h).

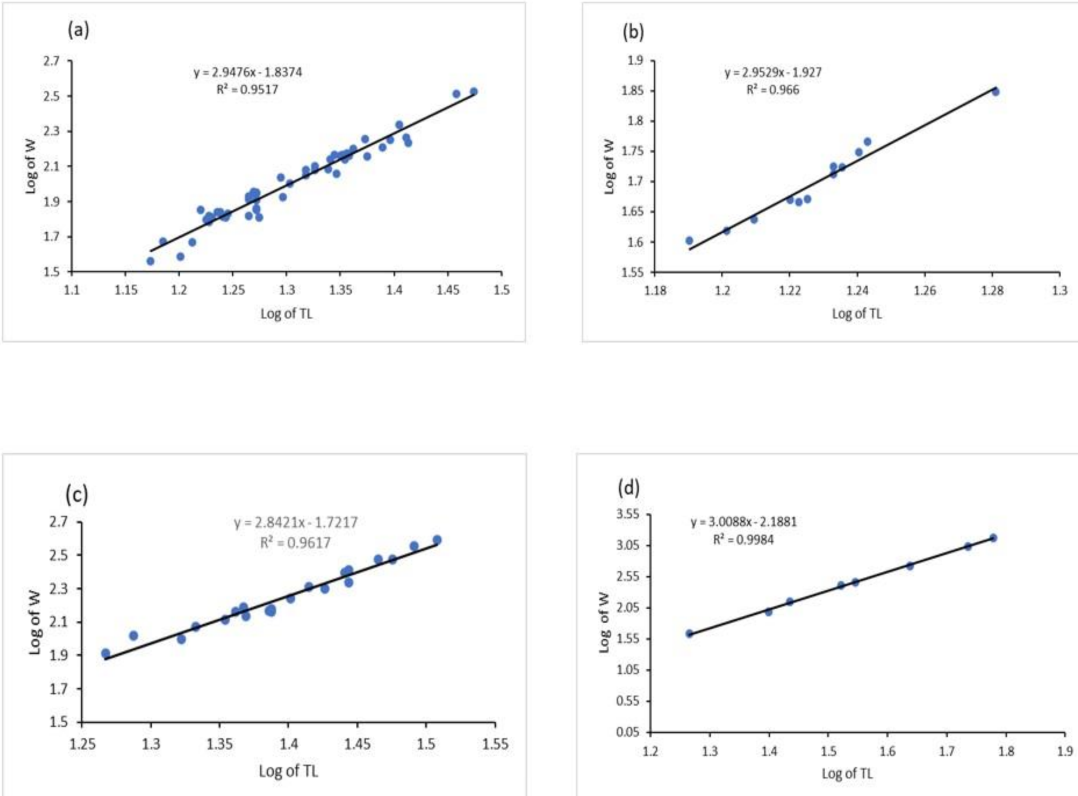

Figure 4.13: Log TL- Log W relationship of (a) *O. niloticus* (b) *S. victoriae* (c) *L. niloticus* and (d) *C. gariepinus* in Kisumu Bay, Lake Victoria

