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OCCURRENCE AND DISTRIBUTION OF MICROPLASTICS IN MAJOR WATER BODIES IN BOTSWANA

By

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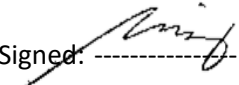
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Abstract

Microplastics (MPs) are ‘emerging’ contaminants that threatens freshwaters and may have negative impact on the aquatic organisms. However, despite plastic waste being a major source of these contaminants, there is no data available on the plastic and microplastics pollution in Botswana’s freshwater systems even though it is slowly becoming an eyesore. Therefore, the role of this study was to assess the distribution of microplastics in surface water, the amount of MPs ingested by freshwater species (*Tilapia sparrmanii*, *Oreochromis niloticus*, *Coregonus kiyi*, *Coptodon rendalli* and *Corbicula fluminea*) as well as characterize MPs based on their morphologies. This study was done in three study areas, the Okavango Delta which is the largest inland wetland hosting wildlife, tourism, and supporting the socio-economic lifestyle of inhabitants, the Gaborone dam and the Dikgatlong dam as the two largest major reservoir of freshwater in the country. Samples were strategically collected from the Okavango delta, Gaborone dam and Dikgatlong dam, such that they represent the whole study areas. The samples were analyzed for microplastic contents by wet peroxide oxidation, potassium hydroxide for aqua fauna, density separation, extraction, and visual identification with microscope.

Results showed that the weight of MPs for surface water samples from Okavango delta were the lowest between study location, varying from 138.18 to 381.67 $\mu\text{g m}^{-3}$ and abundance of 10 to 23 particles L^{-1} , with significant difference observed between sampling sites in both variables. Microplastics recovered from the *Tilapia sparrmanii* of the delta had MPs of 0.3 to 0.6 count per site and mostly present in intestines>gills >stomach. Fibers were mostly discovered in water while fragments were dominant in fish samples with translucent color dominant in both sample matrices. The MPs in size 1-2 mm and 2-3 mm were prevailing both in water and fish

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samples. In Gaborone dam, the abundance and weight of MPs were slightly higher than those in Okavango delta and Dikgatlhong dam. Surface water had MPs ranging from 36 to 76 particles L^{-1} while their weight varied from 760 to 1943 $\mu g m^{-3}$. The significantly high abundances were found in deep areas (S3 and S6) located near the boundary wall of the dam than in shallow areas (S1 and S5). The MPs were also discovered in fishes (*Oreochromis niloticus* and *Coregonus kiyi*) samples which varied from 1.0 to 3.5 count per sampling site, and *Oreochromis niloticus* ingested more microplastics than the other fish species. The dominant forms of MPs in Gaborone dam are the translucent and red colors in the form of fibers and fragments, in size ranges of 1-2 mm and 2-3 mm. In Dikgatlhong dam, the weight of MPs varied from 400 to 1500 $\mu g m^{-3}$ while abundance ranged from 16 to 46 particles L^{-1} , with S5 (dam tower, towards the boundary wall) having the highest in both variables for surface water. The highest counted MPs in *Coptodon rendalli* fish samples differed from 0.3 to 2.2 count per sampling site. The dominant forms of MPs are translucent and small sized (1-2 mm) in fish while fibers were the most common shape discovered in surface water.

Overall, the findings of this study clearly indicate that geographical location, population density, hydrodynamics and economic activity have contributed significantly to the abundance of MPs within freshwater systems. In this case, Gaborone dam which is about 11km away from the capital city had high levels of microplastics recovered than in Okavango delta and Dikgatlhong dam. These two study areas are less exposed to more human activities like the Gaborone dam hence they had low microplastic levels. The Okavango delta is 930 km away from the capital city in north-west direction while Dikgatlhong dam is 457 km in north-east direction. Further, the population around the two dams (Okavango delta- 130 000, Dikgatlhong dam is closest to Robelela village- 924) are much smaller compared to the numbers in the proximity of Gaborone dam (more

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than 500 000 people in the city and surrounding). With these differences in the population density, a significant variation in terms of microplastics generated and reaching the freshwater system is expected to be high as proven by the results. In addition, the hydrodynamics experienced as the delta has inflow from the highlands of Angola, continuous flow in most of the years and it empties into Lake Ngami. Meanwhile, the Dikgatlhong dam is at the downstream of Shashe and Tati Rivers but its overflow drifts into the Ramokgwebana River through Shashe River into Limpopo River and empties into Indian Ocean. Therefore, this could be attributing to the low microplastic accumulation in these areas. The data has shown that indeed there are microplastics present in major water bodies but their abundance are lower than those in other areas of Africa. Nonetheless, this baseline information will provide insights on the loads of microplastics in surface water and the quantities ingested by fishes in the Okavango delta, Gaborone dam and Dikgatlhong dam, hence the need for monitoring. In addition, in the dam, accumulation of MPs in the downstream implied that the dams may serve as sink for the MPs. The data will guide in developing evidence based and effective policies that address plastic and microplastic pollution in freshwater systems.

Keywords: Microplastic abundance, distribution, fish species, clams, Gaborone Dam, Okavango Delta, Dikgatlhong Dam.

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Chapter 1

Introduction

1.1 Background of the study

Plastic evolution dates back to the 1950s, when its production was relatively small and manageable (UNEP, 2022). However, due to their societally beneficial applications and lightweight, durable nature, the demand for plastic increased, and it became one of the most widely used materials (Wang et al., 2019; Dalu et al., 2021). That has resulted in a drastic increase in the generation of plastic waste over the years. Life without synthetic polymers or plastics seems unimaginable in today's world, hence the exponential growth in large-scale global production of plastics since the 1950s, reaching a 359 million tons annual yield in 2018 (Geyer et al., 2017; Shanmugam et al., 2020). Therefore, the combined increase in plastic production and the longer degradation rates (up to 1000 years) of plastic result in plastic accumulation in the natural environment over time, which eventually reaches the aquatic systems. These polymers have become the most inescapable and abundant sources of marine and freshwater pollution in existence. Nonetheless, efforts have been made to reduce plastic waste that reaches landfills or that is lost in the environment through recycling. These prompted efforts, however, seem to be insufficient and overwhelming because plastic waste still reaches the aquatic system in large quantities (Windsor et al., 2019; Campanale et al., 2020; Thompson et al., 2009). Additionally, with the current global production and waste management trends, it is projected that by the year 2050, about 12 billion metric tons of plastic waste will end up in landfills or in the natural environment (Bouwman et al., 2018). According to Sadan and De Knock (2021), the largest producers of plastics are China (28%), North America (19%), and Western Europe (19%), while

Africa produces around 5% of the global plastic volume. In terms of consumption, the same countries are leading, with China at 20%, North America at 21%, Western Europe at 18%, and Africa with only 4% (Ryberg et al., 2018). Even though the African continent contributes a small fraction to the global stage, the increase in imports of manufactured plastic products and packaging is still a concern. The continent's most economically important countries, which include South Africa, Nigeria, and Egypt, are the highest producers and importers of plastic polymers and products. Jambeck et al. (2018) noted that coastal African countries produced an estimated 4 million metric tons of mismanaged plastic waste in 2020, including contributions from landlocked countries. In 2015, the continent generated a total of 19 million metric tons of plastic, of which 17 million metric tons were mismanaged, compared to the global amount of 60–99 million metric tons of mismanaged plastic waste (Sadan and De Knock, 2021).

Over time, this pervasive plastic waste found in the marine, freshwater, or natural environment wears out and disintegrates, forming even smaller plastic particles called microplastics. Microplastics have received considerable attention from researchers and the global media since the first article was published by Thompson in 2004 (Dris, 2016). They are considered "emerging" contaminants due to the concern that they might be ingested by smaller aquatic organisms, causing physical damage to these organisms. Microplastics are also capable of leaching toxic additives and plasticizers, as well as providing adsorptive surface for hydrophobic persistent organic pollutants (POPs) occurring in water, which could be passed on to humans through the food chain (Hylton, 2017).

1.2 Statement of Problem

Botswana has become one of the world's fastest-growing economies since the discovery of diamonds, gradually transitioning from the world's poorest to an upper-middle-income country. The diamonds became the country's cornerstone, and the living conditions of the people have since transformed and improved significantly. According to a World Bank Blog (2020), there was a steady decline in 2011 Purchasing Power Parity, where people living on less than \$1.90 a day decreased from 29.8% to 18.2% between 2002–2003 and 2009–2010, respectively. In addition, the country's gross domestic product (GDP) had an average annual increase rate of 4.6% between 1994 and 2011, while in 2013 it increased to 5.9%. The changes are mainly attributed to a combination of increasing agricultural incomes, growing industrialization, and demographic changes. Despite its middle-income status and positive economic progress over the years, the changes were not without their challenges. With these developments came the need to meet the increasing demands of the growing population, i.e., more access to health services, sanitation, water, and all sorts of goods and packages (plastic and plastic products included). Consequently, a rise in solid waste generation emerged. Today, plastic waste has become an eyesore in Botswana, even though there is currently no specific, comprehensive data on mismanaged plastic waste in the country. Only general records are kept for solid waste collected and disposed of in landfills within the country, and this excludes rural dumping sites as well as illegal dumping sites all over. This effort is supported by major instruments such as the Waste Management Act and the Botswana Strategy for Waste Management of 1998. Waste is lumped together as "solid waste" since it is not segregated at the source. From the total amount of waste collected in 2017, general waste constituted about 52.26% of the waste. In 2017, Francistown landfill collected the most waste (83,160.26 tons), while Gamodubu landfill collected the least (76,314.15 tons). It is noteworthy

that the category of paper and plastic was also the most salvaged type of solid waste in 2017 at 838.27 t (Statistics Botswana, 2021).

Botswana implemented a plastic bag levy in 2007 to address the country's massive plastic waste problem. However, this was not enough to at least reduce the fast-growing rate of plastic waste generation as more plastic materials continued to be used and discarded inappropriately. In further efforts, the country joined some of the African countries that called for an end to plastic packaging. The plastic grocery bags are made from ethanol and chlorofluorocarbons, which are detrimental to the environment and human health (Averda News, 2018). However, this decision was rather put on hold in 2018 by the government after realizing that there was a need for thorough consultation with all stakeholders regarding the ban (Adeyanju et al., 2021). The use of plastic and plastic products increases due to their convenient societal and economic benefits. Nonetheless, their disposal is not accounted for by either the manufacturers or the consumers.

1.3 Aims and objectives

The main goal of this research is to investigate the occurrence and distribution of microplastics in Okavango delta, Gaborone dam and Dikgatlhong dam. The specific objectives are:

Specific Objectives:

1. To determine the distribution of microplastics in surface water of Okavango delta, Gaborone dam and Dikgatlhong dam.
2. To quantify the amount of microplastics ingested by the common fish species and clams

3. To examine the physical morphologies of microplastics present in surface water, fish species and clams.

1.4 Significance of the study

Microplastics tend to release toxic chemicals, such as plasticizers (phthalates and bisphenol A), into the environment. They also act as transport vectors, as they have the potential to uptake and release persistent organic pollutants (PAHs, PCBs) and heavy metals (copper, lead, and zinc), which are very detrimental to human health and aquatic organisms. Further, once ingested by aquatic organisms, microplastics disrupt nutrient absorption, thus inhibiting their growth and reproduction and reducing their survival chances. Therefore, the presence of these contaminants in water bodies poses an environmental threat; hence, it is imperative to understand their abundance in freshwater systems for monitoring purposes. This data will serve as a baseline for future research and monitoring of microplastics in the Okavango Delta, Gaborone Dam, and Dikgatlhong Dam, as well as assist in developing evidence-based decisions in attempts to curb microplastic pollution and the impacts it brings to freshwater species and human health.

1.5 Scope of the study

The data from this study is an initial baseline data, therefore the findings are inconclusive. Further, due to various sampling methods and equipment used in other studies, as well as the amount of literature available on freshwater microplastics, comparison with other global studies is very narrow. Low to average loads of microplastics were observed in this study.

Chapter 2

Literature Review

2.1 Plastic origin and Plastic Pollution

The word "plastic" comes from a Greek word, "plastikos," which means being able to be shaped or molded by heat (Hiskey, 2010). The first synthetic plastic material that was manufactured in a laboratory was Bakelite (phenol-formaldehyde resin), invented by the Belgian chemist Leo Baekeland in 1907, which marked the commencement of the global plastics industry. Plastic further became important as it was used as a secret material during World War II, as the polyethylene properties were crucial in developing radar, while acrylic was used in manufacturing protective screens due to its shatter-resistant properties. Plastic is a synthetic or semi-synthetic material made up of organic polymers and consisting of a chain of monomers bonded together. This polymer comprises other additives that enhance its properties and make it more durable, such as plasticizers, stabilizers, impact modifiers, anti-microbials, antioxidants, blowing agents, anti-block agents, and anti-static agents. The additives help prevent breakdown from heat, increase flexibility, reduce degradation in plastics, prevent adhesion between layers of plastic, and reduce the build-up of static electricity. With these properties, plastic has resulted in a massive presence in the environment, hence becoming an environmental threat as plastic pollution. Moreover, with the exponential growth of population, so do the demands for plastic, which increase without considering the consequences after being used and discarded, and this has led to plastic accumulation in the environment. Plastic waste generated is carelessly disposed of and mismanaged, thus leading to plastic pollution, which varies in size as some are macro (large) and micro (small) plastics.

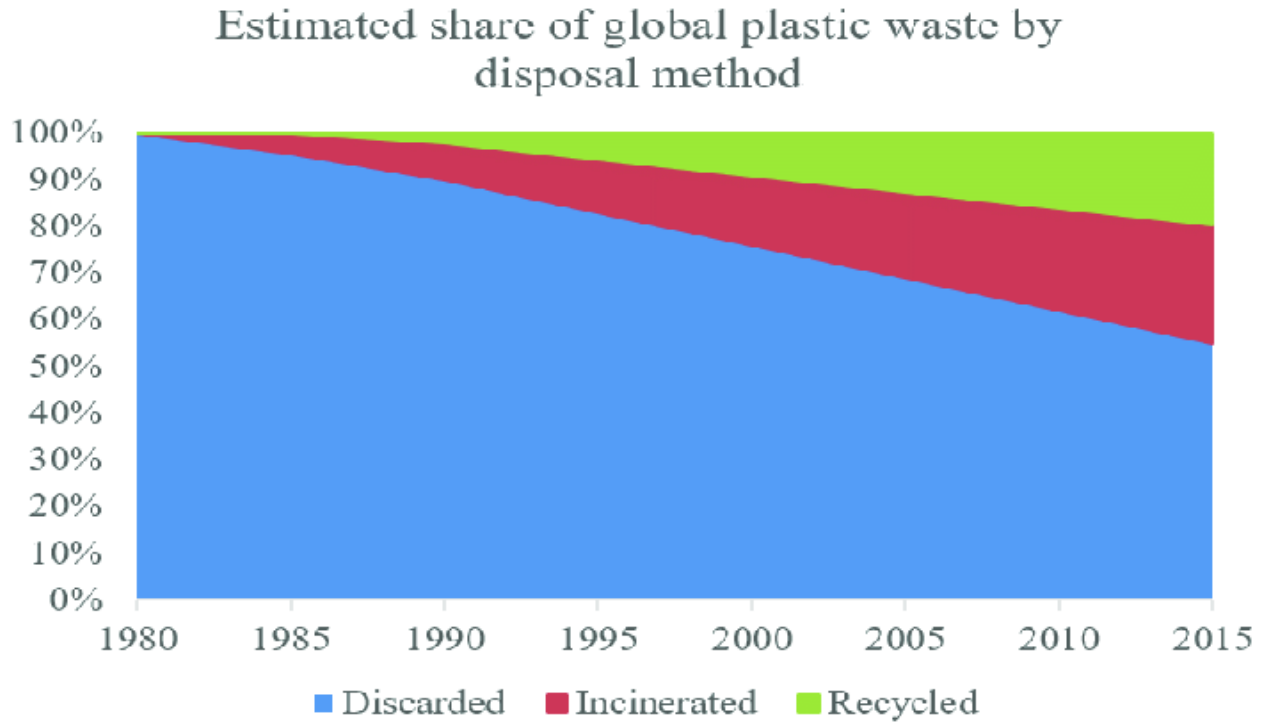


Figure 2.1: Estimated global plastic waste disposal from 1980 to 2015 (Source: Jiang et al., 2019)

Variations in the plastic additives used have also led to preference over which types of plastic to use. i.e., they are tailor-made to meet specific requirements depending on their use and properties. Each type of plastic is selected for a specific application depending on its strength, flexibility, or weight, and this ensures that they are effective and safe when used. The most common types of plastic used are shown below, and these are the kinds that contribute more to secondary sources of microplastics that are carelessly thrown everywhere in the environment.

 PET	 HDPE	 PVC	 LDPE	 PP	 PS	 OTHER
POLYETHYLENE TEREPHTHALATE	HIGH-DENSITY POLYETHYLENE	POLYVINYL CHLORIDE	LOW-DENSITY POLYETHYLENE	POLYPROPYLENE	POLYSTYRENE	OTHER
WATER BOTTLES; JARS; CAPS 	SHAMPOO BOTTLES; GROCERY BAGS 	CLEANING PRODUCTS; SHEETINGS 	BREAD BAGS; PLASTIC FILMS 	YOGURT CUPS; STRAWS; HANGERS 	TAKE-AWAY AND HARD PACKAGING; TOYS 	BABY BOTTLES; NYLON; CDS 

Figure 2.2: Some of the most used types of plastics on daily basis (Source: Plastic for Change, April 2021)

2.2 Microplastics in terrestrial and aquatic environments (freshwaters)

A series of recent studies have indicated that, like marine environments, microplastics have been found in abundance in freshwater matrices. It has been well reported in the literature on the increasing interest in research on microplastics in freshwaters as well as the rising number of publications, which has grown from less than 100 reports in the year 2010 to over 1000 reports by 2020 (Mendoza and Balcer, 2019). Despite global interest, there has been little progress in Africa in terms of microplastics in freshwater (Alimi et al., 2020). As a result, this is cause for concern because Africa is regarded as having the highest generation and mismanagement of plastic waste.

