

### UNIVERSITY OF NAIROBI

## MICROPLASTIC POLLUTION ALONG THE KENYA COAST IN THE WESTERN INDIAN OCEAN (WIO), REGION

BY

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I 80/51036/2016

# A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (MARINE BIODIVERSITY AND FISHERIES MANAGEMENT) IN THE SCHOOL OF BIOLOGICAL SCIENCES OF THE UNIVERSITY OF NAIROBI

2022

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#### DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination or award of a degree. Where other people's work or not my own work has been used, this has been properly acknowledged and referenced.

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### **DEDICATION**

To my late mum, Martha Nyaera, who advocated for girl child education and encouraged me to pursue higher education but never lived to witness and *aiririate* as she leads in song and dance. To my niece, the late Eng. Ann Brita Mogoi, who was an inspiration to the Mogois but was snatched from us through the tragic Ethiopian air crush while persuing her PhD.

#### ACKNOWLEDGEMENTS

First, I thank the Almighty God for the opportunity and grace to undertake this programme to completion. I wish to thank my supervisors; Prof. Agnes W.N. Muthumbi, Prof. John Onyari, Dr. Edward Kimani and Dr. Deborah V. Robertson-Andersson for guiding me all the way with positive criticism during proposal writing, data collection, manuscript, and thesis preparation. I am particularly grateful to Prof. Muthumbi and Prof. Onyari for their relentless push to ensure I kept moving even when it seemed impossible. The study was funded by National Research Foundation under the programme titled NACOSTI/STI/KE-SA/5/003. I would like to acknowledge most sincerely the support given by KMFRI Director for allowing the use of institution equipment for sampling and the biological laboratory space and equipment for sample processing. I acknowledge the support given by Dr. Robertson-Andersson of the MACE science laboratory at the University of KwaZulu-Natal (UKZN), for allowing sample processing using laboratory equipment and materials and guiding through some technical procedures. I also wish to specially thank Prof. Onyari for connecting me with Dr. Reuben Bosire of a Science laboratory in the U.S.A. who guided in polymer identification procedures and allowed the use of laboratory equipment, Dr. George Ong'amo of the UON and Mr. David Gitahi for guiding in data analysis. I am indebted to the field assistants; Benard Kimanthi, Justus Andati, Gilbert Owatto, and Gilbert Amisi for assisting in sample collection in the creeks in the Indian Ocean, and John Nyaberi in the collection of fish samples from beach markets, Kilonzi and Mary for assisting during laboratory sample processing. My sincere gratitude goes to Mathew, Samoe and Alice Mutua, the UON technical staff for their assistance. My comrade; Winnie Awuor is appreciated for her company and moral support during field activities. Last but not least my special thanks go to the Nyaeras and Mogois for their encouragement, patience, advice and financial support. May God bless you all.

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## ABBREVIATIONS AND ACRONYMS

BPA	Bisphenol A
DSC	Differential Scanning Calorimetry
EDCs	Endocrine disrupting compounds
EEZ	Exclusive Economic Zone
FTIR	Fourier Transform Infrared
HDPE	High density polyethylene
IAME	International Association of Marine Economics
КОН	Potassium Hydroxide
KIPPRA	Kenya Institute of Public Policy Research and Analysis
KMFRI	Kenya Marine and Fisheries Research Institute
KWS	Kenya Wildlife Services
LDPE	Low density polyethylene
LLDPE	Lower low-density polyethylene
LWR	Weight-Length relationship
MDPE	Medium density polyethylene
MMT	Million Metric Tons
MPs	Microplastics
NaCl	Sodium Chloride
NEMA	National Environmental Management Agency
NP	Nonylphenol
PE	Polyethylene
PET	Polyethylene terephthalate
POPs	Persistent Organic Compounds
PP	Polypropylene
PS	Polystyrene
SGR	Standard gauge railway
Тс	Crystallization Temperature

Tg	Transition Temperature
Tm	Melting Temperature
UN	United Nations
UNEP	United Nations Environment Programme
UNDP	United Nations Development Programme
WIO	Western Indian Ocean

#### ABSTRACT

Plastics enter the ocean inform of either large debris or microplastics that are a product of breakdown from the large debris or are primarily microplastics such as beads used in beauty products. Plastic pollution impacts in oceans are remarkable world over. Microplastics (MPs) are tiny plastic particles measuring between 0.1µm and 5000 µm, make an important part of plastic pollution and form a pathway to the aquatic food web including humans. Consuming contaminated fish may transfer MPs additives into humans and being endocrine disruptors, the additives can cause cancers, brain tumours and many developmental disorders. And although this is a global problem, there are limited studies along the Kenyan Coast in the Western Indian Ocean (WIO). This study looked at MPs in the surface waters, sediments and fish within three sites (Tudor, Port Reitz and Mida Creeks) that were sampled in January/February and September 2018. Microplastic samples from surface water were collected by towing neuston nets of 500 µm (large) and 250 µm (medium) mesh sizes and sieving 50 litres of seawater through a 20 µm net (small) size. Sediment samples were collected from the intertidal zone using a 3.6 cm diameter corer up to 10 cm deep. Fish were obtained from fishermen on site and local landing beaches. Samples were digested in 10 % Potassium Hydroxide and, microplastics extracted by Thompson's improved density separation protocol using super saturated Sodium Chloride (NaCl) solution (1.2g cm<sup>-3</sup>) pre-filtered over 0.8 um membrane. Total concentrations of MPs in both water and sediments, was highest in Tudor Creek followed by Port-Reitz and finally Mida. In the water column the smallest MPs (20-250µm) recorded the highest concentration while in the sediments, MPs of the large size (500-4999 µm) were the most abundant. In fish, MPs concentration was highest in demersal fish followed by pelagic fish and in both types, omnivores recorded highest MPs concentrations followed by carnivores and finally grazers. Polyethylene (PE) polymers were the most abundant (63.9 %), followed by polypropylene (PP) (27%), while 9.1 % were unknown. Based on the results, it can be concluded that the marine surface water, sediments and fish along the Kenya coast are polluted with MPs. Although fish are economically important as human food, there is a high risk of transferring MPs in their tissues into humans where MPs additives may cause endocrine disruption, cancers, reproductive and growth and development disorders. The results demonstrate the extent of exposure to the MPs of the region's ecosystems and provides the impetus for policy development regarding the management and disposal of plastic waste to protect and save oceans rich in biodiversity for sustainable development. Fish gutting and removal of gills regardless of size during preparation is recommended in order to reduce the possible consumption of MPs from fish guts and gills. Bulky sampling of all the three MPs sizes to increase estimation accuracy is recommended for future monitoring programs.

## CHAPTER ONE 1.0 GENERAL INTRODUCTION

#### **1.1Background information**

There has been increasing global production of plastics since the middle of the 20<sup>th</sup> century accompanied by an accumulation of plastic litter in the marine environment (Barnes et al., 2009). Plastics are synthetic organic polymers derived from the polymerization of monomers extracted from oil or gas (Rios et al., 2007) and are light, inert, corrosive resistant and durable, attributes which together with cheap manufacturing techniques has led to their massive production (Plastics Europe, 2010; Bussiere et al., 2013) and almost inexhaustible application (Andrady, 2011). Plastics are used in the production of electronics, personal cleaning products, clothing, footwear, packaging, building and construction materials and often end up in the environment, waterways and oceans where they are broken up to form microplastics (MPs). Microplastics are tiny plastic particles measuring between 0.1 µm and 5000 µm, although there is still no universally accepted definition of MPs (Andrady, 2011; Frias and Nash, 2019). In this study microplastics were considered as ranging from 20 to 5000 µm. Microplastics are subdivided into larger (1-5 mm), and smaller (20 µ-1 mm) size categories (Hanke et al., 2013). Microplastics in the environment are either primary, that is, originally manufactured to be that size (such as nurdles, fibers, pellets or granules) for use; e.g. in pharmaceutical components (Patel et al., 2009), as materials for plastic production (Turner and Holme, 2011), and in cosmetics, scrubbers and air blasting (Zitko and Hanlon, 1991) or secondary, if they originate from the breakdown of large plastic debris forming fragments (small irregular shaped particles) or films (Andrady, 2015; Free et al., 2014; Lusher et al., 2013). Gradual degradation of plastics through physical and chemical processes adds MPs into the marine waters thereby increasing their concentration (Ter Halle et al., 2016; Galgani et al., 2015; Koelmans et al., 2015)

While plastics have far reaching societal benefits, their waste disposal is a big challenge since they resist degradation and therefore accumulate in the environment (Thompson, 2006; Gregory, 2009; Sivan, 2011; Enyoh *et al.*, 2019). Indiscriminate disposal of municipal waste contributes approximately 10% of plastic waste worldwide (Barnes *et al.*, 2009). Most of the plastic waste ends up in landfills where it takes centuries to degrade and decompose while some get into the marine environment causing great environmental concern (Gregory, 2009; Eriksen *et al.*, 2014). It is estimated that about 4.8-12.7 million metric tons of plastic waste gets into the ocean annually

(Jambeck *et al.*, 2015) some of which remain in surface waters. Macroplastics in marine environment present an aesthetic issue with economic repercussions for the tourist industry, significant environmental concerns and a hazard for numerous marine industries such as shipping, fishing aquaculture and energy production, as plastics may entangle and damage equipment (Wilcox *et al.*, 2016; Barnes *et al.*, 2009).

Through physical, biological and chemical processes overa long period of time, plastics degrade into microplastics (Flint *et al.*, 2012; Thompson 2015; Enyoh *et al.*, 2019). Together with primary microplastics, degradation of large plastics increases the concentration of microplastics in marine ecosystems (Andrady, 2011; Galgani *et al.*, 2015; Koelmans *et al.*, 2015; De Halle *et al.*, 2016). In the marine environment, microplastics are distributed by wind and ocean currents and the distribution is strongly influenced by the microplastic density (Schwarz *et al.*, 2019). Aging as well as bio-fouling processes alter microplastic density leading to their sinking and settling in sediments (Ryan, 2015; Chubarenko *et al.*, 2016; Halle *et al.*, 2016). Virgin polymer density is altered during the manufacturing process where addition of inorganic fillers increases the density while foaming of a polymer decrease its density (Harrison *et al* 2011).

According to Sutherland *et al.*, (2010) and Rands *et al.*, (2010), microplastics are everywhere and ubiquitous in the marine environment, and a potential hazard to living organisms (Turner, 2018). Owing to their lower density, microplastic particles mostly float at sea surface and earlier research estimates that 0.022-8654 items m<sup>-3</sup> are suspended in the water column (Hidalgo-Ruz *et al.*, 2012; Lorder *et al.*, 2015). Sub-tidal sediments seem to represent a sink for microplastics (1800-125000 items m<sup>-3</sup>) while beaches can accumulate floating, neutrally buoyant as well as sinking plastics (185-8000 items m<sup>-3</sup>) (Hidalgo-Ruz *et al.*, 2012).

When leached, microplastics release into the environment their derivatives such as Nonylphenol (NP) and Bisphenol A (BPA) which are endocrine disrupting compounds (EDCs) (Vandenberg *et al.*, 2010; Wang *et al.*, 2017). They are known to cause growth of brain tumours, cancers, reproductive disorders including embryonic development, gonadal formation and sex differentiation, digestion, metabolic and growth disorders (Cosmetic ingredient review, 2012; Wang *et al.*, 2017). The EDCs bind to or block hormone receptors thereby triggering or preventing hormonal response (Mackey *et al.*, 2001; Hotchkiss *et al.*, 2008).

Microplastics are bio-available to a great diversity of organisms through food webs since they mimic prey particles and sediment grains causing some organisms to mistake them for prey while filter feeders may incorporate them as prey (Wright et al., 2013; Setälä et al., 2014; Cole et al., 2015; Hong et al., 2018). Microplastics of considerable quantities in the gut lower the amount of food an organism ingests as well as the feeling of hunger which reduces the drive to search for food (Van Franeker, 1985; Van Cauwenberghe et al., 2015). This can cause a decrease in growth, reproduction ability and ability to evade predators. (Van Franeker, 1985; Van Cauwenberghe et al., 2015). Ingested microplastics can block the entire digestive system or cause abrasion and ulcers (Wright *et al.*, 2013) in MPs, smaller invertebrates or larvae leading to death (Kühn *et al.*, 2015; Lusher, 2015). Owing to their tiny size and hydrophobic nature, MPs adsorb a lot of Persistent Organic Particles (POPs) and trace metals aided by microbials on their surface, MPs adsorb a lot of Persistent Organic Particles (POPs) and trace metals (Van Cauwenberghe et al., 2015; Staniszewska et al., 2016) from the environment. Microplastics may lead to severe ecological implications since they release additives upon degradation and accumulate persistent organic pollutants (POPs) which they pass to organisms that ingest them (Teuten et al., 2009; Rochman et al., 2013; Staniszewska et al., 2016; Mancusio et al., 2019). These pollutants tend to interfere with the natural hormone functions, cause mutations and cancer (De Witte et al., 2014). Therefore, ingesting microplastics may introduce toxins into food chains from where they can bio-accumulate (Teuten et al., 2009) and move up the food chains (Bessling *et al*, 2013; Koelmans *et al.*, 2013), ultimately to humans. Plastic additives have been found to cause adverse biological effects at very low concentrations (ng/L or mg/L) in amphibians, crustaceans and molluscs (Oehlmann et al., 2009).

Microplastic pollution is probably the most hazardous but the least known area of marine pollution (Koelmans *et al.*, 2014). Current knowledge assessing the presence, concentration and shape of microplastics in the ecosystem of the WIO along the Kenya coast and the risk of microplastics to marine fish is insufficient. So far, studies on MPs in the marine environment in Kenya are limited to the study by Kosore *et al*, (2018) who studied MPs in surface water and in zooplanktons at Gazi Bay and oceanic waters in the Exclusive Economic Zone (EEZ), Awour *et al.*, (2020) who investigated the presence of MPs in benthic invertebrates. Following the report by the National Environmental Management Agency (NEMA) in conjunction with the United Nations Environment Programme (UNEP), and the Kenya Institute of Public Policy Research and Analysis (KIPPRA), that showed that supermarkets alone contribute tens of millions of plastic bags into the

environment annually, the Kenya Government effected a ban in February 2017 on the use of low weight plastic bags. The results of this study will provide data for future monitoring of the effects of the ban.

This study investigated the presence, concentration and categories (by shape) of MPs in the surface water and sediments within the coastal creeks in Kenya, and their ingestion by fish to evaluate the extent of microplastic pollution in order to increase understanding of the microplastic challenge in the marine ecosystem of the WIO along the Kenya Coast and the possible risk to human health. This will inform on the basis of the February 2017 ban on low weight plastic bag production and use in Kenya and the formulation of plastic waste management and disposal policies to protect the ecosystems which are rich in marine biodiversity (Cole *et al.*, 2014; Rochman, 2016).

#### **1.2 Problem statement**

Plastic production has increased rapidly since the 1950s due to increased demand for plastic products (Lusher et al., 2017; Ritchie and Roser, 2018). Owing to indiscriminate disposal of plastic waste into the environment, about 4.8-12 Million metric tons of plastic waste gets into the ocean annually (Jambeck *et al.*, 2015). It is not known how much plastic Kenya produces annually but the Kenya Association of Manufacturers' Report (2019), estimates plastic consumption in Kenya to be 0.03 kg per person daily which translates to a total of 1,200,000 kgdaily, translating to 432 million kilograms annually. Plastics degrade into MPsthrough physical, chemical, and biological processes (Brown et al., 2007). Microplastics sorb POPs and transport the toxins into marine invertebrates and vertebrates including fish if ingested (Teueten, 2009; Frias et al., 2010; Staniszewska et al., 2016), and also leach out their chemical additives such as NP and BPA into organisms (Koelmans et al., 2014; Staniszewska et al., 2016). Bioaccumulation and bio-magnification occurs higher up trophic levels, and subsequently to humans. Microplastics cause corrosion and blockage of digestive and circulatory systems, tissue damage, reduced feeding among others while the chemicals from plastics are endocrine disruptors, may cause brain tumours and cancer, reproductive cell deformity, interfere with sex determination, embryo development, and growth disorders, decreased survival and low fecundity among others (Hermabessiere et al., 2017). This implies adverse health as well as economic effects. There is dearth of knowledge of MPs in the environment off the Kenyan coastline or

their presence in commercially caught and consumed fish species. This study sought to address this information gap.

#### 1.3 Justification of the study/rationale

There is dearth information on microplastic presence, abundance and categories along the Kenya coast. The previous study in Kenya focused on MPs in the deep-sea surface waters and ingestion by zooplanktons at Gazi Bay and the EEZ (Kosore *et al.*, 2018), and ingestion of microplastics by invertebrates (Awour *et al.*, 2020). In this study, microplastic occurrence and abundance in the surface water, sub-tidal sediments and fish within creeks sheltered from strong ocean currents and within a Marine Reserve was assessed to establish the effect of water flow on the distribution of microplastics and the possible sources.

Fish is a source of protein, omega 3 fatty acids which are useful in brain and nerve development, fish oil for treatment of common cold and vitamin D deficiency diseases (Robertson *et al.*, 2018). A decline in fish quantity and quality causes a decrease in the fishing industry leading to loss of jobs, shortage of protein food leading to deficiency diseases and a strained economy (Brink *et al.*, 2009; Mouat *et al.*, 2010). Assessing the level of microplastic pollution will shed light on the extent of possible damage of MPs on marine fish hence seek alternative methods of plastic waste disposal to reduce the amount that gets into the environment and find alternatives to plastic products.

The United Nations Sustainable Development Goals 2030 target 14.5 seeks to prevent and significantly reduce marine pollution by 2025 (UNDP, 2015). This study provides relevant information on identity of sources, categories and levels of plastic pollution in the region that will help the United Nations member countries and the region towards meeting this target by identifying the focus for intervention.

#### **1.4 Research questions**

- i) Are there microplastics present in the creek waters along the Kenya Coast?
- ii) Are microplastics present in the creek sediments along the Kenya Coast?
- iii) Are microplastics found in locally available marine fish species?

iv) Which microplastics polymer types occur in the surface water, sediment and fish from the creeks along the Kenya coast and in what proportions?

#### **1.5 Research Hypotheses**

1. There are no microplastics in the marine waters in sheltered creeks along the Kenya coast

- 2. There are no microplastics in the marine sediments along the Kenya coast
- 3. There are no microplastics in the locally available marine fish along the Kenya coast.

4. Both low and high density polymers occur in surface water, sediments and fish along the Kenya coast

# 1.6 Objectives1.6.1 General objective

# To investigate the presence, abundance, and categories of microplastics present in the marine surface waters, sediments and fish species along the Kenya coast.

#### **1.6.2 Specific objectives**

- i) To assess the presence, concentration and categories of microplastics in the marine surface water
- ii) To assess the presence, concentration and categories of microplastics in the marine sediments
- To assess the presence, concentration and categories of microplastics in the gut, gills and muscle tissues of common marine fish
- iv) To identify the microplastic polymers present in the marine ecosystem along the Kenya coast.

#### **1.7 Structure of the Thesis**

This thesis deals with objective (i) that is, presence, concentration and categories of microplastics in the marine surface water in chapter four, objective (ii) presence, concentration and categories of microplastics in the marine sediments is dealt with in chapter five, objective (iii) presence, concentration and categories of microplastics in the gut, gills and muscle tissues of common marine fish in chapter six, and objective (iv) microplastic polymers present in the marine ecosystem along the Kenya coast in chapter seven. The general discussion conclusions and recommendations are given in chapter eight.

#### **CHAPTER TWO**

#### 2.0 LITERATURE REVIEW

#### **2.1 Introduction**

Plastics are a major pollutant recognized as a global threat (Avio *et al.*, 2016: Law, 2017; Sharma and Chatterjee 2017: UNEP, 2018), joining other marine stressors such as climate change, habitat destruction, acidification, and overfishing (Amaral-Zettler *et al*, 2015). Plastics are light in weight, inert, durable and corrosion resistant (Plastics Europe, 2010). Since the production of the first modern plastic in 1907, plastic production has increased rapidly and is estimated to hit 33 billion tons by 2050 (Zalasiewicz *et al.*, 2016).

Despite plastic waste being a global threat with legislation in place to regulate its management, about 10% of plastics produced ends up in the oceans (Gregory, 2009: Lozano and Mouat, 2009). On a global scale, Jambeck *et al.*, (2015) estimated that about nine million metric tons of plastic is produced annually, most of which are single-use. Eriksen *et al.*, (2014) estimated that of the total plastics produced in 2010, about  $2.7 \times 10^8$  kg ended up in ocean part of which sink into the sea bottom or get stranded on shore sediment and some are ingested by fish (Ter Halle *et al.*, 2016; Koelmans *et al.*, 2014). Plastic debris get into the ocean when large amounts of plastics waste such as packaging material and food containers (Fig.2.1), waste textile industries, oil refining industries, shipping and sewage treatment plants are released into the ocean without stripping off the plastics. The durability of plastic primarily makes it highly resistant to degradation hence problematic in waste disposal (Sivan, 2011).



Figure 2.1 A heap of plastic debris on the shore of the Indian Ocean along the Kenya Coast at Nyali-Beach after beach cleaning in September, 2018 (own observation).

Various reviews have addressed the source, abundance and negative effects of plastic litter (Koelmans *et al.*, 2014; Browne, 2015; Galgani *et al.*, 2015; Thompson, 2015). Plastics that enter the marine environment through indiscriminate disposal are of great concern (Sivan, 2011: Barnes *et al.*, 2009). The consequences in sea water are painfully visible with plastic debris injuring and killing marine reptiles, birds, fish and marine mammals due to entanglement and ingestion thereby reducing ability of the organisms to feed (Bessling *et al.*, 2013; Foekema, 2013). Plastics debris transport non-native marine species such as bryozoans, fish, polychaete worms, hydroids, barnacles and molluscs across the ocean into new habitats leading to invasion. Through biofouling and aggregation, some plastics sink and accumulate in sediments (Ter Halle *et al.*, 2016) where they smother the seabed preventing gaseous exchange and create artificial hard grounds (Moore, 2008).

To humans, plastics are a potential health issue, for example blockage of drainage pathways by plastic bags leading to flooding (Vethaak and Leslie, 2016), provide mosquito breeding grounds (Gubler and Clark. 1996), or can be colonized by microbes (Zettler *et al.*, 2013) and potential pathogens (Kristen *et al.*, 2016). Flooding can damage property, drown humans and animals, lead to disease outbreaks such as malaria, cholera, dysentery thereby causing great loss and pain to humans (Opere, 2013; Okaka and Odhiambo, 2018). Through physical, chemical and biological processes, macroplastic degrade into MPs (Browne *et al.*, 2007).

Microplastics are tiny plastic particles measuring between 0.1  $\mu$ m and 5000  $\mu$ m (Andrady, 2011) and are pollutants in their own right (Ryan *et al.*, 2009). They are a major problem in both fresh and marine water systems, and is now recognized as an increasing global threat to marine biodiversity (Guzzetti *et al.*, 2018). Increased use of plastics has steadily increased their abundance in oceans in the recent past (Borrelle *et al.*, 2020; Boyle and Ormeci; 2020; Law *et al.*, 2010; Geyer *et al.*, 2017; Lusher *et al.*, 2017; Jambeck *et al.*, 2015). Positively and neutrally buoyant MPs remain in the upper reaches of the water column while the negatively buoyant MPs sink to settle in the sea beds. Over a period of time biofouling further increases the density of the small particles, causing them to sink. A decrease in water temperature and sunlight deeper down the water kills biofouling thus decreasing the density of MPs and creating an endless circulation of MPs between surface waters and the deep ocean (Kaiser *et al.*, 2017; Andrady, 2011; Ye and Andrady 1991). Microplastics may therefore remain in the marine ecosystem for a long time (Eerkes- Medrano *et* 

*al.* 2015), and may pose a health hazard to organisms (Cole *et al.* 2011; Wright *et al.* 2013; Ivar do Sul *et al.* 2014; Van Cauwenberghe *et al.* 2015; Eerkes- Medrano *et al.* 2015; Galloway, 2015; Koelmans *et al.* 2015; Lassen *et al.* 2015; Lusher, 2015; Oberbeckmann *et al.*, 2015; Duis and Coors, 2016; Auta *et al.*, 2017; Anbumani and Kakkar, 2018).

Microplastics have been shown to sorb POPs present in small quantities in marine waters due to their large surface area and hydrophobicity (Moore, 2008; Hong *et al.*, 2018). If consumed, the nanoscale plastic additives (Bussiere *et al.*, 2013), chemicals, and metals desorb and transfer into tissues of marine organisms and move up the food chain eventually to humans (Teueten, 2009; Frias *et al.*, 2010; Staniszewska *et al.*, 2016).

Sediments and beaches are a sink for microplastics with as much as 8,205 items per m<sup>2</sup> encoutered on the beaches in South Korea during the dry season and 27,606 items /m<sup>2</sup> during the rainy season, and  $1512 \pm 189$  particles m<sup>-2</sup> dry weight on the Adriatic Sea beach (Lots *et al.*, 2017). However, there are no agreed standard methods of sampling microplastics and units of measurement making comparison of data from different regions difficult (Rochman *et al.*, 2017; Wang and Wang, 2018).

#### 2.2. Primary microplastics

Primary Mpsare small plastic particles manufactured (Andrady, 2011; Cole *et al.*, 2011; Duis and Coors, 2016) for use, especially in facial cleansers (Zitko and Hanlon, 1991), as materials for plastic production (Turner and Holme, 2011), in cosmetics and toothpaste (Zitko and Hanlon, 1991; Fandal and Sewell, 2009) or as blasting media (Gregory 1996; Derraik, 2002; Betts, 2008), and as vectors in medicine (Patel *et al.*, 2009). In human medicine, ingestible and inhalable medicine containing MPs have been used to deliver anticancer, cardiovascular and other drugs to the organs of humans and other farmed animals (Browne, 2015; Kreuter, 2014) because MPs can translocate from the lungs or guts into the circulatory system (Corbanie *et al.*, 2006). In fish, MPs carrying antigens have been used in Atlantic salmon aquaculture (Browne, 2015; Kreuter, 2014). Browne (2015) reported that a variety of polymers are used as vectors in medicine ranging from biodegradable to longer lasting polycarbonates and polystyrenes.

The use of exfoliating cleansers also called micro-exfoliates or micro-beads has increased exponentially, since the patenting of microplastics in cosmetics in the 1980s (Fendall and Sewell, 2009). Primary MPsvary in size, shape and composition depending on the product (Fendall and

Sewell, 2009). For the blasting technology, primary microplastics like acrylic, Polyester or melamine scrubbers are blasted at boat nulls, machinery and engines to remove rust and paint (Browne *et al.*, 2007). Repeated use of the scrubbers diminishes them in size losing their cutting power and often become contaminated with heavy metals such as lead, chromium and cadmium (Derraik, 2002). When synthetic products are washed, they shed fibers which can enter the sea. It is estimated that about 100 particles  $L^{-1}$  are released into the sea through wastewater (Fendall and Sewell, 2009).

#### 2.3 Secondary microplastics

Secondary MPsare tiny microplastics derived from the degradation of larger plastic debris on land and at sea (Ryan et al, 2009; Andrady, 2011; Cole et al., 2011; Duis and Coors, 2016) through physical, chemical and biological processes (Browne et al., 2007). Due to high oxygen availability and and exposure to direct sunlight, plastics on the beach degrade rapidly, becoming brittle and form cracks while in the sea however, the cold saline condition may hinder photo-oxidation process (Andrady, 2011: Barnes et al., 2009: Moore, 2008). Wave action, abrasion and turbulence aid plastic fragmentation (Browne et al., 2009). The process continues until the plastics are reduced to MPs (Rios 2007: Ryan et al., 2009). Microplastics may degrade further to form nanoplastics. Microplastics from the degradation of plastic products form a greater proportion while some come from industrial feedstock resin pellet. In this study, MPs of between 20 to 5000 µm size range were considered regardless of their group category. The size range falls within the definition of microplastics by Andrady, (2011) and Barnes et al., (2009), and has been used by Brown et al., (2010), Mathalon and Hill, (2014), Wegner, (2018) among others. The type of plastics (as determined by MPs categories) in the marine environment and their abundance, may provide evidence of their source (direct or indirect), important in formulating proper and effective mitigation measures. Information on the extent of microplastics pollution in the WIO along the Kenya coast is useful in finding solutions to the growing problem.

#### 2.4 Sources and transfer of microplastics into the marine environment

Plastic litter can enter the marine environment directly through entry points including rivers (Lebreton *et al.*, 2017), beaches and agricultural run-off, or land-based sources, or sea-based sources such as platforms, fishing piers and ships (Maximenko *et al.*, 2019, for a recent review) or

indirectly through wastewater systems (Moore et al., 2008; Fendall and Sewell, 2009). According to Andrady, (2011) and Thiel et al., (2013), 80% of marine litter is from terrestrial source which comprise primary and secondary plastic leachates from waste refuse sites. These kinds of plastics have a higher potential to enter the marine environment via rivers and wastewater systems since about half the world's population lives within fifty miles of the coast (Thompson, 2006; Moore, 2008). Through industrial and domestic drainage systems, microplastics used in cosmetics and air blasting media may enter the waterways (Derraik, 2002). A large proportion of wastewater passes through wastewater treatment plants, while macroplastics and some small plastic particles are trapped (Fandall and Sewell, 2009). Plastics in river systems are then transported out to the ocean. Several research studies have shown that plastic debris is driven into the ocean by the high unidirectional flow of freshwater systems (Browne et al., 2010; Cole et al., 2011: Anderson et al., 2016; Lots et al., 2017). The perennial rivers (Mwache, Cha shimba and Mwambone) and seasonal rivers (Kombeni and Tsalu) feeding the Mombasa creeks may be the major sources of land-based debris into the marine ecosystem. Identifying the source and mode of transport of plastics in the marine environment will help in formulating policies that can curb the escalating plastic pollution problem in the region.

Wind and buoyance driven surface currents in the open ocean have a great influence on the transport of microplastics (Cozar *et al.*, 2014: Iwasaki *et al.*, 2017: Larceda *et al.*, 2019: Wichmann *et al.*, 2019). In the coastal areas, barotropic currents are the major transporters and accumulation of plastics (Zhang, 2017), as most plastics enter the ocean in coastal environments (Cole *et al.*, 2011). Most of the plastics released into the ocean stay near the coastline for a long time (Lebreton *et al.*, 2012) causing high concentrations of MPs in the near-shore areas (Desforges *et al.*, 2014; Auta *et al.*, 2017). Kosore *et al.*, (2018) reported 275 particles m<sup>-3</sup> offshore compared to 33 particles m<sup>-3</sup> nearshore in the Indian Ocean along the Kenya coast. This may imply that plastics at the Kenyan coast could be from both inland as well as offshore sources. The type of plastic polymers in the marine environment and their abundance, may provide evidence of their source and will help in finding solutions to the growing problem through informed waste management policies.

#### 2.5 Microplastics in biota

The presence of MPs in the sea is ubiquitous since they have been found floating in the Arctic and Antarctic waters, Atlantic, Pacific and Indian Oceans, and in deep sea sediments (GESAMP, 2015). Ingestion of MPs by marine organisms is widespread (Devriese *et al.*, 2015; Li *et al.*, 2016; Nelms *et al.*, 2018; Awuor *et al.*, 2020). Globally, there is growing concern linked to the presence of plastics in seawater and the potential impacts of these particles in the marine trophic web through ingestion by several marine organisms, ranging from planktons to top predators. Vegter *et al.*, (2014) estimates that about 170 marine invertebrate and vertebrate species ingest MPs with others such as crabs having them trapped in the gills (Cole *et al.*, 2015; Weiden and Cowie, 2016; Karlsson *et al.*, 2017). Wild caught fish have been found to contain MPs (Lusher *et al.*, 2013; Rochman *et al.*, 2015; Lusher *et al.*, 2016; Nelms *et al.*, 2018)) of polymers such as PE, PP, alkyd resin, rayon, polyester, nylon and acrylic, polyamide, polystyrene, PET and polyurethane (Neves *et al.*, 2015; Rummel *et al.*, 2016). Most studies on microplastic ingestion by organisms have been done in Europe, U.S.A, Brazil and China but there are fewer data from Asia, Africa and especially the Kenya coast along the WIO.