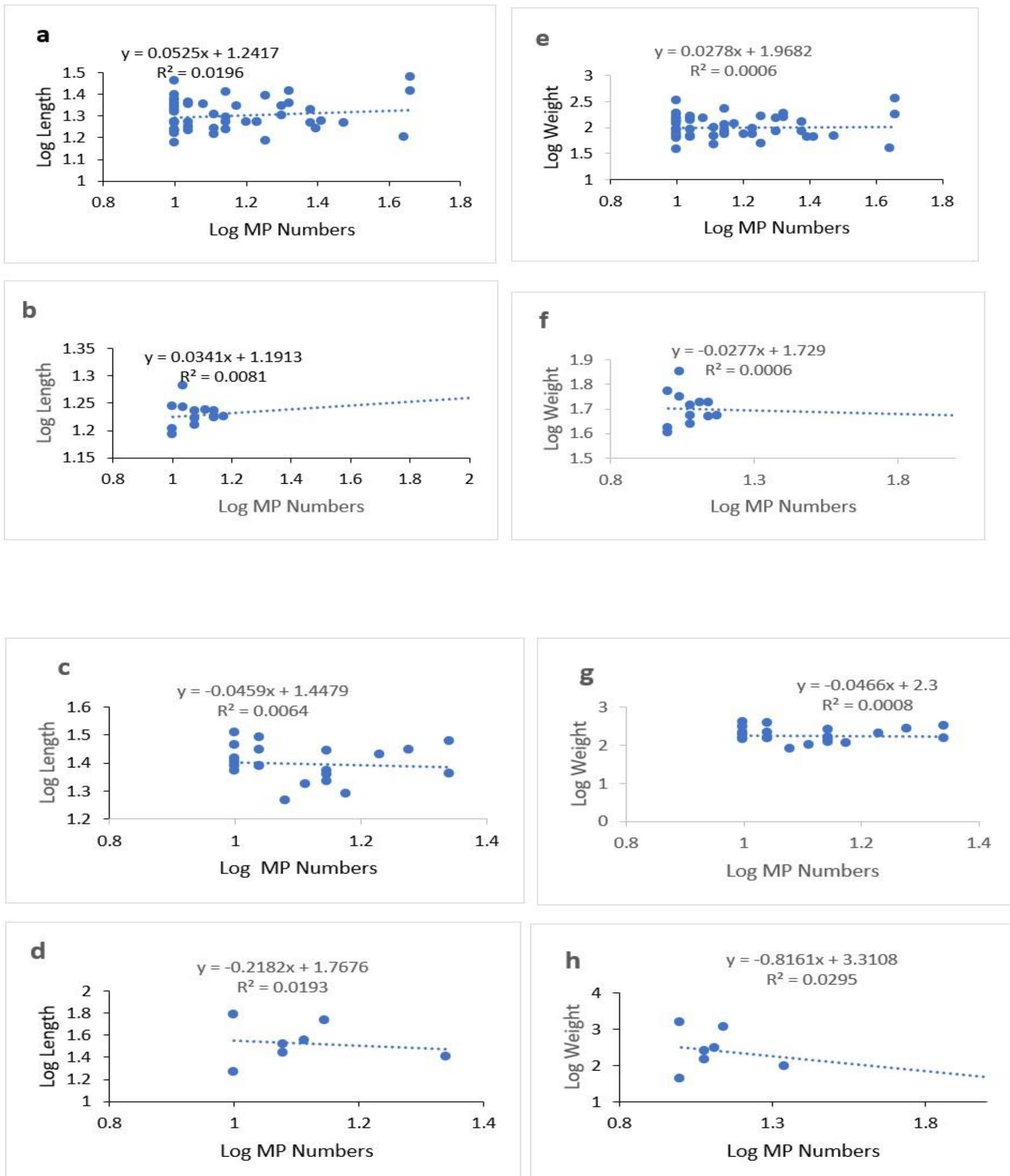

Figure 4.14: Bivariate plots of fish length against microplastic numbers (a–d; (a) *O. niloticus* (b) *S. victoriae* (c) *L. niloticus* and (d) *C. gariepinus* and fish weight against microplastic numbers (e–h; (e) *O. niloticus* (f) *S. victoriae* (g) *L. niloticus* and (h) C. *gariepinus*

CHAPTER FIVE

DISCUSSION

5.1 Water quality parameters

The earth's geological structure and the adjacent anthropogenic activities both affect the lake's water quality (Vasistha & Ganguly, 2020). Conductivity changes, which influence both overall water quality and aquatic life, are frequently the earliest detectable signs of deterioration in the ecological state of water systems (Borowiak *et al*., 2020). Conductivity during the study ranged between 103.8 – 179.5 µS/cm. These values showed a similar trend to previous studies where the values ranged between 73.5 - 184.0 µS/cm (Roegner *et al*., 2020). Meremo *et al*. (2022) reported similar mean conductivity values within the same range of 170.9 ± 22.2 - 176.3 ± 25.7 µS/cm at different sites within Kisumu Bay, Lake Victoria. The higher electrical conductivities at the Kichinjio and River Nyalenda Mouth were ascribed to large chemical inputs of dissolved ions from the city's runoff and from various farm lands in the catchment area.