With freshwater systems considered a major pathway for the transport of microplastics from terrestrial to aquatic environments, most of the largest freshwater bodies in Africa support the food and water needs of most of the inhabitants, yet the extent of microplastic pollution in these systems is unknown. To date, there has not been any study from the central African region regarding microplastics, while South Africa is the only country from southern Africa that has reported all the studies (on sediments, surface water, and organisms) on microplastics' distribution and abundance. Due to these limited studies and the use of various measurement units, the comparison of microplastics abundance and distribution in different regions of the continent seems difficult. Nonetheless, Jambeck et al, (2018) has reported the highest abundance of microplastics found from South-eastern bays of South Africa and Ox Bow Lake in Nigerian in Yenogoa Nigeria at 1215 and 8369 particles m^{-3} , respectively, countries which are the highest manufacturers of mismanaged plastic waste per capita in Africa. This backs up Zhang et al.'s (2022) finding that population density and industrialization are related to the abundance of microplastics in freshwater.

2.3 Sources of microplastics

As long as there is human existence, their ways of living will continue to impact the natural environment, resulting in the generation of plastic and microplastic waste. Previous studies have proven that most domestic and industrial activities are the common sources of microplastics in freshwater. According to Wagner and Lambert (2018), primary microplastics, which are defined as engineered plastic materials, only represent a smaller fraction of these environmental contaminants as compared to the ones breaking away from the already existing large plastic debris. Plastic pellets used in raw plastic in industry or plastic particles used in cosmetics such as body scrubs and exfoliators are examples of primary particles that will eventually end up in water systems and then effluents from wastewaters as carriers into freshwaters (Bouwman et al., 2018).

It is worthy to note that sources of primary microplastics vary in terms of how they are lost to the environment, whether intentionally or unintentionally. Plastic particles used in cosmetics (personal care products), such as microbeads, are intentionally put on these products for a purpose and released into wastewater. However, other sources generate unintentional primary microplastics in small or large incidents along the plastic value chain through abrasions of large plastic materials during manufacturing, transport, use, maintenance, and recycling, or through spills during production, processing, transport, use, maintenance, and recycling (Friot and Boucher, 2017). These sources include synthetic textiles, tyre abrasions, paint coatings, atmospheric deposition, sludge, fertilizers, and fishing gear. In addition, plastic pellets, building paints, and fishing gear have also been added to the list of sources of microplastics, with a projected release of approximately 1000–80 000 metric tons annually (Viveknand et al., 2021). Nonetheless, sources for secondary microplastics include plastic particles leaching from landfills, uncontrolled dumpsites, or those that are inappropriately disposed of and end up in terrestrial or aquatic environments. These particles are eventually transported and deposited in freshwaters by storm water runoff and wind. Interestingly, Wang et al. (2021) suggested that seasonal variations also play an important role in the microplastic abundance found in freshwaters, as most microplastics are land-based, but they are transported into rivers and oceans during rainy seasons and sink mostly during dry seasons. When microplastics reach freshwater, some float on the surface while others sink due to their higher density than freshwater, while others sink due to attached persistent organic pollutants, microorganisms, and heavy metals that adsorb to their surface (Brennecke et al., 2015).

2.4 Impacts of Microplastics in freshwater aqua fauna

Microplastics tend to disrupt the feeding systems of aquatic organisms. Due to their small sizes, they are mistaken for food by aquatic organisms. This was proved by a recent laboratory

trial showing that invertebrates such as polychaeta worms, barnacles, amphipods, and sea cucumbers can ingest microscopic plastic particles (Claessens et al., 2013; Graham and Thompson, 2009). Therefore, once they are ingested, they can cause internal blockage or damage to the digestive system, while some smaller plastics translocate to the circulatory system of these organisms, including bivalves like *Mytilus edulis* (Razeghi et al., 2021). In addition, once microplastics accumulate in these aquatic organisms, they affect their growth, reproduction, and longevity (Xiang et al., 2022). Moreover, researchers have demonstrated the transfer of microplastics in aquatic food webs (Farrell and Nelson, 2013; Razeghi et al., 2021). Due to their minute size, microplastics resemble prey in the lower trophic levels of organisms; hence, they are captured and consumed. Therefore, these plastic particles are transferred from algae to zooplankton and then to fish (Tirkey and Upadhyay, 2021). It is also worthy of note that, with their large surface area to volume ratio, microplastics tend to provide high association potential for environmental pollutants such as persistent organic pollutants (polychlorinated biphenyls, dichlorodiphenyltrichloroethane), heavy metals, as well as releasing their plasticizers and additives to the environment (Brennecke et al., 2016). That is, microplastics introduce foreign pollutants into freshwaters via adsorption, and these pollutants are carcinogenic and mutagenic to aquatic organisms.

2.5 Impacts of Microplastics to human health

Humans may be exposed to microplastics in various ways, including through ingestion and inhalation, where they may be transported to different organs in the body, thus causing damage. Scientists from the Brazil recovered microplastics in the lungs of 13 out of 20 patients who participated in the study in 2021 (EcoWatch, 2022). However, even though these scientists could not tell the possible damage that microplastics can cause in humans, a study by Wright and Kelly

(2017) mentioned that high exposure to these pollutants may result in their accumulation in the body. Furthermore, once deposited in human tissue and body parts, they can alter the immune system or cause some other clinical complications. It is worth noting that microplastics have additives as well as provide an adsorptive surface to other pollutants, such as persistent organic pollutants and heavy metals, both of which are very detrimental to human health. When these pollutants reach high concentrations in the human digestive system, they can cause birth defects, diabetes, kidney failure, and some are even carcinogenic.

2.6 Sampling techniques and identification methods

Various techniques for sampling, pre-treatment, digestion, extraction, and identification methods have been used for microplastic analysis, which makes their results irreconcilable due to different sample volumes and measuring units used. Therefore, studies are objectively driven due to a lack of a standardized method, and most of them have adapted the proposed method by the National Oceanic and Atmospheric Administration (Masura et al., 2015). Sampling methods differ depending on the environmental matrix and sample volume. Depending on their scope, studies have used volume-reduced sampling, such as the use of Manta trawls along transect, while others preferred sieving in-situ. It is challenging to use a manta trawl during dry seasons when it comes to accessibility, but in the case of covering a large sampling area, especially during wet seasons, it is ideal to use it (Stock et al., 2019). However, due to a lack of resources or affordability, it is not always easy to use such sampling tools in other study areas, even though it can be challenging during dry seasons due to accessibility. On the other hand, sampling for freshwater biota can be done using gill nets, mantas, or traps, depending on the research questions. Since the sample matrices contain other materials from the environment, such as organic matter, researchers employ various methods to remove the unwanted materials from the samples. Therefore, in order to

remove organic matter from the sample matrix, studies have explored different techniques, including the use of acid or alkaline oxidative digestion as well as enzyme degradation, which have been recently investigated as potential effective alternative treatments. However, these techniques have their own shortcomings, as the acid has been found to cause the dissolution of some plastics such as polystyrene (PS) and polyethylene (PE). Also, some alkaline digestives cause the degradation of microplastics, while the emerging approach to using enzymes could be expensive in terms of finding appropriate enzymes to digest different organic compounds and time-consuming to apply at a large scale (Bellasi et al., 2020). Following organic matter digestion, some researchers simply filter out microplastics and carry virtual identification. On the other hand, a lot of studies used the density separation method after organic matter digestion using a saturated salt solution suited for the study (Dehghani et al., 2017 and Razeghi et al., 2020). Though it has a lower density than other microplastics, sodium chloride (1.2 g cm^{-3}) is the most commonly used separating solution in many studies because of its affordability. However, some of the polymers with densities higher than those of the sodium chloride solution (PVC and PET, with densities of 1.16 to 1.58 g cm^{-3} and 1.37 to 1.45 g cm^{-3} , respectively) may not be recovered as they would have settled at the bottom. So, in this case, the sediments and other mineral components that settle at the bottom would not be discarded but also visually observed to check for any heavy microplastics. On the other hand, Dehghani et al. (2017) and Harris (2020) recommended the use of zinc chloride and sodium iodide because of their high-density properties as well as a good rate of recovery, even though zinc chloride has proven to be corrosive and environmentally hazardous (Claessens et al., 2013; Rodrigues et al., 2020).

Literature has shown that in other studies, microplastics are viewed under a microscope immediately after the extraction process by the separation method. Vacuum filtering is used to

collect these polymers using filters (varying in pore size, efficiency, and wet strength), which are determined by the scope of the study and are usually made of glass fiber or aluminum oxides to minimize contamination (Zarfl, 2019). Also, it is worthwhile to note that some of the filters (very small pores, weak wet strength) can make it difficult to remove oxidation residues, which tend to accumulate and might obscure the microplastics during counting, leading to underestimation. However, before being observed, dyes such as Rose Bengal and Nile Red can be used on samples to facilitate the observation process (Harris, 2020).

Besides the use of a microscope for visual characterization, it is also crucial to further confirm the potentially identified microplastics that have been virtually recognized. Therefore, techniques such as Fourier transform infrared (micro-FTIR) and Raman spectroscopy are the most commonly used since they can provide size ranges down to 1 μ m and particle numbers, thus giving a comprehensive quantitative and qualitative analysis of the microplastics (Zarfl, 2019). Additionally, the two methods are complementary in terms of molecular structure, as Raman allows a clearer response for non-polar bonds while polar groups are better identified with FTIR. However, chemical characterization can also be done using Pyrolysis-Gas Chromatography-Mass Spectrometry (Pyr-GC-MS), X-Ray Fluorescence (XRF), and Scanning Electron Microscopy combined with energy-dispersive X-ray spectroscopy (SEM-EDS) to analyze the chemical

2.7 Existing knowledge gaps

More studies need to be carried out in Africa to establish the level of microplastic pollution in freshwater that supports the socio-economic lifestyles of most inhabitants. Every country should at least have a baseline of information regarding these emerging contaminants for monitoring purposes and to increase the chances of comparison across studies. Also, sources of microplastics

should be given attention in order to quantify their individual contributions to microplastic pollution in freshwater. There is a need to explore the mechanisms for persistent organic pollutants and heavy metal adsorption because they are detrimental to human health, and the levels at which they are a danger to human health should be further investigated. Extensive studies are also needed in the filtering process prior to visual counting because some oxidation residues tend to accumulate more during filtering, especially when using filter paper, which might lead to an underestimation of microplastics if they are obscured by residues. There is a dire need for a standardized method for microplastic analysis to make research on microplastics less complex because researchers are currently using various microplastic analysis.

Chapter 3

Materials and Methods

3.1 Description of Site 1 - Okavango delta

The Okavango Delta is in the northern part of Botswana (Ngami district), spread on top of the alluvial fan in the western branch of the East African Rift Valley (Milzow et al., 2009). The delta has a population of around 130 000 (Statistics Botswana, 2022) and is in the proximity of the Maun town, which is regarded as the tourism hotspot because of the influx from both local and international tourists it experiences throughout the year. The main source of water in the delta is the Okavango River, which originates in the highlands of Angola and flows southward through Namibia's Caprivi Strip into the terminal wetlands of semi-arid Botswana. The Cubango River flows south, then south-east through Angola, where it joins its largest tributary, the Cuito River, to form part of the Angola-Namibia border. The two rivers become one, and they form the famous Okavango River, which carries its waters into Botswana, ultimately bringing the Okavango Delta to life. The Okavango River flows into the panhandle through Mohembo village, stretching to Seronga village before fanning out into a combination of twisting channels and lagoons lined by thick beds of papyrus and reeds (Junk et al., 2006). The Panhandle, which is approximately 15 km wide and 90 km long, expands in the permanent swamp areas where it splits into three major river systems: Nqoga-Maunachira-Khwai, Jao-Boro, and the Thaoge River, which has since been blocked and is drying up (Murray-Hudson et al., 2006). The Jao-Boro River then splits into Boro and Xudum, which eventually join the Thamalakane River, which discharges into Lake Ngami (drainage base). The delta has permanently flooded swamplands that are surrounded by bogs that flood seasonally depending on water levels. Also, there are many islands (where most lodges and

safaris are located, close to extensive permanent lagoons) that vary in terms of sizes, with the Chief's Island being the largest, and salt islands with barren white centers that are too saline for plant growth. The water supply from the Cubango and Cuito rivers in Angola reaches the delta between March and June, then peaks in July (Milzow et al., 2009).

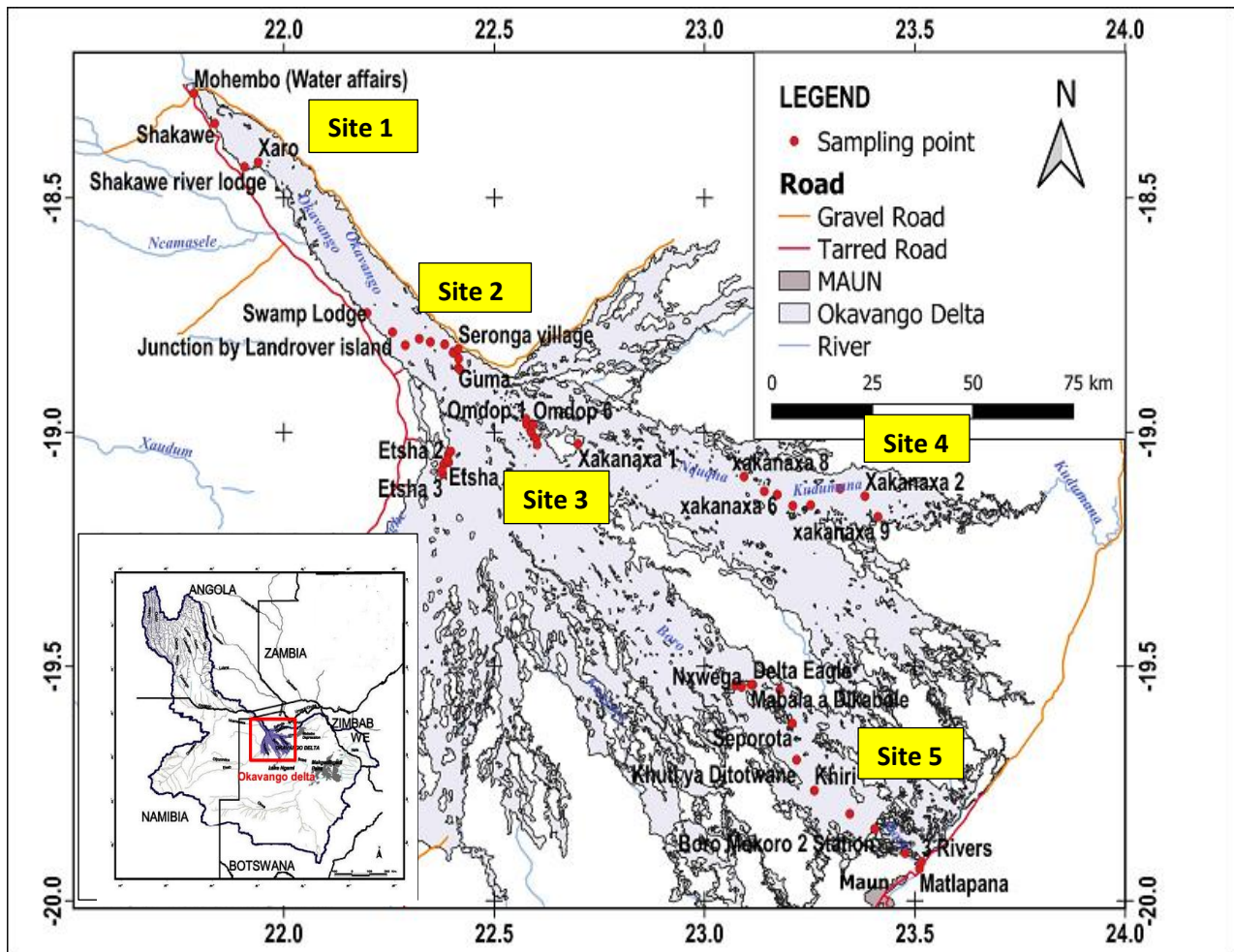


Figure 3.1: Map of the study area showing location of different sampling sites in the river system of Okavango Delta, Botswana.

An estimated 870 000 metric tons of sediment are transported from the Angolan highlands and deposited on the alluvial fan each year. From these sediment deposits, roughly 50% is from

solutes contained in the inflowing water, 22% is from particle sediment (mainly bed load and some suspected load), and 28% is from Aeolian inputs (Garstang et al., 1998). Furthermore, the location of deposition of 200 000 t of particles and 420 000 t of solutes annually varies due to different transport processes while entering the wetland through the inflow channels (flow velocities range from 0.5 to 1.5 m s⁻¹), which are flanked by dense aquatic vegetation (Garstang et al., 1998). The delta's local annual flow from rainfall is around 6140 million m³ of water, with an additional 9200 million m³ supply from the Angolan tributaries (Nash et al., 2006). However, with high evapotranspiration rates within the delta, almost more than 95% of the wetland flow and local precipitation is lost to the atmosphere, while a small quantity flows into regional groundwater. Interestingly, even though there is a high evapotranspiration rate, the delta's water is fresh, not salty, because trees on the edges of the islands have created a barrier of natural filters between the inner part of the islands and the floodplain (Ramberg et al., 2006).