Microplastics can sorb a million times the level of toxicity of the surrounding water (Zettler *et al.*, 2013; Wang *et al.*, 2016). The widespread occurrence of microplastics and their ubiquity within the marine environment poses great danger to marine life as well as humans higher up the trophic level (Rands *et al.*, 2010; Sutherland *et al.*, 2010). Because microplastics are very small in size, they are bio-available to living organisms throughout the food webs (Bessling *et al.*, 2013; Koelmans *et al.*, 2013). Marine invertebrates and vertebrates ingest MPs through the mouth and gills as shown by the presence of MPs in suspension feeding organisms. The chemical additives such as plasticizers used to improve the quality of plastic products and POPs are transported by MPs into organisms where they can leach out or desorb posing a health risk to the organisms (Bakir *et al.*, 2014; Koelmans *et al.*, 2014) and bio-magnification occurs.

Bio-magnification is the bio-accumulation and bio-transfer and concentration of chemicals in tissues of higher organisms through successive trophic levels (US Environment protection Agency, 2010). It is not clear how exposure and bio-accumulation of plastic additives and other environmental pollutants occur in marine life and their subsequent transfer to humans (Clark, 2014; Carbery *et al.*, 2018). Marine zooplanktons mistake the tiny MPs for food (Selck, 2012),

hence enter the food chain at lower trophic levels. Small fish species ingest MPs which may affect their health or kill them through their toxic chemicals or additives or predisposes larger fish to the MPs (Rochman *et al.*, 2009). When larger fish or other predators feed on contaminated fish the dose gets bio-magnified in subsequent feeders (Rochman *et al.*, 2013). Persistent bio-accumulative and toxic substances (PBTS) found on plastics globally, bio-accumulate in food webs (Teuten *et al.*, 2009: Hirai *et al.*, 2011) and are linked with several adverse effects including endocrine disruption, decreased fish populations and reduced species evenness and richness (Johnston and Roberts, 2009; McKinley and Johnston 2010). This implies that organisms higher up the trophic level are potentially exposed to the risk associated with PBTS transported via ingested plastics.

Microplastics of considerable quantities in the gut lower the amount of food an organism ingests as well as the feeling of hunger which reduces the drive to search for food (Hong *et al.*, 2018). This can cause a decrease in growth rate, reproductive ability and ability to evade predators (Van Cauwenberghe *et al.*, 2015). Ingested MPs can also cause blockage of gastrointestinal tract, abrasion and ulcers leading to death (Lusher, 2015). Microplastics accumulate in tissues and migrate into the circulatory system causing blockage (Browne *et al.*, 2008). Earlier research has shown that in *Mytilus edulis* (L.), particles accumulate in the gut and move into the haemolymph (Browne *et al.*, 2008), cause formation of granulocytoma and lyzomal destabilization with increased exposure time (Von Moos *et al.*, 2012). *Mytilus galloprovincialis* (Larmack, 1819) exposed to polyethylene (PE) and polystyrene (PS) shows molecular and cellular alteration (Avio *et al.*, 2015a), while in the common goby fish polyethylene was shown to reduce the production of AchE involved in neurotransmission signaling (Oliveira *et al.*, 2013) and in Japanese medaka fish micron sized PE particles were shown to cause endocrine disruption (Rochman *et al.*, 2014).

Microplastics in tissues of invertebrates and vertebrates transfer up the trophic level to humans. The presence and abundance of microplastics in organisms provides evidence and the extent of microplastic pollution in the area to inform on the need to develop proper plastic waste disposal policy formulation. Humans are exposed to plastic chemicals in water and food materials (Rochman *et al.*, 2015) and long-term exposure may cause a range of diseases not easily detected or attributed to any one cause (Grandjean and Landrigan, 2006). Plastic additives (nanoplastics) cause endocrine disruption, brain tumours and cancers, developmental disorders such as gonadal development, disruption of sexual maturity, fertility and reproductive abnormalities such as

deformed sperm production amongst others (Hermabessiere *et al.*, 2017). Chemicals like Bisphenol A (BPA) affect gene expression related to thyroid hormone axis. Despite MPs being a global threat to marine life as well as humans, little research has been done on the presence and concentration of MPs in locally harvested fish in the WIO along the Kenya coast and information is lacking.

Some of the risks associated with marine microplanktons is the incorporation of the particles and the adsorbed chemicals into the food web through trophic transfer (Setälä *et al.*, 2018). The smaller the MPs particle the more the likelihood of being ingested by marine animals and being transferred in food webs (Hong *et al*, 2018). Small MPs have a large surface area thus, can adsorb much more POPs which accumulate on the MPs and are transferred to many marine organisms (Hermabessiere *et al.*, 2017) where they could become toxic at high levels. Thus, it is therefore prudent that assessment of MPs considers those less than 300  $\mu$ m (normal size of the manta trawl net mesh size) as well.

#### 2.6 Identification of microplastics polymers

Microplastic polymers can be identified by several methods including Differential Scanning Calorimetry (DSC) (Onyari *et al.*, 2008; Coutene-Jones *et al.*, 2017; Wloch *et al.*, 2019), Fourier Transform Infra-red (FT-IR) (Ehrenstein *et al.*, 2004; Mitchell *et al.*, 2013; Petrovich, 2015; Maes *et al.*, 2017) colour, and Thermo-gravimetric Analysis (TGA) (Peltzer and Simoneau, 2013). DSC is fast, very sensitive and easy to use and it allows for detailed analysis of a polymer (Schindler *et al.*, 2016). Although the DSC is a better analysis technique, it is not without demerits where the curve measurement depends on the measurement signal could be given multiple interpretations (Schindler *et al.*, 2016). Differential scanning calorimetry determines the quality of a polymer, whether of low density, high density, amorphous or semi-crystalline (Petrovich, 2015). It identifies the chemical composition and organic additives, analyzing the two simultaneously (Ehrenstein *et al.*, 2012). The DSC allows measurement of glass transition temperature ( $T_g$ ) and crystallization temperature ( $T_c$ ) as the polymer is being heated as well as melting temperature ( $T_m$ ) (Schmack *et al.*, 2000; Roes *et al.*, 2007; Coutene-Jones *et al.*, 2017). A polymer shows a second melting peak

due to differentiating densities throughout a polymer sample (Ehrenstein *et al.*, 2012; Petrovich, 2015). The melting point reflects as endothermic peak while crystallization point reflects as exothermic peak (Petrovich, 2015).

FT-IR characterizes polymer composition by revealing differences in functional groups between various polymers. It does not provide detailed information about a polymer structure for example; it cannot show why Lower low-density polyethylene (LLDPE) exhibits characteristics of highdensity polyethylene (HDPE) and low-density polyethylene LDPE (Petrovich, 2015). The IR rays are absorbed by bonds in a molecule (Mitchell et al., 2013) to form two peaks which occur for the O-H and C=O. Peaks in lower energy areas on the FT-IR graph are known as the fingerprint region and each compound has a unique fingerprint region. Contamination of a polymer may result in no fingerprint region in a sample polymer. Differences in functional group peaks can be caused by colour, additives or branching. FT-IR has better sensitivity because it measures all frequencies simultaneously (Mitchell et al., 2013). It uses less energy compared to the DSC (Pandita et al., 2012) and is fast (1-2 seconds per scan) (Ehrenstein *et al.*, 2012; Petrovich, 2015). However, it gives limited information for most samples and the molecule must be active in the IR region. The sample liquid, gas or solid is placed in a beam of infrared light (Schindler et al., 2016). Polymer identification is important in quality control of raw materials (Ehrenstein et al., 2004). Use of two or more methods for polymer identification reduces multiple interpretation and increase chances of correct identification of polymers for proper determination of their sources.

NMR identifies and quantifies the type of branching present in a polymer and provides molecular structure information (Peez *et al.*, 2019). It also provides information on the dynamics, reaction state and chemical environment o molecules. NMR is fast, size independent and has high accuracy (Krishnan, 2019). Samples are prepared by complete dissolution in the specimen tube using appropriate deuterated solvents and temperature. It requires long experimental time (more than 23 hours) and large amounts of samples to acquire reliable quantification due to its low sensitivity. Most polymers are not soluble in common solvents but dissolve in deuterated benzene which is carcinogenic (Peez *et al.*, 2019).

Colour may provide information useful in identifying and determining the source of marine plastics. Colour is used for preliminary identification of plastic pellets (Abu-Hilal and Al-Najjar, 2009); Polypropylene (PP) pellets are transparent (Abu-Hilal and Al-Najjar, 2009), high density

polyethylene (PE) pellets are white, low density PE opaque while ethyl vinyl acetate corresponds to clear and almost transparent pellets (Ismail *et al.*, 2009). Also, colour signifies the period of stay of plastics on sea water surface, photo-degradation and the extent of weathering (Turner and Holmes, 2011). Long time exposure of PE enhances polymer oxidation turning the pellets yellow in colour. Colour may also be useful in identifying the conditions that objects have been subjected to such as biofilm development, weathering or preferential feeding strategies of organisms (Abu-Hilal and Al-Najjar, 2009). However, this method can be hindered by visual deficiencies such as colour blindness, and change of plastic colour due to weathering. Currently, there is no scheme for colour designation for plastic litter but the European Marine Observation and Data Network proposes the use of either the 12 basic colour terms or eight colour classification scheme (Galgani *et al.*, 2017). This study used the latter scheme. Uniform colour frequencies in locations far apart may mean that plastic colours are uniformly distributed in the surface water (Boerger *et al.*, 2010) within the study site. Colour may provide information on the source of microplastics and the level of degradation which can be an indication of the length of time of exposure to weather elements.

## CHAPTER THREE 3.0 MATERIALS AND METHODS

#### 3.1 Study site description

The study was carried out in three sites, two (Tudor and Port-Reitz Creek) in Mombasa County and one (Mida Creek) in Kilifi County along the Kenya Coast (Figure 3.1). The marine coastal climate is influenced by two seasonal monsoons; the Southeast monsoon lasting from April to October is associated with high rainfall (55-272mm) and temperature range of 20-31 <sup>o</sup>C and the shorter Northeast monsoon (November-March) that is drier with an average annual rainfall of 8-84 mm and hotter 23-32 <sup>o</sup>C (Obiero and Onyando, 2013). The creeks experience semi-diurnal patterns of tide averaging between 0.6 and 1.0 m at the neap tide and 2.5 and 4.5 m at spring tide (Nguli, 2006). Salinity (32-35psu) and water conductivity (53Ms/cm) is higher during the dry spell and low during the rainy season (Kitheka, 1998).

Tudor creek passes under Nyali Bridge and is bordered by Makupa causeway which dissects it into Tudor to the East and Port-Reitz to the West (Kitheka et al., 1999). The creek lies between  $04^{0}40'$ S and 39<sup>0</sup> 00' E (Nguli *et al.*, 2006) and covers a surface area of approximately 20 km<sup>2</sup>. The creek has a long and deep inlet measuring up to 20 meters long that connects a shallower inner basin to the open ocean (Nguli et al., 2006). Tudor creek comprises of three parts; the marine mouth (30 m deep), the middle section (less than 5 m deep) and the upstream (< 1 m deep) which splits into different channels. The creek experiences semi-diurnal tides with an average range at the entrance being 3.2 m and 1.1 m at spring and neap tides respectively. Tudor creek is fed by two main seasonal rivers; Kombeni and Tsalu which arise from around Mariakani town, 32 km Northwest of Mombasa (Kitheka et al., 1999). An estimated 0.9 m<sup>3</sup>s<sup>-1</sup> of water is discharged into the creek with the highest discharge occurring between April and June (1.8 m<sup>3</sup>s<sup>-1</sup> (Wakwabi and Mees, 1999). There are many densely populated informal settlements and villages (such as Mushomoroni, Mikindani, Coast General Hospital and Kenya Meat Commission) around the creek that have come up due to rapid urbanisation resulting from the need for labour in the manufacturing industries, service industries and the Port of Mombasa (Okuku et al., 2011; Maritim et al., 2016). The inner basin is fringed by mangrove forests mainly Rhizophora mucronata and Avecennia marina and mudflats (Wainaina, 2010; Nguli et al., 2006). Tudor creek passes under Nyali Bridge

and is bordered by Makupa causeway which dissects it into Tudor to the East and Port-Reitz to the West (Kitheka *et al.*, 1999).

Port-Reitz creek lies to the south of Mombasa Island ( $04^0 04'$  S and  $39^0 39'$  E) and occupies an area of 1480 km<sup>2</sup> (Kamau, 2002). It experiences a semi-diurnal tidal pattern with the average tide range of 1 and 2.5 ms<sup>-1</sup> at the neap and spring tides respectively (Kamau, 2002). Port-Reitz creek receives freshwater from rivers Mwache, Cha Shimba, and Mwambone (Kitheka *et al.*, 1999). Mwache river discharges 215million m<sup>3</sup> of water annually (UNEP, 1998). The creek is surrounded by highly populated villages such as Dongo Kaya, Dunga Nusa and Ngala (Maritim *et al.*, 2016), Shimanzi, Makunde and Kibarani (Kamau, 2002). The creek is surrounded by heavy human activities ranging from settlement, industrial (kipevu power generation station, Kenya ports authority and heavy commercial transport deports) to municipal waste dumping site (Kibarani dumpsite). Port-Reitz creek is fringed by mangrove forests mainly *Rhizophora mucronate, Ceriops tagal* and Avecennia marina on the inner basin (Wainaina, 2010; Nguli *et al.*, 2006).

Mida creek is a tidal inlet located at the North Coast of Kenya in Kilifi County at longitude 39<sup>0</sup> 58'E and latitude 03<sup>0</sup> 22 S' (Gang and Agatsiva, 1992; Kitheka et al., 1999). Mida creek lacks river inflow, instead the creek receives fresh water through seepage from underground and storm water runoff (Kitheka et al., 1999; Osore et al., 2004). In addition, the creek is in a marine reserve, forming part of the Watamu Marine National Park and Reserve (KWS, 1997; Osore, et al., 2004; Alemayehu, 2017) and therefore believed to be semi-pristine and free from any form of pollution and so was considered as control. Mida creek receives an average annual rainfall of between 600-1000 mm with a rainfall season starting in May all the way to September (GOK, 1989). The creek has a constricted narrow entrance and rough bottom that generate modified currents that show significant spatial-temporal variations, although they are generally gentle (Kitheka, 1998). The speed of currents at the entrance is high reaching 3.2 m/s during spring tide that reduce to 2.0 m/s in the middle and 1.0 m/s at the lower region (Kitheka, 1998). The narrow opening prevents faster turnover of water, consequently water in the creek is higher in salinity than the open sea (Yap and Landoy, 1986). At low tide, currents are barotropic with minor deviations with changing tidal elevation. In the main creek channel, the flow is flood-dominated compared to the backwater region (Kitheka, 1998). Next to Sudi Island, the tides are asymmetrical with ebb flow being dominant compared to flow. Water conductivity is highest (53 Ms/cm) during the dry spell

and low during the rainy season (Kitheka, 1998). The ecosystem consists of mangrove forests mainly *Rhizophora mucronate* and *Ceriops tagal* (Kairo *et al.*, 2002) being dominant.

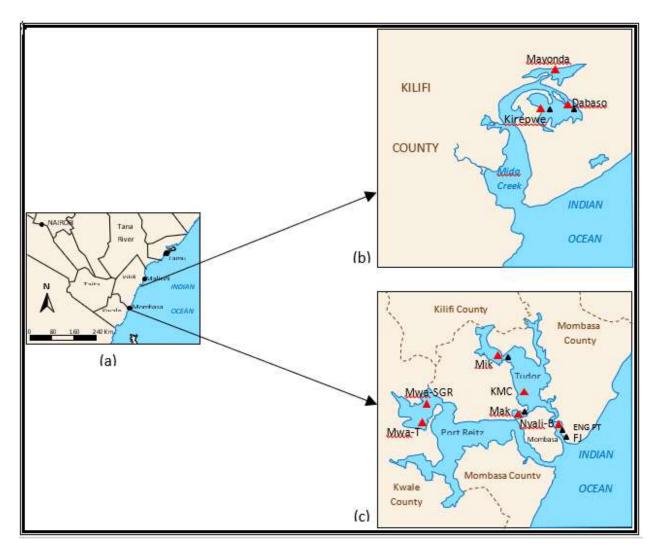


Figure 3.1 Map of Kenya showing the sampling sites and stations a) Kenya Coastal region b) Mida Creek c) Mombasa Island with Tudor and Port-Reitz Creeks

Key: Mwa-SGR: Mwache-SGR, Mwa-T: Mwache Tsunza, Mak: Makupa, Mik: Mikindani,
K.M.C: Kenya meat commission, Nyali-B: Nyali bridge, ENG PT: English Point, FJ: Fort Jesus.
▲ showing Fish collection points. ▲ showing surface water and sediment sample collection points

## **3.2 Sampling strategy**

At each of the three sites, Tudor, Port Reitz, and Mida creeks three stations were identified for sampling and the GPS location noted using a handheld GPS (version; Mitac mio168) with WGS-84 terrestrial reference system (Table 3.1). These were Mikindani, Kenya Meat Commission (K.M.C) and Nyali-bridge (Nyali-B) in Tudor; Makupa, Mwache-Tsunza (Mwache-T) and Mwache – SGR in Port-Reitz and Kirepwe, Mayonda and Dabaso in Mida Creek. At each station, nearshore surface water and inter-tidal sediment samples were collected in January/February 2018 during the dry period and September 2018 during short rains (Fig. 3.2). In Mida, sampling was done in only one station (Kirepwe) during the first sampling campaign and in three stations during the second sampling campaign. Sediments samples were collected from the intertidal zone (between the waterline and hightide water mark) during low tide. Physico-chemical parameters such as water conductivity ( $\mu$ S cm<sup>-1</sup>), salinity (ppt) and temperature (°C) were measured at each station using a multi parameter meter (YSI ProDSS): (Appendix 1).

Site	Station	Southing	Easting
Tudor	Mikindani	4° 41 ~ 51 ~	39° 21″ 12′
	Nyali-B	4° 2′′48.1′	39° 40″ 27.4′
	K.M.C	4° 1´´34.7´	39° 38´´47.5´
Port-reitz	Makupa	4° 2´´16.5´	39° 38″ 50.1′
	Mwache-T	4° 2′′ 47′	39° 40´´26.7´
	Mwache SGR	4° 1′′53.6′	39° 48″ 47′
Mida	Kirepwe	4° 3´´23.5´	39° 48′′ 47′
	Mayonda	3° 19′′ 33.2'	39° 59´´47´
	Dabaso	3° 20″ 39.8′	39° 59″ 12.8′

 Table 3.1 Sampling sites, stations and GPS coordinates

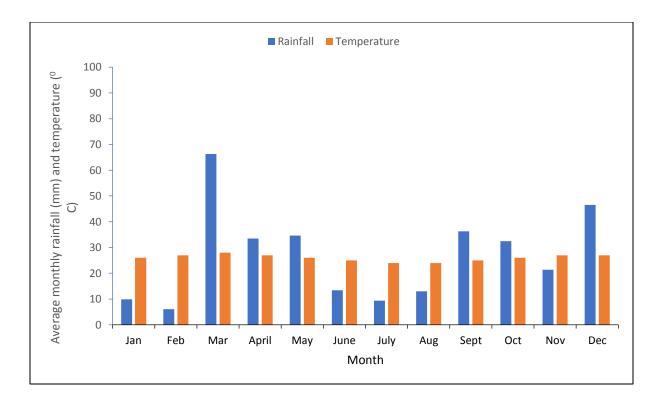


Figure 3.2 Average monthly rainfall and temperature in Mombasa County during the sampling period (January/February and September, 2018) (weather and climate) <u>https//(tcktcktck.org)</u>/mombasa/september-2018

#### 3.3 Data analyses

Data was processed using the Statistics and Data (STATA) version 15. The data was checked for normality using Shapiro-Wilk W test for normal data. Data sets that were not normally distributed were log transformed after which the parametric test of ANOVA was used for data with normal distribution, while non-parametric test (Kruskal-Wallis) was used for data that failed the normality test. The total mean concentration and the concentrations of the different categories and colours of microplastics in surface water and sediments were assessed between sites (Tudor, Port-Reitz and Mida Creeks) and between stations in each of the sites. For fish, mean total concentrations of microplastics in different species were compared using one-way ANOVA and the pairwise multiple comparisons done using Turkey's test. The assessment of microplastics in the guts, gills and muscles was done in accordance with procedure and ethical guidelines for animal experiments in the University of Nairobi and KMFRI. The tests were considered significant at p < 0.05.

# CHAPTER FOUR 4.0 MICROPLASTICS IN SURFACE WATERS IN CREEKS ALONG THE KENYA COAST

## 4.1 Introduction

Plastic production is steadily increasing and is estimated to hit 33 billion tons by 2050 (Zalasiewicz *et al.*, 2016) which may result to a considerable amount of plastics reaching the oceans. Gradual degradation of plastics through biological and chemical processes adds MPs into the water hence the need to investigate the extent of MPs pollution along the Kenya coast to inform on policy formulation on plastic waste management and disposal. The ocean surface water is habitat to a wide range of species, from various fish and cetaceans to rafters and the neuston community (Thiel and Gutow, 2005; Thiel *at al.*, 2006). Owing to their small size (0.1 µm- 5 mm) MPs are bioavailable to a great diversity of organisms since they mimic prey particles and sediment grains causing some animals including filter feeders to incorporate them as prey (Hong *et al.*, 2018). Microplastics transport POPs to biota from the environment which even in low levels could harm or kill organisms, ultimately causing a decrease in biodiversity (Invar do Sul and Costa, 2014).

Bioaccumulation and biomagnification of MPs to higher trophic levels have also been observed where copepods and polychaete larvae ingesting 10  $\mu$ m of polystyrene transfer the particles to mysid shrimps (Setälä et *al.*, 2014). Research has shown that MPs concentrations of > 12.5  $\mu$ g/L decrease survival and lower fecundity in *Tigriopus japonicas* Mori 1938 (Lee *et al.*, 2013), 0.25 mg/L cause analogous embryonic development in sea urchin *Lytechinus variegatus* Lamark 1938 (Nobre *et al.*, 2015), reduced body mass in langoustine *Nephrops norvegicus* L. 1758 (Weiden and Cowie, 2016), reduced feeding behaviour in brine shrimp *Artemia franciscana* Kellogg 1906 (Bergami *et al.*, 2016) and cause tissue damage in the blue mussel *Mytilus edulis* L. 1758 (Von moos *et al.*, 2012).

Most coastal cities in the world have many plastic users such as manufacturing, packaging, building and construction, textile, food processing industries, fishing and tourist activities (Frere *et al.*, 2017). Some industries in some parts of the world release their effluents into the sea, major contributors being coastal cities of China, Indonesia, Philippines and even Africa (Ocean Conservancy Report, 2017). Industrial effluent together with plastic discarded introduce MPs into the oceans (Okuku *et al.*, 2011). Mombasa City is a busy coastal port with dense human settlement, many industrial plants, fishing and tourist activities and thus produces waste including plastic

waste (see plate 2.1). Research studies and documented evidence on the presence of MPs in the surface waters of the WIO along the Kenya coast are few (see chapter 2), resulting in a severe deficit of information on the extent of microplastic pollution. Kenya's Exclusive Economic Zone (EEZ) was found to have at least 33-275 particles m<sup>-3</sup> (Kosore *et al*, 2018) an estimated concentration that is lower than estimated concentrations of some of the most polluted parts in the world such as Geoje Island, South Korea: 16000  $\pm$  14000 items m<sup>-3</sup> (Song *et al.*, 2014), China sea: 4137.3  $\pm$  2461.5 items m<sup>-3</sup> (Zhao *et al.*, 2014) and the West coast of Sweden: 150-2400 items m<sup>-3</sup> (Noren, 2007). The microplastic concentrations in Kenya was close to the concentrations detected in the North Sea and East Pacific (275  $\pm$  255 m<sup>-3</sup>) (Desforges *et al.*, 2014). Following the report by the National Environmental Management Agency (NEMA) in conjunction with the United Nations Environmental Programme (UNEP), and the Kenya Institute of Public Policy Research and Analysis (KIPPRA), that showed that supermarkets alone contribute tens of millions of plastic bags into the environment annually, the Kenya Government effected a ban on the use of low weight plastic bags in February 2017 (NEMA, 2017).

This study aimed at providing an assessment of the presence of MPs in surface waters within Tudor, Port-Reitz creeks in Mombasa city and Mida creek within a less urban environment along the Kenya coast being within a Marine National reserve (KWS, 1997) which was the control. The data and information generated will form a baseline of the status (concentration of MPs) of the Kenyan Coast in 2017 when the Kenya Government effected the ban on use and production of low weight plastic bags (NEMA, 2017). The study will also provide data and information to aid in the formulation of plastic waste management policies to protect marine ecosystem (Rochman, 2016).

#### **4.2 MATERIALS AND METHODS**

## **4.2.1** Water sampling

Microplastic samples were collected using nets of three different mesh sizes from each station (Fig 3.1). For the large (500-4999  $\mu$ m) and medium (250-499  $\mu$ m) sizes, samples were collected from the surface water by towing neuston nets (of 500 and 250  $\mu$ m sizes) fitted with a flow meter for 10 minutes and replicated three times each according to Hidalgo-Ruz *et al.*, (2012). The nets were kept as close to the surface as possible in order to capture any MPs on the water surface. The large size net mouth area was 0.2 m<sup>2</sup> while that of the medium size was 0.07 m<sup>2</sup>. The boat moved at a

speed of between 0.5-1.5 knots sampling between 525.5–13298.2 m<sup>3</sup> of water for the large net size and 177.1–550.2 m<sup>3</sup> for the medium net size. For the small (20-249  $\mu$ m) size microplastics, fifty litres of seawater were drawn with a metal bucket, filtered through a 20  $\mu$ m neuston net. Material on the net was rinsed into glass bottles using sieved seawater and corked with aluminium foil-lined lids. The samples were transferred to the laboratory and stored in a refrigerator at -6 °C awaiting processing. To minimize contamination in the field, hand gloves were worn throughout, glass or metal equipment used, and sample bottles covered with aluminium lined lids immediately after filling. Samples of towing gear material were examined under a microscope at 40 x magnification for microplastics and any similar microplastics in the water samples were not included in the total counts.

## **4.3 Microplastic Extraction Process**

Sieving: The samples of the large size were sieved through 5000  $\mu$ m (to remove MPs sizes larger than 5mm) and collected on 500  $\mu$ m sieves to get MPs between 500 and 4999  $\mu$ m. Materials on the 500  $\mu$ m sieve was retained for further analysis. The samples for the medium size were sieved through 500  $\mu$ m and collected onto a 250  $\mu$ m sieve to remove particles above 500  $\mu$ m. The material on the 250  $\mu$ m sieve was retained for further analysis. The 20  $\mu$ m samples were sieved using the 250  $\mu$ m and collected onto a 20  $\mu$ m sieve. The material above 250  $\mu$ m sieve was discarded while that on 20 $\mu$ m sieve was retained for further analysis.

*Digestion:* The sieved samples of different size categories were digested in 50 ml 10 % Potassium hydroxide (KOH) at 60° C for fourteen hours in order to digest organic matter ((Foekema *et al.*, 2013; Eriksen *et al.*, 2013; Rochman *et al.*, 2015; Dehaut *et al.*, 2016; Kuhn *et al.*, 2017; Lusher *et al.*, 2017; Thiele *et al.*, 2019 modified protocol). Potassium Hydroxide was preferred because it is cheap, effective, readly available, allows recovery of microplastics of a single digit micron size and has limited impact on polymers (Foekema *et al.*, 2013; Rochman *et al.*, 2015; Dehaut *et al.*, 2015; Dehaut *et al.*, 2016). After fourteen hours, the digested samples were then sieved through the respective sieve sizes 500, 250 and 20  $\mu$ m) and thoroughly rinsed with distilled water, transferred to individual glass beakers using distilled water. Super saturated sodium chloride (NaCL) solution pre-filtered over a 0.8  $\mu$ m membrane was added in the ratio of 1mg sample to 5ml solution, stirred thoroughly with a glass rod for five minutes, covered with aluminium foil then left for 12 hours. The samples were then filtered through a vacuum pump fitted with a cellulose nitrate membrane millipore HA

 $0.8 \,\mu\text{m}$ . The membrane filters were placed in a membrane dish-holder, covered and dried at 40 °C for 12 hours (modified protocol) after which the samples were ready for MPs enumeration and characterisation.

## 4.4 Microplastic identification, enumeration and characterisation

The dry membrane filters were examined under a stereo-dissecting microscope (Fig 4.1) at X40 magnification as described by Hidalgo-Ruz et al., (2012), Desforges et al., (2015) and Devriese et al., (2015). Counting and identification of MPs majorly by shape and colour (Fig. 4.2) (Crawford and Quinn, 2017) was done following the selection and identification rules of Masura et al., (2015) and (Hidaigo-Rutz et al., (2012) (no cellular or organic structures visible, fibres with uniform thickness throughout their entire length and either a clear or homogeneous colour all through). Any flat, twisted fibres with uneven diameter and surface scales were considered natural textile fibres (Stanton et al., 2019) and excluded from the counting. Suspected MPs were then prodded and picked with tweezers (0.12 mm wide and 0.03 mm) (Hidalgo-Ruz et al., 2012; Lots et al., 2017. Plastic particles sprung on prodding while non-plastics broke. Further confirmation of plastics was done using the hot needle test as outlined by Claessens et al., (2013), Lusher et al., (2013), De Witte et al., (2014) and Devriese et al., (2015). Microplastics were characterized by shape as fibre (thread-like, microfibers, filaments or strands), film (sheet-like soft fragments), fragment (irregular shaped particles, crystals, fluffs or granules) or foam with the help of a modified microplastic chart (Hidalgo-Ruz et al., 2012; Shim et al., 2016; Graca et al., 2017; Sarkar et al., (2019), enumerated and their colour noted.

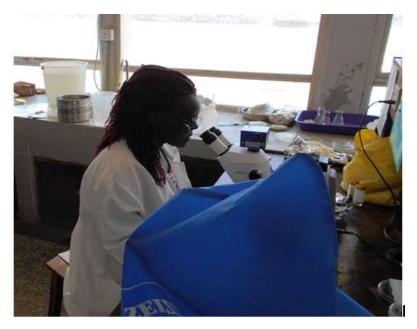
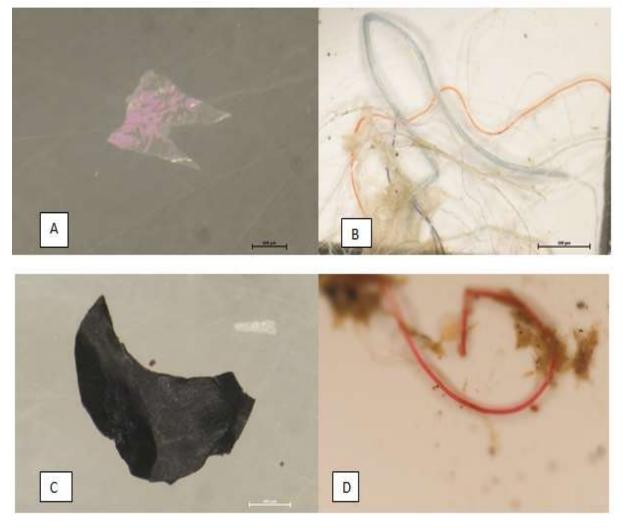
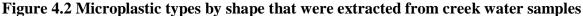


Figure 4.1 Examining microplastics under a microscope





A-Pink film in surface water sample from Makupa, B-Red, brown, blue and white fibers in surface water sample from Mikindani, C-Black fragment in surface water from K.M.C, D-Red fiber in surface water from Dabaso

# 4.5 Quality control check

Owing to the very light weight and mobility of MPs, caution was observed while analyzing samples to guarantee no contamination of samples by particles from the air. Sample processing and analysis were done in a clean room with windows and door closed and limited foot traffic.