One governing factor for the dynamics of aquatic environments is water temperature, which affects the metabolism and reproduction of organisms and speeds up the rate at which organic matter degrades (Fasil *et al*., 2011). The lowest mean temperature was recorded at the Open water (27.30 ± 0.51 °C), while the high mean temperature at the Kichinjio site (27.97 ± 0.70°C) could be attributed to shallow water depth coupled with high turbidity. The high turbidity is evidenced by lower mean secchi depth (0.51 ± 0.03 m) at Kichinjio compared to other sites. This observation compares with Miruka *et al*. (2021) whose findings revealed high water temperatures in turbid waters as a consequence of the water being able to absorb and retain solar energy. Consequently, this site had a low mean dissolved oxygen concentration (5.43 ± 0.33 mg/L). A plausible explanation is that high water temperature decreases gas solubility such as oxygen (Bhateria & Jain, 2016).

The pH obtained ranged between 7.09 and 7.99. These pH values are comparable to what has been reported within the Bay for example Meremo *et al*. (2022) reported a pH range of 7.5 ± 0.4 - 7.9 ± 0.6. Adhiambo (2017) reported a slightly higher pH range of 7.2 (+ 0.384) and 8.4 (+0.332) within the Bay.

The Kichinjio site water was found to be rather turbid with low transparency being recorded (0.51 ± 0.03m). In contrast, the Open water site had higher water transparency (0.79 ± 0.13). This could be attributed to dilution of lake water in the pelagic zone and low algal biomass. These findings are in line with Lung'ayia *et al*. (2001) observation that water transparency increased at offshore sites compared to nearshore sites. Meremo *et al*. (2022) sampled in the same season and reported similar findings within Kisumu Bay where near shore sites had lower water transparency than off shore sites.

The dissolved oxygen concentrations ranged between 5.06 – 5.99 mg/L, which is well above 5 mg/L which indicated that the water in the Bay was plausibly good for aquatic organisms to thrive. Levels between 3 - 5 mg/L have been reported to be lethal for most organisms that live in water (Edokpayi *et al*., 2016). Misiko *et al*. (2014) reported a similar trend within Kisumu Bay where low dissolved oxygen were obtained at the River Kisat's inlet and high dissolved oxygen concentration at an offshore sampling site.

5.2 Levels of microplastics in water and different fish species

5.2.1 Microplastics in water

Microplastics (MPs) abundance in urban waters is intimately tied to human activities; high population density usually results in high MP abundance (Rodrigues *et al*., 2018). The human-related factors are responsible for the high levels of microplastic contamination recorded in Taihu Lake, China by Naqash *et al*. (2020) where microplastic concentrations in surface water samples, ranged between 3.4 to 25.8 items per litre. These findings concur with the surface waters results obtained at Kisumu Bay, Lake Victoria which is adjacent to Kisumu City. The high abundance of microplastics obtained in this study can be attributed to influence of several factors such as fishing, runoff from the city, tourism and wastewater discharge among others. The MPs concentrations at Kisumu Bay compare with Xu *et al*. (2021) findings in Gehu Lake Basin, China. They found a MPs concentration ranging between 1.51- 22.22 particles/litre in the urban lake.