Site 1 and Site 2 are located along the meandering Okavango River in the Panhandle. Site 3 is the section where the Okavango River diverts into main river system channels at the heart of the delta, with some of its sampling points being along the Thaoge River on the west terminus, which is in a series of lagoons and extensive floodplains near its upper end. The Thaoge River, which used to carry more food to Lake Ngami during the nineteenth century, has now stopped flowing due to vegetation blockages and sedimentation changes (Kurugundla et al., 2018). Site 4 lies along the Khwai River, with wide permanent food plains in its middle. It reaches and discharges downstream to less defined floodplains. Site 5 is located alongside the Boro River, which is a single, more or less confined downstream channel that empties into the Thamalakane River (Kurugundla et al., 2018). A total of 156 surface water samples and 15 intact fish samples were collected from this study area.

3.2 Description of Site 2- Gaborone dam

Gaborone dam is situated on the Notwane River in the south-eastern part of Botswana, covering an area of about 4300 km² with a water capacity of 144 000 000 m³ (Kenabatho and Parida, 2005). It is a part of the Limpopo River Basin and a major source of water for the capital city and its surrounding areas. Gaborone Dam is located approximately 11 kilometers south-east of the country's capital. The catchment experiences 475–525 mm of summer rainfall annually, with maximum temperatures ranging from 19 °C to 40 °C in summer, while winter nights are extremely cold with temperatures below 0 °C, as well as estimated annual evaporation rates of about 2000 mm (Matlhodi et al., 2019).

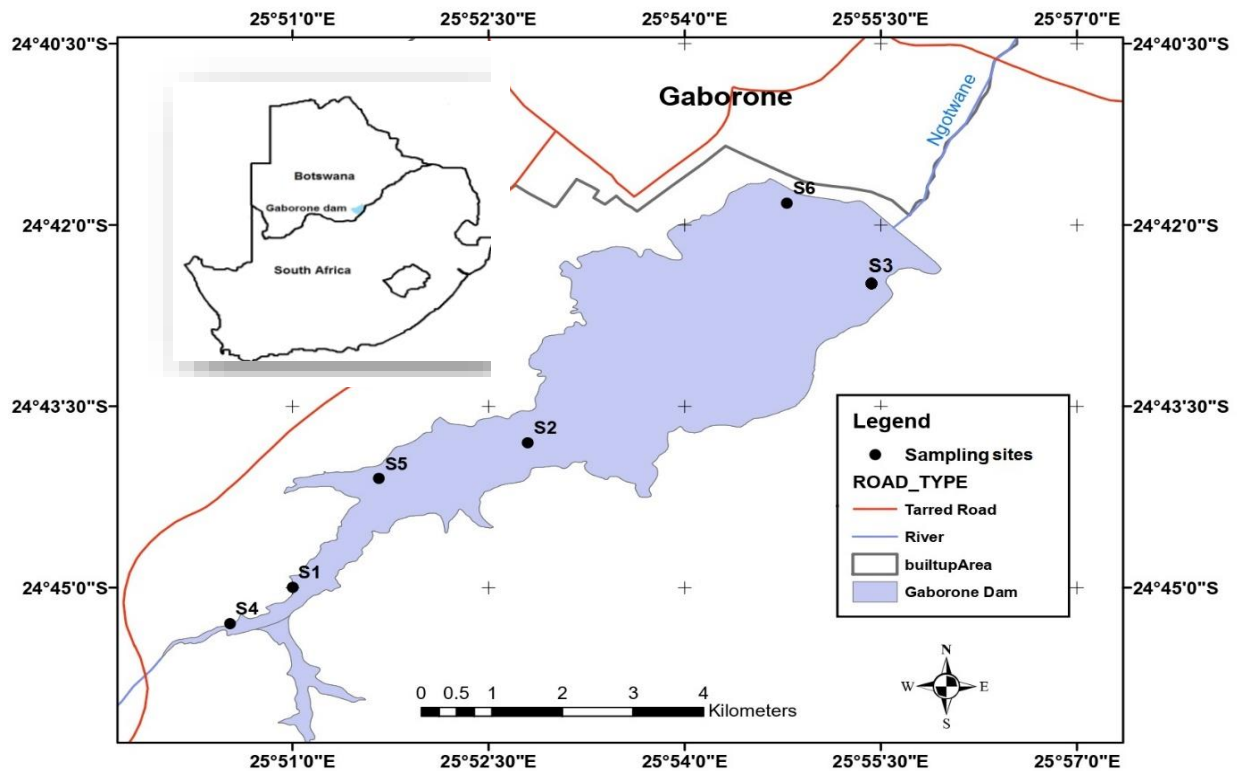


Figure 3.2: Map of the study area showing location of different sampling sites from Gaborone Dam, Southeast, Botswana.

With more than 200 000 people in the greater Gaborone area (Gaborone city and surrounding areas) and a rapid increase in population, the water from the dam is in high demand for domestic use and for agricultural purposes. The catchment is also a tourist attraction and a water sports site for local water sports clubs such as the Yacht Club, which provides sailing, canoeing, and rowing for the youth as well as the locals. Additionally, on the south bank of the dam lies the Kalahari Fishing Club, offering picnic sites and a boat jetty.

Six sampling sites were selected such that adequate representative samples across the dam were taken. From each site, ten surface water samples were collected following the river's flow direction, which flows north-easterly while eighteen intact fish and eighteen clams were obtained. Sample collection was from the shallow areas at the far end of the south-east where the river flows into the dam (S1 and S4), the middle area (S2 and S5), and the deep areas (S3 and S6), which are closer to the boundary wall.

3.3 Description of Site 3- Dikgatlhong dam

Dikgatlhong dam is located near the village of Robelela, which is in the central district of Botswana. This is the largest dam in the country, which was completed in 2011 and has a water holding capacity of 400 million cubic meters, covering an area of 7810 km² with 15.6 km² of surface area (Water Utilities Corporation report, 2015). The dam receives water overflows from the Shashe and Ntimbale dams, which are both fed by the Shashe and Tati Rivers, respectively. These rivers continue eastwards towards Robelela, where they meet and feed the Dikgatlhong dam. Their confluence is right in the middle of the dam; hence it was given the name Dikgatlhong, which in Setswana means "a meeting point." In this regard, the dam is where Shashe meets the Tati River. The overflow from this dam drifts onto the Ramokgwebana River, located along the Botswana-

Zimbabwe border, and into the Limpopo River. Through the North-South Water Carrier, the water from Dikgatlhong dam augments the water supply in the southern part of Botswana, especially the Greater Gaborone area, which has a large population in the country. In addition, the dam provides water to major villages in the central district, such as Palapye, Serowe, and Mahalapye.

The dam's five sampling sites were chosen based on their accessibility during the sampling period. Even though the dam is recharged by two rivers, the Shashe River and the Tati River, accessing the Shashe River side was quite a challenge due to strong waves and currents during the sampling period; thus, samples were collected more on the northern side (Tati River side) than the southern side (Shashe River side) of the dam. S1 is the site located at the center of the dam (by the dam tower); S2 is at the inlet of the Tati River into the Dikgatlhong dam; S3 is situated in the center of the Tati River channel; S4 is towards the edge of the dam, away from the boundary; and S5 is located just by the Dikgatlhong dam boundary wall. From each site, ten water samples were collected and three intact fish samples were obtained.

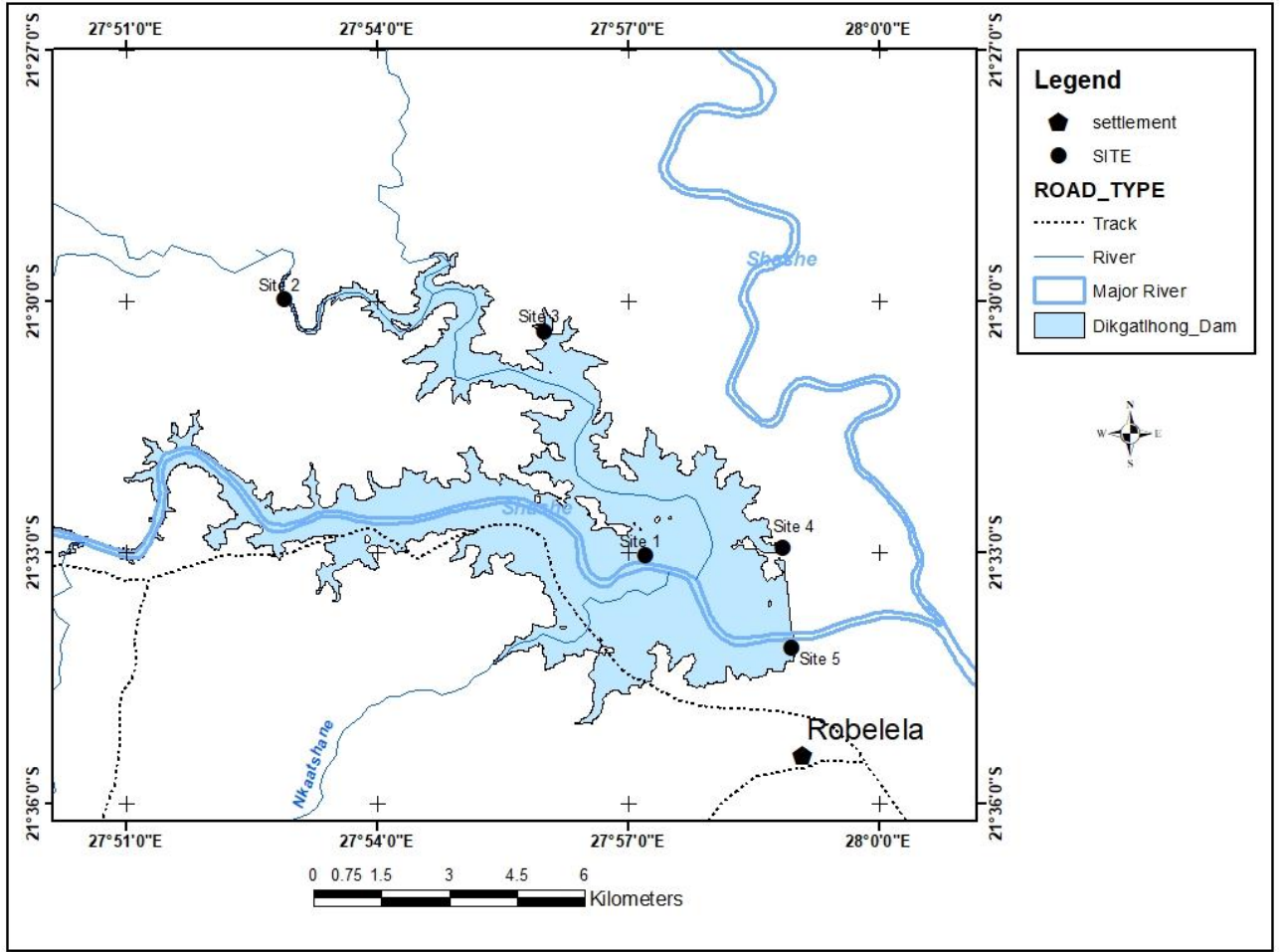


Figure 3.3: Map of the study area showing location of different sampling sites from Dikgathong Dam, Central district, Botswana.

According to Statistics Botswana (2022), Robelela village has a total population of 924. Summer maximum temperatures experienced in this region exceed 42 °C, while winter minimum temperatures are as low as -5 °C. The long-term average annual rainfall is 369.4 mm (1966–2001), with peak rainfall years receiving 916.7 mm (1999–2000) and low rainfall years receiving 135.5 mm (1998). The area falls into the floor of the Limpopo Valley, which is a broad geological trough

formed by the Limpopo mobile belt and is an outlier of the Karoo system. Hills and valleys in the area are formed by the sandstones, shales, and mudstones of the Cave Sandstone Stage.

3.4 Surface water quality parameters

For each site in all study areas, basic surface water quality parameters i.e. electrical conductivity (EC), pH, total dissolved solids (TDS), and dissolved oxygen (DO) were measured using a portable handheld multi-parameter probe (HI-98194 pH/EC/DO multi-parameter).

3.5 Sample collection

Samples were collected from all these study areas at different time intervals: April–May 2021 in the Okavango Delta; November 2021 in Gaborone Dam; and June 2022 in Dikgatlhong Dam. Even though samples collected from the Okavango Delta and Dikgatlhong Dam were collected during the winter, samples from Gaborone Dam were collected during the summer season due to delays in issuing permits to access the dam as well as the release of funds by the university.

In the Okavango Delta, a total of 156 surface water samples were collected from 5 sampling sites, and 15 whole intact fish samples were collected from all sampling sites. Meanwhile, in Gaborone Dam, 6 sampling sites were identified and a total of 60 surface water samples, 18 intact fishes and 18 clams were collected. However, due to accessibility challenges, 5 sampling sites were identified and a total of 50 surface water samples and 15 fish samples were collected from the Dikgatlhong Dam. Samples were collected such that adequate representation was obtained across all sites, despite challenges encountered.

All sample containers and tools were washed with distilled water and sealed to avoid contamination prior to sample collection. Also, nitrile gloves and cotton clothing were worn throughout the sampling process. Samples that contained microplastic loads were collected by passing 20 L of surface water through a stack of stainless steel 5 mm and 32 μ m sieves. The residues retained on the 32 μ m sieve were rinsed with distilled water (a squirt bottle was used) into a 250 ml glass jar and stored in a cooler box for further laboratory analysis. The sampling tools were cleaned with distilled water after each sampling to minimize cross-contamination. Similarly, three whole live fish with intact digestive organs from each sampling site were obtained and euthanized by the pithing method adopted from the American Veterinary Medical Association (AVMA, 2020) Guidelines for the Euthanasia of Animals- 2020 Edition, immediately after capture to prevent regurgitation or stomach inversion (Lusher et al., 2017). Thereafter, individual fish were placed in a glass container covered with aluminum foil and stored in a cooler box filled with ice while in transit to the laboratory. Upon arrival at the laboratory, basic measurements were taken for each fish, including total length (cm) and body weight (g). Subsequently, fish samples were dissected to isolate the stomach, gills, and intestines, which were each weighed. Metal scissors and a scalpel were used to dissect fish samples from the anus to the upper part near the gills to obtain the stomach, gills, and intestines, which were then placed separately in well-labelled, clean glass beakers and covered with aluminum foil. The samples were kept frozen until they were analyzed.

3.6 Pre-treatment of the sample matrix

In the laboratory, microplastics (MPs) were extracted from the sample matrix according to the method proposed by Masura et al. (2015) with minor modifications. Wet peroxide oxidation (WPO) was used for the digestion of organic matter in the sample matrix before the isolation of

MPs. In brief, each sample was treated with 5 ml of a 0.05 M ferrous sulfate (FeSO_4) solution and about 20 ml of 30% hydrogen peroxide (H_2O_2) for 8 h at room temperature inside the fume hood. Hydrogen peroxide was added initially at 5 ml and subsequently added 2 ml every 30 min until the organic matter in the sample matrix was fully oxidized, as indicated by the clear fumes and solution in the reaction vessel. After which, 20 ml of a 5 M sodium chloride (NaCl) solution was added to the digested sample to separate the microplastics from any other particles, like sediments, that may have been left after digestion. The mixture was then transferred to a separating funnel and allowed to settle for 12 hours. The supernatant consisting of microplastics was collected in a 32 μm sieve, and the microplastics left in the sieve were washed with distilled water (using a squirt bottle) to remove any unwanted materials such as sodium chloride particles attached to the MPs. The retained microplastics were transferred using distilled water into a clean, oven-dried beaker and loosely covered with aluminum foil, then oven-dried overnight at 60 °C to evaporate moisture. It is crucial to always keep samples covered to minimize airborne contamination in the laboratory. Also, the "unwanted" solutions collected from the density separation process were sieved and concentrated to check if microplastics with a higher density than the sodium chloride solution could have possibly settled at the bottom of the density separator. Sample preparation for microplastic analysis in fish was done following the methodology described in Bahri et al. (2020) with modifications. For 2 hours, fish organs were thawed at room temperature. Samples were placed in a fume hood, and 30 ml of 10% potassium hydroxide was added to each beaker with a stir bar inside. The beakers were then spun at 350 rpm for an hour on a stir plate. After an hour, 5 ml of 30% hydrogen peroxide was added to the samples and spun again at 350 rpm for 30 min while covered for minimal exposure to airborne microplastics, and samples were allowed to sit for 2 h to ensure complete digestion of the organs. The sample mixtures were then sieved through a

32 μm sieve, and the retained residues were put back into the beakers for hydrogen peroxide digestion. In each beaker, 20 ml of 0.05 M iron sulfate (FeSO_4) and 20 ml of 30% hydrogen peroxide were added using different pipettes, and the mixtures were spun at 350 rpm for 30 min. Similarly, hydrogen peroxide was added until all the visible unwanted materials were oxidized, producing clear fumes and solution from the beaker. Thereafter, the samples were subjected to MP isolation and purification similar to the matrix obtained from surface water. After obtaining the MPs in the oven-dried beaker, 20 ml of ultra-pure water was added and left for an hour for microplastics to detach from the beaker, and a 0.5 ml representative sample was taken to prepare microscope slides, which were covered with coverslips and underwent physical identification with a dissecting microscope (Leica TL5000 Ergo Transmitted Light Base).