Microplastic contamination through exposure to air was reduced by covering samples with aluminium foil and glass covers, and the use of distilled water for rinsing glassware and metal equipment (Hidalgo-Ruz *et al.*, 2012; Liebezeit and Liebezeit, 2014). The working surface was thoroughly cleaned with 70 % ethanol on non-shedding paper three times and allowed to dry before use (Cole *et al.*, 2014). Hand gloves were used and cotton laboratory coats were worn over natural and synthetic fibre clothes throughout. Sample fibres from clothing, and any potential contaminants from ropes and mesh screen were analysed alongside the surface water, by setting up long term blanks (1 blank per samples analysis). A dampened filter paper (30 mm diameter, Whatman No. 1) (Courtene –Jones *et al.*, 2017; Lusher *et al.*, 2017) was placed in a petri dish and left exposed during the processing and analysis period. The counts per blank were subtracted from the total count in each sample to correct ground contamination.

#### 4.6 Data analyses

Data for mean MPs concentrations of the three size categories from the different sites was analysed using the Statistics and Data (STATA) version 15. The data was checked for normality using Shapiro-Wilk W test for normal data. Since all the three sets of data were not normally distributed, the data was log transformed. Both the large size and small size MPs data became normally distributed while the medium size did not achieve normality. Thus, for testing for significant difference the parametric test of ANOVA was used for the data on large and small size MPs while non-parametric test (Kruskal- Wallis) was used for the data of the medium size MPs. The total mean concentration and the concentrations of the different types and colours were assessed between sites (Tudor, Port-Reitz and Mida Creeks) and between stations in each of the sites. The tests were considered significant at p < 0.05.

#### 4.7 Results

#### 4.7.1 Physico-chemial parameters of water

The physical factors of the ocean surface water within the creeks did not vary significantly between seasons and sites (ANOVA:  $F_{1,2} = 1.93$ , p> 0.05). Ocean surface water temperature was higher (23.6 ± 0.7 °C) in Tudor t, than Port-Reitz (21.9 ± 0.8 °C) and Mida (22.2 ± 0.9 °C). Salinity was almost similar in all the sites with Tudor recording a mean of 34.7 ± 0.1 psu, Port-Reitz 34.5 ± 0.1

psu and Mida  $34.4 \pm 0.1$  psu. Similarly, conductivity was similar across sites with Tudor having  $55810.1 \mu$ S cm<sup>-1</sup>, Port-Reitz  $55985.1 \mu$ S cm<sup>-1</sup>, and Mida  $55682.1 \mu$ S cm<sup>-1</sup>.

## 4.7.2 Small size (20-249 μm) microplastics category

## 4.7.2.1 Concentrations in the surface water

The overall (±SE) mean MPs concentration of the small size was 2897.7 ± 232 microplastic particles per cubic meter (mp m<sup>-3</sup>) of water. Mean MPs concentrations was slightly higher (3364 ± 431 mp m<sup>-3</sup>) during the first sampling campaign compared to the second (2534 ± 223 mp m<sup>-3</sup>) but the difference was not significant ( $F_{2,45} = 0.52$ , p = 0.6). The mean concentration in Tudor was slightly higher (3161.3 ± 363.7 pm m<sup>-3</sup>), than Port-Reitz 2883.3 ± 485.4 pm m<sup>-3</sup> and Mida 2523.3 ± 211.8 pm m<sup>-3</sup> but the difference was not significant (ANOVA; F (2,45) = 0.52, p = 0.6)

There was no significant difference in the concentrations between stations (ANOVA; F  $_{8,39} = 1.8$ , P = 0.1). Mikindani in Tudor had the highest concentration of 4520 ± 425.7 mp m<sup>-3</sup>, followed by Makupa in Port -Reitz (3736.7 ± 893 mp m<sup>-3</sup>) while Mwache-T in Port -Reitz (2040 ± 311.7 mp m<sup>-3</sup>), and Dabaso in Mida (2100 ± 177.8 mp m<sup>-3</sup>) had much lower concentrations (Table 4.1).

## 4.7.2.2 Categories of microplastics (based on shape) in the surface waters.

Three categories of MPs based on shape were encountered in the surface waters identified as fibres, fragments and films. Generally, in the small size MPs category, fibres were the most abundant type (2703  $\pm$  226 mp m<sup>-3</sup>) accounting for 93%, followed by fragments (164.6  $\pm$  20.4 mp m<sup>-3</sup>) accounting for 6%, then films (33.5  $\pm$  9.2 mp m<sup>-3</sup>) accounting for only 1% and the differences were statistically significant (ANOVA; F<sub>2.45</sub> = 5.61 p-value = 0.01).

There was no significant difference (p> 0.05) in the concentration of the different MPs categories between sites. In Tudor fibres had a mean of  $2931.7 \pm 358.5$  mp m<sup>-3</sup>; Port-Reitz a mean of  $2716 \pm 474.4$  pm m<sup>-3</sup> and Mida had a mean of  $2340.8 \pm 186.1$  mp m<sup>-3</sup>. Fragments in Tudor had a mean of  $197.8 \pm 31.9$  pm m<sup>-3</sup>, Mida a mean of  $176.7 \pm 39.8$  pm m<sup>-3</sup> and Port-Reitz a mean of  $123.3 \pm 146.3$  pm m<sup>-3</sup>). The mean concentration of films was significantly higher (Chisq.<sub>2,41</sub> = 8.5, p-value = 0.01) in Port-Reitz ( $53.3 \pm 22.8$  mp m<sup>-3</sup>) compared to Tudor ( $32.2 \pm 6.1$  mp m<sup>-3</sup>), and Mida ( $5.8 \pm 4.4$  mp m<sup>-3</sup>.

There was no significant difference (p>0.05) in the mean concentration of MPs categories between stations (Table 4.1). Mikindani had a mean of  $4245 \pm 437.1$  pm. m<sup>-3</sup> fibres, Makupa  $3575 \pm 867.7$  pm. m<sup>-3</sup>, while Dabaso had amean of  $1966.7 \pm 225.2$  mp. m<sup>-3</sup> and Mwache-T a mean of  $1816 \pm 255.6$  pm. m<sup>-3</sup>. On the other hand, fragments were most abundant in Kirepwe with a mean of  $253.3 \pm 58.3$  mp. m<sup>-3</sup>, Mikindani  $245 \pm 33.8$  mp. m<sup>-3</sup> and Nyali-B  $235 \pm 49.0$  mp. m<sup>-3</sup> while films were most abundant in Mwache-T with a mean of  $131.3 \pm 57.5$  mp. m<sup>-3</sup> (Table 4.1).

Site	Station	Total MPs	Fiber	Fragment	Film
Mida	Dabaso	$\overline{2100\pm177.8}$	$1966.7 \pm 225.2$	$126.7 \pm 67.7$	$6.7 \pm 6.7$
	Kirepwe	$2776.7\pm391.3$	$2515\pm342.4$	$253.3\pm58.3$	$8.3\pm8.3$
	Mayonda	$2440 \pm 180.4$	$2366.7\pm155.1$	$73.3\pm29.1$	$0\pm 0$
Port Reitz	Makupa	$\underline{3736.7\pm893}$	$\overline{3575\pm867.7}$	$146.6\pm81.6$	$25 \pm 8.9$
	Mwache-SGR	$2873.3\pm1105$	$2758 \pm 1081$	$131.6\pm47.2$	$3.3 \pm 3.3$
	Mwache-T	$2040\pm311.7$	$1816\pm255$	$91.7\pm53.9$	$131.3\pm57$
Tudor	K.M.C	$\overline{2328.3\pm658}$	$2180\pm651.9$	$113.3 \pm 68.4$	35 ± 13.6
	Mikindani	$\underline{4520 \pm 425.7}$	$4245\pm439.1$	$245\pm33.8$	$30 \pm 11$
	Nyali-B	$2636.7\pm415.1$	$2370\pm414.6$	$235\pm49.0$	$31.7\pm8.7$
Total		$\overline{2897.7\pm232}$	$2703.3 \pm 226$	$164.6 \pm 20.4$	$33.5 \pm 9.2$
F		1.81	1.81	0.94	1.93
DF		8	8	8	8
P-VALUE		0.1038	0.103	0.5	0.09

Table 4.1: Mean ( $\overline{x} \pm SE$ ) concentration (m<sup>-3</sup>) of the different microplastics by shape of the small size microplastics in different stations

## 4.7.2.3 Microplastic colours of the small size category

Eight colours of MPs were encountered among the small size MPs category. Overall, the concentrations of different microplastic colours were significantly different and white colour was the most dominant (2015.2 ± 203.6) accounting for 69.6 %, followed by black (453.5 ± 159.7 mp. m<sup>-3</sup>) 15.6 %, blue (239.6 ± 35.6 mp. m<sup>-3</sup>) 8.2 %, brown (76.9 ± 15.2 mp. m<sup>-3</sup>) at 2.7 %, green (53.3 ± 10.2 mp. m<sup>-3</sup>) at 1.8 %, red (48.5 ± 9.6 mp. m<sup>-3</sup>) at 1.7 % and finally purple (6.3± 3.4 mp. m<sup>-3</sup>)

and grey (4.4  $\pm$  2.10 mp. m<sup>-3</sup>) at 0.2 % each. By site, the mean concentration of MPs of different colours did not differ significantly (p< 0.05) but white in Tudor had the highest mean of 2348.3  $\pm$  312 mp. m<sup>-3</sup> accounting for 74 %, Port-Reitz a mean of 2012  $\pm$  424 mp. m<sup>-3</sup> (71 %), and Mida a mean of 1519  $\pm$  169.7 mp. m<sup>-3</sup> (60 %) (Fig. 4.3). The mean concentration of MPs of black colour was highest (348  $\pm$  81.1 mp. m<sup>-3</sup> (20.2 %) in Port-Reitz while blue had the highest mean (250.6  $\pm$  36.4 mp .m<sup>-3</sup> (17 %) in Mida (Figure 4.3).

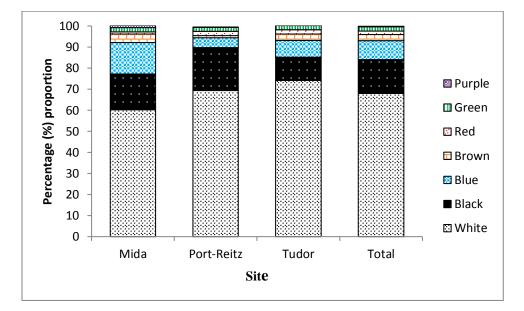


Figure 4.3 Mean ( $\overline{x} \pm SE$ ) concentration (m<sup>-3</sup>) of microplastic particles of different colours in different sites

Microplastics of different colours dominated in different stations. The mean concentrations of white and blue MPs were significantly different ( $F_{8,41} = 5.44$ , P < 0.01,  $F_{8,41} = 2.86$ , P = 0.013 respectively) between stations. Makupa had the highest mean concentration ( $3475 \pm 934$  mp. m<sup>-3</sup>) for white accounting for 93 % and the lowest concentration of black MPs accounting for 3.2 %. On the other hand, Mwache-SGR had the lowest concentration ( $1050 \pm 468.3$  mp. m<sup>-3</sup>) of white accounting for 36.5 % and the the highest concentration of ( $1431.7 \pm 1256.4$  mp. m<sup>-3</sup>) of black MPs accounting for 49.9 %. Blue coloured MPs were most abundant in Kirepwe ( $660 \pm 160.7$  mp. m<sup>-3</sup>) accounting for 23.8%. (Figure 4.4).

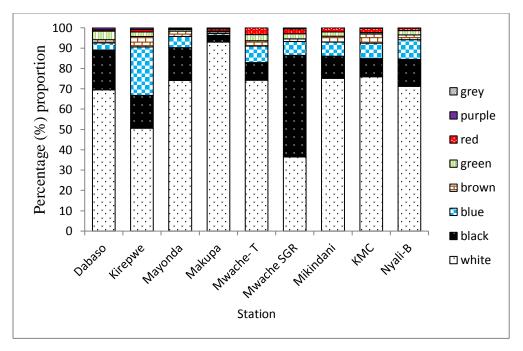


Figure 4.4 Percentage proportion of microplastic particles of different colours in the small size category in different stations

4.7.3 Medium size (250- 449 µm) microplastics category

## 4.7.3.1 Concentrations of the medium size microplastics

The overall mean concentration of the medium microplastics was generally lower compared to the small size MPs category at  $3.1 \pm 0.4$  mp. m<sup>-3</sup> of water. The MPs concentrations between the two sampling campaigns (January and September) were significantly different (chisq 1= 29.3, p-value < 0.01) being  $1.1 \pm 0.2$  mp. m<sup>-3</sup> in the first campaign and  $4.5 \pm 0.4$  mp. m<sup>-3</sup> during the second. Mida recorded higher mean concentration ( $4.2 \pm 0.58$  mp. m<sup>-3</sup>) (chisq  $_{2,45} = 6.4$ , p- value = 0.041), followed by Port-Reitz ( $2.7 \pm 0.71$  mp. m<sup>-3</sup>) and finally Tudor ( $2.6 \pm 0.45$  mp. m<sup>-3</sup>) and the difference between sites was significant. Post hoc analysis showed that the mean concentration for Mida differed significantly from that of Tudor and Port-Reitz while the latter two sites were not different (chisq  $_{2,45} = 6.4$ , p- value = 0.041). By station, the mean concentration of microplastics was higher in Dabaso ( $6.2 \pm 0.53$  mp. m<sup>-3</sup>), while Mwache –T ( $1.0 \pm 0.2$  mp. m<sup>-3</sup>) had the lowest and he differences between stations were different (ANOVA; Chisq  $_{8,41} = 20.87$ , p <0.01) (Table 4.2). Post hoc showed that Dabaso, Mayonda and Mwache -SGR had higher concentrations of MPs compared to other stations.

**4.7.3.2** Microplastic shapes of the medium size microplastics category in the surface waters. The mean concentration of medium size microplastic fibres was significantly higher  $(2.7 \pm 0.3 \text{ mp.} \text{m}^{-3})$  (Chisq<sub>2,45</sub> = 7.7, p-value = 0.02) compared to fragments  $(0.3 \pm 0.1 \text{ mp.m}^{-3})$  and films  $(0.1 \pm 0.01 \text{ mp.m}^{-3})$  (Chisq<sub>2,39</sub>, = 11.8, p-value = 0.002). The mean concentration of fibres was significantly higher (Chisq<sub>2,45</sub> = 7.7, p-value = 0.02; Chisq<sub>2,39</sub>, = 11.8, p-value = 0.002 in Mida creek (4 ± 0.6 mp.m^{-3}) compared to Port-Reitz (2.3 ± 0.6 mp.m^{-3}) and Tudor (2.2 ± 0.4 mp. m^{-3}) creeks while the latter two sites were not significantly different. The mean concentration of fragments was significantly higher in Port-Reitz ( $0.4 \pm 0.4 \text{ mp.m}^{-3}$ ) and Tudor creek ( $0.3 \pm 0.1 \text{ mp.m}^{-3}$ ) compared to Mida creek ( $0.1 \pm 0 \text{ mp.m}^{-3}$ ) (Chisq<sub>2,45</sub> = 7.7, p-value = 0.02; Chisq<sub>2,39</sub>, = 11.8, p-value = 0.02; Chisq<sub>2,39</sub>, = 11.8, p-value = 0.02; Chisq<sub>2,45</sub> = 7.7, p-value = 0.02; Chisq<sub>2,39</sub>, and Tudor creek ( $0.3 \pm 0.1 \text{ mp.m}^{-3}$ ) compared to Mida creek ( $0.1 \pm 0 \text{ mp.m}^{-3}$ ) (Chisq<sub>2,45</sub> = 7.7, p-value = 0.02; Chisq<sub>2,39</sub>, = 11.8, p-value = 0.002 respectively). The mean concentration of films was relatively low across all sites and showed significant difference (Chisq<sub>2,45</sub> = 11.792, p< 0.01).

Within the stations, fibres were significantly more abundant in Dabaso  $(5.8 \pm 0.1 \text{ mp.m}^{-3})$ , Mayonda  $(5.2 \pm 0.3 \text{ mp.m}^{-3})$  and Mwache SGR  $(5.4 \pm 1.6 \text{ mp.m}^{-3})$  than in Mwache-T  $(0.9 \pm 0.2 \text{ mp.m}^{-3})$ . Fragments were significantly more abundant in Mwache-SGR  $(0.8 \pm 0.2 \text{ mp} \text{ m}^{-3})$  than in Kirepwe, Mayonda, Mwache-T and K.M.C, at  $0.1 \pm 0.03 \text{ mp.m}^{-3}$  while films were significantly higher in Mayonda  $(0.2 \pm 0.1 \text{ mp.m}^{-3})$  with none being encountered in Mwache-T. There was a significant difference in the mean concentration of fibres, fragments and films between stations within the three creeks (ANOVA; Chisq<sub>8,39</sub> = 20.69, p = 0.008; Chisq<sub>8,29</sub> = 17.95, p-value = 0.02; Chisq<sub>2,29</sub> = 18.9, p = 0.02) respectively (Table 4.2).

Site	Station	Total mps	Fiber	Fragment	Film
Mida	Dabaso	$6.2 \pm 0.5$	$5.8 \pm 0.5$	$0.2 \pm 0.1$	$0.14 \pm 0.1$
	Kirepwe	$2.7\pm0.6$	$2.5\pm0.6$	$0.1 \pm 0.1$	$0.09\pm0$
	Mayonda	$5.5\pm0.3$	$5.2\pm0.3$	$0.1\pm0.0$	$0.22 \pm 0.1$
Port Reitz	Makupa	$1.8 \pm 0.6$	$1.6 \pm 0.5$	$0.2 \pm 0.1$	$0.05 \pm 0.0$
	Mwache-SGR	$1 \pm 0.2$	$0.9\pm0.2$	$0.1\pm0.0$	$0\pm 0$
	Mwache-Tsunza	$5.4 \pm 1.6$	$4.5\pm1.4$	$0.8\pm0.2$	$0.07\pm0.0$
Tudor	KMC	$2.3 \pm 0.7$	$2.1 \pm 0.7$	$0.1 \pm 0.1$	$0.1 \pm 0.0a$
	Mikindani	$3.6 \pm 1$	$2.9\pm0.8$	$0.7\pm0.3$	$0.09\pm0.1$
	Nyali-bridge	$1.7 \pm 0.4$	$1.5\pm0.3$	$0.2\pm0.1$	$0.04 \pm 0.0$
	Total	3.1 ± 0.4	$2.7 \pm 0.3$	$0.3 \pm 0.1$	$0.08 \pm 0.0$
	F	20.866	20.686	17.953	18.912
	df	8	8	8	8
	P -value	0.0075	0.0075	0.0215	0.0153

Table 4.2: Mean  $(\bar{x} \pm SE)$  concentration  $(m^{-3})$  of the total microplastics and the different types by shapes of the medium size microplastics in different stations

# 4.7.3.3 Microplastics colours of the medium size microplastics category.

Unlike the small sized MPs, only six colours were encountered in this size with purple and grey missing. Overall, white was most abundant  $(2.1 \pm 0.3 \text{ mp.m}^{-3})$  followed by black  $(0.5 \pm 0.1 \text{ mp.m}^{-3})$  and blue  $(0.3 \pm 0.1 \text{ mp.m}^{-3})$ . By site, the percentage proportion of the white MPs was almost the same across all the sites at 70 % (Figure 4.3) with that of other colours being only 30 %. Green was encountered in small proportions in Mida Creek while it was missing in the other two sites (Figure 4.5).

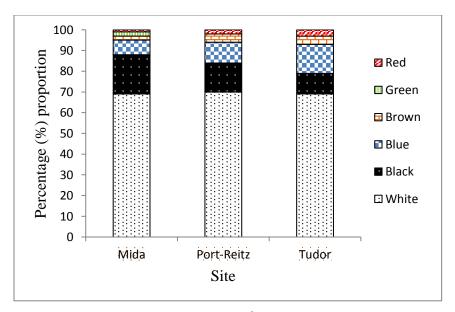


Figure 4.5 Mean percentage concentration (m<sup>-3</sup>) of the MPs of different colours in the medium size category in different sites

By station, Dabaso, Mayonda and Mwache-SGR recorded the highest mean concentration for white  $(4 \pm 0.1)$ . Black MPs were abundant in Dabaso  $(1.6 \pm 0.3)$  with none being recorded in Makupa  $(0 \pm 0)$ . Microplastics of grey colour were recorded only in Makupa  $(0.04 \pm 0)$  (Table 4.3). The concentrations were significantly different between stations for white and black coloured MPs  $(Chisq_8=18.911, p = 0.01; Chisq_8=24.516, p = 0.001)$  respectively.

Site	Station	White	Black	Blue	Brown	Green	Red	Purple	Grey
Mida	Dabaso	$4 \pm 0.6$	1.6± 0.3	$0.3 \pm 0.2$	$0.1 \pm 0.1$	$0.1 \pm 0.1$	$0.1 \pm 0.1$	$0\pm 0$	$0\pm 0$
	Kirepwe	$2.1 \pm 0.4$	$0.4\pm0.2$	$0.02\pm0.1$	$0.0 \pm 0$	$0\pm 0$	$0\pm 0$	$0\pm 0$	$0\pm 0$
	Mayonda	$4\pm0.5$	$0.9\pm0.4$	$0.4 \pm 0.1$	$0.1\pm0$	$0.1 \pm 0$	$0.1 \pm 0$	$0\pm 0$	$0\pm 0$
Port Reitz	Makupa	$1.2 \pm 0.4$	$0\pm 0$	$0.3 \pm 0.0$	$0.1 \pm 0$	$0\pm 0$	0.1±0	$0\pm 0$	$0.04 \pm 0$
	Mwa- T	$0.7\pm02$	$0.1\pm0$	$0.1 \pm 0.0$	$0.0 \pm 0$	$0\pm 0$	$0.1 \pm 0$	$0\pm 0$	$0\pm 0$
	Mwa -SGR	$3.6\pm1.2$	$1.0\pm0.3$	$0.5\pm0.2$	$0.1 \pm 0$	$0.1 \pm 0$	$0.1 \pm 0$	$0.1 \pm 0.1$	$0\pm 0$
Tudor	K.M.C	$1.6 \pm 0.6$	$0.1 \pm 0.1$	0.3±0.1	$0.1 \pm 0.1$	$0\pm 0$	$0.1 \pm 0$	$0.1 \pm 0$	$0\pm 0$
	Mikindani	$2.2\pm0.5$	0. $7 \pm 0.3$	$0.5\pm0.2$	$0.1 \pm 0$	$0\pm 0$	$0.1 \pm 0$	$0\pm 0$	$0\pm 0$
	Nyali-B	$1\pm0.3$	$0.1 \pm 0.1$	$0.03\pm0.1$	$0.1\pm0$	$0.1 \pm 0$	$0.1 \pm 0$	$0\pm 0$	$0\pm 0$
	Total	$2.1 \pm 0.3$	$0.5 \pm 0.1$	$0.3 \pm 0.1$	$0.1 \pm 0$	$0.1 \pm 0$	$0.1 \pm 0$	$0 \pm 0$	$0\pm 0$
	Chisq.	18.911	24.516	F=1.63	8.253	8.842	7.902	0.82	
	df	8	8	8,36	8	8	8	8	
	P-value	0.017	0.002	0.15	0.41	0.35	0.44	0.59	

Table 4.3: Mean ( $\overline{x} \pm SE$ ) concentration (m<sup>-3</sup>) of the different colours of the middle size microplastics category in different stations

## 4.7.4 Large Size (500 μm- < 5 mm) Microplastics Category

## 4.7.4.1 Concentrations of the large size microplastics

The concentrations of the large size MPs category were generally less than 1 MPs particle per m<sup>3</sup> in all the sites. Overall, mean concentration of the large size MPs was  $0.6 \pm 0.1$  mp.m<sup>-3</sup> of water. There was a significant difference (F<sub>1,46</sub> = 41.82, p< 0.05) in the MPs concentrations between sampling periods. During the first sampling campaign the overall concentration was  $0.33 \pm 0.04$  mp.m<sup>-3</sup> while during the second the concentration was  $0.80 \pm 0.05$  mp.m<sup>-3</sup> of water. Mida Creek had a higher mean concentration of MPs ( $0.8 \pm 0.1$  mp.m<sup>-3</sup>) followed by Port-Reitz ( $0.6 \pm 0.1$  mp.m<sup>-3</sup>) then Tudor ( $0.5 \pm 0.1$  mp.m<sup>-3</sup>). Kruskal Wallis test showed significant difference (F<sub>2,45</sub> = 4.97, p-value = 0.01) in mean microplastic concentration between sites. Post hoc test showed that the mean concentration in Mida was different from that in Port-Reitz and Tudor but the latter two were not different.

## 4.7.4.2 Large size microplastics shapes in the surface waters.

Like the middle size MPs category, the mean concentration of the large size MPs category was low, with fibres having a higher mean concentration  $(0.5 \pm 0.1 \text{ mp.m}^{-3})$  compared to fragments  $(0.1 \pm 0 \text{ mp.m}^{-3})$  and films  $(0.04 \pm 0.01 \text{ mp.m}^{-3})$ . By site, fibres were significantly more abundant in Mida  $(0.7 \pm 0.1 \text{ mp.m}^{-3})$ , and lower in Port-Reitz and Tudor  $(0.4 \pm 0.1 \text{ mp.m}^{-3})$  each, fragments were significantly more abundant in Mida and Port-Reitz  $(0.1 \pm 0 \text{ mp.m}^{-3})$  and lowest in Tudor  $(0.04 \pm 0.01 \text{ mp.m}^{-3})$ , while films varied in concentrations from  $0.1\pm 0.02 \text{ mp.m}^{-3}$  in Port-Reitz to  $0.04 \pm 0.02 \text{ mp.m}^{-3}$  in Mida and  $0.03 \pm 0.0 \text{ mp.m}^{-3}$  in Tudor. There was a statistically significant difference in the mean concentration of fibres and fragments between sites (ANOVA;  $F_{2,45} = 6.22$ , p-value = 0.004;  $F_{2,45} = 5.21$ , p-value = 0.01) respectively. Post hoc test showed that the mean concentration of fibres in Mida was different from Port-Reitz and Tudor and the latter two were not different, while the mean concentration of fragments in Tudor was different from Mida and Port-Reitz and the latter two were not different. Films did not show significant variation in mean concentration between sites (p> 0.05).

By station, Dabaso, Kirepwe, and Mayonda had a mean concentration of fibres  $(0.7 \pm 0.1 \text{ mp.m}^{-3})$ , followed by K.M.C, Mwache-SGR and Nyali-B  $(0.5 \pm 0.1 \text{ mp. m}^{-3})$  and Mikindani  $(0.2 \pm 0.1 \text{ mp. m}^{-3})$ . Fragments varied with a mean concentration of  $0.2 \pm 0.1 \text{ mp. m}^{-3}$  in Makupa abundant while films recorded a similar but low concentration of  $0.1 \pm 0.03 \text{ mp.m}^{-3}$  in K.M.C., Kirepwe, Makupa, Mwache-T and Mwache-SGR (Table 4.4). There was no significant difference (p> 0.05) in the mean concentration of MPs shapes between stations.

	Station	Total mps	Fibre	Fragment	Film
Mida	Dabaso	$0.8 \pm 0.1$	0.7 ± 0.1	0.1 ± 0	$0.02 \pm 0$
	Kirepwe	$0.9\pm0.2$	$0.7\pm0.0$	$0.1 \pm 0.1$	$0.06\pm0.1$
	Mayonda	$0.8 \pm 0.1$	$0.7\pm0.0$	$0.1\pm0$	$0.02\pm0$
Port Reitz	Makupa	$0.6 \pm 0.1$	$0.4 \pm 0.1$	$0.2 \pm 0.1$	$0.05 \pm 0$
	Mwa-SGR	$0.6\pm0.2$	$0.5 \pm 0.4$	$0.1 \pm 0$	$0.06\pm0.1$
	Mwa-T	$0.6 \pm 0.2$	$0.4 \pm 0.2$	$0.1 \pm 0.1$	$0.06\pm0.1$
Tudor	KMC	$0.6 \pm 0.7$	$0.5 \pm 0.1$	$0.03 \pm 0$	$0.05 \pm 0$
	Mikindani	$0.3\pm0.1$	$0.2\pm0.1$	$0.04 \pm 0.1$	$0.03 \pm 0$
	Nyali-B	$0.5\pm0.1$	$0.5\pm0.1$	$0.1 \pm 0.1$	$0.02\pm0$
	Total	$0.6 \pm 0.1$	$0.5\pm0.0$	$0.1 \pm 0$	$0.04 \pm 0$
	F	1.78	2.04	2.23	1.16
	df	8	8	8	8
	P-value	0.112	0.0673	0.0553	0.3598

Table 4.4: Mean ( $\overline{x} \pm SE$ ) concentration (m<sup>-3</sup>) of the different types by shape of the large size microplastics in different stations

### 4.7.4.3 Microplastics colours of the large size microplastics category

Like the small size MPs, eight colours were encountered with low mean concentrations across sites (Table 4.5). However, the mean concentrations between sites were significantly different (p< 0.05). Post hoc test showed that Mida varied from Port-Reitz and Tudor and the latter two were not different. By station, the mean concentrations were also low with white having a higher mean concentration ( $0.4 \pm 0$  mp.m<sup>-3</sup>), followed by black ( $0.8 \pm 0$  mp.m<sup>-3</sup>), while grey recorded zero in most stations. There was a significant difference (p< 0.05) for blue and brown microplastics between stations (Table 4.5). Kirepwe had a higher concentration of blue MPs compared to other stations, while Makupa had a higher concentration of brown MPs compared to other stations.