The high concentration of microplastics at the Kichinjio site could be linked to industrial waste dumping as well as other plausible contamination sources such as Kisumu city runoff. The site is also near River Kisat's inlet, which is associated with direct sewage discharge into the lake (Miruka *et al*., 2021). River Kisat also flows through an area dominated by industrial activities (Kiema *et al*., 2017). Long *et al*. (2019) reported that wastewater treatment plants (WWTPs) daily effluent discharge released MPs into Xiamen Bay at a projected rate of $\sim 6.5 \times 10^8$ MPs. A plausible explanation for activities that would have contributed to the MP load were fishermen washing and mending their fishing nets along the shores of the lake beaches. This finding compares with a study carried out by Ngupula *et al*. (2014) where macroplastics were found during trawl surveys on Lake Victoria, mostly from fishing gillnets, long-line twines, carry bags, and cloth. Chen *et al*. (2021) reported that discarded fish nets and lines could disintegrate to produce microplastics. Additionally, laboratory simulations by Chen *et al*. (2018) and Montarsolo *et al*. (2018) replicating environmental conditions where fishing nets were washed showed microplastics shedding from the nets.

A significant concentration of microplastics were observed at River Nyalenda Mouth. The high microplastics concentration could be associated with the river flowing through Nyalenda which is an informal settlement. This finding is supported by a study undertaken by Sibanda *et al*. (2017) who reported that due to poor road network accessibility, the Nyalenda informal settlement receives little or no waste collection services from the city or private collectors. This is a source of MPs as River Nyalenda flows along the informal settlement. However, the river mouth had a lower MPs concentration compared to the Kichinjio site. This could be attributed to the effect of biofiltration by the wetland at its mouth. Although wetlands can act as a sink or/and source for microplastics. This result agrees with Sharley *et al*. (2017) who reported that many pollutants are carried by stormwater runoff and can collect in urban wetlands and reach hazardous quantities over time. Concurrently, Paduani (2020) indicated that plastic may enter the wetland system and become entangled in or excluded by plants, break into tiny fragments, mix with natural colloids, and sink into the sediment, or become resuspended and transported out of the system to the recipient water body. This finding aligns with a study carried out by Liu *et al*. (2022) that an urban natural wetland (Lalu wetland) acted as a sink for microplastics with abundances in the surface water in the wetland ranging between 0.06–3.05 MPs/L.

The lowest concentrations of microplastics were recorded at the Open water site. A possible explanation could be that the site was offshore far from human activities. This is in line with results reported in the surface waters of the Laurentian Great Lakes where samples near the shorelines had higher microplastic concentration compared to offshore samples (Eriksen *et al*., 2013). The concentration could also be attributed to the dilution effect as the site had a greater depth and was situated a distance from the shore. Normally terrestrial environments are the primary source of microplastics. These findings compare with Wu *et al*. (2019) findings that the diluting effect of marine water and away from the proximity of the sampling sites at the coast resulted in a gradual reduction in the concentration of microplastics.

The decrease in microplastic density and concentration at the Airport Area site suggests that the anthropogenic activities of the less developed Usoma settlement next to the site did not have a large impact on the waste that reached the lake. However, without a formal waste management system, this relatively small community generates a disproportionate volume of improperly disposed trash. A similar case was reported in Lake Hovsgol in a near-pristine freshwater system (Free *et al*., 2014). Microplastics are more likely to enter these rural and underdeveloped locations through the breakdown and fragmentation of consumer plastics blown or washed into the water from shore (Ryan *et al*., 2009). The findings of this study are comparable to those of Egessa *et al*. (2020), who reported a high abundance of micro-, meso-, and macro-plastics measured as particles/kg dry sediment of the shoreline sediment and in lake sediment in the northern Lake Victoria, Uganda.