The use of catalyzed hydrogen peroxide with ferrous sulfate (uncooled) was used because it has been proven by Lenz et al. (2021) that it causes minor changes and alteration to microplastics, thus it does not hinder their recognition. As a result, it is safe to use for treatment as long as temperatures are not elevated and time exposure is not prolonged, especially when Raman or FTIR spectroscopy is used.

3.7 Visual identification and quantification

A dissecting microscope (Leica TL5000 Ergo Transmitted Light Base) was used to observe the prepared microscope slides at a magnification of X40. Based on previous identification approaches, we recorded microplastic morphologies (color, shape, and size) as well as took photographs for the identified microplastics (Hidalgo-Ruz et al., 2012; Huang et al., 2020; Rodrigues et al., 2018). Further, in cases where there is uncertainty on whether the identified particle is plastic or not, a hot needle test was employed. A very hot needle was moved closely to

a potential microplastic while still under the microscope, and, without blocking the view, the microplastic melted or curled once in contact with the needle, whereas non-plastic did not (De Witte et al., 2014). In addition, biological structures such as cellular or organic structures were used to distinguish between plastic and non-plastic materials. The units for water samples were the mean micrograms per cubic meter ($\mu\text{g m}^{-3}$) and amount of microplastics per liter (particles L^{-1}), while the units for fish samples were based on the number of counted microplastic particles per organ as well as particles in fish per site

3.8 Quality Assurance/Control

To minimize contamination during sampling, fiber-free clothing was worn, and all materials were washed thoroughly using distilled water before use as well as covered. Also, blanks were used to establish any possible level of contamination during sample collection as well as laboratory analysis. One field blank was used for each sampling site to determine any contamination that might have occurred during field sampling, while five method blanks were used in the laboratory to examine contamination that might have resulted from laboratory preparations and procedures.

The field blanks collected an average of 1.5 ± 0.3 microplastics, while the laboratory blanks collected an average of 3.3 ± 0.8 microplastics. Even though no sample was left uncovered for more than 30 minutes during analytical procedures and counting, as a conservative measure, these averages were subtracted from all sample counts for analysis and reporting.

3.9 Statistical analysis

For the number of MPs in surface water, one-way ANOVA was used to determine significant variation between sites, and two-way ANOVA was used to determine variations of

MPs' morphological properties (color, shape, and size) between different sites. Similarly, for fish samples, a one-way ANOVA was also used to determine the variation of the total MP load and the MPs present in the gills, stomach, and intestines. A two-way ANOVA was used to determine variations in MPs' morphological properties between different organs. When significant differences exist at the 5% level, Tukey's test is employed for mean comparison. The data were analyzed using IBM SPSS Statistics for Windows, Version 26.0.

NB: All the methodology employed in this study have been used in the Okavango Delta article which is already published and is a part of this project [Ditlhakanyane, B. C., Ultra, V. U., Mercy, J., & Mokgosi, S. (2022). Microplastic load in the surface water and *Tilapia sparrmanii* (Smith , 1840) of the river systems of Okavango. *Environmental Monitoring and Assessment*, 2018. <https://doi.org/10.1007/s10661-022-10263-w>]

Chapter 4

Results and Discussion

4.1 Results

4.1.1. Surface water parameters for Site 1- Okavango Delta

The results for various surface water parameters that were measured during sample collection are presented in the table below (Table 4.1). Significant variation in the surface water quality parameters was observed across all sampling sites. The pH ranged from 7.06 to 7.60 across sampling sites, and sites 1 and 4 have significantly higher pH than other sites. This is followed by sites 2 and 5, which are higher than site 3.

Table 4.1: The averages for surface water parameters from five sampling sites in the Okavango delta, Northwest, Botswana.

Sampling site	pH	Electrical Conductivity ($\mu\text{S}/\text{cm}$)	Dissolved Oxygen (mg/L)	Total Dissolved Solids (mg/L)
S1	7.60a	39c	4.66a	19c
S2	7.20a	37c	3.92b	19c
S3	7.06c	33c	4.18b	16c
S4	7.47a	54b	4.39ab	27b
S5	7.26b	93a	3.44c	46a
<i>p</i> -value	0.001**	0.000***	0.003**	0.000***

Means within the same column followed by the same letter (s) are significantly different from each other based on Tukey's test at significance level of 5%; **= highly significant; ***= very highly significant.

The EC ranged from 33 to 93 $\mu\text{S cm}^{-1}$. Sites 1, 2, and 3 have lower EC compared to sites 4 and 5 while it increased by 21 $\mu\text{S cm}^{-1}$ in site 4 and by 39 $\mu\text{S cm}^{-1}$ in site 5 compared to site 3. Similarly, the same trend was observed for TDS wherein sites 3, 2, and 1 have TDS varying

between 16 and 19 mg L⁻¹. Based on site 3, the TDS increased by 11 mg L⁻¹ in site 4 and by 19 mg L⁻¹ in site 5. As for the DO, the lowest value was observed in site 5 compared to other sites, while the highest value was in site 1.

4.1.2 Abundance and Distribution of Microplastics in Surface Water of Okavango Delta

As depicted in Table 4.2 below, there was significant variation observed between sampling sites in terms of the weight and the frequency of microplastics recovered from the surface water samples.

Table 4.2: Summary of the results of analysis of variance of different microplastic parameters of the surface water from the five sampling sites in Okavango delta, Northwest Botswana.

Parameters	Source of variation	Mass of Microplastics	Frequency of Microplastics	Color	Shape	Size
Surface water	Sampling site	0.046*	0.000***	0.036*	0.047*	0.000***
	Color/shape/size	NA	NA	0.000***	0.000***	0.000***
	Sampling sites x (color/shape/size)	NA	NA	0.251 NS	0.183 NS	0.144 NS

NS not significant, NA not applicable

*Significant

***very highly significant

In addition, there was a significant difference amongst the physical morphologies of microplastics in terms of the color, shape, and size of microplastics recovered in the surface water of the Okavango River and the major tributary channels. Further, there was no significant variation between sampling sites and physical morphologies, as shown in Table 4.2 above.

The recovered microplastics recovered were weighed and their average weight ranged from 138.2 to 381.7 µg m⁻³ (Figure 4.1), with site 4 from Xakanaxa which is located within the Moremi Game Reserve having the highest weight. This was significantly higher than the weight of

microplastics found near the end of the panhandle (site 2), which measured $138.18 \mu\text{g m}^{-3}$, and downstream of the Boro River (site 5), which measured $162.7 \mu\text{g m}^{-3}$. The weight of microplastics at the inlet of the delta (site 1) and the heart of the delta (site 3) were not significantly different from neither site 2, site 4, nor site 5. The weight of microplastics in site 2 is the lowest and is 36% lower than in site 4.

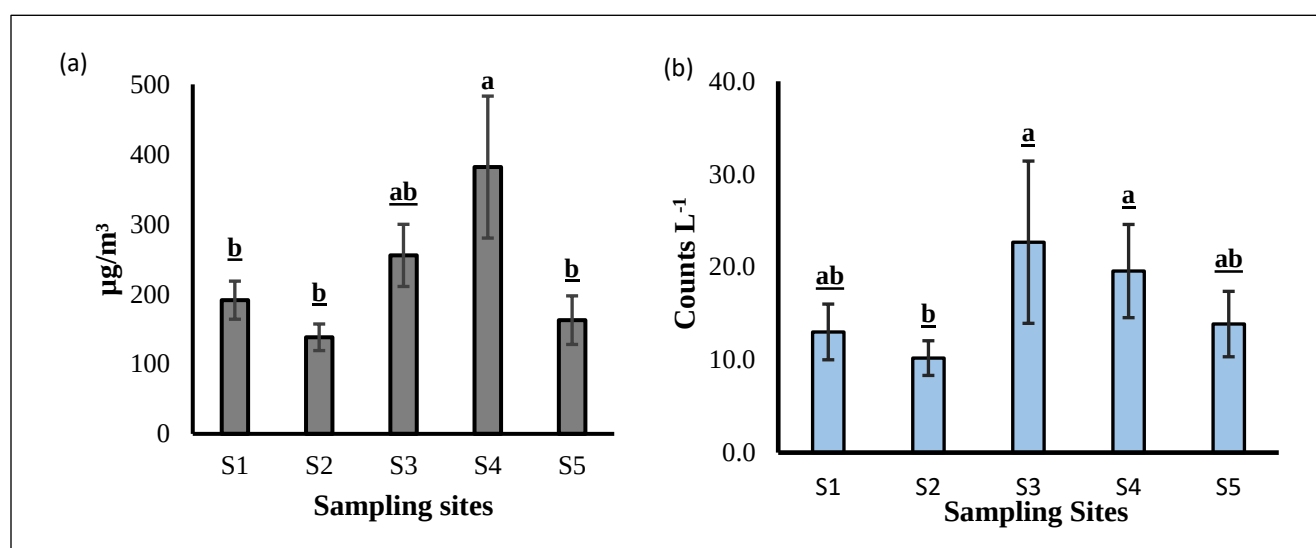


Figure 4.1: The average (a) weight and (b) counts, of microplastics from five sampling sites of Okavango delta, Northwest, Botswana. Samples were collected in April-May 2021. Bars with the same letters are not significantly different from each other based on Tukey's test at 5% significance level. Average values used to calculate percentages and standard deviation employed to indicate variability between morphologies.

The abundance varied from 10.2 to 22.7 particles L^{-1} , with the highest abundance found in the heart of the delta and the lowest abundance was discovered towards the end of the panhandle (Figure 4.1a). The microplastic abundance at the heart of the delta (site 3) and in the eastern part of the delta in the Xakanaxa area (site 2) was significantly higher than at the inlet of the delta, down the panhandle, as well as downstream of the Boro River (site 1, site 2, and site 5,

respectively). However, microplastic abundance was not significantly different for the samples from the delta inlet, downwards the end of the panhandle, and downstream of the Boro River.

4.1.3 Abundance of Microplastics in *Tilapia sparrmanii* from Okavango Delta

The overall frequency of microplastics recovered in fish varied significantly between sites as well as between the fish organs with no interaction observed between the fish and the mass of microplastics (Table 4.3). The color, shape and sizes of microplastics recovered in *Tilapia sparrmanii* samples from the Okavango delta varied significantly between fish organs and between the different physical morphologies but there was no interaction between sampling sites and these morphologies.

Table 4.3: Summary of the results analysis of variance of different microplastic parameters of the fish (*Tilapia sparrmanii*) from the five sampling sites in Okavango delta, Northwest, Botswana.

Parameters	Source of variation	Mass of Microplastics	Frequency of Microplastics	Color	Shape	Size
Fish (<i>Tilapia sparrmanii</i>)	Sampling site	0.207 NS	0.020*	0.157 NS	0.103 NS	0.175 NS
	Fish organs		0.019*	0.006**	0.012*	0.006**
	Color/shape/size		NA	0.010*	0.047*	0.041*
	Sampling site x fish organ		0.789 NS	0.638 NS	0.724 NS	0.631 NS
	Sampling site x color/shape/size		NA	0.246 NS	0.316 NS	0.444 NS
	Fish organ x color/shape/size		NA	0.489 NS	0.081 NS	0.864 NS
	Sampling site x fish organ x color/shape/size		NA	0.483 NS	0.123 NS	0.505 NS

NS not significant, NA not applicable

*Significant

***very highly significant

In terms of abundance, more microplastics were found in fish samples from site 5 compared to other sampling sites while fish samples from site 3 had the least microplastics (Figure 4.2a). On

the other hand, it was discovered that the intestines was the organ with the highest abundance of microplastics, followed by gills and stomach in decreasing order (Figure 4.2b). The mean abundance of microplastics found in the intestines was significantly higher than those found in the stomach of the fish samples.

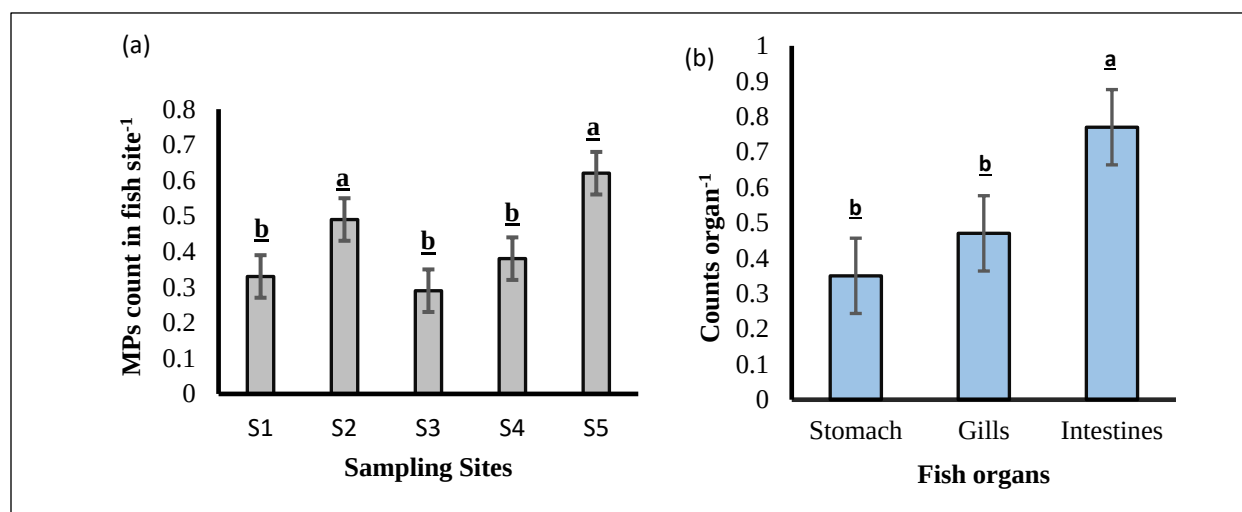


Figure 4.2: The average (a) count per site and (b) count per organ of microplastics recovered in fish samples (*Tilapia sparrmanii*) of the Okavango delta, Northwest, Botswana. Based on Tukey's test at 0.05 significance level, bars with the same letters are not significantly different from each other and the whiskers indicate the standard deviation which shows variability.

4.1.4 Physical Morphologies of Microplastics in Surface Water and *Tilapia sparrmanii* from Okavango Delta

4.1.4.1 Color

The colors of the discovered microplastics were red, blue, black, and transparent as well as yellow (Figure 4.3a). The most abundant colors were translucent and black which were significantly higher than microplastics colored blue, red, and yellow. Translucent and black comprised about 34.9% and 31.2% respectively while blue, red, and yellow contribute about 18.0%, 14.9%, and 1%, respectively.

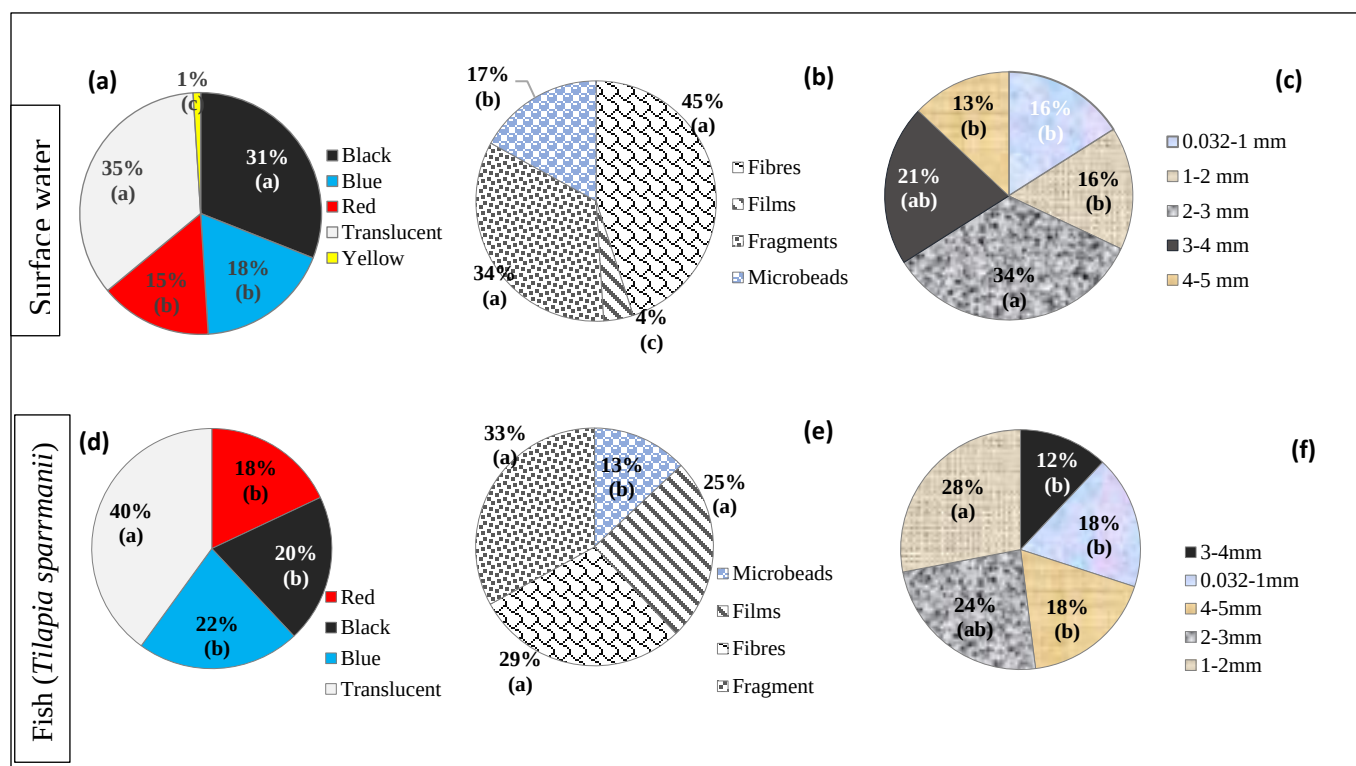


Figure 4.3: Physical morphologies of microplastics recovered from surface water and fish (*Tilapia sparrmanii*) of the Okavango Delta, Northwest, Botswana. Bars with the same letters are not significantly different from each other, based on the Tukey's test at 5% significance level. Average values used to calculate percentages and standard deviation employed to indicate variability between morphologies

The results showed that ingested microplastics discovered from fish organs were translucent, blue, black, and red (Figure 4.3d). Translucent microplastics dominated in fish samples with 40.0% and were significantly higher than blue, black, and red microplastics which contributed 22.1%, 20.0%, and 17.9%, respectively.