Site	White	Black	Blue	Brown	Green	Red	Purple	Grey
Mida	0.52±.06	.8±.2	0.2±.05	0.03±.01	0.02±.01	0.02±0	0.01±0	0.02±.01
Port-Reitz	$0.31 \pm .06$	.11±.3	0.1±.02	$0.04 \pm .02$	0±0	0.02±0	$0.01 \pm 0$	0.01±0.01
Tudor	$0.29 \pm .04$	.05±.1	0.1±.01	0.03±.01	$0.01\pm 0$	0.1±0	$0\pm 0$	0±0
Total	0.36±.03	.8± .01	0.1±.02	0.03±.01	0.01±0	$0.2 \pm 0$	$0.01 \pm 0$	0.01±0
F	$X^2 = 8.588$	2.10	1.20	0.06	0.88	1.47	6.13	1.88
df	2	2,35	2,44	2,32	2,13	2,31	2,7	1,4
p- value	0.0137	0.1371	0.3095	0.9403	0.4383	0.251	0.0291	0.2417
Station	White	Black	Blue	Brown	Green	Red	Purple	Grey
Dabaso	0.67 ±0	$.08 \pm .01$	0.03±.01	$0.02 \pm 0.01$	$0.02 \pm .01$	0.03±0	1.0±0	$0.04 \pm .04$
Kirepwe	$0.46 \pm 0$	$.05 \pm .03$	$0.28\pm.09$	$0.03 \pm .01$	$0.01 \pm .01$	$0.02 \pm 0$	$0.01 \pm .01$	0±0
Mayonda	$0.48\pm0$	. 14± .05	$0.08 \pm .02$	$0.04\pm0$	0.03±.01	0±0	$0.02 \pm .01$	$0.04 \pm .04$
Makupa	0.23±0	.13±.05	$0.11 \pm .02$	$0.08 \pm .04$	0.01±0	0.02±0	0.04±.02	0.03±.02
Mwache-T	0.3±.13	$.09 \pm .04$	$0.11 \pm .05$	$0.01 \pm 0$	0±0	$0.03 \pm .0$	$0\pm 0$	0.01 ± .01
Mwache-SGR	0.4±.12	$.10 \pm .05$	$0.05 \pm .03$	$0.04 \pm .02$	0±0	0.01±0	$0\pm 0$	0±0
КМС	0.4±.03	.05 ±.02	0.0 5±.02	$0.03 \pm .02$	0.01±0	0±0	0.02±.01	0 ±0
Mikindani	0.17 ±. 05	$.03 \pm .01$	$0.04 \pm .02$	$0.03 \pm .01$	$0.01 \pm .01$	0.01±0	$0.01 \pm .01$	$0\pm 0$
Nyali-bridge	$0.3 \pm .07$	$0.05 \pm .03$	$0.11 \pm .02$	$0.02 \pm .01$	$0\pm 0$	0±0	$0.01 \pm .0$	$0\pm 0$
Total	0.4±.03	0.08±.01	0.0 1±.02	0.03±0	0.01±0	0.01±0	$0.02\pm0$	$0\pm 0$
F	0.06	0.67	3.18	1.63	0.26	2.35	0.95	0.98
df	8,32	8,29	8,38	8,26	6,9	4,5	8,25	3,2
P -value	0.9403	0.7103	0.0073	0.0165	0.9405	0.1867	0.4935	0.5412

Table 4.5: Mean ( $\overline{x} \pm SE$ ) concentration (m<sup>-3</sup>) of the different colours of the large size microplastics in different sites and stations

### 4.8 Discussion

The presence of MPs in marine surface waters along the Kenya Coast clearly provided evidence for widespread MPs pollution of the ocean waters. Seasonal changes affected MPs distribution in surface water differently. The average monthly rainfall in January/February sampling period was low (6.1-9.8 mm), and relatively high (36.3 mm) in September 2018 (Kenya Meteorological Department, 2018; figures Appendix 5 and 6). Higher mean concentrations were encountered in the first and second sampling seasons, for the small size MPs category but the concentrations were not significantly different (p> 0.05). The mean concentrations for both the medium and large size categories were higher in the second sampling season and were significantly different (p< 0.05). The lack of significant difference between the sampling seasons for the small size MPs may mean that land-based sources may not be the major sources of MPs in the creeks or no new arrivals through runoff or wind (Veerasingam *et al.*, 2016). This could be attributed to the calm ocean conditions with low wind, wave intensity and similarity in physico-chemical factors (Maes *et al.*, 2017) that prevailed during the sampling seasons hence uniform distribution of the small size MPs in the sites. The average salinity of 34.2 ppt during the first and second sampling periods could have increased water density making MPs buoyant hence the high concentrations.

Microplastics were found in all the sites including Mida creek, a National Marine Reserve thought to be safe from pollution by industrial effluents, sewage disposal and fishing activities. The relatively high concentration of MPs in this site suggests that the MPsmay not necessarily be all from adjacent land activities. The higher concentration of the large size microplastics category in Mida compared to Tudor and Port-Reitz suggests that the MPs were close to their source. The MPs could be originating mainly from the tourists to the Marine National Park, the inhabitants of Uyombo and Dabaso village, and Kirepwe and Sudi Islands surrounding the creek, disposing domestic waste into the ocean, similar to earlier research linking human population density and plastic pollution (Dai *et al.*, 2018). The human inhabitants release domestic effluent into the ocean and throw plastic waste which could be seen floating on the water surface (personal observation) thereby contributing to the pollution. It also suggests that plastic debris have not stayed within the creek waters for long and little degradation has occurred probably due to the regular cleaning done removing anthropogenic litter from the creek waters (personal observation). The trend of MPs occurrence is similar to those observed for the Mediterranean Sea, the Northeast Pacific Ocean and the open ocean waters (Goldstein *et al.*, 2013; Cozar *et al.*, 2015). A lot of boat and dhow

fishing activities go on in the creek waters (personal observation) which could be contributing to the high concentration of MPs in the waters in Mida.

Makupa in Port-Reitz had a high MPs concentration compared to other stations within the site. This could be attributed to the fact that Kibarani dumpsite is near Makupa where municipal waste has been dumped for many years (Eriksen et al., 2014). Water flow within the station is limited hence MPs are not carried away by ocean currents. High MPs levels have been linked to anthropogenic activities like aquaculture, fishing and coastal tourism in other parts of the world (Frere et al., 2017; Dai et al., 2018). Population density and the level of urbanization and waste infrastructure have also been linked to high accumulation of MPs in different regions of the world (Lebreton et al., 2012; Pedrotti et al., 2016). This could be the case with Port-Reitz creek bordering a suburb area on the mainland which hosts oil refineries and housing estates and surrounded by densely populated villages such as, Dongo Kaya, Dunga Nusa, and Ngala. The relatively high concentration of MPs of small and medium size categories in Mwache-SGR in Port-Reitz could be attributed to Port activities as well as high population density. Mwache-T, also in Port-Reitz, has high population density (IAME, 2018) that is more of rural than urban thus, low MPs concentrations probably due to less usage of plastics but may also be as a result of flushing by the many river channels such as river Mwache, Cha Shimba and Mwambone, and frequent ocean waves and currents (Kitheka et al., 1999).

Mikindani in Tudor is an outlying township in the mainland along Nairobi highway within the heavy industries at Changamwe and accommodates the working population who work in the Port of Mombasa, town centre and in the industries (IAME, 2018). Tudor creek is fed by two major seasonal rivers; Kombeni and Tsalu which arise from near the town of Mariakani (Kitheka *et al.*, 1999). The rivers collect surface runoff with plastic and other waste debris from the mainland and discharge them into the creek. Rapid urbanization has led to the development of informal settlements near the Coast General Hospital and Kenya Meat Commission (IAME, 2018) that may be adding onto the MPs brought in by the seasonal rivers and ocean currents through the release of raw domestic waste hence high MPs concentrations.

Microplastics sampling by different researchers has been done using different methods and therefore comparisons of concentrations become difficult. Lusher *et al.*, (2015) recovered between 0.02 and 100 particles m<sup>-3</sup> from the Northeast Atlantic Ocean by pumping and sieving surface water

with a 250 µm sieve. De Lucia et al., (2014) recovered 0.11-119 MP particles m<sup>-2</sup> from surface water in the Mediterranean Sea using a 333µm manta net. In the central part of Kenya Exclusive Economic Zone (EEZ), Kosore et al., (2018) recovered an average of 110 MPs particles m<sup>-3</sup> by sieving water samples through a 250 µm stainless steel mesh. In this study, an estimated average of 3.1 MPs particles m<sup>-3</sup> were recovered by towing a net of 250  $\mu$ m net and a lower average of <1 MPs particle  $m^{-3}$  by towing a net of 500 µm. The recoveries of MPs from 250 µm and 500 µm neuston nets were very low. pointing to the possibility of there being much lower concentrations of large and medium MPs particles in the water surface compared to the small size MPs. The levels of the small size category were high as expected due to the high densities and the many anthropogenic activities within the creeks (Okuku *et al.*, 2011; own observation). Thus, the manta trawl with 300 µm mesh size that has been proposed for sampling of MPs in the water column (Viršek et al., 2016) and used in many studies (Tamminga et al., 2018) may be underestimating the MPs of  $< 250 \,\mu\text{m}$  in the water column (Dai *et al*, 2018). Bulk sampling method could be efficient for sampling small size MPs in the water column. On the other hand, there is the possibility that the effect of currents generated by towing the nets could be causing the MPs to be pushed out of the nets through the large mesh size. Kang et al., (2015) found that MPs less than 2 mm were two orders of magnitude higher in concentration in the hand net compared to the towed net.

In this study MPs concentrations of three size categories (large, medium and small MPs) were estimated using different net mesh sizes and sampling strategies. Although the small size MPs samples were collected by scooping water using a stainless-steel bucket, the concentrations were several orders of magnitude higher compared to the medium and large sizes. The medium and large size MPs samples were collected by towing neuston nets for ten minutes where less than 10 MPs and less than 1 MPs particles per cubic meter were recovered, respectively. However, owing to the heterogeneous distribution of MPs on the sea surface (Eriksen *et al*, 2018) towing nets on the sea surface helps to overcome the heterogeneity. Owing to the great contrast in mean concentrations of MPs between the small size and the others, it may be critical to test the efficiency of bulk sampling vis-a-vis use of towed plankton or manta trawl nets for all sizes of MPs and compare the recovery. The challenge of heterogeneity of MPs distribution can be overcome by taking several replicate samples at different points.

It is also worth noting that the Bulk sampling method used for smaller size MPs was fast, less laborious, easy, economical and efficient in MPs recovery. However, it was not used in the sampling of all MPs sizes since information on its use was limited. The net towing method used in this study was easy but may have generated currents which could have pushed out MPs through the net openings leading to underestimation of MPs. This was supported by the fact that there was no relationship between the water volume sampled and MPs concentration from the corelation results of the data generated. Keeping the net mouth close to the surface was challenging requiring constant monitoring making it laborious.

Microplastic categories by shape were similar to those found in other regions of world marine waters with fibres being the most dominant category (>90 %) (Dai *et al*, 2018) across sites for the small size MPs category. This could be as a result of release from fishing nets and ropes or washing of synthetic textiles (Napper and Thompson, 2016), while few were fragments and films from packaging material (Kowalski *et al.*, 2016) since microplastic particles are associated with specific materials. Fibres of the medium and large size categories were higher in Mida compared to Port-Reitz and Tudor suggesting more fishing activities and washing of synthetic textiles in the creek. It also suggests that the source of the MPs is close to the creek and that the MPs may have undergone little degradation (Dai *et al.*, 2018).

A greater proportion of MPs in this study were white, followed by coloured MPs. This is in line with earlier research findings in the Hawaii islands (Young and Elliott, 2016), the North Pacific Ocean and Bering Sea (Boerger *et al.*, 2010) and Wuhan, China Sea (Wang *et al.*, 2016). Colour is used for preliminary identification of plastic pellets and polypropylene (PP) Ismail *et al.*, 2009). Polyprpylene pellets produced for plastic manufacturing are transparent, high density polyethylene (PE) pellets white, low density PE opaque while ethyl vinyl acetate corresponds to clear and almost transparent pellets. However, MPs colour inherited from their plastic products can change due to degradation. The results imply plastics along the Kenya coast are from sea-based activities such as fishing and tourist activities and being an open sea, the plastics could also be coming from far and wide. It may be interesting to investigate how colour affects the environmental fate and ecological effects of microplastics.

# **4.9** Conclusions

The creeks along the Kenya coast, are polluted mostly by MPs of the 20 - 250  $\mu$ m size range. Microplastics of the 250 – 5000  $\mu$ m size range occur in low concentrations. The analysis showed that physiographic factors did not influence the distribution of microplastics.

Tudor creek is more polluted with MPs compared to Port-Reitz and Mida creeks. Makupa in Port-Reitz was the most polluted station perhaps due to the Kibarani dumping site within the vicinity. Mida creek located within the Marine National Park, a protected environment and considered a control was also contaminated with MPs.

Microplastics fibres were the most abundant in the creek surface waters accounting for greater than 90 %, followed by fragments and films accounting for 10 %. The bulk of the microplastics recovered were white suggesting that fishing and shipping activities may be one of the main sources of MPs in the coastal waters.

# 4.10 Recommendations

- Further study to test the recovery of MPs of all three sizes using bulk sampling method because it is fast, less laborious, economical and increases accuracy estimation.
- Monitoring MPs pollution during the dry and rainy seasons and during the Northeast and Southeast Monsoon, when fishing activities are different along the Kenyan coast to establish the effect of the 2017 ban on the production and use of light plastics in Kenya
- Set up educational measures to create awareness and promote the decrease of plastic use, encourage recycling and evaluate disposal facilities.
- The Kenya Government should revive the sisal processing and paper making industries to produce alternatives to plastics to reduce plastic use hence reduce the amount of plastics entering the marine ecosystems.

#### **CHAPTER FIVE**

# 5.0 MICROPLASTICS IN SEDIMENTS IN THE CREEKS ALONG THE KENYA COAST

#### **5.1 Introduction**

Plastics are anthropogenic pollutants originating from land to the Earth's oceans in the water column, along the shorelines and deep-sea sediments (Moore et al., 2001b; Eriksen et al., 2013; Eerkes-Medrano et al., 2015). Microplastics (MPs) are everywhere in urban and remote areas (Van Cauwenberghe et al., 2013) and come from diverse sources. About 80% of MPs in the marine environment are telluric, 18% from fishing industries (Cole et al., 2014) and the rest from effluent discharge. Effluent discharge sources include; wastewater treatment plants, overflow of waste water sewage from urban areas during heavy rains (Eriksen et al., 2013), run-off from sludge applied on agricultural land (Imhof et al., 2013), fresh water bodies (Woodall et al., 2014), and ship yards and tourism (Zalasiewicz et al., 2016). The fate of MPs is not clear but research has shown that sediments accumulate MPs (Corcoran et al., 2015). Microplastics were first reported in beach sediments in the late 1970s and comprised resin pellets on beaches in Bermuda, Lebanon, New Zealand, Spain and Canada (Shiber, 1982; Gregory, 1983). Extreme abundances of between 20000-100000 pellets m<sup>-1</sup> were reported by Gregory on New Zealand beaches, (1978). Microplastics have also been reported in deep sea sediments from the Atlantic Ocean, Mediterranean Sea and Indian Ocean (Woodall, et al 2014; Fischer et al., 2015). Low density plastics such as PE and PP are likely to float on surface water while heavy plastics like polystyrene (PS), polyester (PES), polyvinyl chloride (PVC), and polyamide (PA) are likely to sink. Addition of minerals to PE and PP during production and biofouling increases plastic density causing them to sink (Alomar et al., 2016). However, the connection between MPs abundance and sediment grain size is not clear (Vianello et al., 2013; Desforges et al., 2014). It is also not clear how concentrations in the water column relate with the concentrations in the sediments.

Different categories of benthic invertebrates with different feeding methods ingest microplastics (Moore, 2008; Hong *et al.*, 2018). These include; Annelids (surface deposit and filter feeders), Cnidaria (predators), Molluscs (surface deposit, filter and suspension feeders), Arthropods

(deposit feeders) and sea cucumber (detrivore) (Brown *et al.*, 2008; Lusher *et al.*, 2017; Naji *et al.*, 2018). Microplastics may be detrimental since they adsorb toxic organic contaminants (Teuten *et al.*, 2007; Rochman *et al.*, 2013), are ingested by benthic organisms (Van Cauwenberghe and Jansen, 2014), and change heat transfer and water movement in sediments (Carson *et al.*, 2011). Microplastic pollution has been added to marine ecosystem stressors such as illegal, unreported and unregulated fishing acidification, climate change impacts, and pollution (Thompson *et al.*, 2004; Tin *et al.*, 2009; Obbard *et al.*, 2014).

The occurrence of MPs in Coast ecosystems has recently raised considerable interest in Kenya indicated by the number of studies in the recent past concentrating on the water column and the living organisms (personal observation based on researchgate search). The studies include Kosore *et al.*, (2018) who studied MPs in surface water and zooplanktons at Gazi Bay and the EEZ, Awour *et al.*, (2020) who investigated presence of MPs in benthic invertebrates and the current study. High levels of MPs concentrations have been detected in the sediments in different marine environments (Hidalgo-Ruz *et al.*, 2012; Liebezeit and Dubaish, 2012; Rochman, 2016). Data on microplastics in the marine sediments within the creeks in the WIO is limited. Following the report by the National Environmental Management Authority (NEMA) in conjunction with the United Nations Environmental Programme (UNEP), and the Kenya Institute of Public Policy Research and Analysis (KIPPRA), as stated in Chapter4, the Kenya Government subsequently effected a ban in February 2017 on the use of low weight -single use plastic bags to reduce further deterioration of the environment by plastic wastes (Kimani *et al.*, 2018).

This study investigated the occurrence of MPs in sediments within the coastal creeks (Tudor, Port-Reitz and Mida) in Kenya. This will form a baseline of the February 2017 ban on low weight plastic bag production and use in Kenya, for future monitoring of the effect of the ban, and the formulation of plastic waste management and disposal policies to protect the near shore ecosystem from MPs pollution (Cole *et al.*, 2014; Rochman, 2016). Future assessments can be compared with these results to establish whether the ban is making a difference in the extent of MPs pollution in the coastal sediments.

## **5.2 MATERIALS AND METHODS**

#### **5.2.1 Sediment sampling**

At each sampling station (Fig 3.1) three replicate sediment samples for MPs and three for sediment granulometry analysis were obtained upto a depth of 10 cm using a 3.6 cm diameter hand corer giving a total of nine samples per site. In the field, hand gloves and cotton laboratory coats were worn throughout, and the samples were kept in glass sample bottles covered with metal lids lined with aluminum foil to avoid contamination. Samples were then transferred to the laboratory (Coppock *et al.*, 2017; Courtene-Jones *et al.*, 2017) for further analysis.

#### 5.2.2 Sample processing and microplastic extraction.

The dried sediments were analyzed for grain size distribution using standard sieve sizes (63  $\mu$ m, 125  $\mu$ m, 250  $\mu$ m, 500  $\mu$ m 1 mm and 2 mm) stack together to obtain sediment fractions of silt, very fine sand, fine sand, medium sand, coarse sand and very coarse sand (Mudroch *et al.*, 1997; Das, 2009; Reagan *et al.*, 2015).

For microplastics analysis, dry samples were homogenized, weighed and separated over a series of sieves (5 mm, 500  $\mu$ m, 250  $\mu$ m, and 20  $\mu$ m mesh sizes) stacked on a shaker to obtain three categories of sediments; small size (20 – 249  $\mu$ m), medium (250 – 499  $\mu$ m) and large (500 - 4999  $\mu$ m). Sediments on the 5mm sieve were discarded because they were outside the <5 mm microplastics range. The sediments on each sieve were weighed and placed in 800 ml beakers into which 10 % KOH was added in the ratio of 1 g sediment to 5 ml KOH, stirred thoroughly for five minutes using a glass rod and digested as described in section 4.2. The sample was left overnight to cool.

To the digested sample in the beaker, super-saturated pre-filtered NaCl solution  $(1.2g/cm^3)$  was added in the ratio of 1 g sediment to 10 ml NaCl solution, stirred for 10 minutes using a glass rod then covered with aluminum foil and left for 12 hours to settle (Thompson *et al.*, 2004; Van Cauwenberghe, *et al.*, 2015 modified protocol). The procedure of density separation was repeated three times for each sample to ensure all MPs were recovered. The sedimentation time was adjusted to six hours (De Witte *et al.*, 2014; Besley *et al.*, 2017) for the second and third time. The supernatant was filtered using a vacuum pump fitted with 10 µm pore size net filter. Filters were kept in lidded filter dish holders, dried at 40  $^{\circ}$ C for 12 hours before being examined under a microscope as described in Chapter four.

#### 5.2.3 Microplastic identification, enumeration and characterization

Microplastics were identified as described in section 4.3 and in addition to non-plastics, sand and salt crystals broke with a glass sound and were pushed aside. The enumeration and charecterizatiion of MPs were done as described in section 4.3. Similarly, quality controls were done as described in section 4.4.

### 5.3 Data analyses

Data was analyzed using the Statistics and Data (STATA) version 15. The data was checked for normality using Shapiro-Wilk W test. The small sized MPs data was normally distributed, thus no need for transformation. The medium sized did not show normality even after transformation, thus non-parametric method of data analysis was used. The large size MPs data became normally distributed after log transformation. Thus, for testing for significant difference the parametric test of ANOVA was used for the data on large and small size while non-parametric test (Kruskal-Wallis) was used for the data of the medium size MPs. The total mean concentration and the concentrations of the different shapes and colours were assessed between sites (Tudor, Port-Reitz and Mida Creeks) and between stations in each of the sites. Linear regression was used to determine whether a relationship existed between sediment grain size and the concentration of MPs. The tests were considered significant at p < 0.05.

#### **5.4 Results**

## 5.4.1 Grain size distribution

The six sediment grain size classes were grouped into; small (silt, very fine and fine sand), Medium (medium sand) and large (coarse and very coarse sand) sand sizes (Reagan *et al.*, 2015). During the first sampling campaign, the small size sediment grains occurred in high mean percentage proportions in Port-Reitz and Mida where, 70.5 % and 70 % of the total sediments were recorded respectively but the proportions were low in Tudor (47.9 %) (Fig.2). During the second sampling campaign, Tudor had the highest percentage proportion (86.6 %) of the small size sediment grains followed by Port-Reitz (75.6 %) and finally Mida (57.2 %). The mean percentage proportion of the medium size sediment grains was less than 20 % of the total sediments in all the sites during both sampling campaigns except in Mida where 39 % was recorded during the second campaign. Like the small grain size, the mean percentage proportion of the large sediment grain size was less

than 20 % in all the sites during both sampling campaigns except in Tudor where 33 % of the total sediments was recorded during the first sampling campaign (Fig.5.1).

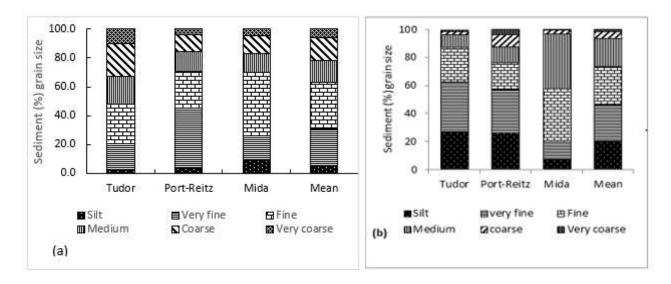


Figure 5.1 Distribution of sediment grain sizes (% mean) in the different sites (a) First sampling season (b) Second sampling season.

Among stations, Mwache-T and Mwache-SGR in Port-Reitz had high mean percentage proportions (>70 %) of the small sediment grain size during both sampling campaigns, while Mikindani, K.M.C and Nyali- B stations in Tudor had more (>80 %) of the small sediment grain size during the second campaign compared to the first (Fig. 5.2). Kirepwe had a higher mean percentage proportion (66 %) of the medium size sand during the second sampling campaign compared to the other stations while Mikindani recorded a higher mean percentage proportion (39.7 %) of the large size grains (Figure 5.2)

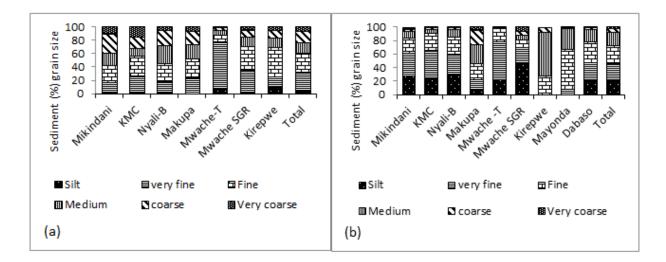


Figure 5.2 Distribution of sediment grain sizes (% mean) in the different stations (a) First sampling campaign (b) Second sampling

# **5.4.2 Overall Concentration of Microplastics**

Overall, the mean concentration of MPs in the Kenya coast was  $21.7 \pm 1.6 \text{ mp.cm}^{-2}$  being significantly (F<sub>2,45</sub> = 4.057, p = 0.029) higher in Tudor (27.3 ± 1.7 mp.cm<sup>-2</sup>) compared to Mida (20.2 ± 1.4 mp.cm<sup>-2</sup>) and Port-Reitz (17.7 ± 1.6 mp.cm<sup>-2</sup>) but did not differ significantly (P = 0.228) between the latter two sites). The large size MPs category had the highest mean concentration (26.7 ± 0.91 mp.cm<sup>-2</sup>) that was significantly different from the medium size category (15.9 ± 0.9 mp.cm<sup>-2</sup>) and the smallest size category (22.8 ± 0.83 mp.cm<sup>-2</sup>) (Fig.5.3).

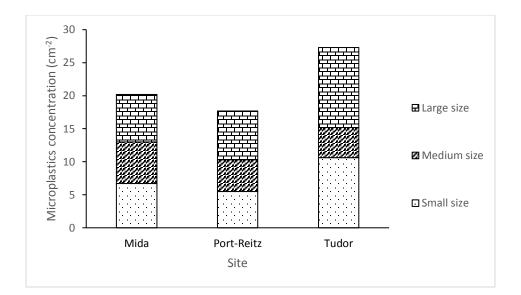


Figure 5.3 Mean concentrations (cm<sup>-2</sup>) of microplastics of all size categories in the different sites

## 5.4.3 Concentration of the Small Size (20-249 µm) Microplastics Category.

The overall ( $\pm$ SE) mean MPs concentration of the small size category was 7.7  $\pm$  0.6 MPs particles per centimeter square (mp.cm<sup>-2</sup>). The mean MPs concentrations were significantly (F<sub>2,45</sub> = 9.75, P < 0.01) higher in Tudor (10.6  $\pm$  0.6) compared to Mida (6.7  $\pm$  0.0 mp.cm<sup>-2</sup>) and Port-Reitz (5.5  $\pm$ 1.2 mp.cm<sup>-2</sup>) but did not differ (p = 0.64) between the latter two sites (Mida and Port-Reitz) (Fig.3). Between sampling campaigns, the concentration was significantly higher (ANOVA, F<sub>1,46</sub> = 14.46, p < 0.01) during the second sampling campaign (9.5  $\pm$  0.5 mp.cm<sup>-2</sup>) compared to the first (5.4  $\pm$  1 mp.cm<sup>-2</sup>). This could be as a result of the significantly higher (9.7  $\pm$  1.1 mp.cm<sup>-2</sup>) concentrations that were observed in Port-Reitz (t = 8.38, p = < 0.01) during the second campaign (Sept, 2018) compared to the first in Jan/Feb (1.3  $\pm$  0.4). Incidentally, all three stations in Port Reitz recorded significantly higher concentrations during the second campaign compared to the first campaign (Table 5.1). In Tudor, significantly higher concentrations were recorded in K.M.C during the second campaign, while in Mida higher concentrations were recorded in Kirepwe, the only station where both sampling campaigns were done (Table 5.1).

In Tudor MPs concentration was significantly (t = 18.306, p < 0.01) higher (12.0  $\pm$  1 mp.cm<sup>-2</sup>) during the first sampling campaign in Mikindani compared to Nyali-B (9.7  $\pm$  1.7 mp.cm<sup>-2</sup>) and K.M.C (7.5  $\pm$  1.1 mp.cm<sup>-2</sup>) but the latter two stations did not differ significantly. In Port-Reitz during the second sampling campaign, the MPs concentration was significantly (t = 4.979, p < 0

.01) higher in Makupa ( $13.8 \pm 0.8 \text{ mp.cm}^{-2}$ ) compared to Mwache-T ( $8.4 \pm 0.8 \text{mp.cm}^{-2}$ ) and Mwache-SGR ( $6.8 \pm 0.6 \text{ mp.cm}^{-2}$ ) but the latter two stations did not differ significantly (Table 5.1). In Mida during the second sampling campaign, the MPs concentration was significantly higher in Kirepwe.

Table 5.1: Mean concentrations (cm <sup>-2</sup> ) of the small size microplastics in different stations
and the sampling seasons (*Not sampled during first campaign).

Site		Total	ntrations (cm <sup>-</sup>	,		
Site	Station	mean	Jan 2018	Sept 2018	F	р
Mida	Dabaso	$6.8\pm0.3$	0*	$6.8 \pm 0.3$	0	0
	Kirepwe	5.8 + 1.2	$4.7\pm2.3$	$6.9\pm0.6$	116.5	0.01
	Mayonda	$8.4\pm0.5$	0*	$8.4\pm0.5$	0	0
	p-value	0.132	0.037	0.172		
	Mean	$6.7\pm0.7$	$4.7\pm0.2$	$7.3\pm0.4$	2.27	0.054
Port Reitz	Makupa	$7.7\pm2.8$	$1.6 \pm 1$	$13.8\pm0.8$	8.7	0
	Mwache-T	$4.5\pm1.8$	$1.8\pm0.3$	$8.4\pm0.8$	6.59	0.048
	Mwache-SGR	$4.3\pm1.2$	$0.5\pm0.1$	$6.8\pm0.6$	5.64	0
	p-value	0.047	0.61	0.006		
	Mean	$5.5\pm1.2$	$1.3\pm0.4$	$9.7 \pm 1.1$	8.38	0.00
Tudor	Mikindani	$12\pm0.7$	$12 \pm 1$	$12 \pm 1.2$	0	1
	K.M.C	$10.1\pm1.3$	$7.5\pm1.1$	$12.7\pm0.7$	5.73	0.046
	Nyali-B	$9.7\pm0.8$	$9.7\pm1.7$	$9.7\pm0.4$	0.02	1
	p-value	0.589	0.041	0.102		
	Mean	$10.6\pm0.6$	9.7±0.9	$11.5\pm0.6$	1.58	0.619
	Overall mean	$7.7\pm0.6$	$5.4 \pm 1$	$9.5 \pm 0.5$	14.46	0.001
	p-value	0.003	0.024	0.001		

#### 5.4.4 Concentration of the Medium Size (250-449 µm) Microplastics Category

The overall mean ( $\pm$ SE) MPs concentration of the medium size category was 5.1  $\pm$  0.5 mp.cm<sup>-2</sup>. The MPs concentrations were not significantly different (Chisq. = 2.7<sub>46</sub>, p = 0.253) between sites, although the mean MPs concentration was slightly higher in Mida (6.3  $\pm$  0.8 mp.cm<sup>-2</sup>), followed by Port-Reitz (4.8  $\pm$  1 mp.cm<sup>-2</sup>) and Tudor (4.6  $\pm$  1 mp.cm<sup>-2</sup>). Like the small MPs category, the mean MPs concentration was significantly higher (Chisq. = 13.994, p< 0.01) during the second sampling campaign (7  $\pm$  0.7 mp.cm<sup>-2</sup>) compared to the first (2.8  $\pm$  0.5 mp.cm<sup>-2</sup>). Similar to the small MPs category, the difference was due to the significantly higher concentrations that were observed in Port-Reitz (Chisq. = 7.61, p = < 0.01) during the second sampling campaign (Sept. 2018) 8.3  $\pm$  0.9 mp.cm<sup>-2</sup> compared to the first in Jan/Feb 2018 (1.4  $\pm$  0.4 mp.cm<sup>-2</sup>).

Between stations within the same sites there were variations in the concentrations between the first and second campaign. For instance, in Tudor, Kenya Meat Commission, recorded a significantly (Chisq. = 4.56, p = 0.007) lower MPs concentration during the second sampling campaign compared to the first campaign (Table 5.2). The mean MPs concentrations were significantly (Chisq. = 4.863, p < 0.01) higher in Mikindani (11.9  $\pm$  1) during the second sampling campaign compared to K.M.C (2.1  $\pm$  0.8) and Nyali-B (0.8  $\pm$  0.4). Kirepwe in Mida had significantly (Chisq. = 5.47, p = 0.001) higher MPs concentration (7.9  $\pm$  0.9) during the second sampling campaign compared to the first (2.1  $\pm$  0.8). In Port-Reitz, the mean MPs concentrations were significantly different between stations during the second sampling campaigns with Makupa recording higher concentrations (11.6  $\pm$  1), followed by Mwache-SGR (7.5  $\pm$  0.4) and Mwache-T (5.8  $\pm$  0.4) (Table 5.2).