5.2.2 Microplastics in fish

Generally, the fish recorded lower concentrations of microplastics as compared to water. Microplastics were detected in the fish studied from the waters of Kisumu Bay, Lake Victoria. The findings provide further evidence that the four fish species were all susceptible to microplastic ingestion with a frequency of occurrence range of (59.26 – 75%). These findings align with those of Browne *et al*. (2011) who reported that cities can act as important sources of macroplastics and MPs, consequently being available for ingestion by the fishes captured near highly populated metropolitan areas. The average number of ingested MPs in *O. niloticus* was 5.57 ± 8.87 items/individual. This value was lower than that reported for *O. niloticus* in the Nile River (Cairo, Egypt) (7.5 ± 4.9 items/individual) by Khan *et al*. (2020). A possible explanation of these differences could be the variance in freshwater ecosystems and plastics sources and management. Kisumu Bay is adjacent to an urban centre with various economic activities such as industries, wastewater discharge, fishing activities, agriculture, businesses and local and international tourism. These activities compare with Sun *et al*. (2021) findings in freshwater basins in Guangdong province, China. They demonstrated that a variety of wild fish species ingested MPs. In addition, they also revealed a relationship between microplastic dispersion and abundance and the growth of the economy, tourism, industry, agriculture, and fisheries.

The high uptake rate of MPs for *L. niloticus* at Open Water site would have been influenced by the feeding habits of this species since it has multiple food sources in the pelagic zone (Basooma *et al*., 2023). This showed that the availability of MPs for ingestion was influenced by the sites this fish species occupied. This showed that the availability of MPs for ingestion was influenced by the sites this fish species occupied. The benthic *C. gariepinus* had an average of 3.28 ± 4.11 MP particles/fish in its gastrointestinal tract. This could be explained by the species dwelling and feeding at the benthic zone where the denser MPs sink into the sediments by the combined effects of buoyancy and gravity. This aligns with findings by Merga *et al*. (2020) which demonstrated that more fish species, including the benthic *C. gariepinus* and the benthopelagic *Cyprinus carpio* and *Carassius carassius*, consumed more plastic particles. Dahms *et al*. (2022) reported a higher average value of 7.47 particles/fish in *C. gariepinus* in the upper Vaal River, South Africa. The study highlighted that *C. gariepinus* being a top predator made it difficult to ascertain whether the MPs are ingested directly or indirectly. Contrary, *S. victoriae* had low MP particles/fish. A possible explanation of this has been aligned to its feeding behaviour among other characteristics. However, the effects of MPs on *S. victoriae* are not fully understood. Egessa *et al*. (2020) reported that MPs in the surface waters of Lake Victoria pose risks to fish and their natural food notably invertebrates, coupled with the potential link to human health.

Some of the effects of MPs on fish include intestinal abrasion, lesions, and a false sense of satiation, which leads to malnutrition and hunger (Ahrendt *et al*., 2020). While the environmental impact of MPs received great attention from the scientific community, regulators, and society in general, the health risk for humans arising from dietary exposure to MPs has yet to be examined (Barboza *et al*., 2018; Campanale *et al*., 2020). There is a lack of reference values for dietary MPs makes it difficult to assess and control their risk (Rubio-Armendáriz *et al*., 2022). Scientists and Food Safety Authorities face challenges and opportunities related to the presence of MPs in foods.

5.2.3 Characterization of microplastics in fish and water

Fibre-shaped MPs were dominant in both fish and water. This could be explained by the presence of more secondary sources than primary sources of MPs such as rope materials from fishing gear and domestic wastewater. The findings suggest that MP shape is a crucial factor influencing ingestion rates by the studied fish species, with fibrous MPs being more readily ingested than fragmented MPs. This is supported by Singh and Sharma, (2008) who reported that fishing lines and nets are a major source of secondary MPs. Other shapes such as films, foams and pellets were not encountered. Khan *et al*. (2020) also observed fibres as the most common MP shape found in the gastrointestinal tracts of *O. niloticus* and *C. gariepinus* in the River Nile (Cairo, Egypt). Similar findings were reported by Pazos *et al*. (2017) that a high percentage of fibres ingested by the fish in the coastal freshwater of the Río de la Plata estuary. However, Browne *et al*. (2011) reported that water near sewage discharge locations contained fibre quantities similar to those found in synthetic clothes.