4.1.4.2 Shape

Microplastics were classified into four categories: fibers, fragments, microbeads, and films (Figure 4.3b). It is noteworthy that fibers and fragments dominated the entire water samples occupying 45.1% and 34.4% respectively. Fibers were the most abundant type of microplastic followed by fragments, microbeads whereas films were the least common shape discovered in surface water samples.

Meanwhile, fragments (32.6%) were the most dominant forms of microplastics discovered from all organs whereas microbeads were the least discovered forms of microplastics, constituting only 12.5%. Further, fibers and films were also recovered from the fish samples, constituting 29.5% and 25.3%, respectively (Figure 4.3e)

4.1.4.3 Size

The microplastics identified under the microscope were divided into five categories following their sizes: 0.032–1 mm, 1–2 mm, 2–3 mm, 3–4 mm, and 4–5 mm. Regardless of sampling site, size range 2–3 mm was the most dominant microplastics followed by 3–4 mm (20.9%), 1–2 mm (15.8%), <1 mm (15.8%) while size range of 4–5 mm (13.6%) was the least range discovered in surface water (Figure 4.3c).

In fish samples, the size range 1–2 mm was 41% significantly higher than size 3–4 mm as the most ingested size of microplastics, while microplastics of size 2–3 mm contributed 24.2% with 4–5 mm and <1 mm each contributing 17.9% (Figure 4.3f).

NB: All the results for Site 1- Okavango Delta has already been published even though it is a part of this project [Ditlhakanyane, B. C., Ultra, V. U., Mercy, J., & Mokgosi, S. (2022). Microplastic load in the surface water and *Tilapia sparrmanii* (Smith , 1840) of the river systems

of Okavango. *Environmental Monitoring and Assessment*, 2018. <https://doi.org/10.1007/s10661-022-10263-w>]

4.2.1 Surface water parameters for Site 2- Gaborone dam

Surface water parameters were measured during sample collection from the Gaborone dam, as depicted in Table 4.4, with significant difference between sites. The pH for surface water in Gaborone dam ranged from 8.26 to 8.62, where site 3 was significantly higher than all sites while site 4 had the lowest pH.

Table 4.4: The averages for surface water parameters from six sampling sites in the Gaborone dam, South-east Botswana.

Sampling sites	pH	Electrical Conductivity ($\mu\text{S}/\text{cm}$)	Dissolved Oxygen (mg/L)	Total Dissolved Solids (mg/L)
S1	8.40ab	231b	5.64c	115b
S2	8.57a	237ab	6.25ab	118ab
S3	8.62a	238ab	6.48a	119ab
S4	8.26b	255a	6.00b	128a
S5	8.54a	244a	6.41a	122a
S6	8.54a	238ab	6.65a	119ab
<i>p</i> -value	0.002**	0.006**	0.004**	0.003***

Means within the same column followed by the same letter (s) are significantly different from each other based on Tukey's test at significance level of 5%; **= highly significant; ***= very highly significant.

In terms of EC, site 4 was significantly higher while site 1 had the lowest EC of $231 \mu\text{S cm}^{-1}$. Site 6 had the highest DO which was 1.01 mg L^{-1} significantly higher than DO in site 1. Similarly, site 1 also had the lowest TDS of 115 mg L^{-1} , while site 4 had the highest at 128 mg L^{-1} .

4.2.2 Abundance and Distribution of Microplastics in Surface Water from Gaborone dam

The weight of the recovered microplastics were significantly different between sampling sites while no significant variation was observed in the frequency of microplastics between sites (Table 4.5).

Table 4.5: Summary of the results for analysis of variance for different microplastic parameters of surface water collected from six sampling sites of Gaborone dam, Southeast, Botswana.

Parameters	Source of variation	Mass of Microplastics	Frequency of Microplastics	Color	Shape	Size
Surface water	Sampling sites	0.012*	0.003**	0.000***	0.000***	0.008**
	Color/shape/size	NA	NA	0.000***	0.000***	0.000***
	Sampling sites x (color/shape/size)	NA	NA	0.191 NS	0.133 NS	0.111 NS

NS not significant, NA not applicable

*Significant

***very highly significant

***very highly significant

Moreover, a significant difference amongst the physical morphologies of microplastics was observed, in terms of the color, shape and size of microplastics recovered in the surface water of the Gaborone Dam.

In terms of weight, the highest weight of microplastics was detected in S3 at $1943.0 \mu\text{g m}^{-3}$ from the deep area of the dam, while the lowest weight was found in samples from S4 at $760.0 \mu\text{g m}^{-3}$ from the shallow area. Generally, the weight of MPs from the deep areas (S3 and S6) was significantly higher than the weight of MPs from the shallow areas (S1 and S4). In addition, the middle areas (S2 and S5) had a moderately higher weight of MPs than those from shallow areas, which are S1 and S4 (Figure 4.4a).

Results in Figure 4.4b shows the abundance of the potential MPs, which varied from 36.0 to 76.0 particles L⁻¹. The lowest microplastic abundance was recovered from the shallow area of the dam in S1, and the highest abundance was from S3. Overall, the lower abundance of MPs observed were from the shallow areas (S1 and S4), while an increasing trend was observed in water samples from the middle areas (S2 and S5), moving towards the deep areas (S3 and S6), which are closer to the boundary wall of the dam.

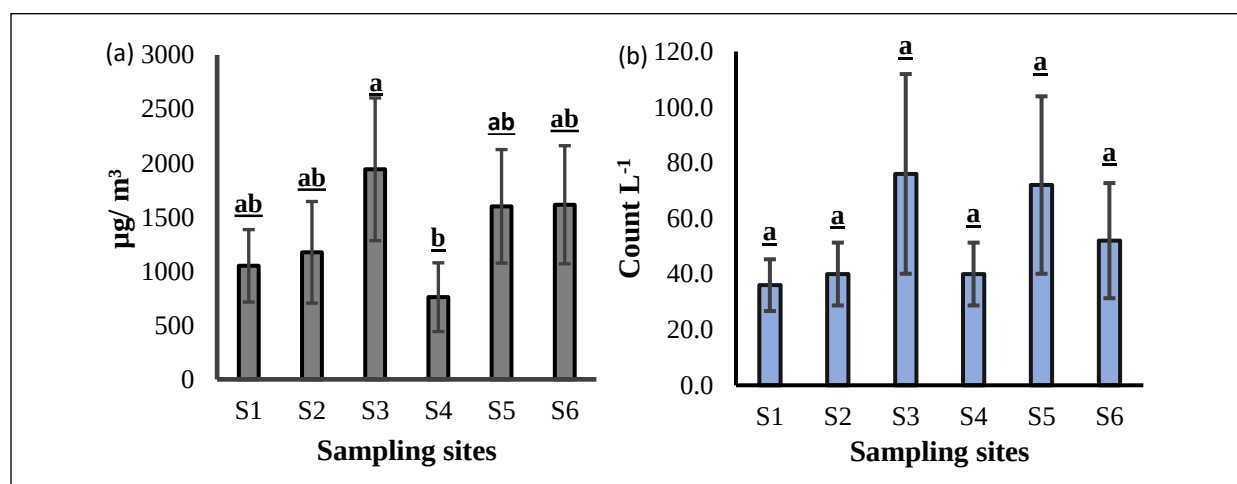


Figure 4.4: The average (a) weight, and (b) counts of microplastics in surface water from six sampling sites of Gaborone dam, Southeast, Botswana. Samples were collected in November 2021. Based on Tukey's test at 0.05 significance level, bars with the same letters are not significantly different from each other.

4.2.3 Abundance of Microplastics in fish (*Oreochromis niloticus* and *Coregonus kiyi*) and clams (*Corbicula fluminea*) from Gaborone Dam

Table 4.6: Summary of the results for analysis of variance for different microplastic parameters of fishes (*Oreochromis niloticus* and *Coregonus kiyi*) collected from six sampling sites of Gaborone dam, Southeast, Botswana.

Parameters	Source of variation	Mass of Microplastics	Frequency of Microplastics	Color	Shape	Size
Fish	Fish organs	NA	0.004**	0.001**	0.000***	0.005**
(Oreochromis niloticus & Coregonus kiyi)	Color/shape/size	NA	NA	0.000***	0.000***	0.009**
	Sampling sites x fish organ	NA	0.867 NS	0.013*	0.001**	0.057 NS
	Sampling sites x color/shape/size		NA	0.268 NS	0.316 NS	0.579 NS
	Fish organ x color/shape/size		NA	0.030*	0.001**	0.637 NS
	Sampling sites x fish organ x color/shape/size		NA	0.466 NS	0.342 NS	0.219 NS

NS not significant, NA not applicable

*Significant

***very highly significant

***very highly significant

According to the results presented in Table 4.6 above, there was significant difference between fish organs and the frequency of microplastics as well as amongst physical morphologies. Also, interaction between sampling sites, fish organs and physical morphologies varied significantly.

Since two different fish species were used for microplastic analysis in Gaborone Dam, the data was presented based on the type of fish species. Generally, the amount of microplastics recovered in fish species from varied from 1.0 to 3.5 count per site (Figure 4.5a). According to the

results presented below, microplastics were recovered in all fish samples from S2, S3, S5 and S6 whereas in S1 and S4, MPs were only recovered in *Oreochromis niloticus* fish. The highest count of microplastics per site was found in *Coregonus kiyi* of S3 while *Oreochromis niloticus* from S4 had the highest MPs. In terms of the color, the most dominant found in *Coregonus kiyi* was the red (31 %) microplastics while translucent microplastics were prevailing in *Oreochromis niloticus* at 34 % and the yellow MPs were the least recovered in both species at 4 %, each (Figure 4.5b).

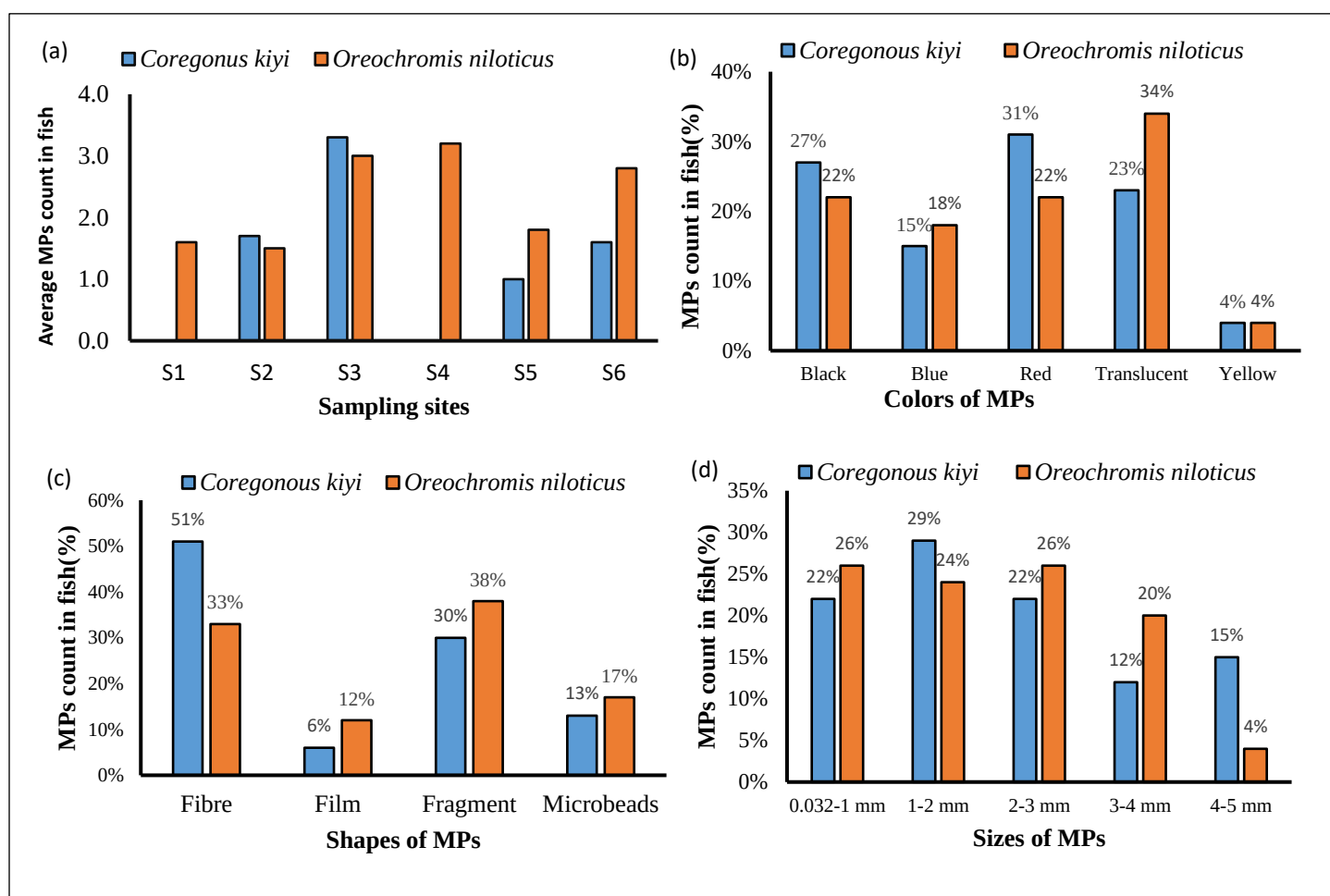


Figure 4.5: Microplastics (a) counted in two fish species (*Oreochromis niloticus* & *Coregonus kiyi*) across sampling sites and the variation of microplastics physical morphologies (b) color, (b) shape and, (c) size for the fish samples from Gaborone dam.

More fibers were in *Coregonus kiyi* at 51 % and the least form was film at 6 % while the *Oreochromis niloticus* had more fragmented microplastics at 38% with the film microplastics lower at 12 % (Figure 4.5c). In terms of the sizes of MPs, 1-2 mm were dominant in *Coregonus kiyi* (29 %) while the size ranges 0.032-1 mm and 2-3 mm were both dominant in the *Oreochromis niloticus* fish at 26%, each.

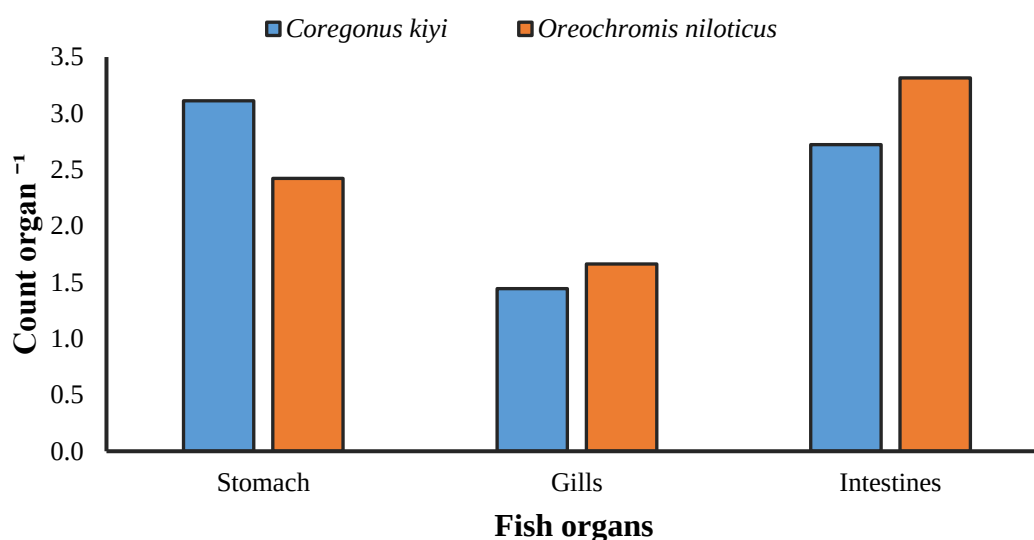


Figure 4.6: The average count per organ of microplastics recovered in fish samples (*Oreochromis niloticus* and *Coregonus kiyi*) of the Gaborone dam, Southeast, Botswana.