		Mean concentrations (cm <sup>-2</sup> )					
Site	Station	Total	Jan	Sept	Chisq	р	
Mida	Dabaso	$7.3\pm0.6$	0*	$7.3\pm0.6$	0	0	
	Kirepwe	$5 \pm 1.2$	$2.1\pm0.8$	$7.9\pm0.9$	5.47	0.001	
	Mayonda	$8.1 \pm 0.6$	0*	$8.1 \pm 0.6$	0 0		
	p-value	0.143	0	0.54			
	Mean	$6.3\pm0.8$	$2.1\pm0.8$	$7.7\pm0.4$	8.7	0.04	
Port Reitz	Makupa	$6.4 \pm 2.4$	$1.3 \pm 0.9$	11.6 ± 1	9.62	0.000	
	Mwache-T	$3.9\pm0.9$	$2.1\pm0.8$	$5.8\pm0.4$	5.47	0.0102	
	Mwache- SGR	4.1 ± 1.5	$0.8 \pm 0.4$	$7.5 \pm 0.4$	6.28	0.000	
	p-value	0.527	0.54	0.01			
	Mean	$4.8 \pm 1$	$1.4 \pm 0.4$	$8.3\pm0.9$	7.61	0.000	
Tudor	Mikindani	$7.7 \pm 1.2$	$3.4 \pm 1.2$	11.9 ± 1	8	0.000	
	K.M.C	$4.5 \pm 2$	$6.9\pm0.4$	$2.1\pm0.8$	4.56	0.007	
	Nyali-B	$1.8\pm0.5$	$2.8\pm0.8$	$0.8 \pm 0.4$	4.87	0.011	
	p-value	0.029	0.002	0.001			
	Mean	4.6 ± 1	$4.4\pm0.8$	$4.9\pm1.8$	1.58	0.619	
	Overall mean	$5.1 \pm 0.5$	$2.8 \pm 0.5$	$7\pm0.7$	13.99	0.0002	
	p- value	0.253	0.016	0.001			

Table 5.2: Mean concentration (cm<sup>-2</sup>) of the medium size microplastics in different stations and different sampling seasons (\* Not sampled in Jan/Feb)

# 5.4.5 Concentration of the Large Size (500 $\mu$ m< 5mm) Microplastic Category

The overall mean (( $\pm$ SE) MPs concentration of the large size category was 9.1  $\pm$  0.8 mp cm<sup>-2</sup>. The mean MPs concentration was significantly higher (ANOVA, F<sub>1,46</sub> = 8.7, P <0.01) during the second sampling campaigns (11  $\pm$  0.7 mp.cm<sup>-2</sup>) compared to the first (6.8  $\pm$  1 mp.cm<sup>-2</sup>). Similar to the other two size categories, relatively higher concentrations were observed in Port-Reitz (F<sub>1,45</sub> =

5.4, P = 0.01) and Mida ( $F_{1,11}$  = 7.006, p = 0.001) during the second sampling campaign (Sept.2018) (10.3 ± 0.6 mp. cm<sup>-2</sup> and 9.4 ± 0.3 respectively) compared to the first in Jan/Feb 2018 (4.5 ± 1.2 mp.cm<sup>-2</sup> and 1.5 ± 0.7 mp.cm<sup>-2</sup> respectively) (Table 3). In Tudor the MPs concentrations (10.7 ± 2.3 and 13.4 ± 1.8) did not vary significantly (F = 1.3, p = 0.531) between the sampling campaigns. The mean MPs concentrations were significantly ( $F_{2,45}$  = 5.4, P < 0.01) higher in Tudor (12.1 ± 1.4 mp.cm<sup>-2</sup>) compared to Mida (7.2 ± 1.0 mp.cm<sup>-2</sup>) and Port-Reitz (7.4 ± 1.0 mp.cm<sup>-2</sup>) but did not differ significantly (p > 0.05) between the latter two sites (Mida and Port-Reitz). In Tudor, significant ( $F_{2,25}$  = 5.19, p = 0.047 and  $F_{2,25}$  = 4.87, p = 0.014) differences were observed in the MPs concentrations with Mikindani recording higher (17 ± 2.6 mp.cm<sup>-2</sup> and 18.5 ± 1.5 mp.cm<sup>-2</sup>) MPs concentrations during the first and second sampling campaigns compared to Nyali-B (10.9 ± 23.1 mp.cm<sup>-2</sup> and 10.2 ± 3.7 mp.cm<sup>-2</sup>) and K.M.C (4.3 ± 2.5 mp.cm<sup>-2</sup> and 11.6 ± 1.2 mp.cm<sup>-2</sup>) (Table 5.3).

	Mean concentration (cm <sup>-2</sup> )							
Site	Station	Total	Jan	Sept	t	Р		
Mida	Dabaso	$9.5\pm0.3$	0*	$9.5\pm0.3$	0	0		
	Kirepwe	$5.3\pm4.2$	$1.5\pm0.7$	$9.1\pm0.2$	6.91	0.003		
	Mayonda	$8.8 \pm 1$	0*	$8.8 \pm 1$	0	0		
	p-value	0.001	0.183	0.54				
	Mean	$7.2 \pm 1$	$1.5\pm0.7$	$9.4 \pm 0.3$	7.006	0.001		
	Makupa	$10.1\pm0.8$	$8.8\pm0.9$	$11.4\pm1.1$	1.13	0.824		
Port- Reitz	Mwache-T	$6.8 \pm 1.8$	3.1 ± 1.3	$10.6\pm0.9$	5.46	0.048		
	Mwache-SGR	$5.4\pm1.7$	$1.7 \pm 0.4$	$9\pm1.2$	11.42	0.013		
	p-value	0.01	0.001	0.062				
	Mean	$7.4 \pm 1$	$4.5 \pm 1.2$	$10.3\pm0.6$	5.84	0.041		
	Mikindani	$17.7\pm1.4$	$17 \pm 2.6$	$18.5\pm1.5$	1.01	1		
Tudor	K.M.C	$8\pm2$	$4.3 \pm 2.5$	$11.6\pm1.2$	5.91	0.04		
	Nyali-B	$10.6\pm2.2$	$10.9\pm3.1$	$10.2\pm3.7$	0.01	1		
	p-value	0.007	0.047	0.0149				
	Mean	$12.1\pm1.4$	$10.7\pm2.3$	13.4±1.8	1.3	0.531		
	Overall mean	9.1±0.8	6.8±1.3	11±0.7	8.7	0.024		
	p-value	0.008	0.026	0.001				

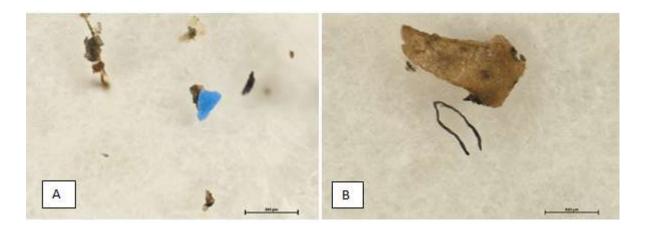
Table 5.3: Mean concentration (cm<sup>-2</sup>) of the large size microplastics in different stations and sampling seasons (\* Not sampled during first campaign).

### 5.4.6. Relationship between concentration of MPs and sediment grain size

A linear regression analysis showed no relationship between the concentrations of small size MPs ( $F_{5,21} = 0.499$ , p > 0.05), medium size MPs ( $F_{5,21} = 1.179$ , p > 0.05) and large size MPs ( $F_{5,21} = 0.320$ , p > 0.05), respectively, and sediment grain size distribution. However, a relationship was observed between the concentration of MPs and the small size sediment grains at the KMC station, Tudor Creek.

### **5.4.7** Distribution of microplastic categories (shapes)

Overall, three MPs categories (fibres, fragments and films) based on shapes (Fig 5.4) were encountered with fibres being the most dominant accounting for more than 90 % of the total MPs, followed by fragments (< 10 %) and finally films (< 3 %) in the different sites (Fig.5.5).



## Figure 5.4 Microplastic types by shape extracted from creek sediment samples

A-Blue fragment in sediment sample from Makupa, B-Brown film and black fiber in sediment sample from Mikindani

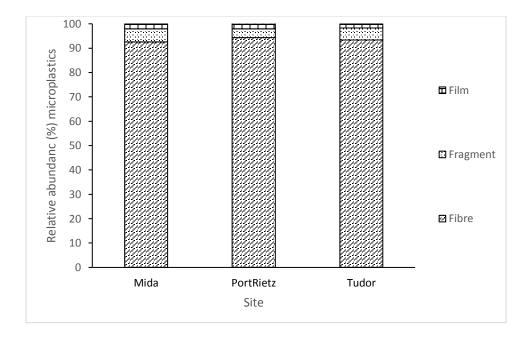


Figure 5.5 Relative abundance (%) of the different microplastic shapes in the different sites

# **5.4.8** Distribution of the different categories (shapes) of microplastic in the different size categories of microplastics

Among the small size category of MPs, the distribution of the three categories of MPs (fibres, fragments and films) followed the general distribution where, fibres were the most abundant accounting for 93.5 % of the total microplastics, followed by fragments accounting for 5.2 %, and films accounting for 1.3 % and the differences were significant (p< 0.05) (Fig.5.6).

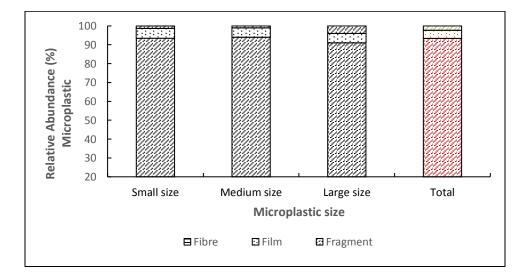


Figure 5.6 Relative abundance (%) of the different microplastic shapes in the different size categories.

Between stations, the percentage mean proportion of fibres was highest in Makupa (98 %) and lowest in K.M.C (89 %). Fragments had a higher percentage proportion in K.M.C (9 %) and lowest in Makupa (1 %), while films occurred in significantly lower proportions and were absent in several stations (Fig. 5.7).

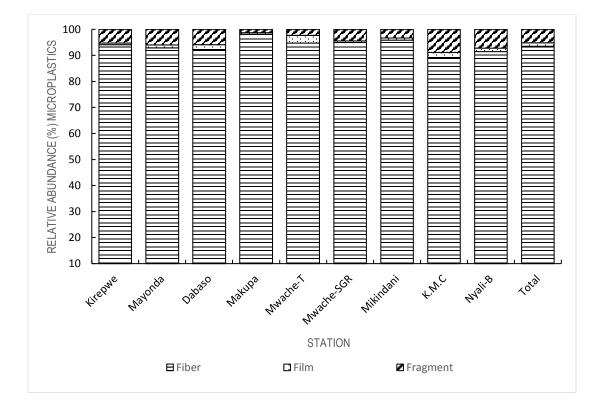
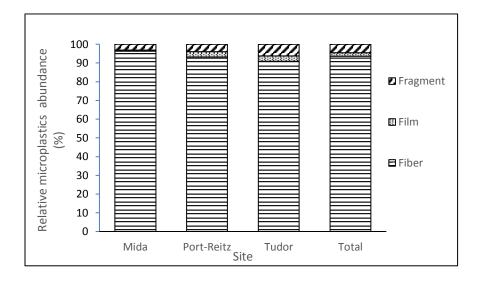
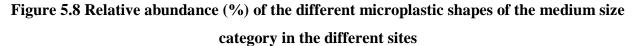


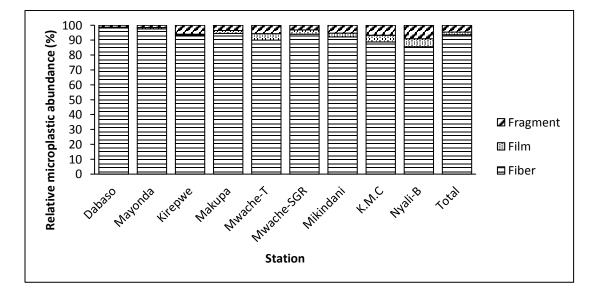
Figure 5.7 Relative abundance (%) of the different microplastic shapes in the different stations.

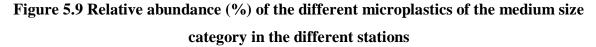
Like in the small size category of MPs, the distribution of the three categories of MPs (fibres, fragments and films) among the medium size category of MPs followed the general distribution where, fibres were the most abundant accounting for 94 % of plastic particles, followed by fragments accounting for 5 %, and films accounting for 1 % and the differences were significant (p < 0.05). The overall concentrations of the MPs categories did not show significant variation between sites (p > 0.05). The percentage proportion of fibres was 96.4 % in Mida, 93.2 % in Port-Reitz, and 90.9 % in Tudor. Films and fragments had relatively low percentage proportions (< 10 %) across the sites (Fig.5.8).



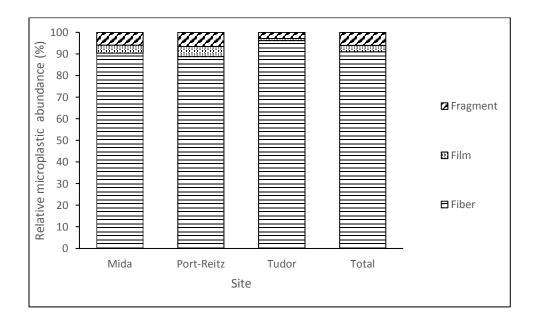


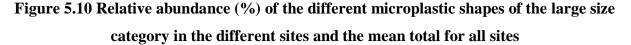
Between stations, the percentage mean proportion of fibres was highest in Makupa (98 %) and lowest in K.M.C (89 %). Fragments had a higher percentage proportion in K.M.C (9 %) and lowest in Makupa (1 %), while films occurred in significantly lower proportions and were absent in several stations (Fig. 5.9).





Among the large size category of MPs, the distribution of the three categories of MPs (fibres, fragments and films) followed a general distribution where, fibres were the most abundant accounting for 91 % of the total MPs, followed by fragments accounting for 5 %, and films accounting for 4 % and the differences were significant (p < 0.05). Tudor had a significantly higher proportion of MPs fibres (ANOVA;  $F_{2,47} = 4.81$ , p = 0.01) accounting for 96 %, compared to Mida (90 %) and Port-Reitz (89 %) but the latter two sites did not differ. Post hoc test showed that the mean percentage proportion in Tudor was different from that in Mida and Port-Reitz but the latter two were not different from each other. The percentage proportions of films and fragments were relatively low (< 10 %) in all the sites ((Fig. 5.10).





Within stations, the mean concentration of fibers and films were significantly different ( $F_{2,47} = 4.58$ , P < 0.01) and  $Chisq_{.8} = 22.8$ , p = < 0.01) respectively. Dabaso and Mayonda had significantly higher mean percentage proportions of fibers accounting for 97.9% each, followed by Mikindani (96%), while Kirepwe (92%) recorded significantly lower percentage proportion of the total particles per station (Figure 5.11). The relative abundances of films and fragments were low, while

some stations like Dabaso and Mwache-SGR recorded zero film microplastic particles (Figure 5.11).

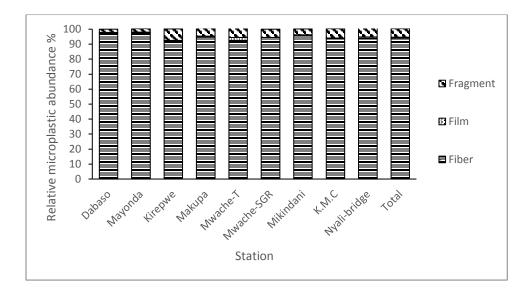


Figure 5.11 Relative abundance (%) of the different microplastics in the large size category in different stations

# **5.4.9** Distribution of the different colours of microplastic in the different size categories of microplastics

Overall, MPs occurred in seven different colours with white being the most dominant ( $11.7 \pm 0.5$  mp.cm<sup>-2</sup>) and accounting for 51.7 % of the total MPs, followed by black accounting for 18.1 %, and green accounting for 11.5 %. The rest of the colours together accounted for 18.7 %.

Among the small size MPs, the concentrations of the different MPs colours were significantly different (p < 0.05) with white colour being the most dominant ( $3.4 \pm 0.5 \text{ mp.cm}^{-2}$ ) and accounting for 43.4 % of the total, followed by green (23 %), black (19.1 %) and the rest together accounted for 14.6 % (Fig.5). In terms of distribution of colours by site, Port Reitz had the highest proportion (55.6 %) of the white MPs, followed by Mida (42.4 %), and finally Tudor (38.5 %). The next most dominant MPs colour was black which was represented by 22.4 % in Mida, 18.8 % in Port-Reitz and 16.7 % in Tudor. Green MPs were high in Tudor being higher than black accounting for 20.1 %, while in the other sites they had lower proportions than black being 16.1 % in Mida and 9.3 % in Port-Reitz. For the rest of the colours, they had less than 10 % occurrence in the different sites except blue which had 15.4 % in Tudor and 14.1 % in Mida (Fig.5.12).

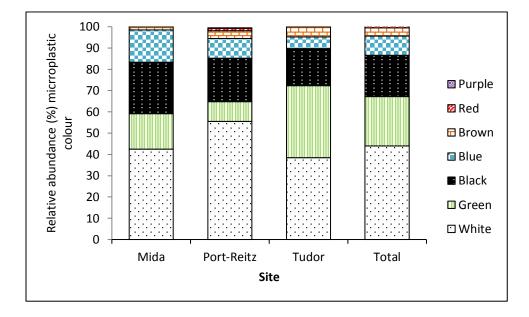


Figure 5.12 Relative abundances (%) of the different microplastic colours in the different sites and the total mean proportion in all the sites.

The distribution of the different colours within the stations followed a similar pattern like in the sites where white was the most dominant followed by black, green and blue. However, it was only in Makupa and Dabaso that white MPs was represented by more than 50 % of all MPs. The lowest occurrence (28-31 %) of white MPs was in Mikindani and Kirepwe which had relatively higher proportions (38-40 %) of green MPs compared to the other stations (Fig.5.13).

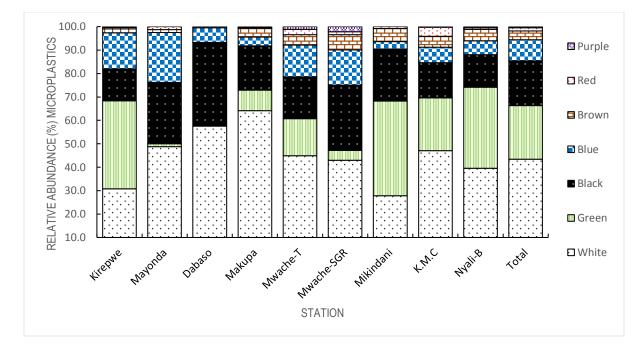


Figure 5.13 Relative abundance (%) of the different colours of microplastics in different stations and the total mean proportion for all the stations

Among the medium size MPs, the concentrations of the different colours were significantly differed (p < 0.05) with white colour being the most dominant ( $2.5 \pm 0.4$  mp. cm<sup>-2</sup>) accounting for 51.9 % of the total, followed by black (20.4 %), blue (11.1 %), and the rest together accounted for 16.6 %. In terms of distribution of colours by site, Port-Reitz had the highest proportion (55.3 %) of the white MPs, followed by Mida (48.6 %), and finally Tudor (30.8 %). The next most dominant MPs colour was black which was represented by 25.1 % in Mida, 19.8 % in Port-Reitz and 17.3 % in Tudor. Blue MPs were high in Mida accounting for 12.5 % followed by Tudor (11.5 %) and finally Port-Reitz (9.9 %). Brown MPs accounted for 11.5 % in Tudor, 5.9 % in Port-Reitz, and 4.7 % in Mida. Red, green, and purple colours had less than 5 % in the different sites (Figure 5.14).

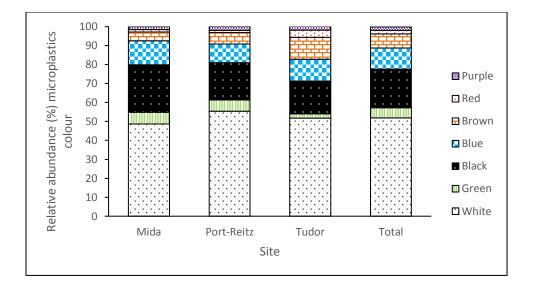
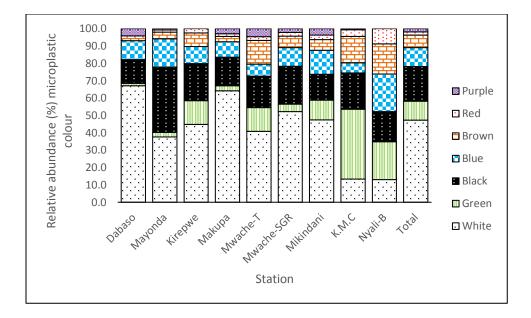
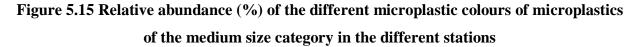


Figure 5.14 Relative abundance (%) of the different microplastic colours of the medium size category in the different sites

Between the stations, the mean concentrations of MPs of the same colour did not differ significantly (p > 0.05) for all colours. White colour was predominant accounting for 47.3 %, followed by black (20 %), blue and green (10.9 %) each, brown (7.3 %), and finally red and purple (1.8 %) each. The percentage proportion for white microplastics was 67.1 % in Dabaso, followed by 64.2 % in Makupa, 52.2 % in Mwache-SGR, 47.5 % in Mikindani, 44.8 % in Kirepwe, 40.9 % in Mwache-T, 37.7 % in Mayonda, 13.4 % in K.MC, and 13 % in Nyali-B. Black colour in Mayonda accounted for 37.7 %, in Mwache-SGR 21.7 %, in Kirepwe 21.4 %, in K.M.C 21 %, and the rest of the stations had percentage proportions below 20 % (Figure 5.15). The percentage proportions for the rest of the colours were low with no red MPs in Dabaso and no purple MPs in Kirepwe and Nyali-B (Fig.5.15).





For the large size MPs, overall, the concentrations of the different colours were significantly different (p < 0.05) with white colour being the most dominant ( $5.8 \pm 0.6$  mp. cm<sup>-2</sup>) accounting for 64 % of the total, followed by black (18.8 %), blue (9.9 %) and the rest of the colours together accounted for 7.3 %. In terms of colour distribution by site, Tudor had the highest proportion (67.7 %) of the white MPs, followed by Port-Reitz (65.8 %), and finally Mida (52 %). The next most abundant colour was black which was represented by 16.1 % in Tudor, 22.6 % in Mida and 20.5 % in Port-Reitz. Blue MPs were high in Mida accounting for 17.3 % of the total MPs, followed by Tudor (9.3 %) and finally Port-Reitz (6.8 %). For the rest of the colours, they had less than 5 % occurrence in the different sites (figure 5.16)

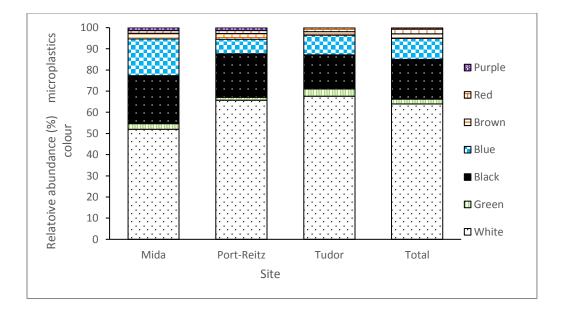


Figure 5.16 Relative abundance (%) of the different colours of the large size microplastics category in the different sites

Between different stations, the mean concentration of MPs varied significantly (p < 0.05). White MPs were significantly more abundant in Mikindani ( $12.7 \pm 7.9 \text{ mp.cm}^{-2}$ ) accounting for 72.5 %, followed by Dabaso (67.7 %), while Kirepwe (41.5 %) recorded significantly lower percentage proportion. Makupa had 34.5 % of the black MPs, Kirepwe 32.1 %, Mwache-SGR 24.1 %, Mwache-T 22.1 %, Nyali-B 18 %, Mayonda 15.4 %, K.M.C 16.6%, Mikindani 14.8 %, and Dabaso 14.6 %. Blue MPs in Mayonda accounted for 21.6 %, in Kirepwe 18.9 %, in Makupa 14.2 %, while the lowest mean percentage proportion was recorded in Mwache-SGR (7.4 %). The percentage proportions of the rest of the colours were very low across the stations (Fig. 5.17).

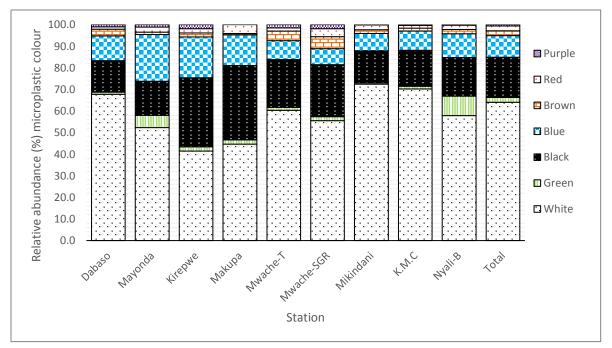


Figure 5.17 Relative abundance (%) of the different microplastics colours in the large size category in different stations

#### 5.5 Discussion

Studies in MPs occurrence in marine environments has been going on for a while due to the recognition of their importance (Cole *et al.*, 2011). However, in the Western Indian Ocean and Kenya in particular, investigations in concentrations in the sediments has not been done until now. To our knowledge, this is the first report on microplastics occurrence in the coastal creek sediments in Kenya. Our study sampled the top 10 cm sediment layer and used 10  $\mu$ m net mesh filters and recorded MPs concentrations ranging between  $5.1 \pm 0.5$  to  $9.1 \pm 0.8$  particles per cm<sup>2</sup>. The occurrence of microplastics in all sediment samples collected provided evidence of MPs pollution of marine sediments in the creeks along the Kenya coast. Sampling periods coincided with low average monthly rainfall (9.8 and 6.1 mm) in January and February respectively and relatively high (36.3 mm) in September 2018 (Kenya Meteorological Department, 2018; Appendix 1). The high percentage proportion of the small size sand during the second sampling campaign compared to the medium and large sizes across sites can be attributed to the increased water flow from rivers and surface runoff discharging more sediment into the creeks but did not seem to influence the deposition of Mps.

Comparison of my results with findings from different regions is challenging due to the different methods of sediment sample collection, classification differences and differences in units (items/kg) used to express MPs presence (Leads and Weinstein, 2019). Nevertheless, overall MPs concentration in the sediments from the creeks in Kenya were much higher (17.7-27.3 MP particles/cm<sup>2</sup>) than most other studies which have record less than 1 MP particle per cm<sup>2</sup>. For examples, Lee *et al.*, (2013) recorded between 2-92 particles per m<sup>2</sup> which translates to -0.009 MPs/cm<sup>2</sup> from the Sea of Japan, while Kaberi *et al.*, (2013), had 57-602 MP particles per m<sup>2</sup> which translates to 0.062 MP particles/cm<sup>2</sup> from the Kea Island of Greece, Hidalgo-Ruz and Thiel, (2013), recorded 805 particles per m<sup>2</sup> that is only 0.085 MP particless/cm<sup>2</sup> from the coastal beaches of Eastern Chile, to name a few. Establishment of standardized sediment sampling techniques and units of measurement of MPs will be important to enable comparisons.

Microplastic concentrations differed significantly (p < 0.05) between the sampling campaigns with a higher MPs concentration occurring during the second campaign when there was higher rainfall. This may imply more MPs from land probably due to increased water flow through rivers, surface runoff and wastewater effluent discharging large quantities of water and more macro-plastic debris breaking down (Veerasingam et al., 2016). Similar results have been reported elsewhere. For example, Lima et al., (2014) reported almost three times higher microplastic loads during the wet season in a Brazilian estuary, Gundogdu et al., (2018) recorded 14 times higher MPs loads in Mersin Bay, Turkey, following flooding. Microplastics of the large size category were more abundant in both sampling campaigns compared to the medium and small size MPs categories probably because they attract microbial films become heavy and sink into the sediments and are not easily resuspended due to attainment of high density. This could explain the low concentrations of large MPs (500-4999 µm) size categories in the water column encountered (see sub-section 4) in the same sites, suggesting that possibly this size category tends to sink into the sediments rather than remain in the water column. The results point to the possibility of a greater number of large size MPs particles in the sediments than the small and medium size MPs particles probably because coarse sand grains tend to have large interstices, allowing smaller MPs to pass through so easily thus getting buried deeper rather than accumulating on the upper sediment depth (Harris, 2020) although this was not studied. Fine sand grains on the other hand are more cohesive making MPs deposition difficult unless sediments are in suspension (Alomar et al., 2016). This could also mean

temporal resuspension of MPs by turbulences within the creeks as in the Baltic Sea (Bunke, 2017) and the high salinity of water in the creeks limiting accumulation of small size MPs in sediments.

A linear regression analysis showed no relationship between MPs concentrations and sediment grain size distribution (P > 0.05), a finding supported by several studies that have also reported no clear relationship between MPs concentration and sediment grain size (Harris, 2020; Alomar et al., 2016; Khan and Prezant, 2018; Dadson, 2020; Mathalon and Hill, (2014). On the other hand, several studies have reported a clear connection between MPs concentration and the size of sediment grains (Harris, 2020), with Enders et al., (2019) reporting a correlation between MPs of high density material (>1 g cm<sup>-3</sup>) and sediment grain size while Ling *et al.*, (2017) reported a relationship between MPs concentration and sediment grain size, although this did not apply to fibres. Therefore, there is need to understand how edaphic factors influence MPs deposition. The results imply that sediment composition does not control MPs distribution in our study area (Dadson et al. 2020) except at KMC station and only for the small size sand grains. A high percentage proportion of small size sediment grains could be a consequence of erosion, transport and deposition of sediments by increased surface runoff, or wastewater and domestic waste release from the nearby urban settlement and also because Tudor Creek is enclosed within a narrow channel, experiences low tides and limited water flow which could be influencing deposition and settlement of MPs together with sediments.

The distribution of MPs could be as a result of input, density and biological interactions with microbial films and physical processes like wave action and tidal patterns (Mathalon and Hill, 2014). The poor correlation between MPs sizes and sediment grain deposited in the same location could be due to the differences in density and grain shape (Harris, 2020). In light of Enders *et al.*, (2019), a no connection between microplastic concentration and sediment grain size in our study may suggest that all the polymers were of low-density (< 1 g/cm<sup>3</sup>), and they were not available for hydraulic sorting along with sediment grain size, hence their transport is not associated with sediment deposition. This could also mean temporal resuspension of MPs by turbulences within the creeks as in the Baltic Sea (Bunke, 2017) and the highwater salinity in the creeks limiting accumulation of MPs in sediments. Microlastics could have been transported via ingestion, biofouling, flocculation or some other mechanism (Cole *at al.*, 2016; Enders *et al.*, 2019; Harris, 2020).