The clear-coloured MPs may be related to accidental ingestion or photo-oxidation of MPs. This could be expounded by Martí *et al*. (2020) that the discolouration of MPs is caused by intensive photo-oxidation, although, small fractions of red, black and green coloured particles were also identified in Kisumu Bay. The size of sampled microplastic in this study was relatively small with the particles being less than 1mm. This could be due to the breakdown of macroplastics by ultraviolet radiation and wind action. The results concur with those of Xiong *et al*. (2018) that microplastics in Qinghai Lake (China), an urban lake ranged between 112 µm to 5 mm. Similarly, nine lakes from the Argentine Patagonian Region recorded a size class of between 38 µm and 1 mm (Alfonso *et al*., 2020). The four fish species studied fed on a smaller size range of MPs similar to those identified in the surface water. This could be attributed to the particles having sizes similar to their food items. These results compare to the findings of a study undertaken by (Merga *et al*., 2020). They found MP particle size range of 0.2 - 5 mm in *O. niloticus* and *C. gariepinus*. Galafassi *et al*. (2021) found that dorsal muscles of various fish species contained more little fragments (less than 100 µm) than larger ones (101–5000 µm). Although the exact mechanisms of this translocation are still unclear, their results appear to validate the possibility that MPs can be transferred to other organs in wild fish.

5.2.4 Relationship between environmental variables and microplastics abundance

Electrical conductivity influenced microplastic abundance. This was possibly ascribed to the strong interrelationship between electrical conductivity and pollution. Similar findings by Birami *et al*. (2022) reported a significant correlation between microplastic levels and electrical conductivity in Anzali an urban wetland in north of Iran. The results showed a positive correlation between pH and microplastic abundance. This could be attributed to water pollution which is a source of this pollutant having the potential to drastically alter the pH levels of bodies of water, resulting in increased acidity or alkalinity. Similar findings were reported by Nkosi *et al*. (2023) that the abundance of microplastics was positively correlated with pH. The results obtained show that temperature had a negative correlation on the microplastic abundance. This result disagrees with what Yonkos *et al*. (2014) reported that temperature impacts the distribution of microplastics because it affects both the MPs breakdown mechanism and the hydrodynamic interactions of water.

5.3 Identification of the chemical composition of microplastics in fish and water

Morgado *et al*. (2021) reported that there is a possibility to examine spectra manually by recognizing distinctive bands visually, comparing the wave numbers of bands to databases of characteristic bands of different polymers or automatically by utilizing a suitable mathematical comparison of particle and reference spectra. The Marine Strategy Framework Directive (MSFD) Technical Subgroup on Marine Litter recommends not classifying samples as synthetic plastic material if a match has a similarity of less than 60% and only accepting those with a similarity of more than 70%. The visual selection of MPs was validated by ATR-FTIR spectroscopy. The similarity match between the sample and the reference database is influenced by environmental factors such as biochemical reactions and abrasions.Those that had a similarity of more than 70% from the database were selected. Polystyrene (PS), Poly (vinylidene fluoride-co-hexafluoropropylene) (PVDF) and Poly (perfluorobutadiene) microplastic particles were identified.