The average counted microplastics per organ ranged from 1.4 to 3.1 in *Coregonus kiyi* while in *Oreochromis niloticus* fish organs microplastics recovered ranged from 1.7 to 3.3 plastic particles as shown in Figure 4.6 above. For both fish species, the least recovered microplastics were from the gills and the highest recovery for *Coregonus kiyi* was from stomach whereas in *Oreochromis niloticus* more microplastics were found in the intestines than any other organs.

Table 4.7: Summary of the results for analysis of variance for different microplastic parameters of clams (*Corbicula fluminea*) collected from six sampling sites of Gaborone dam, Southeast, Botswana.

Source of variation	Mass of Microplastics	Frequency of Microplastics	Color	Shape	Size
Sampling sites	NA	0.481*	0.152NS	0.448 NS	0.337 NS
Clams		0.030*	0.256NS	0.732 NS	0.364 NS
Color/shape/size		NA	0.000***	0.015*	0.009**

NS not significant, NA not applicable

*Significant

***very highly significant

***very highly significant

From Table 4.7, the significance difference was observed between sampling sites and frequency of microplastics as well as between clams and the frequency of microplastics. Further there was an interaction among the physical morphologies of microplastics. Regardless of the site, a very high level of significant variation was observed amongst the colors of microplastics as well as amongst the sampling sites.

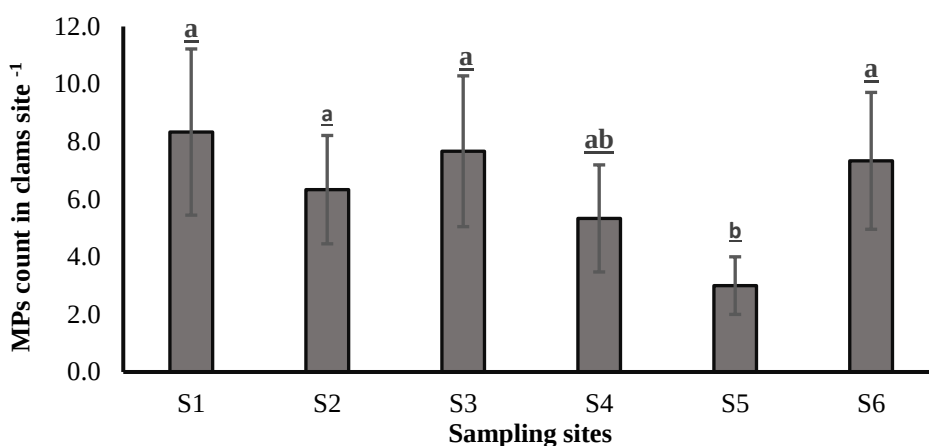


Figure 4.7: Microplastics counted in clams from the six sampling sites. Based on Tukey's test at 0.05 significance level, bars with the same letters are not significantly different from each other and whiskers indicate the standard deviation which shows variability.

4.2.4 Physical Morphologies of Microplastics in Surface Water, fish (*Oreochromis niloticus* & *Coregonus kiyi*) and clams (*Corbicula fluminea*) from Gaborone dam

4.2.4.1 Color

Black and translucent microplastics were the most dominant colors of microplastics, each at 28%, followed by the blue MPs at 23%, red at 14%, and white at 5%, while the least MPs were in yellow at 2% (Figure 4.7a). Both black and translucent MPs were 14 times more significant than the yellow microplastics.

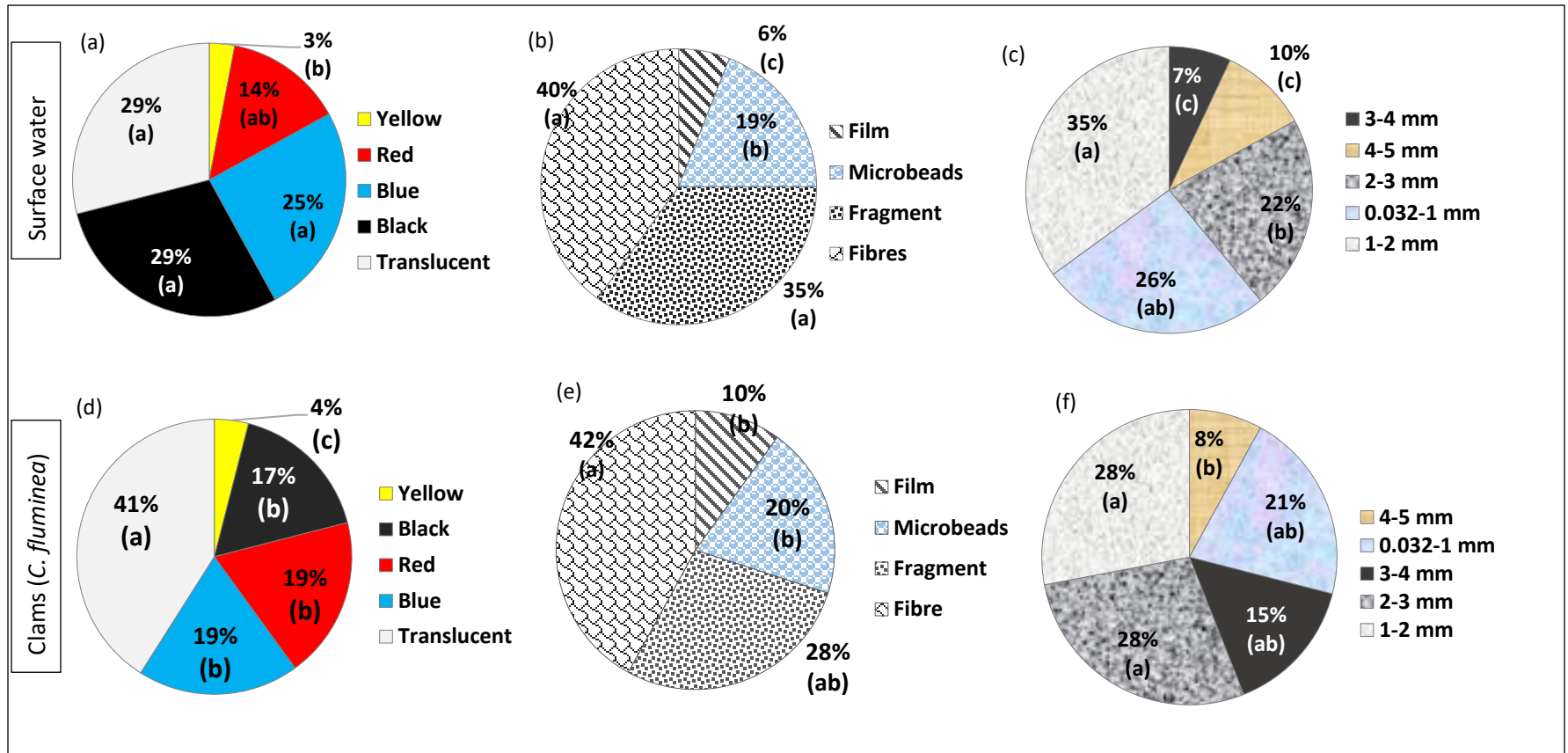


Figure 4.8: Physical morphologies of microplastics recovered from surface water, fishes (*Oreochromis niloticus* - *O. niloticus* & *Coregonus kiyi* - *C. kiyi*) and clams (*Corbicula fluminea* – *C. fluminea*) of the Gaborone dam, Southeast, Botswana. Bars with the same letters are not significantly different from each other, based on the Tukey's test at 5% significance level. Average values used to calculate percentages and standard deviation employed to indicate variability between morphologies.

The most dominant color found in fish species varied as per the type of fish. Red microplastics were the most recovered microplastics in *Coregonus kiyi* at 31 % while black, translucent, blue and yellow contributed 27%, 23%, 15% and 4%, respectively (Figure 4.5b). However, in *Oreochromis niloticus* fish, translucent were the most common microplastics at 34%, with red, black, blue and yellow constituting 22% (red and black, each), 18% and 4%, respectively.

Likewise, translucent MPs accounted for larger proportions in clams at 41%, followed by 19% for both blue and red MPs each, black at 17% and yellow at 4% (Figure 4.7d).

4.2.4.2 Shape

Microplastics in the form of fibers were prevalent in *Coregonus kiyi* constituting 51%, followed by fragments at 30%, microbeads at 13% and film at only 6% and significantly higher than film microplastics, at 40% and 6%, respectively. On the other hand, fragments were the most common forms of microplastics found in *Oreochromis niloticus* at 38%, followed by fibers at 33%, microbeads at 17% and film at 12% (Figure 4.5c).

For the clams, a similar trend was observed in terms of microplastics ingested. Fibers dominated the shapes, accounting for 42%, while fragments, microbeads, and films accounted for 28%, 20%, and 10%, respectively (Figure 4.8e).

4.2.4.3 Size

The results indicated that the size range of 1-2 mm prevailed in *Coregonus kiyi* fish species compared to other sizes, accounting for 29%, followed by the 0.032-1 mm and 2-3 mm, 4-5 mm while 3-4 mm was the least as they constituted 22% (0.032-1 mm and 2-3 mm, each), 15% and

12% respectively. In the *Oreochromis niloticus*, sizes 0.032-1 mm and 2-3 mm were dominant at 26% each, followed by 1-2 mm at 24%, 3-4 mm at 20% and 4-5 mm at 4% (Figure 4.5d).

On the other hand, the dominant sizes of microplastics ingested by clams were 1-2 mm and 2-3 mm, at 28% each, while size 4-5 mm was the least at only 8% (Figure 4.8f). Moreover, the different size proportions of the potential discovered MPs in clams include sizes 3-4 mm (21%) and 0.032-1 mm (15%).

4.3.1 Surface water parameters for Site 3- Dikgatlong dam

The following surface water parameters were measured in situ during sample collection and the results are as depicted in Table 4.8 below.

Table 4.8: The averages for surface water parameters from five sampling sites in the Dikgatlong dam, Central district, Botswana.

Sampling sites	pH	Electrical Conductivity ($\mu\text{S}/\text{cm}$)	Dissolved Oxygen (mg/L)	Total Dissolved Solids (mg/L)
S1	8.25a	148a	2.31a	110a
S2	8.30a	151a	2.27a	119a
S3	8.24a	149a	2.30a	118a
S4	8.20ab	148a	2.32a	111a
S5	8.17b	148a	2.31a	112a
<i>p-value</i>	0.003**	0.000***	0.000***	0.000***

Means within the same column followed by the same letter (s) are significantly different from each other based on Tukey's test at significance level of 5%; **= highly significant; ***= very highly significant.

Based on the results shown in Table 4.8, there was significance variation observed between the sampling sites. The pH ranged from 8.17 to 8.30 across sampling sites, with sites 2 having significantly higher pH than other sites while site 5 had the lowest. The EC ranged from 148 to 151 $\mu\text{S cm}^{-1}$ and was constant across all sites except for site 2 which had the highest EC compared to other sites. Similarly, there was not much difference in terms of TDS between the sites which

was also almost the same across and varied from 110 to 119 mg L⁻¹. However, in terms of DO, the values obtained were very low ranging from 2.27 to 2.32 mg L⁻¹, where site 4 was significantly higher than all other sites.

4.3.2 Abundance and Distribution of Microplastics in Surface Water from Dikgatlhong dam

Table 4.9 represent the significant variation which was tested for different parameters, between the sampling sites and between the observed physical morphologies of recovered microplastics from Dikgatlhong dam.

Table 4.9: Summary of the results for analysis of variance for different microplastic parameters of surface water and fish (*Coptodon rendalli*) from five sampling sites in Dikgatlhong dam, Central district, Botswana.

Source of variation	Mass of Microplastics	Frequency of Microplastics	Color	Shape	Size
Sampling site	0.012*	0.861 NS	0.000***	0.000***	0.008**
Shape/size/color	NA	NA	0.001**	0.003**	0.001**
Sampling site x (color/shape/size)	NA	NA	0.205 NS	0.333 NS	0.231 NS

NS not significant, NA not applicable

*Significant

***very highly significant

***very highly significant

A significant variation amongst the physical morphologies of microplastics was observed, in terms of the color, shape and size of microplastics recovered in the surface water of the Dikgatlhong Dam.

In terms of weight, the highest weight of microplastics was found in S5 at 1500.0 µg m⁻³ from the deeper area of the dam, while the lowest weight was found in samples from S4 at 400.0

$\mu\text{g m}^{-3}$ which is located on the edge of the dam. Generally, the weight of MPs from the deep areas (S1 and S5) was significantly higher than the weight of MPs from the shallow areas (S1 and S4). In addition, the middle areas (S2 and S5) had a moderately higher weight of MPs than those from shallow areas, which are S1 and S4 (Figure 4.8a).

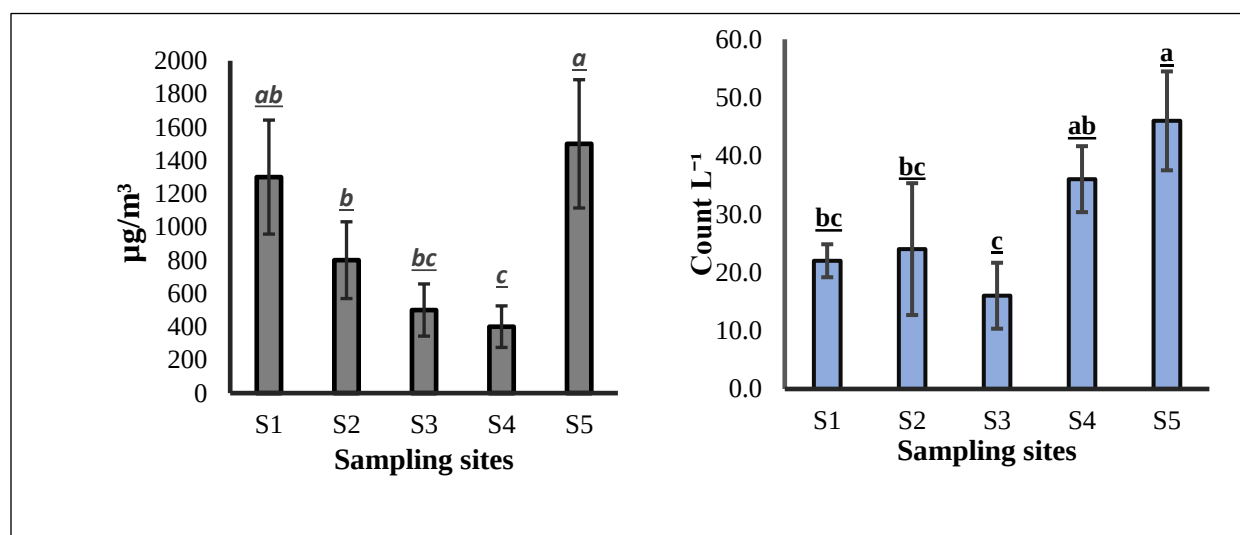


Figure 4.9: The average (a) weight, and (b) counts of microplastics in surface water from five sampling sites of Dikgatlhong dam, Central district, Botswana. Samples were collected in June 2022. Based on Tukey's test at 0.05 significance level, bars with the same letters are not significantly different from each other and whiskers indicate the standard deviation which shows variability.

According to the results in Fig 4.8b, the abundance of the potential MPs varied from 16.0 to 44.0 particles L^{-1} . The lowest microplastic abundance was recovered from S3 in the middle of the channel, and the highest abundance was from S5. Overall, the lower abundance of MPs observed were from S3 and S1, while an increasing trend was observed in water samples from the S4 and S5, moving towards the boundary wall.

4.3.3 Abundance of Microplastics in *Coptodon rendalli* fish from Dikgatlhong Dam

The color, shape and size of microplastics recovered in *Coptodon rendalli* samples of the Dikgatlhong Dam varied significantly between fish organs and frequency of the microplastics. Also, there was significant difference between fish organs and the physical morphologies of microplastics as well as amongst the morphologies (Table 4.10).

Table 4.10: Summary of the results for analysis of variance for different microplastic parameters of fish (*Coptodon rendalli*) collected from five sampling sites of Dikgatlhong dam, Central district, Botswana.

Parameters	Source of variation	Mass of Microplastics	Frequency of Microplastics	Color	Shape	Size
Fish (<i>Coptodon rendalli</i>)	Fish organs	NA	0.009**	0.001**	0.003**	0.000**
	Color/shape/size		NA	0.000***	0.000***	0.001**
	Sampling site x fish organ		0.310 NS	0.115NS	0.207NS	0.076 NS
	Sampling site x color/shape/size		NA	0.188 NS	0.316 NS	0.689 NS
	Fish organ x color/shape/size		NA	0.080NS	0.101NS	0.233 NS
	Sampling site x fish organ x color/shape/size		NA	0.466 NS	0.212 NS	0.411 NS

NS not significant, NA not applicable

*Significant

***very highly significant

***very highly significant

The abundance of microplastics in fish from each sampling site varied from 0.3 to 2.5 count per site, whereas the MPs recovered from the fish organs varied from 0.1 to 0.35 count per organ (Figure 4.10a and 4.10b, respectively). The highest count of microplastics per site was found in S1, and the least was from S2. In terms of the count of MPs per organ, the highest was recovered in the gills and the lowest count was found in the intestines.