Tudor Creek was more highly polluted with MPs than Mida and Port Reitz creeks. Mida Creek is geographically remote from Tudor Creek but Port Reitz Creek is nearby and is only separated by a narrow channel. Port Reitz Creek has fast-flowing rivers that could transport sediments and MPs offshore. In contrast, Tudor Creek is narrow, especially at Nyali-B, with deep water and weak currents that might result in a low flushing rate and could lead to increased retention of MPs introduced by runoff in September that might sink into sediments. The seasonal rivers feeding Tudor Creek collect surface runoff transporting plastic and other waste debris from the mainland (Kitheka et al. 1999). The urban development surrounding Tudor Creek (IAME, 2018; Mombasa County Government, 2018) may be adding to the MPs introduced by seasonal rivers and ocean currents through the release of raw domestic waste. Mikindani had a high concentration of MPs in surface waters (see chapter four) indicating a possible correlation between surface water and sediment MPs concentrations. A positive correlation between the concentration of MPs and human population density has been shown in several regions of the world (Browne et al. 2010; 2011). Anthropogenic activities such as, shipping, plastic production (such as Multi-Creek International Limited, Africa PVC Industries, Weeco recycling Industry within Mombasa), port activities and sewage treatment could be other sources of MPs (Browne et al., 2010).

Makupa in Port-Reitz had a significantly higher MPs particles probably due to leachates from the Kibarani dumpsite during the rains and the fact that the station is blocked by the Kibarani courseway on one side and partly blocked by a low bridge on the other side that interferes with water mixing and flushing rate (Nguli, 2006). It could also be attributed to the high concentration of MPs in the water column (as reported in chapter four) in Makupa from where they could be settling into sediments. High MPs levels have been linked to anthropogenic activities like coastal tourism, aquaculture and fishing in other parts of the world (Dai *et al.*, 2018). Population density and the level of urbanization and waste infrastructure have also been linked to high accumulation of MPs in different regions of the world (Pedrotti *et al.*, 2016; Lebreton *et al.*, 2012). This could be the case with Port-Reitz creek which is close to suburban areas on the mainland that host oil refineries and housing estates and are surrounded by populated villages such as Ngala, Dongo Kaya and Dunga Nusa (IAME, 2018).

Mida creek, which is within the National Marine Reserve and therefore expected to be free from pollution by industrial effluents, sewage disposal and fishing activities also had MPs pollution in

the sediments. The MPs concentration was high in Mayonda located further interior within calm waters (Kitheka *et al.*, 1999) but also having a high fishing activity (personal observation). This could be attributed to the high concentration of MPs in the water column (see chapter four), the tourist activities in the Marine Watamu National Park and Reserve and the populated villages of Uyombo, Kirepwe and Dabaso. These results support the findings from earlier research linking human population density and plastic pollution (Dai *et al.*, 2018). Dabaso and Kirepwe stations located nearer to the ocean have low MPs concentrations probably because of flushing by the waves carrying MPs in the water column into the ocean.

Microplastic categories as identified by shape were similar to those found in other regions of world marine sediments with fibres being the most dominant category (90 %). Our results are in line with increased global plastic (PE and PP) production (Thompson et al., 2004) and use, suggesting sewage, synthetic textiles, packaging material and fishing gear as important sources of the MPs (Browne et al., 2010; Cole et al., 2014) in the creeks along the Kenya coast. The study findings were in line with other research findings. For example, Gay et al., (2018) recorded higher synthetic fibres in intertidal sediments in Winyah Bay (77.5 %) and Charleston Harbor (76.2 %) in South Carolina compared to fragments and films, Sagawa et al., (2018) reported 90 % fibres in intertidal sediments in Hiroshima Bay and Graca et al., (2017) recorded only fibres in their samples from the Polish beaches. In the current study however, it was challenging identifying clear MPs fibres and short fibres perhaps resulting in underestimation. Tide dominated estuaries have recorded up to 70 % fibres (Alomar et al., 2016)). Few of the MPs were fragments and films probably from packaging material (Kowalski et al., 2016). This could be as a result of the low concentration of MPs fragments and films in surface water within the creeks (chapter four). High fibre density could also be due to zooplanktons selecting against fibres in favour of fragments and films (Amin et al., 2020).

Majority of the fibers were white, which was not a surprise because white pellets are the most common colour manufactured (Redford *et al.*, 1997) and the most widely used class of plastics in the world (Andrady, 2003) followed by other colours (Turner and Holmes, 2011). It could also be because of the preference of clear or transparent plastics for use in plastic products such as packaging bags, synthetic clothing, fishing lines, fishing nets and ropes (McEachern *et al.*, 2019; Cole *et al.*, 2014). This could imply that the fibres may be from the breakdown of worn-out and

abandoned fishing gear, and the release of wastewater from waste treatment plants. A lot of smallscale fishing activities were observed during the study period. Tourism and boat activities are also possible sources of microplastics within the creeks (own observation; Plastics Europe, 2016).

Large grain sediments positively influence benthic microbial biomass increasing biological productivity in shallow water ecosystems (Cahoon *et al.*, 1999). Microplastics in sediments become available to benthic fauna within the sediments (Murray and Cowie, 2011; Rochman *et al.*, 2015) important in nutrient and biochemical recycling processes. Marine microplastics are associated with the risk of their incorporation and adsorption of chemicals into the food web through trophic transfer (Setälä, *et al.*, 2018). Owing to their large surface area and hydrophobic nature, MPs adsorb much more POPs transferring them to many marine organisms ((Hermabessiere *et al.*, 2017; Setälä, *et al.*, 2018), including benthic communities (Coppock *et al.*, 2017). The benthic zone is home to a substantial part of the world's biodiversity. The toxic chemicals, MPs additives and pathogens on biofilms adversely affect reproductive development in marine organisms and even cause death (Cole *et al.*, 2014) leading to decreased biodiversity. Loss of biodiversity may lead to the collapse of marine ecosystem, diminish economic and sustainable development abilities, and undermine environmental principles on earth.

#### **5.6 Conclusions**

Distinctive MPs differences were noted between sampling campaigns suggesting that increased runoff during the rainy season resulted in increased MPs abundances in the creeks probably due to the larger discharges and increased river velocity.

The creek sediments are polluted with MPs which is a serious environmental issue in the Western Indian Ocean along the Kenya Coast with Tudor creek being more polluted compared to Port-Reitz and Mida creeks.

The concentration of microplastics in the creeks showed no relationship with sediment grain size hence MPs and sediments were delivered by different processes.

The concentration of MPs in the sediments is influenced by the concentration of MPs in the surface water in the same station within the site.

The significance in MPs concentrations between sampling seasons implies that MPs in marine sediments are land based. Fisheries, tourism, washing of textile, domestic sewage, population density and coastal infrastructure development are also potential sources of MPs in the sediments

The predominance of fibers suggests packaging, synthetic fabrics from fisheries, and sewage treatment as main sources of MPs in the creek sediments. The bulk of the microplastics recovered were white fibres suggesting that fishing activities may be one of the main sources of MPs in the coastal creek sediments.

Although regular beach cleaning in Mida creek reduces plastic debris, it fails to address the MPs. Separating microplastic contamination from sediments is almost impossible. The potential risk of plastics and microplastics in the marine environment can successfully be decreased only by removing their sources. There is need therefore for continued monitoring of MPs pollution of marine sediments in the creeks to ensure safety and health of benthic organisms.

Mida creek, a crab rearing region is polluted with MPs. It will be prudent to investigate the degree of MPs ingestion by crabs in the area to establish the quality and health of the product and its impact on human health.

Although the quantification of plastic bags was not done, a change in the microplastic concentrations is likely to be observed in future with the removal of the plastic bags effected by the 2017 ban in Kenya. The results of this study provide a baseline for future monitoring of the effect of the Kenya Government ban in 2017 on single use plastic carriers. Future assessments can be compared with these results to establish whether the ban is making a difference in the extent of MPs pollution in the coastal sediments.

#### **5.7 Recommendations**

- There is a need for a critical evaluation of plastic waste disposal policy in the region to curb the problem.
- The Kenya Government should protect the Ocean through legislation on plastic waste management, obligating producers and consumers to contribute to the cost of plastic waste

management; encourage the development of plastic recycling industries by creating assistance programs to those in need of waste management system expertise.

• Further research should test the recovery of MPs of all three sizes using a density separation solution of higher density than NaCl to recover high density MPs.

#### CHAPTER SIX

# 6.0 MICROPLASTICS CONTAMINATION OF COMMON MARINE FISH FROM THE CREEKS ALONG THE KENYA COAST.

#### **6.1 Introduction**

Pollution of the oceans with microplastics and their potential impact along marine food web through consumption of marine products is of increasing concern (Cole et al., 2013; Eerkes-Medrano et al., 2015; Beer et al., 2018). Barnes et al., (2009), defined microplastics as synthetic particles measuring < 5mm in diameter. They can be primary in nature if they are particles manufactured for product production in which case they are referred to as nurdles or they can be secondary if the particles are degraded from microplastic debris (Lusher et al., 2013; Free et al., 2014). Plastic degradation occurs through physical, and ultra-violet radiation processes (Moore, 2008; Andrady, 2011; Rummel, 2014). Microplastics are further classified as fibres, fragments, films, beads or foams based on shape (Claessens et al., 2013; Cole et al., 2013; Mathalon and Hill, 2014; Hartline et al., 2016). Fibres originate mainly from plastic bags, ropes, fishing nets and clothing (Claessens et al., 2013; Hartline et al., 2016), while fragments often originate from plastics that do not unfurl into filamentous threads such as molten plastics or plastic films, filters, and geo textiles (Cole et al., 2011; Claessens et al., 2013). Owing to their bright color and small size, MPs are ingested by invertebrates and some vertebrates, and bio-magnification up trophic levels has been reported (Setälä et al., 2014; Wright et al., 2013). As discussed in the earlier chapters, ingested microplastics may transfer toxins into organisms. For example, Koelmans et al., (2013) reported low concentrations of plastic additives such as Bisphenol A and Nonylphenol in the cod (Gadus morhua) fish, and the lugworm (Arenicola marina) from the North Sea. Goldstein and Goodwin, (2013), Van Cauwenberghe and Janssen, et al., (2014) and Van Cauwenberghe, et al., (2015), found low concentrations of Bisphenol A (BPA) and Nonlyphenol (NP) in barnacles and mussels from the Noth pacific. This however, may be an underestimation because the Endocrine Disruptors Compounds accumulate along the food chain as mussels and barnacles feed on aquatic planktons (Wieters et al., 2008). Nonylphenol has been reported in fishes (Ferrara et al., 2008), mussels (Perna perna) Isobe et al., 2007) and oysters (Crassostrea gigas) (Chen et al., 2006). Basheer et al., (2004) reported accumulation of both BPA and NP in fish, blood cockles (Acadara gronosa), crabs (Portunds pelagicus), pawns (Penaeus monodon), white clams

(*Meretrix meretrix*) and squid (*Lolgo* sp) from a supermarket in Singapore. The oceanic seabird (short-tailed shearwaters *Puffinus tenuirostris*) from the Northern North Pacific Ocean accumulated 16.9 ng/g lipid weight of polybrominated diphenyl ether (PBDE) through plastic ingestion (Tanaka *et al.*, 2013; 2015; Tanaka, 2017; Tanaka *et al.*, 2020). The yellow tail (*Seriola lalandi*) from the North Pacific ingested plastics and PBDE and NP were found in the fish tissues (Gassei *et al.*, 2013) suggesting that the additives were from the ingested plastics (Hirai *et al.*, 2011; Rochman *et al.*, 2013; Teuten *et al.*, 2009. In a laboratory setting, the Japanese medaka (*Oryzia latipes*) exposed to LDPE pellets kept in seawater for two months accumulated significant amount of PBDE's that were associated with liver toxicity and pathology including glycogen depletion and cell necrosis (Rochman *et al.*, 2013).

Microplastic toxins affect organisms along webs as well as the environment. For instance, styrene in polystyrene is an endocrine disrupter, while polyester contains hazardous level of monomers associated with respiratory irritation, cell mutation, and are toxic to aquatic environments (Lithner *et al*, 2011). Polyethylene and polyamides (nylon) although thought to be benign, may adsorb POPs from the environment (Rochman *et al.*, 2013) such as pesticides and polychlorinated biphenyls (PCB's), known to disrupt immunity and cell division (Lauby-Secretan *et al.*, 2013; Hable and Nguyen, 2013). Microplastics toxins in low density polyethylene (LDPE) cause liver stress including: single cell glycogen depletion, necrosis, and fatty vacuolation (Rochman *et al.*, 2013). Microplastics have been known to cause inimical physiological effects, leading to a decrease in feeding ability, energy accumulation, and reproduction for small-size organisms at lower trophic levels (Cole *et al.*, 2013; Sussarellu *et al.*, 2016). However, information on ingestion of microplastics by pelagic fish is not well documented (Romeo *et al.*, 2015) creating a knowledge gap more so, no study has been done on microplastics ingestion by fish along the Kenya Coast. Owing to the toxic effects of microplastic contaminants to organisms along the food webs and the

Owing to the toxic effects of microplastic contaminants to organisms along the food webs and the ever-increasing release of plastics into the ocean, it is important to understand the extent of the problem, to effectively mitigate and take preventive measures. The main objectives of this study were therefore to; a) assess the presence and abundance of microplastics in the gut, gills and muscles of five most common marine fish species from the creeks along the Kenya coast in WIO. b) characterize the microplastics by shape and colour. c) Assess the relationship between microplastic concentration and fish feeding habits and habitat (pelagic or benthic). Considering the importance of the marine trophic web, as prey for big fish and food to humans, this study makes

an important contribution to knowledge of microplastics pollution in fishes in Kenyan inshore areas.

#### **6.2 MATERIALS AND METHODS**

#### 6.2.1 Fish sampling

Common local marine fish species were obtained from local fishermen encountered during MPs sampling from the different stations (Dabaso, Kirepwe in Mida; Makupa in Port-ReitzCreek; Mikindani, Fort Jesus and English Point in Tudor Creek) (Fig 3.1) or from the fish landing sites (Fig. 6.1). Information on the actual fishing ground was obtained from the fishermen to ensure site fidelity and the number and species of fish selected for study depended on their availability in the catch. A total of 225 fish were collected and sorted according to species and placed into ziploc bags that were labeled and then placed in cooler boxes with ice for transportation to the laboratory. In the laboratory fish were washed with deionized water, and rinsed in 70 % ethanol to get rid of any particles affixed to the body surface. The fish samples were randomly assigned into one of the three replicate groups based on species and location. Individual fish lengths (cm) and weights (g) were measured to the nearest 0.1 mm and 0.1g respectively (Karami *et al.*, 2017). The samples were wrapped in aluminum foil to avoid external contamination, placed in ziplocs and stored at  $-40^{\circ}$ C until further analyses.



#### Figure 6.1 Fish landing site from where part of the fish samples were obtained

A: Fish traders waiting for fishermen at the shore at Mkomani fish landing Beach, B: Purchasing fish samples from the local fishermen at the Mkomani fish landing Beach in Tudor Creek.

#### 6.2.2 Fish sample processing and analysis

Sample processing and analysis were done at the Kenya Marine and Fisheries Research Institute (KMFRI) and the University of Nairobi (UON) Laboratories. The fish were dissected by making a cut just below the throat and extending the cut down the ventral side to the anal pore. The gut, gills and flesh were separated, weighed, chopped into smaller pieces and digested using 10 % KOH (1g: 5 ml) (Foekema *et al.*, 2013; Rochman *et al.*, 2015; Dehaut *et al.*, 2016; Kuhn *et al.*, 2017; Thiele *et al.*, 2019) at 60 °C for 14 hours (modified protocol) due to its attributes as described in section 4.2. A further five minutes digestion in 55 % Nitric acid (HNO<sub>3</sub>) solution (10mL/g) was performed to remove any organic material that was still evident (Collard *et al.*, 2015). Acid digestion was done in a fume cupboard, in glass jars covered with watch glasses. The digestates were diluted by adding 100 mL of distilled water before density separation and vacuum filtration to protect the filtration equipment and ease floatation (Collard *et al.*, 2015). The microplastic particles were density separated by adding filtered supersaturated Sodium Chloride (NaCl) solution (1.2g cm<sup>-3</sup>), in the ratio of 1: 3 (sample: salt solution), and left to settle overnight (12 hours) (Thompson *et al.*, 2004; Rochman *et al.*, 2015; Kuhn *et al.*, 2017 modified protocol). The supernatant was filtered by vacuum pump filtration over 0.8µm membrane filters. The filters with

particles were placed in covered glass petri-dishes, and then dried at 40°C for 12 hours before being examined under a dissecting microscope as described in section 4.2 at X40 magnification (Claessens *et al*, 2013; Lusher *et al*, 2013). Suspected microplastics were confirmed using the hotneedle testas described in section 4.3, and further characterization done. The microplastics were characterized by shape and categorized as fiber, fragment or film and their color noted.

#### 6.2.3 Data analyses

Shapiro-Wilk's test was used to test data normality and all data was found to be normally distributed after log transformation. Species abundance and the mean concentrations of microplastics in species during the different seasons were compared using One way ANOVA and the Turkey's post hoc test separated the means. One way ANOVA was also used to compare the concentration of microplastics and the weight of tissues followed by a Turkey's test. A Pearson's correlation was done to determine the relationship between the mean microplastic concentration and mean lengths and weights of the organisms. Species pairwise comparisons were done using the t- test. Fish data from the two seasons was combined and von Bertalanffy growth curves compared.

The assessment of microplastics in the guts, gills and muscles was done in accordance with procedure and ethical guidelines for animal experiments in the University of Nairobi and KMFRI.

#### 6.3 Results

#### 6.3.1 Fish distribution and size

A total of 225 individuals from five different species were obtained, most of which were benthic fish (Nelson, 1994: Sheaves, 2006: Fischer *et al.*, 1990: Lieske and Myers, 2004; Froese and Pauly, 2020), and included *Geres oyena* (Forsskal, 1775), *Acanthopagrus berda* (Forsskal, 1775) and *Terapon jarbua (Forsskal, 1775). Gerres oyena* inhabits coastal waters and is a carnivore (Cyrus and Blaber, 1982), *Acanthopagrus berda (Forsskal, 1775)* is predominantly marine (Nelson, 1994: Sheaves, 2006) with some living in euryhaline estuarine environments (Leu and Chou, 1996), and is an omnivore (Nasir, 2000: Shelta *et al.*, 2018), *Leptoscarus vaigiensis* (Quay & Galmard, 1824) is reef associated grazing fish (Locham *et al.*, 2015) while, *Rastrelliger kanagurta* (Cuvier 1816) is pelagic and omnivore (Collette, 2001).

During the first sampling all five species were encountered and were represented by more individuals than during the second sampling. In the second sampling only four species were encountered (because *L. vaigiensis* that had been encountered at English point was not encountered again probably due to depletion of the species or migration to other suitable grounds for spawning and all species were represented by fewer individuals.

Mida and Tudor Creeks had a higher variety of fish species compared to Port-Reitz. The higher diversity in Mida was mainly because of the high diversity encountered in Dabaso station while in Tudor all three stations sampled had different species. *Gerres oyena* was the most predominant species (91 individuals representing 44 %) and occurred in all stations except Fort Jesus and English Point (Table 2). Data on species diversity from the two sampling campaigns did not vary significantly (ANOVA: F = 0.77, df =1, P = 0.790). Although *G. oyena* was dominant, it did not occur in Port-Reitz Creek during the second sampling campaign.

Site	Station	Species	Feeding	Habitat	Jan	Sept
Sile	Station	species			2018	2018
Mida	Dabaso	G. oyena	Carnivore	demersal	-	16
		A. berda	Omnivore	demersal	31	9
		T. jarbua	Carnivore	demersal	37	6
	Kirepwe	G. oyena	Carnivore	demersal	15	-
Port-Reitz	Makupa	G. oyena	Carnivore	demersal	11	-
Tudor	Mikindani	G. oyena	Carnivore	demersal	31	18
	Fort Jesus	R. kanagurta	Omnivore	pelagic	35	6
	<b>English</b> Point	L. vaigiensis	Grazer	pelagic	10	-

 Table 6.1 Occurrence of different fish species in different sites and stations

#### 6.3.2 Mean lengths and mean wet weights of the individual fish species

The average ( $\pm$  SE) weights and lengths of the different fish species showed wide ranges with the greatest mean weight range being observed in *G. oyena* perhaps because of its occurrence in different stations and seasons. The heaviest *G. oyena* individuals were encountered in Makupa followed by those from Dabaso while Mikindani had the smallest individuals. *Leptoscarus* 

*vaigiensis* individuals were as heavy as the heaviest *G. oyena* individuals from Makupa while while *A. berda* had the smallest individuals (Table 6.2).

Table 6.2 Mean  $((\pm SE)$  lengths and mean wet weights of fish of different species per site and station

		Jan 2018			Sept 2018	
Site	Station	Species	Av. Bw (g)	Av. TL (cm)	Av. Bw (g)	Av. TL (cm)
Mida	Dabaso	G. oyena	-	-	$79.9\pm0.1$	$15.6\pm0.02$
	Dabaso	A. berda	$11.9\pm0.1$	$9.7\pm0.03$	$74.3\pm0.01$	$9.8\pm0.01$
	Dabaso	T. jarbua	$16.3\pm0.17$	$10.8\pm0.01$	$74.8\pm0.1$	$10.2\pm0.01$
	Kirepwe	G. oyena	-	-	$50.4\pm0.04$	$14.9\pm0.1$
Port-						
Reitz	Makupa	G. oyena	$143.8\pm1.4$	$28.2\pm0.06$	-	-
Tudor	Mikindani	G. oyena	$35.6\pm0.7$	$12.8\pm0.2$	$28.6\pm0.01$	$19.4\pm0.03$
		<i>R</i> .	118.6 ±			
	Fort Jesus	kanagurta	0.15	$22.3\pm0.08$	$32.5\pm0.06$	$10.3\pm0.1$
	English	L.	143.1 ±			
	Point	vaigiensis	0.74	$20\pm0.04$	-	-

# 6.3.3 Length-Weight relationships of the fish species

The length-weight relationship of the fish varied widely among species (Table 6.3).

Table 6.3 Table of length-weight Von Bertalanff	y growth curve parameters for the
---	-----------------------------------

## different fish species

Species	Station	a-value	b-value	$\mathbb{R}^2$	n	Non-linear equation
G. oyena	Dabaso	3.1266	0.3675	0.4962	17	$W = 3.1266L^{0.3675}$
G. oyena	Kirepwe	4.2102	0.3275	0.8813	16	$W = 4.202L^{0.3275}$
G. oyena	Makupa	6.3678	0.3042	0.9796	12	$W = 6.3678L^{0.3042}$
G. oyena	Mikindani	11.631	0.0964	0.0127	46	$W = 11.631L^{0.0964}$
R. kanagurta	Fort Jesus	1.5434	1.3915	0.393	41	$W = 1.5434L^{1.3915}$
A. berda	Dabaso	0.0107	3.054	0.9143	40	$W = 0.0107 L^{3.054}$
T. jarbua	Dabaso	0.1226	2.0467	0.4172	43	$W = 0.1226L^{2.0467}$
L. vaigiensis	English Point	0.0273	2.8453	0.9137	10	$W = 0.0273L^{2.8453}$

Y-intercept (a-value), slope of the curve (b-value) and the coefficient of determination (R<sup>2</sup>)

The length-weight data fitted to the Von Bertalanffy growth curve model produced varied growth curves for the different fish species (Figure 6.2).

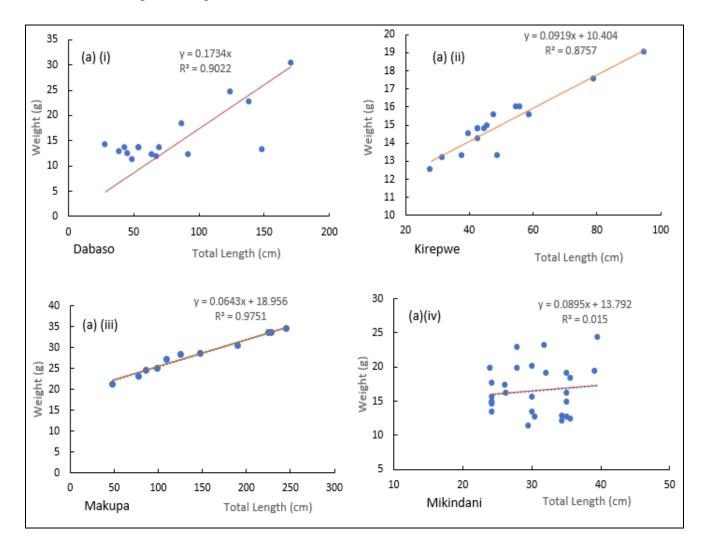


Figure 6.2 Scatter diagrams showing length-weight relationships of; a) Gerres oyena from different stations

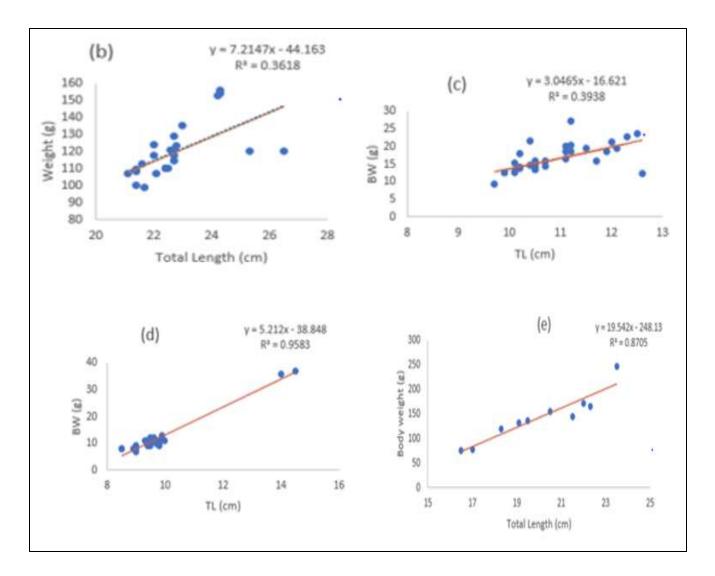


Figure 6.2 Scatter diagrams showing length-weight relationships of; b) *Rastrelliger kanargurta*, c)*Terapon jarbua*, d) *Acanthopagrus berda*, *e*) *Leptoscarus vaigiensis* from the creeks along the Kenya coast

The scatter diagrams obtained from this study are similar to scatter diagrams of species from other global regions available in Google researchgate (Fig.6.3).

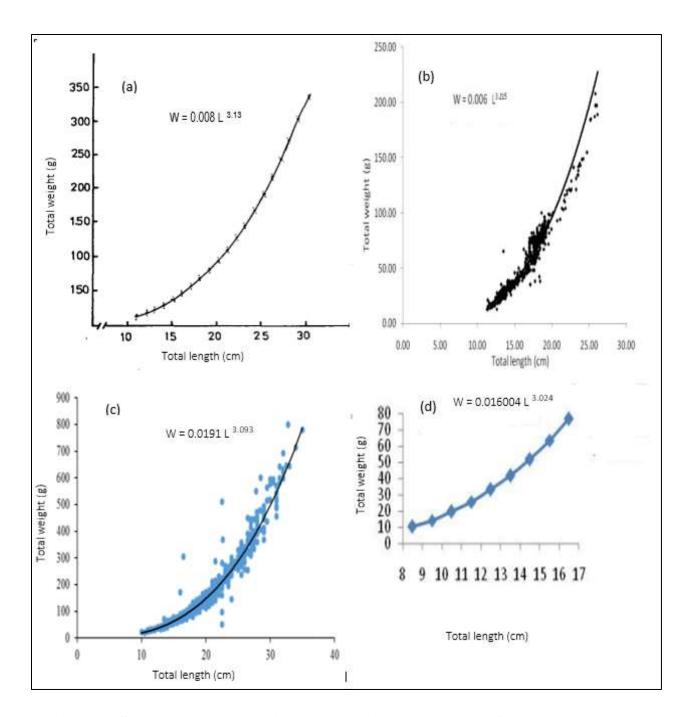
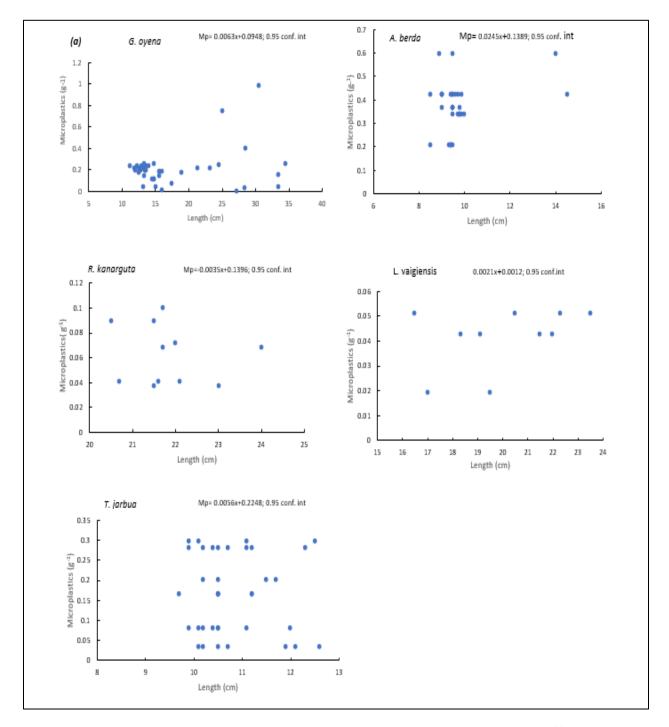


Figure 6.3 Scatter diagrams showing length-weight relationships of; a) *Gerres oyena*, b) *Rastrelliger kanargurta*, c) *Acanthopagrus berda*, d) *Terapon jarbua* from different parts of the world.

A correlation between MPs concentration and body length and weight showed that all the fish species except *R. kanarguta* had increased MPs concentration with increase in body length



(Fig.6. 4 a), while *G. oyena* and *L. vaigiensis* showed a decease in MPs concentration with increase in body weight (Fig.6.4 b)

Fig. 6.4 Relationship between (a) MPs concentration and body length in the different species.

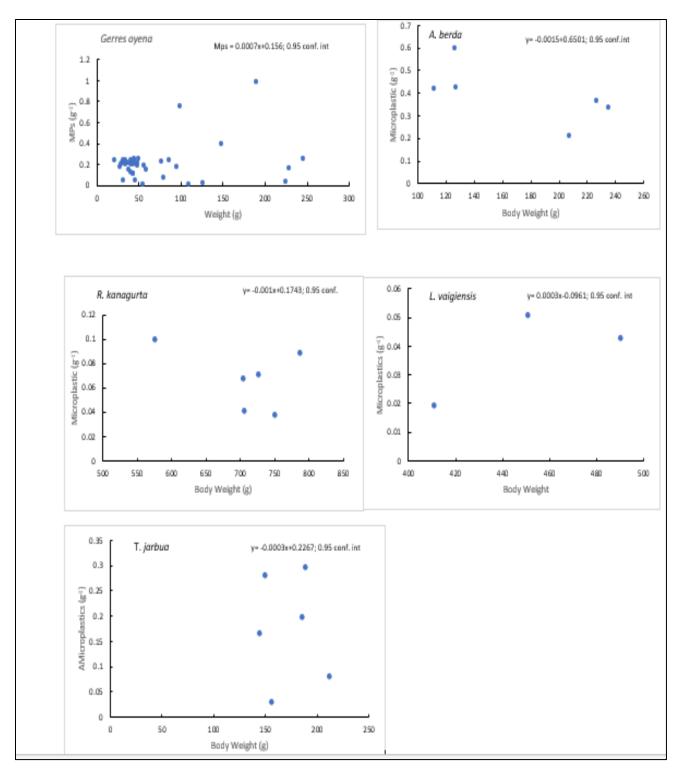


Fig. 6.4 Relationship between (b) Microplastics concentration and body weight in the different species

6.4. General distribution of microplastics in the different fish species per gram tissue Microplastics were observed in all the fish sampled (Fig 6.5). Irrespective of site, station or sampling season, *G. oyena* had a higher mean microplastic concentration per gram tissue ( $0.530 \pm 0.158 \text{ mp.g}^{-1}$  tissue) (Chisq<sub>4</sub> =5504, p = < 0.01) compared to *A. berda* ( $0.48 \pm 0.058 \text{ mp.g}^{-1}$  tissue), *T. jarbua* ( $0.240 \pm 0.04 \text{ mp.g}^{-1}$  tissue), *R. kanagurta* ( $0.132 \pm 0.011 \text{ mp.g}^{-1}$  tissue) and finally *L. vaigiensis* ( $0.04 \pm 0.001 \text{ mp.g}^{-1}$  tissue).