Dallaev *et al*. (2022) reported that PVDF is widely used in the production of components for the petrochemical, chemical, metallurgical, food, paper, fabric, and nuclear industries, as well as in electronics, acoustics, radio engineering, medicine, pharmaceuticals, as well as structural and packaging material for solar cells and piezoelectric components, making it more abundant in the environment. Notably, PS was identified in different marine fish species from the Taif market, Saudi Arabia (Khattab *et al*., 2022). Barnes *et al*. (2009) reported that PS was a prominent litter source on land and marine systems that is easily dispersed by the wind. PS is widely used because of its low weight, excellent insulating properties, and moisture resistance. It is used in the production of plastic bottles, containers, disposable dishes, razor blades, and packaging, making PS an easy target for microplastic pollution in water systems. Zhu *et al*. (2014) reported that Poly (perfluorobutadiene) serves as a synthetic monomer for a variety of substances, including a new kind of fluorine resin, fluorine plastic, and fluorine rubber. Given the properties and use of these two polymers, it is possible therefore to directly link the existence of MPs in fish digestive systems with surface waters in Kisumu Bay. When compared to the pristine PS and Poly (perfluorobutadiene) reference material, the spectra, for instance, had expanded to include wide peaks at 1000-1100 cm^{-1} and 1100 -1300 cm^{-1} respectively. This could be ascribed to degradation, aligning with Ding *et al*. (2019) findings that ingested polymers' spectral characteristics may change due to biochemical processes, natural ageing, and chemical weathering. Given the properties and use of these polymers, it is therefore possible to directly link their MPs existence in fish digestive systems and surface waters in this study.

5.4 Relationship between microplastics and the well-being of fish

The fish length and fish weight generally showed no correlations with microplastic counts in all fish species studied; however, the low R^2 values of the results revealed that these measures do not have a substantial relationship. The condition factors for the fish species studied were all above 1 indicating that the fish species were in good condition except for *C. gariepinus* which was less than 1 exhibiting poor condition according to Eberhardt and Ricker (1977). Although the correlations have limited usability due to other compounding factors, no relationship was observed between fish condition factors and microplastic ingestion. It is still a challenge defining the actual effect of MPs on fish health. Even though there were no significant correlation between fish condition factors and microplastic ingestion, the condition factor of *C. gariepinus* being less than 1 implies that the health condition of this species is relatively poor when compared to the other species.

On the other hand, *O. niloticus* had a condition factor that was slightly above 1 depicting good health although, had a high frequency of occurrence of microplastics. However, *C. gariepinus* had a comparatively low condition factor. The findings of this study did not statistically demonstrate a significant correlation between fish well-being and ingested MPs. These findings relate to Rummel *et al*. (2016) study which had no direct effects on the condition factor between fish species that had ingested plastic and those that had not ingested plastic. Similarly, Foekema *et al*. (2013) reported that the presence of ingested plastic particles did not affect the condition factor (size-weight relationship) of the various fish species from the North Sea. This could indicate that fish condition factor may not be the best assessment characteristics to determine the effect of microplastic on fish health. Other characteristics or parameters including morphological and bioaccumulation studies should be undertaken to give in-depth possible ecological and health effects of MPs in fish and their value chain. In addition, with the new interest of scientific community on how MPs might interact with other contaminants like antibiotics, UV filters and polychlorinated biphenyls (PCBs) (Mohamed & Koelmans, 2019) may be the path to follow for a better understanding of effect of microplastics to fish health.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. The concentration of microplastics differed significantly among the sites showing that anthropogenic activities influenced the abundance of these particles. The study also found that microplastics occurred in the four commercially important fish species studied at different frequencies of occurrence from the waters of Kisumu Bay, Lake Victoria, a developing health concern. Hence, the hypothesis that levels of microplastics in water and four fish species within Kisumu Bay of Lake Victoria do not significantly differ was rejected.

ii. Three polymers of microplastics were identified as analyzed by ATR-FTIR spectroscopy. Poly (perfluorobutadiene) and poly (vinylidene fluoride-co-hexafluoropropylene) were the main plastic polymers found in water. Polystyrene and poly (perfluorobutadiene) were the main plastic polymers found in the four fish species. Characterization of MPs identified fibres were the dominant shapes and clear being the main colour in both fish and water. Therefore, the hypothesis that the composition of microplastics differed in fish and water in Kisumu Bay of Lake Victoria was not rejected.

iii. Positive correlations were observed between microplastic numbers and fish length and between microplastic numbers and fish weight. However, the coefficient of determination (R^2) values were low in all the cases. Therefore, the hypothesis microplastics pollution had no significant influence on the well-being of fish within Kisumu Bay of Lake Victoria was not rejected.