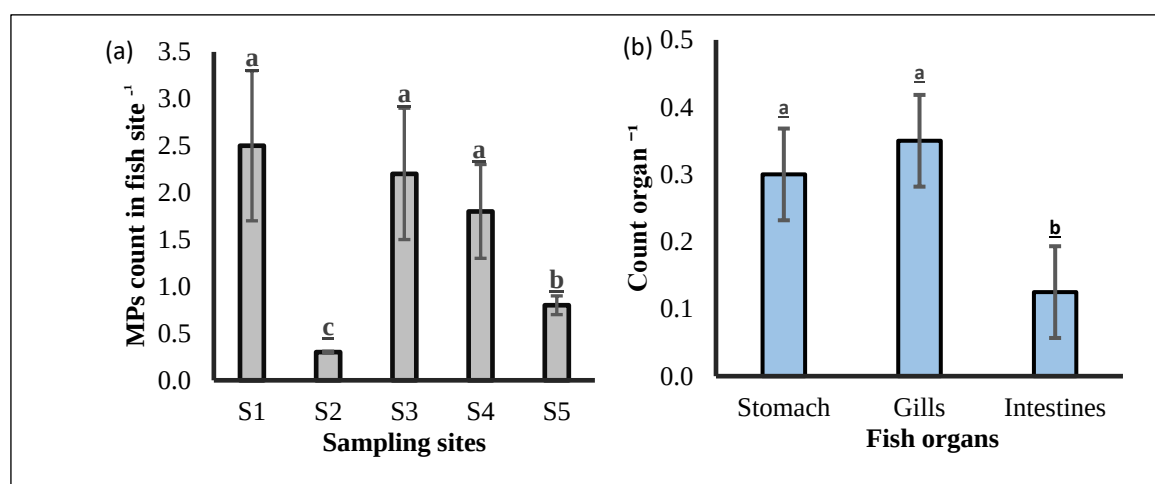


Figure 4.10: The average (a) count per site and, (b) count per organ, of microplastics in the fish samples (*Coptodon rendalli*) of the Dikgatlong dam, Central district, Botswana. Based on Tukey's test at 0.05 significance level, bars with the same letters are not significantly different from each other and whiskers indicate the standard deviation which shows variability.

4.3.4 Physical Morphologies of Microplastics in Surface water and fish (*Coptodon rendalli*) from Dikgatlong dam

4.3.4.1 Color

The discovered microplastics from surface water were in translucent, black, blue and purple color (Figure 4.11a). The most abundant colors were translucent at 42% followed by black at 35%, which were significantly higher than microplastics colored blue and purple, contributing 20% and 3%, respectively.

The results showed that ingested microplastics discovered from fish organs were translucent, black, blue and red (Figure 4.11d). Translucent microplastics dominated in fish samples with 38% and were significantly higher than black, blue and red microplastics which contributed 29%, 19%, and 14%, respectively.

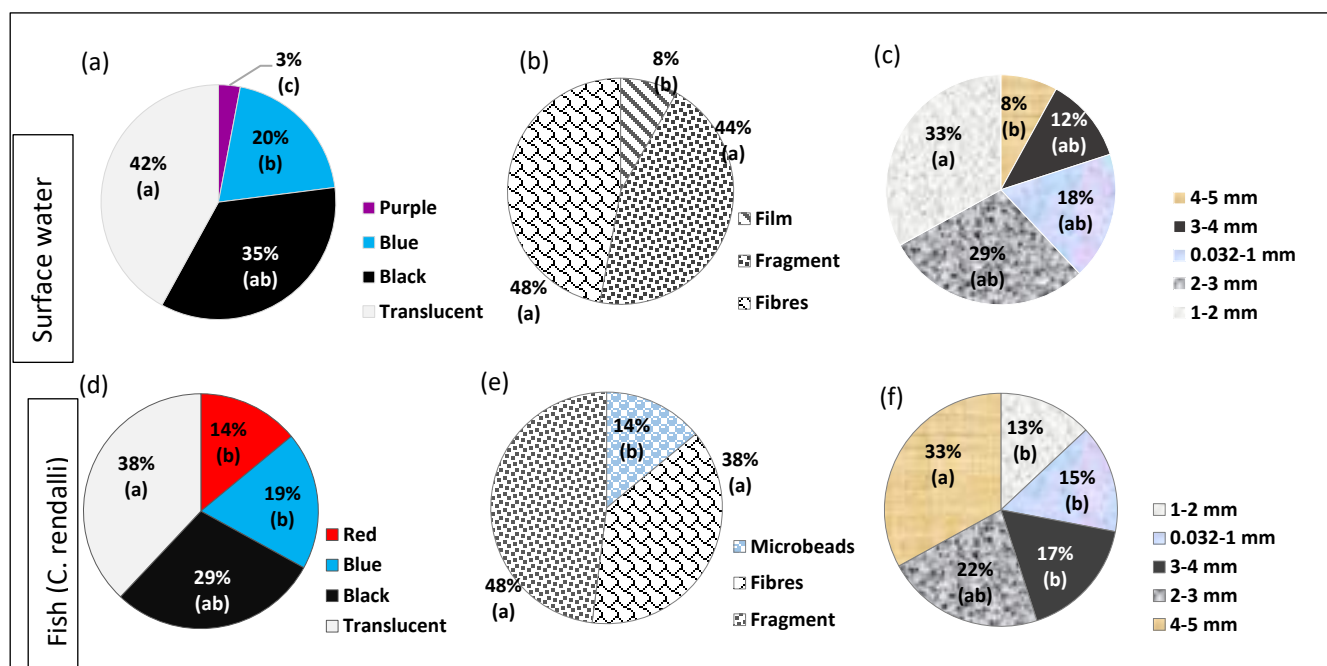


Figure 4.11: Physical morphologies of microplastics recovered from surface water and fish (*Coptodon rendalli*- *C. rendalli*) of the Dikgathong dam, Central district, Botswana. Bars with the same letters are not significantly different from each other, based on the Tukey's test at 5% significance level. Average values used to calculate percentages and standard deviation employed to indicate variability between morphologies

4.3.4.2 Shape

Microplastics were classified into four categories: fibers, fragments, microbeads, and films (Figure 4.11b). According to the results, fibers were the most prevailing forms of microplastics, and fragments were the second most dominant while films were the least recovered and no recovery of microbeads was made.

Meanwhile, in *Coptodon rendalli* fish samples, fragments were the most dominant forms of microplastics at 48% followed by the fibers with 37% and microbeads as the least recovered at 14%, while there was trace of film microplastics (Figure 4.11e)

4.3.4.3 Size

Microplastics identified under the microscope were divided into five categories following the size ranges: 0.032–1 mm, 1–2 mm, 2–3 mm, 3–4 mm, and 4–5 mm. Regardless of sampling site, size range 1-2 mm was the most dominant microplastics followed by 2-3 mm (29%), 0.032-1 mm (18%), 3-4 mm (12%) while size range of 4–5 mm (8%) was the least range discovered in surface water (Figure 4.11c).

On the other hand, the size range 4-5 mm was 20% significantly higher than size 1-2 mm as the most ingested size of microplastics, while microplastics of size 2–3 mm contributed 22% while 3-4 mm and <1 mm contributed 17% and 15%, respectively (Figure 4.11f)

4.4 Discussion

This is the first report of the microplastic load in the surface water of the freshwater systems as well as the quantified microplastics ingested by the common fish in the Okavango Delta, Gaborone Dam, and Dikgatlong Dam. Our study aimed at assessing microplastic loads in three large freshwater systems that are situated in different geographical locations and have different capacities. Therefore, we observed that indeed there is the presence of microplastics in the Okavango Delta, Gaborone Dam, and Dikgatlong Dam as they were recovered in water and fish samples. In this context, our results are of utmost importance as they provide new insights on microplastic load in these freshwater systems for future reference on monitoring microplastic levels, which are detrimental to the freshwater biota and human's health.

Our results from all study areas confirmed that there is a significant difference between the sampling sites and the measured surface water quality parameters (pH, EC, TDS, and DO). Also, there was an interaction between the sampling sites and the parameters. According to Omer (2019), all the values for surface water quality parameters were within the standard limits for both drinking water and the survival of aquatic species. Meanwhile, relatively low mean weights of microplastics and mean abundances in surface water samples were observed from the results across all three study areas. However, compared to previous studies (Ding et al., [2020](#); Yakushev et al., [2021](#)), the overall mean weights of microplastics were higher than those recovered in the Eurasian Arctic, which recorded the highest weight from the Barents Sea ($12.5 \mu\text{g m}^{-3}$). The highest mean weight of microplastics recorded in the Okavango Delta was at site 4, with $381.7 \mu\text{g m}^{-3}$, and it lies in one of the major tributaries of the Okavango River, which is the Khwai River. Also, the site is within one of the tourism hotspots in the Okavango Delta, the Moremi Game Reserve. Nonetheless, even

though site had high weight, overall the Okavango Delta had low microplastic levels despite its location in the tourism hotspot. Since the delta is a World Heritage and Ramsar site, it is crucial to consistently keep its aesthetic beauty a top priority and protect it all costs. Therefore, biodegradable packaging materials are promoted especially in lodges and hotels found within the delta. Also, the community trust entrusted with the protection of the delta has capacitated their community to make it an individual responsibility of removing any foreign materials in the water once they come across, including floating plastics that may have been disposed of inappropriately. These efforts help in reducing the plastic waste found and generated in the delta thus contributing to the low levels of microplastics recovered in this study area. However, the area experiences a lot of movement from wildlife and tourism activities (boat cruising, camping, and game drives), which occur all year round because of its spectacular game viewing and bird watching (Mogobe et al., 2019). Therefore, despite the availability of wheelie bins on site and the reinforcement of the environmental standards to guard against environmental pollution, the area is bound to experience plastic waste generation that is ultimately subjected to mechanical stress from roaming animals as well as moving vehicles. Through rainwater drainage and winds, these plastics are carried from inland into the freshwater systems, where they are further broken down by currents and waves. It is also worth noting that Site 4 is found where the flow velocity of the channel tributary has reduced due to its long travel from the upper part of the delta, and thus microplastics tend to settle and float in that area. According to Hoellein et al. (2019), particles continue to move when agitated by water or could be re-suspended due to a change in velocity; hence, the reduced flow movement led to the re-surfacing of submerged microplastics at site 4. On the other hand, site 2 ($138.2 \mu\text{g m}^{-3}$) had the lowest mean weight of microplastics after site 5 ($162.7 \mu\text{g m}^{-3}$). Since water is flowing from the inlet down the panhandle into the alluvial fan (Milzow et al., 2009), that means the flow

velocity is considerably fast in site 2, thus carrying away most of the microplastics downward into the alluvial fan while less is trapped by the vegetation. Moreover, even though Site 5 lies downstream where the flow velocity is reduced, it still had a lower mean weight of microplastics as well. This could be because of the high fish population since the area is closer to Maun Town, where agricultural activities transpire and most of the nutrients are washed into the water. Therefore, fish reproduction is increased, and most of the smaller microplastics are mistaken for nutrients and ingested by fish, resulting in a lower weight recorded.

In general, the results showed that the mean abundance for the spatial distribution of microplastic in surface water samples fluctuated. There was a mean decrease from 13.0 to 10.2 particles L^{-1} between site 1 and site 2, a drastic increase in site 3, and then a decrease in both site 4 and site 5 (19.6 particles L^{-1} and 13.9 particles L^{-1} , respectively). The observed variations in microplastics abundance in sites may be explained by intensive monitoring for compliance with environmental standards to have a minimal ecological impact by some communities and safaris in various sites. However, it is important to note that even though site 4 had the highest weight of microplastic loads, the results indicated that for the mean abundance of microplastics, site 3 was dominant with 22.7 particles L^{-1} . This suggests that site 4 may have contained a few microplastics with a high density, whereas site 3 had a large number of plastic particles with a low density, resulting in a lower overall weight. Additionally, some of these MPs could be from land-based sources in the Etsha and Jao communities, who also formed a part of Site 3, thus contributing to the highest abundance of microplastics discovered. A similar trend was observed in the West River in the south of China, where an area with high human activities experienced the highest microplastic abundance. Anthropogenic activities may have resulted in the increased abundance of plastic waste and other contaminants in the river (Huang et al., 2020; Wang et al., 2018).

In Gaborone Dam, the weight of microplastics and particles recovered per liter were observed to be high in sampling sites located in the middle and deeper areas of the dam. S3, S5, and S6. These sites had high weights and a high counted plastic particles which could be attributed to the fact that water flows in a north-easterly direction (Keaitse, 2008), so microplastics are bound to be carried in that direction, away from S1 and S4. Also, it is easy for dense MPs in S4 to descend in the thin water column and be buried in the sediments or be ingested by aquatic animals in shallow areas, especially once the water level drops, compared to the deep area (S3) where the water column is thick. Additionally, the main water source to the dam, the Notwane River, rises from the south and passes close to Gaborone in a north-easterly direction, where it will eventually join the Limpopo River in the far eastern part of the country (Keaitse, 2008). Therefore, with this water flow direction, microplastics could be carried along in the same direction and accumulate where there is a boundary wall. In addition, the sampling sites S3 and S6 are closer to a lot of built-up areas, so they are prone to the airborne MPs from the nearby areas, thus accounting for their high abundance. Furthermore, the MPs may have originated from various sources, such as the plastic residues and microplastics from agricultural products (fertilizers, wastewater irrigation, and sludge biosolids) used by farmers located along the Notwane River. During rainy seasons, these plastic particles may be washed into the river and eventually reach the dam, where they may accumulate toward the boundary wall when water flows into the dam. A similar trend was noticed in studies by Zhang et al. (2015) and Park et al. (2020), where MPs accumulated more downstream than upstream of the Three Gorges Dam and Han River, respectively. Therefore, this corresponds with our results, where more MPs accumulated downstream of a dam than upstream. However, there is a need for more research to confirm this trend, where microplastics tend to accumulate more downstream than they do upstream of a dam. Nonetheless, when compared to the recovery

made in the Orange-Vaal River in South Africa, our findings on the frequency of MPs were deemed very low (Weideman et al., 2019). On the contrary, the MPs from this study were higher than the MPs recovered by Mao et al.'s (2020) study in surface water from Wuliangsu Lake, which varied from 3.1 to 11.3 particles L⁻¹.

Meanwhile, the Dikgatlong Dam had the second highest weight and abundance after the Gaborone Dam. This dam is located at the confluence of two rivers, and one of them, the Tati River, passes through high-density areas such as Monarch, Somerset, Bluetown, and the industrial area in Francistown, where plastic waste is bound to be washed into the river system and transported all the way to the dam. It is also worth noting that surface waters from these built-up areas receive no treatment prior to reaching surrounding rivers, so plastic wastes are carried in bulk from inland into the river systems. Also, the Shashe River passes in the localities of villages with more population, such as Tonota, where high waste generation is possible and can reach the river if not disposed of properly. From the results, a high frequency of microplastics was observed in S5 and S4, which are both towards the downstream of the dam, and microplastics tend to accumulate as the water velocity and currents transporting these particles are slowed by the boundary wall. The high abundance in these areas is also attributed to the water flow direction of the rivers. These rivers flow south and past Francistown and the flow changes to a south-easterly direction before reaching the dam, where the Tati River ends and the Shashe River continues until it flows into the Limpopo River. With this flow direction, microplastics are carried away from S2 and S3, transported towards the dam center, S3, while some deposit at S4 on the edge, and the rest accumulates towards the boundary wall in S5.

Compared to previous studies, the mean abundance of microplastics in surface water for all the study areas was higher than what Huang et al. (2020) found in the West River, south of China, which ranged from 3.0 to 9.9 particles L⁻¹ as well as Ding et al. (2019), who reported 3.7 to 10.7 particles L⁻¹. On the contrary, when compared to studies by Naidoo et al. (2015) and Han et al. (2020), these quantities were very low. These differences could be influenced by various factors, such as the scope of the study, the characterization methods used, differences in water capacities, and the recharging systems across the studies.

Originally, plastics were mainly opaque, white, milky white, and almost clear before the addition of any colorants (Turner, 2018). Subsequently, some undergo either external coloring (dying of the surface, i.e., printing, coating, or plating) or internal coloring (kneading colorants are used, i.e., dye or pigment). Therefore, this suggests that externally colored microplastics can easily decolorize and release colorants as well as other additives when subjected to various environmental factors (long retention time in water, exposure to water turbulence) for a long time. In addition, Szaky (2015) has emphasized that clear plastics are highly preferred in the recycled material market and manufacturing. Compared to clear plastics, colored plastics tend to have multiple impurities and consist of complex composite materials such as colorants and hazardous additives, which require extra processing to remove. Therefore, clear plastics become ideal over colored plastics, which are limited to becoming darker shades of the original color. Furthermore, colored plastics result in a drastic decrease in the quality and potential application of a recycled material due to contamination during the recycling process. In this context, translucent parent plastics become dominant, thus resulting in the generation of more translucent microplastics (Li et al., 2020; Prata et al., 2019; Xiong et al., 2018). Our results also concur with these studies because, across all study areas, translucent microplastics were dominant in all sample matrices. Black

microplastics also contributed a significantly higher percent of microplastics discovered after translucent microplastics. This could be because they are almost impossible to recycle, so they end up in landfills or just about anywhere in the environment since they are not accepted by recycling plants (Turner, 2018). Furthermore, the high percentage of black and translucent microplastics could be attributed to the degree of production and utilization of the raw plastics and the type of transformation to which these raw plastics are exposed in the environment. These results are consistent with the findings of Li et al. (2021) and Di and Wang (2018), who also found translucent microplastics to be the most prevalent color of microplastics. Also, a lot more colored microplastics are found in the Gaborone Dam and Okavango Delta than in the Dikgatlong Dam. This may be influenced by the human activities surrounding these freshwater systems, where colorful plastic materials are used and disposed of regularly, thus being carried through surface runoff into the delta and the dams within a short period of time. Unlike the other two study areas, for plastic waste to reach the Dikgatlong Dam, the materials must be transported through the Tati River and Shashe River over an extended period of time and exposed to turbulence in the process, where they might even lose color (especially the externally colored plastics) before reaching the dam. Moreover, local fishermen in these areas usually use transparent or black nets to catch fish, which also contributes to the abundance of microplastics of this nature and color. Abundant translucent MPs have also been found in other studies (Su et al., 2017; Jiang et al., 2019; Huang et al., 2020). These studies have highlighted that the prevailing use of translucent MPs could be due to the wide and extensive use of transparent plastic products, such as packaging bags used daily. Furthermore, the digestion method used could have bleached the colored plastic particles during analysis, resulting in a possible overestimation of the translucent microplastics recovered in the study (Huang et al., 2020).