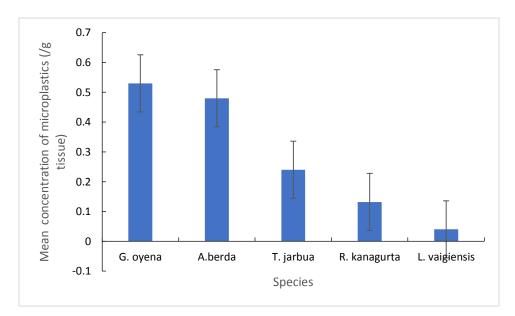


Figure 6.5 Mean ( $\pm$  SE) microplastics concentrations (g<sup>-1</sup> tissue) for the different fish species with standard error bars.

# 6.4.1 Overall mean concentration (g<sup>-1</sup>) of microplastics in the different fish species

The overall ( $\pm$ SE) mean microplastics concentration in different species were below 1 microplastic per gram of tissue in all the species (Table 6.4).

Site	Station	Species	Jan 2018	Sept 2018	Mean conc
Mida	Dabaso	G. oyena	-	$0.18\pm0.041$	$0.18\pm0.041$
		A. berda	$0.16\pm0.003$	$0.52\pm0.01$	$0.480\pm0.058$
		T. jarbua	$0.15\pm0.008$	$0.31\pm0.01$	$0.240\pm0.04$
	Kirepwe	G. oyena	$0.041 \pm 0.032$	-	$0.041\pm0.032$
Port-Reitz	Makupa	G. oyena	$0.1\pm0.034$	-	$0.1\pm0.034$
Tudor	Mikindani	G. oyena	$0.2 \pm 0.02$	$0.21\pm0.011$	$0.209\pm0.051$
	Fort Jesus	R. kanagurta	$0.07\pm0.01$	$0.16\pm0.004$	$0.132\pm0.011$
	English Point	L. vaigiensis	$0.04\pm0.001$	-	$0.04\pm0.001$

Table 6.4: Concentration of MPs  $(x \pm SE)$   $(g^{-1}$  tissue) in the different species in different creeks and stations

Overall, the mean concentration of microplastics showed significant difference (F = 12.69, df = 11, p < 0.01) between species. *Acanthopagrus berda* had the highest mean concentration while *G*. *oyena* from Mida (Kirepwe) and *L. vaigiensis* had the lowest mean concentration of microplastics per gram tissue.

Apairwise comparison in MPs concentration between different stations but same sampling period, and same species but different sampling seasons produced varied results (Table 6.5).

Table 6.5 Pairwise comparison of Mean microplastic concentration ( $x \pm SE$ ) in fish species
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	Stations and seasons	Mean	Std. Err Mean	t- value	df	р			
Same species (Gerres oyena)									
1	Jan - Sept Mik (same stn; diff sampling)	0.014	0.031	0.45	2	0.70			
2	Mik - Mak Jan (diff stn; same sampling)	0.169	0.020	8.51	2	<u>0.01</u>			
3	Mik - Kir Jan (diff stns; same sampling)	0.159	0.029	5.55	2	<u>0.03</u>			
4	Mik - Dab Sept (diff stn; same sampling)	0.033	0.045	0.72	2	0.54			
5	Kir Jan - Dab Sept (diff stns; diff sampling)	0.141	0.056	2.50	2	0.13			
	Other species								
6	R. kanagurta Jan -Sept (FJ) (diff sampling)	0.408	.153	7.62	2	<u>0.012</u>			
7	A. berda Jan – Sept (Dab) (diff sampling)	0.177	.060	6.93	2	<u>0.039</u>			
8	T. jarbua Jan – Sept (Dab) (diff sampling)	0.036	.012	5.98	2	<u>0.040</u>			

Pearson's correration test showed no relationship (r = 0.01,) between the concentration of MPs in surface water and in fish tissues. Significant differences (p < 0.05) were observed in the concentration of MPs in *G. oyena* between Mikindani and Makupa and Mikindani and Kirepwe where Mikindani had higher concentration compared to the other stations (Table 6.5). There was no significant difference in the concentration of MPs in *G. oyena* from Mikindani during the different sampling seasons (t = 0.45, df = 2, p = 0.07). For *R. kanagurta*, *A berda* and *T. jarbua*, significant differences (p < 0.05) were observed in the concentration of MPs between the different sampling seasons where the second campaign recorded higher concentrations than the first campaign.

# 6.4.2 Mean (± SE) concentration of microplastics in different organ tissues in different species

All the fish species contained MPs in their guts, gills and muscle tissues (Table 6.6). There were significant differences (F = 22.725, df = 20, p = 0.002) in mean MPs concentration in the guts between species. Guts of *A. berda* (7.41 ± 0.42) had significantly higher mean MPs concentration, compared to *G. oyena, R. Kanagurta, T. jarbua* and *L. vaigiensis* but the latter three were not significantly different (F = 1.549, df = 20, P = 0.211) (Table 6.6). With respect to the gills, *A. berda* and *G. oyena* had significantly higher mean concentrations of MPs per gram tissue compared to other species but the two were not significantly different (p > 0.05). Generally, the mean concentration of MPs in muscle tissues were lower than 0.1 MPs g<sup>-1</sup> across all stations and species and did not vary significantly (p > 0.05).

Based on station, the guts of *G. oyena* from Dabaso had a significantly higher (F = 12.692, df = 7, p < 0.05) mean concentration (g<sup>-1</sup> tissue) of microplastics compared to those from Kirepwe, Mikindani and Makupa but the latter three were not significantly different (p > 0.05). Similarly, the gills of *G. oyena* from Dabaso had a significantly higher mean concentration of MPs (g<sup>-1</sup>) (F = 13.142, df = 7, p = 0.001) compared to other stations (Table 6.6)

			Microplastics in organs g <sup>-1</sup>				
Site	Station	Species	MPs g <sup>-1</sup>	Guts	Gills	Body	
Mida	Dabaso	G. oyena	$0.181 \pm 0.041$	$*3.557 \pm 0.15$	$*2.599 \pm 0.23$	$0.042\pm0.001$	
	Kirepwe	G. oyena	$0.041 \pm 0.032$	$1.398\pm0.05$	$1.339\pm0.07$	$0.004\pm0.001$	
	Dabaso	A. berda	$0.48\pm0.058$	$*7.41 \pm 0.42$	$*2.82\pm0.08$	$0.081 \pm 0.01$	
	Dabaso	T. jarbua	$0.240\pm0.04$	$1.38\pm0.02$	$1.97\pm0.025$	$0.031 \pm 0.01$	
Port-Reitz	Makupa	G. oyena	$0.1 \pm 0.034$	$0.94\pm0.01$	$1.92\pm0.12$	$0.02 \pm 0.0$	
Tudor	Mikindani	G. oyena	$0.209\pm0.051$	$1.43\pm0.02$	$0.172\pm0.05$	$0.015\pm0.001$	
	Fort Jesus	R. Kanagurta	$0.132\pm0.011$	$1.44\pm0.03$	$0.74\pm0.01$	$0.01\pm0.01$	
	English Point	L. vaigiensis	$0.04\pm0.001$	$0.56\pm0.1$	$0.45\pm0.1$	$0.01\pm0.01$	

Table 6.6 Mean  $(x \pm SE)$  concentration of microplastics in various organs per gram tissue of the different fish species

Asterix indicates significantly higher (p < 0.05) concentration of microplastics

#### 6.5 Microplastic types by shape and colour in the tissues of the different fish species.

Most of the MPs recovered from the fish were fibers (91.4 %) and a small percentage (8.6 %) were film fragments (Fig. 6.6). Similarly, significantly higher (F = 22.721, df = 20, P< 0.001) proportions of fibers and fragments were recovered from fish gills, compared to fibers and fragments from guts, and from fish body (Fig. 6.6).



Figure 6.6 Mean percentage concentration of microplastic shapes observed in organs of different fish species from the creeks along the Kenya coast.

In addition, clear balls of fibers, films and fragments were recorded from the guts of some fish of *R. kanagurta, G. oyena, L. vaigiensis* and *T. jarbua* species (Fig.6.7).

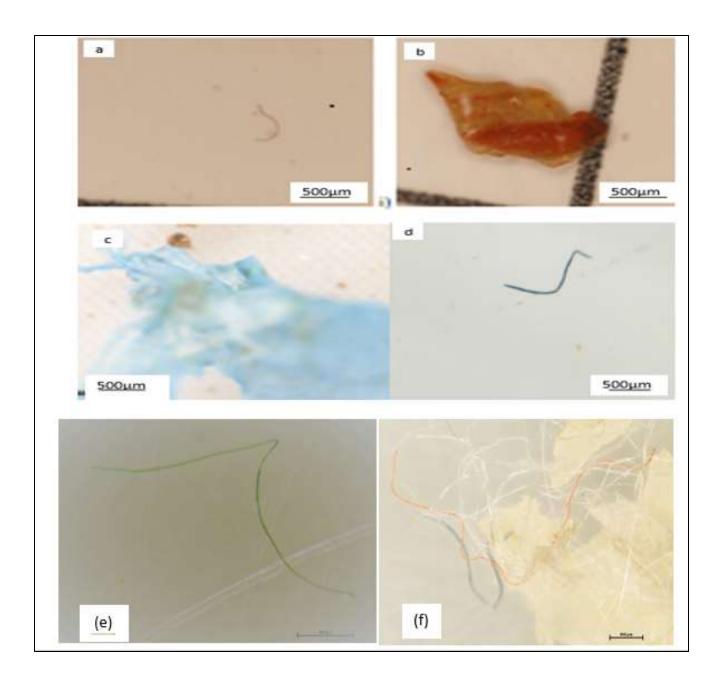


Figure 6.7 Examples of microplastic types by shape recovered from fish tissues: (a) Red fibre from the gut of *R. kanagurta* from Fort Jesus (b) Brown fragment from the gills of *G. oyena* of Makupa (c) Blue film from the gut of *L. vaigiensis* from English point (d) Green fibre from the muscle tissue of *T. jarbua* from Dabaso (e) Green fibre from the gut of *R. kanagurtafrom* Fort Jesus (f) Red, blue and white tangled fibers from the gills of *G. oyena from Makupa* 

The majority of the microplastics recovered from the fish were blue (36.4 %) and black (34.2 %) followed by white (18.4 %), with a low presence of green (6.5 %), red 3.3 %), and purple (0.9 %) (Figure 6.8).

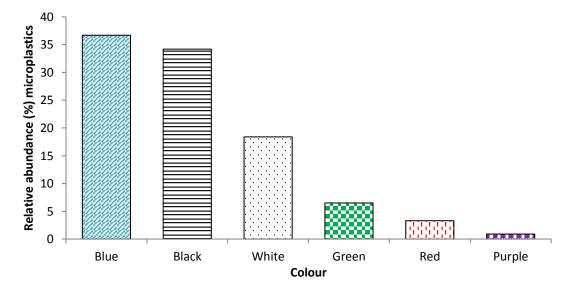


Figure 6.8 Mean percentage concentration of microplastic colours observed in different fish species from the creeks along the Kenya coast

#### 6.6 Discussion

Kosore *et al.*, (2018), Awour *et al.*, (2020) and Kerubo *et al.*, (2020) distinctly show that microplastics are abundant in the Kenya's marine environments and are interacting with zooplankton and macro-invertebrates by way of ingestion. Fish are economically important as well as human food (Barboza, *et al.*, 2018). Some of the risks associated with marine fish are the incorporation of MPs and adsorbed chemicals into the food web through trophic transfer (Setälä *et al.*, 2018). Ingestion of MPs increases toxicity of plastic chemicals such as nonylphenols, bisphenol A and antioxidants in the organisms through leaching (Hermabessiere *et al.*, 2017). It is therefore imperative to assess the interaction of MPs with marine fish, as a potential risk to humans. The study focused on the sites around Mombasa due to the rapid increase in human population and high solid waste from tourism and industrial sectors (Okuku, 2019). Microplastics are present everywhere including Dabaso within Watamu Marine Reserve, a protected area expected to be free from microplastic contamination (Kerubo *et al.*, 2020).

Growth in fish is isometric if body weight increases with increase in total body length (b = 3), positively allometric if the b-value is greater than 3 and negatively allometric if the b-value is far less than 3 (Ricker, 1975; Wootton, 1990). Based on Fish Base data, different fish species attain maturity at different body lengths with *G. oyena* attaining maturity at an average total body length of 22 cm (Roux, 1986), *T. jarbua* 13 cm (Lieske and Myers, 1994), *A. berda* at the range of 20-22 cm (Smith and Smith, 1986), *R. kanagurta* 19.9 cm (Sommer *et al.*, 1996) and *L. vaigiensis* attains maturity at the range of at 16.5-18.4 cm total body length for fish in parks and reserves while in the open the species matures at 13.4cm total body length (Randall, 1986). In this study therefore, only *G. oyena*, from Makupa could be considered mature while the rest of the fish sampled were immature.

The length-weight relationship gave a good fit to the length and weight of *A. berda* Von Bertalanffy growth curve (James *et al.*, 2003; Ontomwa *et al.*, 2018) while data for the length-weight relationship of the other species of fishfish had negative allometry, hence did not obey the cubic law (Wootton, 1990). The length-weight relationships for *A. berda*, indicates isometric allometry as the high coefficient of determination implies proportional increase in weight and length. These results affirm earlier research on *A. berda* from the North Coast of Kenya, (W=  $0.0191L^{2.988}$ ) with a coefficient determination of R<sup>2</sup>= 0.9676 (Anam *et al.*, 2019) and from Shimoni artisanal fishery in Kenya (Ontomwa *et al.*, 2018). The length-weight relationship of *A. berda* 

could have influenced microplastic ingestion during feeding. Isometric growth could be attributed to the phenotype of the species, condition of the fish, the environment and food availability (Ontomwa *et al.*,2018; Anam *et al.*, 2019) which were not investigated because they were not within the scope of the current study.

The length-weight relationship for *G. oyena* and *L. vaingiesis* imply positive correlation and negative allometric growth while the length-weight relationships for *R. kanagurta*, and *T. jarbua* indicate negative correlation and negative allometric growth patterns. These results differ from previous research findings for *G. oyena* from the Gulf of Suez, W= 0.094L<sup>3.11</sup> (Saber *et al.*, 2020), from Caledonia (W=  $0.0120L^{3.232}$ ) (Letourneur *et al.*, 1998) and *G. oyena* from Okinawa Island Southern Japan, W=  $0.035L^{2.89}$  (Kanak and Tachihara, 2006), but agrees with findings for *T. jarbua* from Mindano, Philippines, W=  $0.0006L^{2.8484}$  (Fortaleza *et al.*, 2019) and *L. vaigiensis* from Shimoni artisanal Fishery, Kenya, W =  $0.0000129L^{2.3}$  (Ontomwa *et al.*, 2018), showing that length of fish increased more than weight. The results also differ from findings of *R. kanagurta* from Mangalore India, W=  $0.0045L^{3.2234}$  (Hulkot *et al.*, 2013) and from the North coast Kenya, b value of 3.249 (Akinyi *et al.*, 2018) which indicate positive correlation and allometry. The negative allometric growth can be attributed to several factors including; fullness of the stomach probably by microplastics (own observation), insufficient feeding, age, sex, health condition of the fish, poor food quality and availability, low salinities and poor habitat conditions (Sarre and Potter, 2000; Froese, 2006).

This study established that the common marine fish within the creeks along the Kenya coast, including those from Mida creek expected to be free of microplastic contamination were contaminated with MPs. *Geres oyena* was the most abundant, widespread species and highly contaminated with MPs. Kosore *et al.*, (2018), Awuor *et al.*, (2020) and the current study distinctly show that microplastics are abundant in the Kenya's marine ecosystem and are interacting with zooplankton and macro-invertebrates by way of ingestion. Fish are economically important as human food. Some of the risks associated with marine fish are the incorporation of microplastics and adsorbed chemicals into the food web through trophic transfer (Setälä *et al.*, 2018). Ingestion of microplastics increases toxicity of plastic chemicals such as nonylphenols, bisphenol A and antioxidants in the organisms through leaching (Hermabessiere *et al.*, 2017). It is therefore imperative to assess the ingestion of microplastics by marine fish, as a potential risk to humans.

The study focused on the sites around Mombasa due to the rapid increase in human population and high solid waste from tourism and industrial sectors (UN-Habitat 2008, 2014; Kenya Data Portal, 2019; KNBS, 2019). Microplastics are everywhere including Dabaso within Watamu Marine National Park, a protected area expected to be free from microplastic contamination (see Chapter 4).

Significant variations in microplastic concentrations among species could be explained by differences in habitats and feeding behaviour that affect ingestion of microplastics. For example, G. oyena a demersal fish inhabiting inshore areas and feeds on small organisms and benthic invertebrates (Lieske and Myers, 2004; Froese and Pauly, 2020) recorded relatively high MPs in the tissue, A. berda is also a demersal fish that feeds mainly on macroinvertebrates (barnacles, crabs and oysters) (Fischer et al., 1990) that are known to ingest and accumulate MPs (Neves et al., 2015; Li et al., 2016; Nelms et al., 2018; Awour et al, 2020), while T. jarbua is also a demersal fish feeding mainly on white (Paneaus indica) and brown (Paneaus monoceros) shrimps which may accumulate MPs and pass them on to a higher trophic predator. *Rastrelliger kanagurta* is pelagic and omnivore, feeding on algal material and small invertebrates (Collette, 2001), while L. vaigiensis, is pelagic, reef associated inhabiting seagrass areas and is herbivorous feeding on sea grasses and algae (Sommer, 1996; Locham et al., 2015; Froese and Pauly, 2017). MPs are likely to arrive into the coastal environment through rivers and may have high concentration in the surface waters (see chapter 4) yet pelagic fish tend to accumulate much less compared to the demersal fish. The variation in habitats and feeding behaviour could also explain the correlation between MPs concentration in surface water and in fish.

Small invertebrates accumulate microplastics passing them up trophic levels, thereby increasing microplastics in higher trophic levels (GESAMP, 2015) as could be the case for *G. oyena* and *A. berda*. The high contamination with MPs of *A. berda* from Dabaso both in the gut and gills could not be explained as it was not the site with the highest MPs concentration in the surface water and the sediments (see chapter 4 and 5) although Awuor *et al*, (2020) found that MPs concentrations in the invertebrates in Dabaso were comparable to other sites along the Kenya Coast. On the contrary, Mikindani had recorded relatively high MPs in the sediments (see chapter 5) and yet, *G. oyena* population from that site had not accumulated as high MPs in the gut and gills as was

observed in the population from Dabaso. This suggests that several factors playing together influence MPs contamination in fish and not just the level of contamination of the environment.

Leptoscarus vaigiensis from English Point had the lowest microplastic concentrations in the gut and gills which could be as a result of the fish not spending much time in the creeks being reef associated species (Locham *et al.*, 2015) and only occasionally venturing into the creeks (Locham *et al*, 2015). This could imply that the reefs are less contaminated with MPs compared to the creeks. Our results are consistent with earlier research where similar concentrations have been observed in the digestive tracts of the Mediterranean lantern fishes which are both shallow and deep water-living but pelagic feeders, mainly feeding on mesopelagic fish (Romeo *et al.*, 2016) and the South African catfish which is omnivore with carnivorous tendency (Silva-Canti *et al.*, 2017), but were lower than those obtained in the gastrointestinal tracts of fish from other regions of the world, such as crevalle jack (*Caranix hippos*, Linnaeus, 1766) (Froese and Pauly, 2017). The relatively high microplastic concentrations in both juvenile and mature fish samples indicates that size or age does not influence microplastic ingestion in fish.

Microplastic concentrations were significantly lower in fish muscle tissues but significantly higher in the guts and gills of all the fish species. The high concentration of microplastics in the gills of *A. berda* and *G. oyena* from Dabaso could imply transfer of the contaminants to humans in high concentrations if the fish is consumed whole with gills. For example, in this study, consuming 1 kg whole *A. berda* with gills from Dabaso can transfer about  $2901 \pm 0.09$  microplastics while the same quantity of gutted fish with gills discarded can only transfer about  $81 \pm 0.02$  particles. Therefore, consuming fish gills is risky.

Microplastics were mainly fibers agreeing with Nelms *et al.*, (2018), who reporting similar results in the Atlantic Mackerel with fibers (72 %) being higher than fragments as well as in the digestive tracts of the South African catfish (Silva-Canti *et al.*, 2017). Fibers made up 88 % of the microplastics in five fish species in China (Jabeen *et al.* 2017), 96 % of microplastics in fish from Texas (Fazey and Ryan, 2016) and were predominant in the stomachs of Mediterranean lantern fishes (Romeo *et al.*, 2016). The occurrence of clear balls of fibers in guts of some fish is consistent with earlier research reports of bunched balls of microplastics in the digestive tract of the *Lates niloticus* (Linnaeus, 1758) and the *Oreochromis niloticus*. (Linnaeus, 1758) in Lake Victoria (Biginagwa *et al.*, 2016).

The high percentage of fibers suggests waste water from treatment plants, domestic waste water, fishing ropes and nets, plastic bags, synthetic textiles and other types of materials from tourism activities as the main sources of microplastics (Khan *et al.*, 2018; Graca *et al.*, 2017). The presence of film fragments suggests light weight plastics as the source. It is worthwhile to note that the source of microplastics directly influences their concentration in water bodies and subsequently fishes (Free *et al.*, 2014). Most of the microplastic particles from fish in this study were blue and black. Earlier research reported red, blue, and white elongated fibers in the Gulf of Mexico with no proportions (Philips and Bonner, 2015). Variations in microplastic particle colours implied multiple sources of the pollutants. Further investigation is required to establish the source of microplastics in the demersal and pelagic fishes of the creeks along the Kenya Coast and the subsequent impact on human health.

#### **6.7 Conclusions**

The study established that the common marine fish in the creeks along the Kenya coast ingest microplastics and body size or age did not influence their ingestion.

Growth of the fish species in the study deviated from the norm in literature for the same species which could probably suggest that fish are affected by microplastics in the aquatic environment. Although all the fish species had microplastics in the guts, gills and muscles, muscles had significantly lower values than the gut and gills. But the fact that microplastics are found in the muscle tissue is alarming as it demonstrates gut tissue or gill tissue transfer of microplastics. In retrospect, this study shows that common fish in the creeks along the Kenya coast ingesting microplastics could pose a risk to humans especially if they are consumed whole. All fish regardless of size should be gutted and gills removed before being processed or cooked for human consumption.

Microplastics of different shapes and colours were ingested most of which were blue indicating multiple sources of the pollutants. High microplastic concentrations in demersal and pelagic fish indicates that microplastics in the creeks along the Kenya coast accumulate in the sediments and water column and differences in feeding modes could influence ingestion.

This study has implications for fishery and wildlife management. Understanding of the results could benefit the National and International Governments, environmental advocacy groups such as NEMA, and Intergovernmental organizations.

# 6.8 Recommendation

- . It is the researcher's opinion that based on this work all fish regardless of size should be gutted and gills removed before being processed or cooked for human consumption.
- Monitoring during the Northeast and Southeast Monsoon, when fishing activities are different along the Kenyan coast, needs to be conducted to establish seasonal variations in MPs ingestion by fish.
- There is need to study the sources and effects of microplastics in fish within the creeks along the Kenya coast to establish the possible effects on organisms higher up trophic levels.

#### **CHAPTER SEVEN**

# 7.0 MICROPLASTIC POLYMER TYPES IN SURFACE WATER AND SEDIMENTS IN THE CREEKS ALONG THE KENYA COAST

## 7.1 Introduction

Pollution of oceans by microplastics is a threat that has attracted global attention and more research is focused on the problem leading to many publications on ocean plastic pollution (Ryan, 2015; Lusher *et al.*, 2015; Horton *et al.*, 2017; Hamid *et al.*, 2018; Wang and Wang, 2018; Hale *et al.*, 2020; Horton ad Barnes, 2020). Large plastic pieces are everywhere in ocean waters, marine sediments, on road sides and ocean beaches (Setälä *et al.*, 2014; Suaria *et al.*, 2020). However, information availed on chemical identification and thermal analysis of ocean macroplastic polymers in the Western Indian Ocean (WIO) is scarce and none along the Kenya coast. This study sought to investigate the abundance and type of microplastic polymers in marine sediments and surface water within Tudor, Port-Reitz, and Mida creeks along the Kenya coast. Knowledge of polymer type is essential for assessing plastic pollution in the marine ecosystem, and establish the sources of plastics and MPs in the oceans for information-based policy formulation on plastic production and plastic waste management to save the oceans and aquatic resources.

About 92 % of the plastics found in the ocean are small particles (< 5mm) (Arthur *et al.*, 2009) from the breakdown of macroplastics due to exposure to Ultraviolet (U/V) light radiations, chemical and weathering processes or enter the marine environment directly as fibers, pellets and granules (Andrady, 2015; Cole *et al.*, 2011). Microplastics can harbor a wide range of microbial communities and rafting organisms (Frackowiak *et al.*, 2018), function like disposal carriers of chemical additives accumulated from surrounding waters and persistent organic pollutants (POPs) (Koelmans *et al.*, 2014; Rochman *et al.*, 2015). Owing to their bright color and tiny size, microplastics are ingested by invertebrates and some vertebrates, thereby introducing POPs into the food web (Moore, 2008). Bio-magnification occurs higher up trophic levels (Setala *et al.*, 2014) and the accumulation of harmful POPs is likely to cause damage to organisms and ultimately a decrease in biodiversity.

World over, there is increasing demand for packaged food (Ryan, 2015) leading to increased use of polymers such as polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyamide (PA) and polyethylene terephthalate (PET) for commercial packaging (Gomes *et al.*, 2019; Rani and Kumar, 2019). Of these, PP is the widely preferred packaging material but becomes extremely resistant to biodegradation after the short time of use and resists degradation (Andrady, 2015) raising environmental concern. Polyethylene exists in different densities including; upper high-density polyethylene, high density, medium density, low density and lower low-density polyethylene (Rani and Kumar, 2019). To reduce environmental impact, waste polymers can be managed by incineration for energy generation or chemical recycling (Nemade, *et al.*, 2011; Wloch *et al.*, 2019) to form different products and plasticizers such as PET used in making bottles for soft drinks, textile industry (polyester fabrics), matrix for glass-filled composites, films magnetic recording tape, to mention but a few. The investigation sought to determine the types of polymers in the Kenya marine ecosystems for proper policies on plastic waste disposal and management.

#### 7.2 MATERIALS AND METHODS

The Differential Scanning Calorimetry (DSC) and Nuclear Magnetic Resonance (NMR) analyses were done in science laboratories in the U.S.A, while (Transform Infra-Red (FT-IR) analysis was done in the Chemistry laboratory at the University of Nairobi. Microplastics smpled as in chapter 4 and 5 from surface water and intertidal sediments respectively from the three sites (Port Reitz, Tudor and Mida creeks) were analyzed for polymer types. For surface water MPs, 10 % of the total MPs of the large size per station were analyzed while, 1 % of the small size and 1 % of the medium size MPs per station were analyzed because of their large number and time factor implication. For MPs in sediments whose total number was smaller than in surface water, 10 % of the total MPs per station were analyzed

## 7.2.1 Polymer identification

**Differential Scanning Calorimetry** (**DSC**): Polymer characterization was done using the DSC (Onyari *et al.*, 2008; Courtene-Jones *et al.*, 2017; Wloch *et al.*, 2019). DSC allows the measuring of crystallization temperature ( $T_c$ ), glass transition ( $T_g$ ) and melting temperature ( $T_m$ ) while a

polymeric sample is being heated or cooled (Schmack *et al.*, 2000; Roes *et al.*, 2007; Courtene -Jones *et al.*, 2017; Kataoka *et al.*, 2018). Samples weighing between 0.9-2.7 mg were measured using the DSC Q100 V9.9 model while the DSC profiles were analyzed using aV4.5A TA Universal Analysis Software. Experimental temperatures used ranged between -25 and 250° C at a heating rate of 10° C/min. The characteristic temperatures ( $T_g$ ,  $T_m$ , and  $T_c$ ) were obtained after removing the thermal history of the samples by running first and second DSC scans.

**The Fourier Transform Infra-Red (FT-IR) analysis:** FT-IR is highly reliable in determining the chemical composition of microplastic fragments (Hidalgo-Ruz *et al.*, 2012; Shim *et al.*, 2017). It presents the structural groups and reflects the optical responses of the surface functional groups. It is useful in detecting functional groups, characterizing covalent bonding information and data interpretation for polymers (Fan *et al.*, 2021; Ludwig *et al.*, 2018). The FT-IR shows the composition of a polymer, and the functional groups present giving information about the vibration and rotation of the chemical bonding and molecular structures (Zbyszewski and Corcoran, 2011; Lorder *et al.*, 2015). Like in DSC analysis, there were no sample preparations prior to analysis. FTIR is rapid and quite reliable in identifying polymer types of different MPs (Veerasingam *et al.*, 2020). Subsamples of nine microplastic particles each from surface water and marine sediments were analyzed according to Baker *et al.*, (2014) protocol using a Fourier Transform Infrared (FT-IR) spectrometer (Shimadzu IR Affinity-1S, Japan). Cleaning of the ATR diamond crystal was done using absolute ethanol. Before the analysis of each sample, background scans were performed to eliminate carbon dioxide and humidity for quality spectra. Spectra wavelengths ranged from 4000 cm<sup>-1</sup> to 500 cm<sup>-1</sup> with data interval of 1cm<sup>-1</sup> and spectra resolution of 4 cm<sup>-1</sup>.

Each sample was placed onto the center of the crystal plate and the pressure pump lowered by turning the control knob, compressing the sample against the diamond to ensure good contact between the sample and the ATR crystal. Absorption spectra were recorded and identified by comparison with polymers in the research gate spectra library and in literature (Frere *et al.*, 2016; Veerasingam *et al.*, 2020).

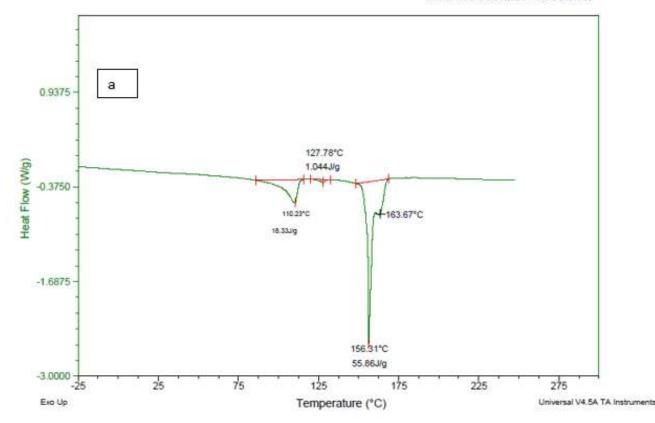
**NMR:** NMR is fast, size independent and has high accuracy. It identifies and quantifies the type of branching present in a polymer and provides molecular structure information (Peez *et al.*, 2018). Sub-samples from all the nine stations were analyzed. Samples were prepared by complete dissolution in the specimen tube using appropriate deuterated solvents and temperature. The

samples obtained from fish (F), water (W) and sediments (S) were dissolved overnight in the deuterated benzene and proton NMR done using a 600 MHz Bruker Avance NEO equipped with a nitrogen cooled TCI cryoprobe (gives 2-4 times better signal-to-noise than regular probes). Spectra obtained were compared with spectra in the literature.