6.2 Recommendations

i. The presence of microplastics in water and fish should warrant education of the local community by the Kisumu County government and other government entities such as the National Environmental Management Authority on how to properly manage waste to protect aquatic ecosystems from pollution. More research should be done to analyze the effectiveness of existing environmental policies on reducing microplastic pollution and designing community-based interventions to increase awareness and reduce microplastic contamination at the source.

ii. Creation of a long-term monitoring program for microplastics in Kisumu Bay will help identify the patterns and modifications in the composition of microplastics over time. Routine sampling and analysis will deliver crucial information a consequently develop a database of microplastics composition in Kisumu Bay.

iii. More research should be done to understand the possible threats of microplastics on fish health. Monitoring initiatives that aid in determining the negative impacts of microplastics on fish health and higher trophic levels should be established and put into place. Additional research is required to fully comprehend the effect and potential transfer of microplastics to humans especially in Lake Victoria which is a main source of fish for food locally, regionally and even globally.

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APPENDICES

Appendix A: Mood's Median Test summary table showing significant differences in microplastic concentrations across sites

Mood Median Test: conc_per_Litre versus SITE

Mood median test for conc_per_Litre

Chi-Square = 18.22 DF = 3 $p = 0.000$

```
                                  Individual 95.0% CIs
SITE               N≤  N>  Median  Q3-Q1  -------+---------+---------+---------
Airport Area        4   5    1.49   0.69      (-------*---)
Open water          9   0    0.85   0.14  (*-)
Kichinjio           0   9    2.15   0.87                    (-*------------)
River Nyalenda Mouth  5   4    1.44   1.04          (-*---------------)
                                  -------+---------+---------+---------
                                      1.20      1.80      2.40
```

Overall median = 1.46

Appendix B: Research permit granted by the National Commission for Science, Technology and Innovation (NACOSTI) to conduct a study in Lake Victoria

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Appendix C: Publication

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Levels and Classification of Microplastics and Their Impact on the Wellbeing of Selected Commercially Important Fish Species in Kisumu Bay, Lake Victoria

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Abstract
Microplastics (MPs) are emerging pollutants of concern in aquatic ecosystems. Fish ingest MPs accidentally during normal feeding because they resemble prey or by ingesting prey that previously consumed them. Despite severe plastic pollution in Africa, some countries, including Kenya have implemented laws to curb this pollution menace. MPs have scantly been studied in African freshwaters. This study provides empirical data and describes the levels of MPs in four commercially important fish species in Lake Victoria. A total of 95 fish samples were collected from four sampling sites (inshore–offshore waters) between March and May 2022. Microscopy and Attenuated Total Reflectance Fourier Transformed Infrared (ATR-FTIR) spectroscopy methods were used to identify MPs. In this study, 62 out of 95 (65.26%) of the gastrointestinal tracts of the sampled fish contained MPs. The four species showed different proportions of detected MPs among the sampled individuals: 75.00% (*Clarias gariepinus*), 75.00% (*Synodontis victoriae*), 71.43% (*Lates niloticus*), and 59.26% (*Oreochromis niloticus*). Polystyrene (PS) and poly (perfluorobutadiene) were the main plastic polymers in the fish samples. The condition factors estimated for *O. niloticus*, *S. victoriae*, and *L. niloticus* were >1 and <1 for *C. gariepinus*, respectively. Positive correlations were observed between microplastic numbers and fish length and microplastic numbers and fish weight. However, the low R^2 values obtained implied no strong relationship exists between these parameters. These findings provide evidence of microplastic contamination in fish in Kisumu Bay.

Keywords Microplastics · Aquatic pollution · ATR - FTIR · Condition factor