Fibers and fragments are the most dominant types of microplastics, and their abundance is higher in all the study areas despite the geographical differences. This suggests that potential sources of these microplastic types are domestic wastewaters resulting from washing processes as well as industrial wastewaters where there is textile manufacturing, thus releasing fibers. Fibers from the Okavango Delta are probably from local communities that carry out washing within or near main river systems. Furthermore, most safaris in the delta generate wastewater, as do indigenous people's fishing nets, which wear out and break down, as well as the deposition of airborne particles (Cole et al., 2011). In addition, some of the fragments and fibers found in the Okavango Delta might be transported through the Cubango and Cuito Rivers, which originate in the highlands of Angola and enter Botswana through the Namibia border and distributed through the Okavango River into the delta. This means they are being transported through a non-point source into the delta. In 2019, the National Solid Waste Agency of Angola announced the worrying situation that the country's commercial establishments distributed a total of 12.4 million plastic bags on a daily basis, yet they were already struggling with the proper disposal of plastic waste. The same could be said for Gaborone and Dikgatlong dams, where fibers and fragments are abundant due to high population densities (for domestic and industrial applications). With Gaborone Dam being in the vicinity of an urbanized area, the fibers could be released from urban effluents, especially the domestic wastewater used in textile and clothing activities. According to Browne et al. (2011), garments can release about 1900 fibers every time they are washed, which contributes to the high fiber content found in surface water. The Notwane River is exposed to millions of liters of wastewater that flows from surrounding areas of Gaborone daily, which could be reaching the dam and carrying the microfibers from the various synthetic industries (Mmegi Online Newspaper, 2015). Also, there is fishing activity going on in the dam, so fibers may break

from fishing gear, fish cages, or nylon ropes used and remain floating in the surface water (Kasamesiri and Thaimuangphol, 2020). Additionally, there are farms along the Notwane River that could be releasing fibers through agricultural waste into the river and eventually reaching the dam. That is, long-term agricultural plastic mulching and the application of sludge biosolids from wastewater treatment plants could result in an accumulation of MPs in the soil and eventually be carried into the river by heavy rainfall (Zubris and Richards, 2005). Thereby, these fibers become available for consumption by freshwater species during active feeding or even via respiratory processes in the case of fish (via gills) since they are in abundance. Similarly, wastewater discharged into the Tati and Shashe Rivers from the second city Francistown, and some surrounding areas may be carried through these river systems until they reach Dikgatlong Dam, where they will accumulate. Furthermore, fragmented plastic particles may be washed away from inland sources and into freshwater systems, where they can accumulate in large quantities. These results correspond to findings by Huang et al. (2020) and Zhao et al. (2014), who discovered fibers as the most common form of microplastics in the West River in southern China and the Yangtze Estuary, respectively. However, a study by Rodrigues et al. (2018) recovered more fragments than fibers in a study done at the Antuà River. On the other hand, the detected microbeads may be mostly from cosmetic products used in homesteads, hotels, lodges, and safaris. Plastic wrappings and bags that are disposed of inappropriately by most people with a single-use plastic preference might be the reason why they reach our freshwaters, and because they are very light, they break easily, hence a low amount is recovered as most of them could have been too small to be detected.

Macroplastics have been and are continuously degrading; hence, small microplastics are formed and discovered in greater numbers than those that are larger, as supported by these results. The size distribution showed that size ranges of 0.03–2 mm, 1–2 mm, and 2–3 mm microplastics

were the most abundant across all study areas in all sample matrices. Plastic parents are broken into smaller particles due to various reasons, such as a lot of movement from wildlife and humans, exposure to UV light and temperatures, the type of plastic polymer, retention time, and the hydrological dynamics of the freshwater systems. Their small sizes make it easier for them to be carried from inland into the water, where they are further subjected to stronger currents and waves, which enhance their weathering process, thus forming smaller-sized microplastics (Huang et al., 2020). With so many people moving around all year, the life of large microplastics may be shortened through further fragmentation. According to a report by United Nations Environment Programme - UNEP, (2018), plastics such as polystyrene or Styrofoam (PS) are lightweight and easy to form into plastic materials; they also break effortlessly, which means they have a very short lifetime, hence they are always found in smaller sizes. It becomes difficult for larger microplastics to be found on the surface of the water in high quantities; probably because of their densities, they tend to sink through the water column, hence being recovered in small quantities. Additionally, sizes of microplastics larger than 0.5 mm are used in making fishing ropes, nets, or lines, indicating that the source of microplastics in the delta emanated from fishing gear as well as equipment used in aquaculture.

Once microplastics reach the freshwater systems, they can be ingested by aquatic organisms, while some sink and get buried in the sediments. According to the results, fish samples from site 5 of the Okavango Delta had the highest frequency of microplastics of all the other sites. This site lies downstream of the Boro River where anthropogenic activities are occurring since it is closer to the major town in the northern district, which is Maun. Therefore, the site is prone to microplastic pollution from agricultural activities and fishing, as well as from the high rate of atmospheric deposition that transpires in Maun. Fertilizers carried inland into the river system

contain microplastics that fish may mistake for food and consume. Nevertheless, even though surface water samples from site 3 had the highest abundance of prospective microplastics, fish samples from the same site had ingested low amounts of microplastics. Meanwhile, microplastics recovered in fish samples from Gaborone Dam were in abundance compared to other study areas. This study area is in a locality that has the largest population in the country, which means more plastic waste is generated, more microplastics reach the dam in abundance, and plenty of them are available to aquatic organisms. Also, these small plastic particles could either be ingested as soon as they reach the dam through the inlet at S4 or as they accumulate towards the boundary wall in S3, like the results have proven. Also, variation in terms of the amount of microplastics recovered in the different fish species used could be attributed to the differences in terms of feeding habits and habitat preference, where in this case fish species like *Coregonus kiyi* prefers cold and deeper areas unlike the *Oreochromis niloticus*. Furthermore, the *Coregonus kiyi* preys on small shrimps and amphipods while the *Oreochromis niloticus* is omnivorous that feeds on phytoplankton, aquatic plants and small invertebrates. Subsequently, these fish species could be ingesting these plastic particles during active feeding or indirectly through trophic transfer, thus the differences in the amount of microplastics recovered in their organs. However, in Dikgatlhong Dam, more microplastics were recovered in fish samples from S1, which is in the dam center, while the least were in S2, at the Tati River inlet. This could be attributed to the intensity of the water flow into the dam, which moves microplastics away from the inlet into the dam. Similarly, these fish could be indirectly feeding on microplastics that are attached to megaplastics, which result from improper plastic disposal in surrounding areas as well as through trophic transfer where their prey have already consumed microplastic (Batel et al., 2016; Romeo et al., 2015). Due to the excretion process from the fish, some of the ingested microplastics may be lost prior to sample collection,

thus low amount is recovered from the digestive organs of these fish. In terms of the recovery made in clams, high numbers were found in clams collected from S1 of the Gaborone Dam, while the lowest amount was found in clams from S5. Since S1 is at the dam inlet, the water column is thin, and as water levels drop, some of the plastic particles can sink through the column and be accessible to the clams in the soft sediments. However, S5 is located near the boundary wall, where the water column is thicker compared to the other sites, so the clams might not be able to have access to the suspended microplastics.

On the other hand, microplastics were further categorized based on the organ they were recovered from which include the stomach, intestines and the gills. More microplastics were recovered in intestines for fish samples from the Okavango Delta, in the stomach and intestines for samples from Gaborone Dam while the Dikgatlong Dam had more of these plastics in the gills. These differences could be attributed to different feeding habits of the different fish species used for analysis, the availability of microplastics in the freshwater systems and their various digestion and excretion processes. A study by Wang et al. (2019), discovered the highest amount of microplastics in the stomachs of blackmouth catshark (*Galeus melastomus*) from the Mediterranean Sea, which corresponds to the amount of microplastics found in *Coregonus kiyi* fish of the Gaborone Dam. In addition, everything that is ingested goes to the digestive tract thus microplastics are also retained in the stomach and intestines after ingestion while some could be trapped in the gills during the water ventilation process by the fish hence they were discovered in all these organs (Wright & Kelly, 2017; Abbasi et al., 2018; Su et al., 2019).

According to Yang et al. (2020), colored microplastics are easily mistaken for food by aquatic organisms, which is contrary to our findings for all the three study areas where colored

microplastics recovered in fish organs are lower compared to translucent and black. This observation could be due to much abundant translucent and black microplastics in surface water than the other colors hence they are consumed by aquatic organisms. Further, these abundant translucent microplastics might become a challenge for the aquatic organisms (fish and clams) to be able to discriminate these plastic particles away from nutrients during the feeding period and end up taking them along with their food, hence they are recovered in higher quantities from fish and clams than any other color.

NB: All the discussion regarding Site 1- Okavango Delta has already been published and it is still a part of this project [Ditlhakanyane, B. C., Ultra, V. U., Mercy, J., & Mokgosi, S. (2022). Microplastic load in the surface water and *Tilapia sparrmanii* (Smith , 1840) of the river systems of Okavango. *Environmental Monitoring and Assessment*, 2018. <https://doi.org/10.1007/s10661-022-10263-w>]

Limitations

- Due to limited accessibility in the center of Gaborone Dam, it became a challenge to collect adequate representative samples from the dam, as rocks prohibited maneuvering of the boat. A similar situation was experienced at the Dikgatlong Dam from the south side of the dam (along the Shashe River), where access was also limited.
- Although changes in seasonal patterns and weather contribute to microplastics carried into freshwaters, our sample collection was not seasonally based due to limited resources in terms of funds; hence, sampling was done only once.
- Lack of adequate resources such as the automated imaging μ Raman spectroscope or μ FTIR available is quite challenging, as some studies end up resorting to what is available, such

Results and Discussion

as the use of one single method (an optical microscope) of analyzing microplastics. This is quite a challenge, as it is limiting in terms of identifying the chemical composition and polymer type confirmation. Furthermore, the method can be limiting in terms of depicting how the digestion method affects MP quality; making it difficult to identify the smallest microplastics and determine their sizes with a microscope.

Chapter 5

Summary, Conclusion and Recommendations

5.1 Summary and Conclusion

It is crucial to monitor microplastic levels in freshwater systems, as well as raise awareness and find suitable solutions on how to reduce the sources and loads of microplastics released into freshwater systems. These plastic particles pose a serious environmental threat as they contain a mixture of chemicals (additives) that can leach into the surrounding environment (Food and Agriculture Organization—FAO, 2017). They also serve as a substrate for toxic chemicals such as persistent organic pollutants and provide a settlement for living organisms like microalgae, bacteria, fungi, or viruses, some of which are potential pathogens. Once ingested by aquatic organisms, they have physiochemical impacts on these organisms, while some are passed to humans through the food chain. Therefore, this study has provided a great deal of information on establishing first-time evidence of the existence of microplastics in some of the major waterbodies in Botswana and proven that they are being consumed by aquatic organisms once they reach the watersheds. However, there is a critical need for long-term and systematic monitoring of these particles, including seasonal variations, to assess how different seasonal events influence the levels of microplastics in freshwater systems. Due to limitations and challenges encountered in this study, these factors were not considered, hence the results are inconclusive.

The results show that there is variation in terms of the quantities of microplastics recovered from the surface water samples and from the digestive organs of different fish species (*Tilapia sarrmanii*, *Oreochromis niloticus*, *Coregonus kiyi*, and *Coptodon rendalli*) as well as the clams

(*Corbicula fluminea*). From the results, a great amount of microplastics in surface water was found in Gaborone Dam with an average range of 36–76 particles L⁻¹, Dikgatlong Dam with 16–46 particles L⁻¹, and the Okavango Delta at 10-23 particles L⁻¹. These differences could be influenced by various factors, such as the water flow velocities, the capacity of the watershed, the type of freshwater, the source of recharge, and human activities surrounding the watershed. All these study areas have different water capacities, with the Okavango being the largest inland wetland, the amount of microplastics found in the area would differ from those found in the dams. The delta receives a continuous flow of water from the Angolan highlands and eventually leaves the delta for Lake Ngami, where there could be high accumulations of these plastic particles. On the other hand, Gaborone Dam being in a vicinity where there are a lot of human activities going on and the population density is high, so the area is prone to high microplastic pollution, as has already been proven by the results. As for Dikgatlong Dam, it is at the confluence of two rivers, one of which passes through the second city, so more plastic is bound to be transported to the dam by these rivers and accumulate due to the boundary walls. Overall, this variation is inevitable due to the many factors that contribute to the accumulation of microplastics in freshwater systems; hence, their results are incomparable.

Once they reach the freshwater systems, either from in-land sources or from fishing gears used, microplastics become available to aquatic organisms, which survive by feeding off the water. Due to their size, it becomes a challenge for organisms to separate these plastic particles from the natural nutrients during feeding, so they are mistaken for food and ingested. Their availability within the freshwater systems also contributes to the amount ingested by organisms, even though some are taken in through trophic transfer. The results show a high abundance of microplastics discovered in fish species from the Gaborone Dam, with an average range of 1.0 to 3.5 counts site⁻¹

¹, followed by 0.3 to 2.5 counts site⁻¹ in Dikgatlhong Dam, and the least recovered at a range of 0.3 to 0.6 counts site⁻¹ in the Okavango Delta. This result indicates that if microplastics are available or released into the waterbodies and aquatic organisms will always be at the receiving end, their systems will be threatened, and they will continue to consume them as they are mistaken for food. Meanwhile, the average count of microplastics in clams ranged from 2.0 to 8.0 after analysis of clam tissues, which were only accessible in Gaborone Dam due to low water levels. These findings suggest that microplastics in freshwater can either sink through the water column due to their density or be deposited in sediments as a result of a drop in water level, where they can be consumed by clams.

Aquatic species use different methods during feeding times to get their nutrients, and once ingested, they go through the digestion process until some of the excess or unwanted material is excreted. The amount of microplastics recovered in the digestive organs or tissues (clams) is bound to vary depending on the organism's feeding habits, digestion and excretion rates, and the size and type of the organism (undergoing trophic transfer process). In this study, a significant variation was observed in terms of the amount of microplastics recovered in the organs of interest, and they differed across sites. Fish samples from the Okavango Delta (*Tilapia sparrmanii*) had more microplastics recovered in their gills than in their stomachs and intestines. This indicates that gills are more capable of accumulating microplastics than respiratory ventilation. For fish samples from Gaborone Dam, two fish species (*Oreochromis niloticus* and *Coregonus kiyi*) were used due to their availability during sample collection and the plastics accumulated more in the stomach and intestines for *Coregonus kiyi* and *Oreochromis niloticus*, respectively. However, the fish samples from the Dikgatlhong Dam had more microplastics collected in the intestines than in the stomach and gills.

Generally, the results revealed that microplastics discovered in both surface water and the digestive system or tissues are in translucent and black colors, respectively, while colored microplastics were less common. There could be various reasons as to why these are dominant, such as the discoloration process plastics undergo due to being exposed to various environmental factors and the fact that more translucent plastics are used daily, hence their waste generation surpasses that of colored plastics. In terms of shape, overall, the fibers and fragments surpassed other forms, which are microbeads and films, as proven by the results. This suggests that domestic and industrial wastewaters, could be the primary source of microplastics in freshwater systems particularly fibers. It also indicates that a lot of plastic waste is fragmenting into smaller pieces and being transported into freshwater systems, where they are eventually ingested by aquatic organisms. Overall, the size distribution of microplastics in this study skews towards small to medium-sized microplastics, which could be from extensive fragmentation of large plastic debris into smaller pieces (Zhang et al., 2015). Also, it is easier for small-sized microplastics to be mistaken for food and ingested, depending on the size of the fish species, as the results have proven that larger ones can take up the large microplastics, such as the *Coptodon rendalli* from Dikgatlhong Dam, which was the largest among all the species used.

5.2 Recommendations

- For future research, there is need to understand how seasonal variations influence the microplastic abundance in surface water and a variety of fish species.
- Long-term and systematic monitoring of microplastic pollution in freshwater systems should be considered when tapping into this kind of research so that there is consistency.

Summary, Conclusion and Recommendations

- There should be room for collaborative work in order to improve the quality of research on microplastics and overcome existing challenges such as lack of resources like the automated μ Raman spectroscope or μ FTIR to characterize the polymer type of microplastics.
- Raising awareness on these emerging contaminants can really help in attempts to cut sources of microplastics or finding solutions and alternatives that can reduce the generated microplastics at a source level in a collective effort which is inclusive of all stakeholders
- Given the gap that exist in our country in terms of plastic and microplastic pollution data, there is a need for capacity development to support monitoring and research on these contaminants to improve knowledge, hence increasing an understanding of the risk involved, as well as their implications on the freshwater systems and potential threat to the human health.

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