## 7.3 Results

## 7.3.1 Microplastic polymer types in surface water

The DSC analyses characterizedfour different classes of synthetic polymers (LDPE, HDPE, MDPE, and PP) in the microplastic samples studied (fig 7.1). The DSC profile exhibited one melting peak for LDPE that occurred at between 110-115° C, while DSC for MDPE showed both main and second temperature peaks falling at 123° C and 126° C respectively. The second melting peak could be due to short-chain (lower molecular weight) polymer segments. The melting peak for HDPE is about fifteen degrees higher than that of LDPE, an indication that HDPE is highly ordered with less branching.



Instrument: DSC Q100 V9.9 Build 303

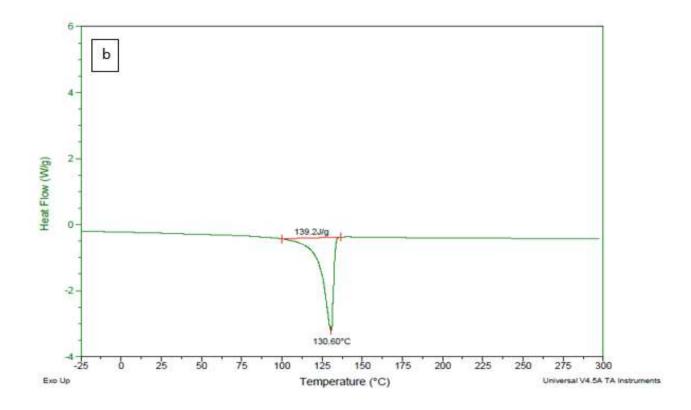


Figure 7. 1: DSC curves (a) MDPE and PP sample SK 7 (b) HDPE sample SK 3 from Tudor creek

The polymers exhibited varied melting peaks with some having two while others had one (Table 7.1).

	Weight			Enthalpy		Enthalpy
Sample	$(mg)T_M1$	$T_{m1}^{\circ}C$	$T_{m2}^{\circ}C$	(J/g)	$T_c \circ C$	(J/g)
HDPE	0.91	129.31		96.84	118.16	77.64
HDPE	1.49	127		37.49	118.93	34.34
HDPE	0.9	127.48		51.72	118.33	51.4
PP	2.09	157.51	165.3	9.89		
LDPE	2.09	115.23		1.72	106.19	1.81
HDPE	2.09	129.91		1.55	119.69	2.32
MDPE	1.8	123.92	128.37	70.24	113.83	20.51

Table 7.1 Important parameters of microplastic polymers from surface water samples.

FTIR characterization of Polyethylene showed two bands at 2914 cm<sup>-1</sup> and 2849 cm<sup>-1</sup>due to the C-H stretch of the methylene group (CH<sub>2</sub>) (Fig.7.2b). This is in agreement with values of 2915 and 2845 cm<sup>-1</sup> C-H stretching vibrations reported by Jung *et al.*, (2018). The peak observed around 1472 cm<sup>-1</sup> is attributed to the CH<sub>2</sub> bending vibration. A medium (1033 cm<sup>-1</sup>) and weak (720 cm<sup>-1</sup>) peaks were observed. The peak at 720 cm<sup>-1</sup> could be assigned to CH<sub>2</sub> rocking vibration of Highdensity polyethylene (HDPE) as observed by Jung *et al.*, (2018). These results are in agreement with earlier research on polyethylene characterization by FTIR (Gulmine *et al.*, 2002, Jung *et al.*, (2018 and Majewski *et al.*, 2016). As observed by Majewski *et al.*, (2016), polyethylene and polypropylene can be qualitatively identified with DSC by their specific endothermic peak temperatures. The spectra were compared with spectra from previously published polymer chemistry literature in libraries (Fig. 7.2) (Peltzer and Simoneau, 2013). The results demonstrated that attenuated total reflectance (ATR FT-IR) analysis can be used to differentiate HDPE and LDPE (Fig 7.3) in line with findings by Jung *et al.*, (2018)

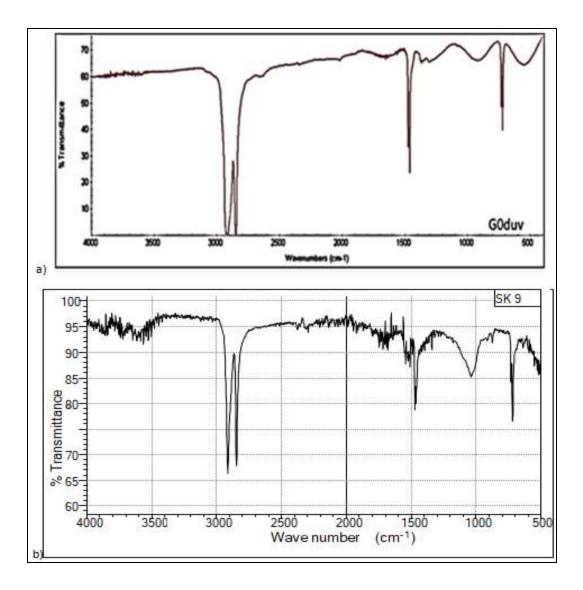


Figure 7.2: ART- FTIR spectra (a) the reference LDPE from search gate library (b) LDPE sample SK 9 from Port-Reitz Mwache-SGR

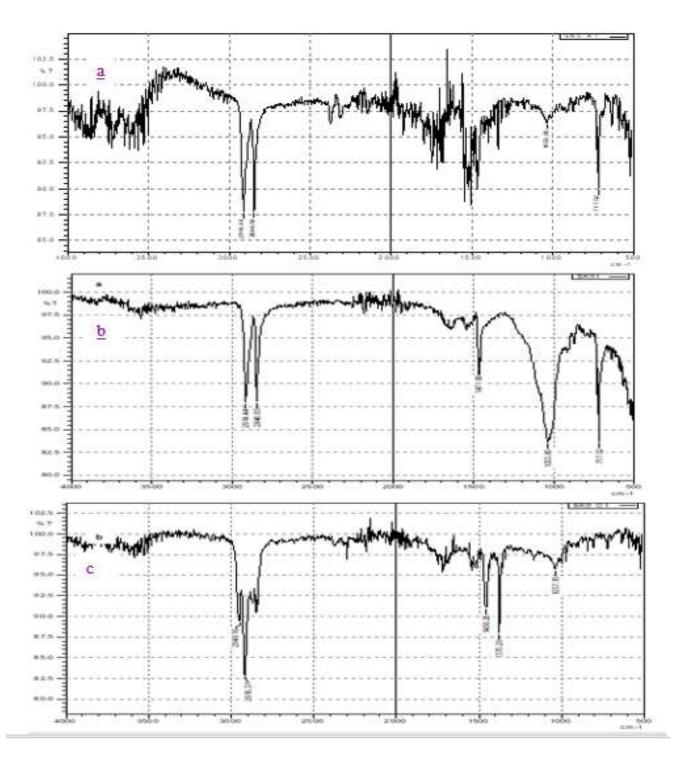


Figure 7.3 ART- FTIR spectra of MPs from surface water from Port: Reitz a: LDPE: Tudor b: HDPE c: PP

#### NMR analysis of microplastic polymer types from surface water, sediments and fish.

The microplastic samples showed slight solubility in benzene-d6 and showed no dissolution in other common NMR solvents. Microplastic samples obtained from fish, sediments and water are presented in Figures 7.4 and 7.5. All the samples analyzed from the two creeks showed the characteristic methylene protons (CH2) chemical shift in the region 1.2 to 1.41 ppm and methyl (CH3) protons signals below 1 ppm. These results are consistent with observations by (Peez *et al.*, 2019; Long *et al.*, 2021).

Further, the proton spectrum for S2 had enough signal to carry out an edited HSQC. The microplastics obtained from sediments (S2) (Fig.7.5) showed chemical shifts (1H, 13C) at 6.92, 130.43ppm and 8.13,129.46 ppm, 4.28,67.75 ppm and 4.18,67.27 ppm, 1.21,23.12 ppm to 1.29,23.92 ppm, 1.51,38.91 ppm to 1.59,38.91ppm, and 0.82,10.87 ppm suggesting presence of phthalates. Duchowny and Adams (2021) reported the 1H NMR spectra of various plasticizers. The aromatic peaks were observed in the region approx., 7 ppm, the  $\alpha$ -CH2 groups next to the ester bond (3 to 4 ppm), aliphatic CH2 (around 1 ppm) and the CH3 chain ends around 0.8 ppm consistent with the general structure of phthalates derived from phthalic anhydrides.

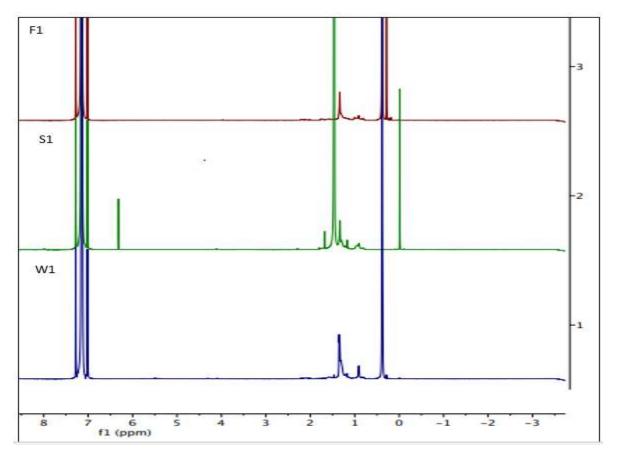


Figure 7.4 NMR proton spectra of microplastics from fish (F1 – Port-Reitz), sediments (S1 - Tudor) and water (W1 - Tudor).

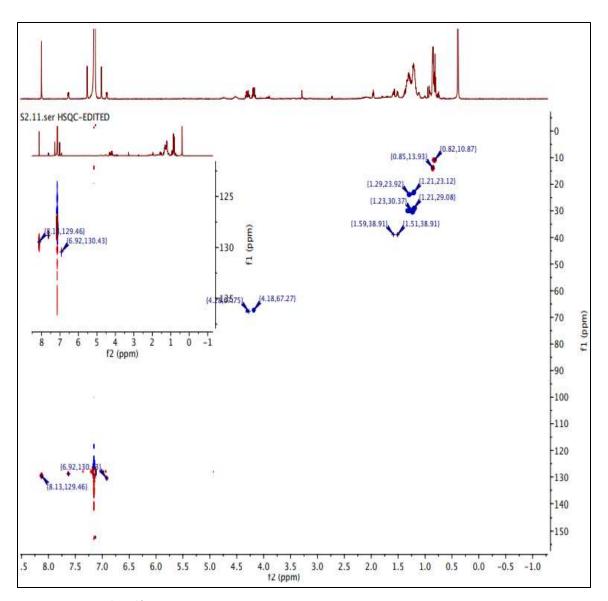


Figure 7.5 2D <sup>1</sup>H–<sup>13</sup>C HSQC of microplastic obtained from sediments (S2 - Mida)

## 7.3.2 Microplastic polymer types and abundance in surface water.

Generally, the concentration of polymers between types showed significant variation (p < 0.05). High Density Polyethylene was more abundant accounting for 38.3 % compared to LDPE 27.1 % and PP 34.6 % of total MPs extracted. These results are similar to research findings from the Italian Minor Islands with polyethylene being more abundant accounting for 20% compared to polypropylene 11% of the total microplastics extracted (De Lucia *et al.*, 2018) and the South Ocean (with Polyethylene accounting for 61% compared to polypropylene 29.2% of the total microplastics extracted (Suaria *et al.*, 2020). Among sites, HDPE was found to account for 33 %;

26 %; 19 % of the total polymer particle extracted in Tudor, Mida, and Port-Reitz respectively, while PP accounted for 33 % of the total microplastic particles extracted in Port- Reitz, compared to 17 and 14 % microplastic particles in Mida, and Tudor respectively (Fig. 7.6). Medium-density polyethylene (MDPE) was very rare only occurring in Tudor creek surface water with 17 % of the total polymer particles extracted.

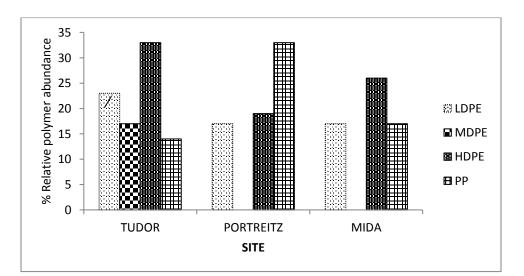


Figure 7.6 Differences in relative abundance (%) of the polymer types in surface water between sites as identified by DCS and FTIR (n=685).

#### 7.3.3 Microplastic polymer types and abundance in sediments

Unlike in surface water samples, three microplastic polymer types were identified with parameters falling within normal range (Table 7.2)

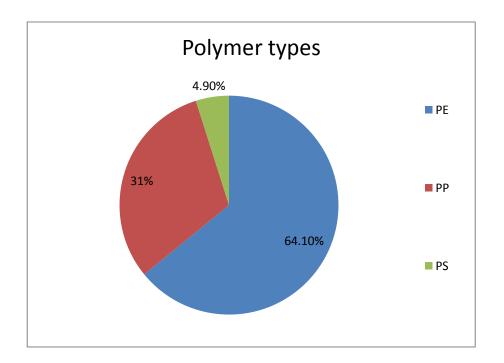
Weight				Enthalpy	Enthalpy	
Sample	(mg) T <sub>M</sub> 1	$T_{m1}^{\circ}C$	$T_{m2}^{\circ}C$	(J/g)	$T_c^{\circ}C$	(J/g)
PP	1.8	157.66	163.95	29.07	101.54	13.65
LDPE	2.7	110.23		18.33	55.58	
HDPE	0.9	127.78		1.04	118.25	1.22
PP	2.7	156.31	163.67	55.86	101.54	13.65
HDPE	3.7	130.6		139.2	119.29	124.6

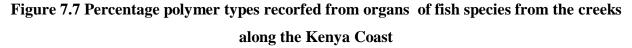
 Table 7.2 Important parameters of microplastic polymers from sediment samples

None of the sediment samples tested contained MDPE plastic polymer. The polymer distribution varied between types and HDPE ( $0.42 \pm 0.01$  particles g-1 d.w.) was more compared to LDPE ( $0.22 \pm 0.002$ ) and PP ( $0.18 \pm 0.01$ ) particles g<sup>-1</sup> d.w. Among sites, the mean concentration of HDPE differed significantly (p< 0.05) and was higher in Tudor ( $0.53 \pm 0.015$  particles g<sup>-1</sup> d.w.) (33 %) followed by Mida and Port-Reitz ( $0.45 \pm 0.041$  and  $0.27 \pm 0.023$ ) particles g<sup>-1</sup> d.w (28 %, 17 %) of the total polymer particles extracted respectively. Similarly, LDPE mean concentrations were higher in Mida ( $0.27 \pm 0.017$  particles g<sup>-1</sup> d.w). The mean concentration for PP showed no significant difference (p > 0.05) but was more abundant in Tudor ( $0.27 \pm 0.127$  particles g<sup>-1</sup> d.w. 17 %)

## 7.3.4 Polymers in fish

Fourier Transform Infra-Red and DSC analyses identified PE, PP and PS as the major polymers observed in the fish samples across species and organ tissues. Of the polymer types identified, Polyethylene (PE) polymers dominated with a relatively higher percentage compared to polypropylene (PP) and polystyrene (PS) (Figure 7.7).





#### 7.4 Discussion

The use of two or more methods for polymer identification reduces multiple interpretations and increases the chances of correct identification. However, FTIR is not able to characterize black particles (Kappler *et al.*, 2016), and gives limited information about the functional groups present in a polymer structure, and the force applied to samples to ensure good contact can dent vulnerable samples (Mitchell *et al.*, 2013).

Polyethylene is used in; i) packaging applications such as pharmaceutical and squeeze bottles, caps and closures, tamper evident liners, trash bags, films for food packaging, bubble wraps, thick shopping bags, laminations, crates, trays, bottles for milk. Juice fruits, caps for food packaging, jerrycans, drums, industrial bulk containers among others, ii) consumer goods such as garbage containers and refuse sacks, housewares, ice boxes, toys among others iii) fibres and textiles such as in ropes, fishing and sports nets, nets for agricultural use, industrial and decorative fabrics among others, iv) pipes and fittings such as pipes for gas, water, sewage, drainage, sea outfalls, industrial application cable protection, steel pipe coating among others, v) automotive products such as fuel tanks, wiring and cables for sheeting of energy, telecommunication cables among oth ers (Patel, 2016). Like PE, in addition to packaging applications PP is used in the production of electronic products, films, graphics art applications, disposable diaper tabs and closures, furniture applications, crates, bottles and pots, translucent parts, houseware, luggage, toys, automotive appliances such as battery cases and trays, bumpers and fender liners, slit films, tape, strapping, staple fibers, ropes and twines, medical application products such as disposable syringes, medical vials, diagnostic devices, intravenous bottles, petri dishes, food trays, pans, pill containers among others, and industrial application products such as acid and chemical tanks, sheets, pipes, returnable transport packaging among others (Patel, 2016). All these plastic products are likely to leak to the environment leading to contamination.

Low density polymers mainly PE and PP were observed in both water and sediment environments, which was no surprise because they are among the polymer types that accounted for 74 % of global plastic production in 2015, and are the leading polymers in plastic production commonly used in short-cycle products (Geyer *et al*, 2017: Plastics Europe, 2017). High density polyethylene polymers predominated both in surface water (38.8 %) and in sediments (51.2 %). High density particles can enter the water column by resuspension of the bottom sediment. Nearshore circulation

and offshore tides influence sediment resuspension in water estuaries contributing to redistribution and discharge of substances between water and sediments. The presence of low-density polymers in sediments can be attributed to change of densities with weathering and biofouling in the water and strong turbulences caused by wind, waves or currents that cause sedimentation of the polymers (Kukulka *et al.*, 2012). Our results show that low density polymers are widely distributed in both water and sediment environments, while high density polymers were not observed, which could imply different transport and deposition mechanisms, while suggesting sewage, synthetic textiles, packaging material and fishing gear as important sources of the MPs (Browne *et al.*, 2010; Cole *et al.*, 2014) in the creeks along the Kenya coast.

Microplastics of different polymers occurred in sediments and surface water samples from all sites including Mida creek, within Watamu National Marine Reserve thought to be safe from pollution by industrial effluents, sewage disposal, and fishing activities. This can be due to the high tourism activity, boat and dhow fishing activities (own observation), densely populated villages such as Dabaso, Ngala, and Kirepwe (IAME, 2018), and the mangrove vegetation cover of tall trees that bind soil particles favoring microplastic accumulation.

Tudor creek is fed by two major seasonal rivers; Kombeni and Tsalu (Kitheka, 1999) which collect runoff with plastic and other waste debris from the mainland and discharge them into the creek. The creek experiences strong waves and currents (Kitheka, 1999) but the shore had a reasonably thick mangrove vegetation cover (own observation) whose roots bind sediments together to hold microplastic particles. Rapid urbanization has led to the development of heavy industries at Changamwe and densely populated informal settlements like the Mikindani, Coast General Hospital, and KMC settlements (UN Habitat, 2014; County Government of Mombasa, 2018) around the creek that may be adding onto the microplastics brought in by the seasonal rivers and ocean currents through the release of raw domestic waste. The many industries in Mombasa Island release their effluents into the sea thereby increasing microplastics in sediments but, the proportions were not determined by this study.

#### 7.5 Conclusions

The results from the study provide evidence that the marine environment along the Kenya coast is polluted with microplastics of different polymer types. The analysis showed that physiographic

factors did not influence the distribution of microplastics. The accumulation of microplastic polymers within the creeks may be of serious concern because of their ability to absorb and concentrate POPs, passing the toxins up trophic levels when ingested by plankton species. Marine planktons form the base of the marine food web and any threat to them may have serious negative impacts in the oceans. There is a need of quantifying the levels and establishing the sources of synthetic polymers PE and PP in the WIO along the Kenya coast and assess the future impacts of soaring microplastic levels on oceans globally.

FT-IR, DSC and NMR analyses indicated that high-density polymers such as polyvinylchloride and polyethylene Terephthalate were not obtained probably due to the medium of extraction used (Sodium Chloride solution) which could have favored low-density microplastic fibers, therefore underestimating the microplastic concentrations found in the region.

This study provides insights into the presence, concentration, and type of microplastic polymers, providing impetusfor monitoring microplastics in the WIO along the Kenya coast. The information also offers a basis for an evaluation of the effect of the Kenya government ban effected in August 2017 on low weight plastic bag production, and use in Kenya (NEMA, 2017). This study is important since the knowledge can be used for proper policy formulation regarding the plastic production, waste management and disposal to save oceans that are rich in biodiversity.

## 7.6 Recommendations

- This study recommends that further research on occurrence and abundance of MPs could be done, using a different medium of extraction and also subject the microplastic samples to GC-MS analysis to detect any presence of other types of polymers present in the marine ecosystems.
- Governments should protect the ocean through legislation on plastic waste management, to enhance sound waste management techniques such as obliging producers and consumers to meet the cost of plastic waste disposal and management, encourage the development of plastic recycling industries by creating assistance programs for those in need of waste management system expertise.
- Produce alternative packaging materials to plastics such as sisal bags and other products like metal drinking bottles, food containers and glass straws to reduce the amount of PE and PP polymers entering the environment.

• The Kenya Government should revive the sisal processing and paper making industries to produce alternative packaging materials to reduce the amount of plastic getting into the ocean.

#### **CHAPTER EIGHT**

## **8.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS**

#### 8.1 General Discussion.

The current study surveyed three sites within creeks in two coastal counties to assess the presence, abundance and types of MPs in the marine ecosystems. Mombasa and Kilifi counties have experienced growth in fisheries, tourism, plastic manufacturing and other manufacturing industries which have led to rapid urbanization and population growth, hence increased domestic waste release and other solid waste into the ocean. Despite this, knowledge on plastic pollution in the creeks within the counties is lacking. The level of plastic pollution in the coastal creeks along the Kenya Coast has not been established. The few studies focused on MPs in surface water and zooplanktons in the Kenya central EEZ and Gazi Bay (Kosore *et al.*, 2018) and MPs in benthic invertebrates within the creeks (Awour *et al.*, 2020), but this study has collected information on the presence, abundance and types of MPs in surface water and sediments within the creeks and the ingestion of MPs by common local fish.

The current study design in which the study area was stratified then random sampling points selected in each station ensured substantial coverage. This was useful in determining the presence, abundance and types/shapes of MPs in the marine ecosystem. The bulk sampling and net towing techniques were good for water sample collection while coring up to 10 cm deep was appropriate for sediment sample collection. Collection of fish samples for MPs analysis from fishermen on site provided a good sample size. Replicate sampling increased accuracy as appropriate sieve sizes ensured maximum extraction of MPs size categories from the collected samples. Digestion of samples with an alkaline solution (10 % KOH at 60 <sup>o</sup>C) effectively removed organic matter from samples making extraction and characterization of MPs easy and minimizing chances of misidentification or underestimation of the small particles (Dehaut *et al.*, 2016).

The physico-chemical factors were similar during the two sampling campaigns and the ocean was calm with low wind and wave intensity (Maes *et al.*, 2017) which could have contributed to uniform distribution of MPs in the sites. The different sampling methodologies used in different parts of the world led to different recoveries. This study used bulk sampling and sieved the water samples through 20 µm net size for the small size particles extracting high concentrations of MPs

from surface water. The high concentration of MPs was attributed to the many anthropogenic activities within the creeks. As MPs concentrations for the medium and large sizes were <5 particle  $m^{-3}$  and <1 particle  $m^{-3}$  respectively, MPs could have been pushed out of the net by currents generated during towing (Kang *et al.*, 2015) making it a non-efficient sampling protocol, although net towing is known to overcome the heterogenicity of the distribution of MPs on the water surface (Eriksen *et al.*, 2018). Stations with populated suburban villages and many anthropogenic activities such as Mikindani and Makupa were noted to have higher concentrations of MPs in the water column compared to others within the sites (Kerubo *et al.*, 2020). The kibarani waste dumpsite at Makupa could have greatly contributed to the high concentrations of MPs in the station.

The presence of MPs in marine sediments confirms sediments as the MPs sinks, and therefore a potential risk to benthic organisms as well as those higher up trophic levels due to bioaccumulation and biomagnification. Microplastic concentrations in sediments were higher in stations with high concentrations of MPs in the water column (Chapter four). The abundance of MPs in surface water and sediments is affected by waterflow within the creeks, the strength of waves and currents, water temperature, ocean salinity, population density and anthropogenic activities (Okuku *et al.*, 2019). Microplastics abundance suspected to be associated with river flow and surface runoff was greater in Port-Reitz compared to Tudor and Mida. This could have been probably because heavy surface runoff and fast river velocity brought in more plastics especially during the second sampling campaign when rainfall was high through the rivers that feed the creek. This was consistent with findings of studies conducted in river Yangtze, China which showed that increased river volume and velocity increased plastic abundance in the water column and ultimately sediments (Lebreton *et al.*, 2017). Proper plastic waste management and disposal will minimize land-based plastics into the ocean.

The predominance of MPs fibres in the ecosystems in the Kenya central EEZ and Gazi Bay (Kosore *et al.*, 2018) and in zooplanktons was confirmed by this study. Information on MPs shapes helps to determine the sources of MPs in the marine ecosystems.

Microplastics were detected in all the fish species with very low concentrations in muscle tissues <0.2 particles g<sup>-1</sup> but high in the gut and gills. Several factors affect the ingestion of MPs by organisms. The factors include MPs abundance, size, density and the organism's feeding mode

(Nerves *et al.*, 2015; Li *et al.*, 2016). The findings confirm the potential risk of consuming whole fish or seafood without gutting to human health (Thompson, 2018).

Chemical identification through DSC, NMR and FTIR analyses characterizesMPs as PE (LDPE, HDPE, MDPE), PP and PS plastic polymers. Information of polymer type can help identify the sources of plastics in the creeks along the Kenya Coast. Polyethylene dominated both surface water and sediment ecosystems as well as fish samples. Polystyrene polymers were found in the local common fish samples only and in small proportions compared to PE and PP. The presence of PE previously in the waters of the Kenya central EEZ and zooplanktons (Kosore *et al.*, 2018) was confirmed by this study, and shows that they are the most dominant polymers in the marine ecosystems along the Kenya Coast. The chemical source of MPs in the ecosystems could be majorly from products such as; fishing nets and lines, plastic bags, food containers, six-pack soda can ring, textile industries, engineering plastics for agricultural machine parts and water bottles. Information on the source of plastics into the ocean is important in developing measures to manage plastic waste and disposal to minimize plastic pollution.

#### **8.2 General Conclusions**

In this study, MPs abundance and distribution in the creeks along the Kenya Coast seems to be associated with a combination of factors including; general water circulation, location and anthropogenic activities. Limited water circulation accumulates MPs in both surface water and sediments within creeks. Microplastics from land brought in by rivers and surface runoff and those from far-off locations brought in by waves, currents and wind remain within the creeks posing a health risk to biota therein. All the creeks along the Kenya Coast including Mida within a National Marine Reserve are polluted with MPs. Preventing entry of plastics into oceans could lower the degree of exposure of the crabs to MPs and reduce the risk human health.

Rapid urbanization and increased anthropogenic activities in the coastal region along the Kenya Coast have increased MPs in the water column. Proper policies on the disposal of solid domestic waste, effluent from waste water treatment plants, industrial effluents, worn out fishing nets and lines, shipping plastic waste, tourism plastic waste could reduce MPs pollution in the ocean.

The presence of MPs in marine sediments is an indication of the risk to the health of the benthic organisms and those higher up the trophic levels through bioaccumulation and biomagnification. Preventing land based plastic sources in addition to proper plastic waste management policies and practices could reduce MPs in marine sediments.

The higher concentrations of MPs in the marine ecosystems of Makupa in Port-Reitz is associated with the Kibarani dumpsite that has been in place for decades of years and releases leachates into the ocean especially during the rainy season.

Ingestion of MPs by common local fish within the creeks along the Kenya Coast poses a health risk to humans if the fish are consumed whole since high concentrations were recovered from the guts and gills of all the fish species investigated. Care should be taken during the preparation of such fish for consumption. The presence of MPs in muscle tissues is evidence of the potential danger of fish to humans. Reduction in the release of plastics into the ocean through actions such as the 2017 ban on use of plastic bags in Kenya could minimize exposure of fish to MPs and therefore humans.

The abundance of white fibres in the marine ecosystems along the Kenya Coast implies that fishing lines and nets are the major sources of microplastics since they are made up of white plastics. Abandoned fishing ropes, lines and nets degrade and pollute the marine ecosystems with MPs. Regular beach cleaning and proper disposal of won out fishing gear could minimize the danger of exposure of organisms to MPs.

Chemical identification of plastics through DSC and FTIR analyses identified MPs as PE (LDPE, HDPE, MDPE), PP and PS plastic polymers. Polyethylene polymers dominated the surface water and sediments as well as fish corresponding with their huge production accounting for > 60 % plastic production and use. The chemical composition of the MPs recovered and the dominance of fibres (< 90 %) is associated with several plastic waste sources including plastic bags, fishing gear, textile industries, food packaging industries and waste water treatment plants.

The results of this study provide a baseline for future monitoring of the effect of the Kenya Government ban on single use plastic carriers of February 2017. Future assessments can be compared with these results to establish whether the ban is making a difference in the extent of

MPs pollution in the coastal waters. The results represent inshore marine environments in the Western Indian Ocean and is one of the first assessments in the region.

# 8.3 General recommendations.

- The high concentration of MPs in the creeks along the Kenya coast raises concern on the need to develop measures on prevention of input or release of plastics into the ocean ecosystems as the most effective way of reducing plastic and MPs pollution. To achieve this, improvement of plastic waste management around the ocean and along the rivers feeding the creeks, increased public awareness and education on the general environment protection, development and Promotion of environment friendly and alternatives to plastics are encouraged.
- Marine plastic pollution control should be through properly formulated effective policies on plastic waste management and disposal to reduce their accumulation in the ocean ecosystems.
- Studies involving bulk sampling techniques are strongly encouraged to test the recovery of all microplastic size categories to reduce underestimation of MPs concentrations in marine surface water. The technique is preferred to net towing as it is economical, easy and fast. Monitoring studies should be conducted during the dry and rainy seasons, and during the Northeast and Southeast Monsoon, when fishing activities are different along the Kenyan coast.
- It is important to understand the environmental variables that may potentially affect MPs abundance and composition in the ocean. Investigation to establish the interaction between various physico-chemical parameters and MPs abundance in surface water and sediments is strongly encouraged.
- This study serves as a first step that identifies and evaluates the extent of plastic pollution in surface water, sediments and fish species within the creeks. The concentrations detected indicate the need to evaluate the accumulation and effects of MPs in fish and other organisms in the marine ecosystems since very small concentrations have been found to affect both invertebrates and vertebrates.

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### APPENDICES

## **APPENDIX 1:** Parameters of surface water at different sampling stations

# First sampling (Jan/Feb 2018)

DATE	STATION	SIZE (µm)	Salinity (psu)	Conductivity	Temperature	Water volume (m <sup>3</sup> )
31/01/2018	Mikindani	500	33.7	55559	29.1	13689.4
31/01/2018	Nyali -B	500	35.9	53139	29.9	6609.1
31/01/2018	K.M.C	500	33.4	54734	28.7	10888.1
1/2/2018	Makupa	500	33.4	55291	28.8	12895.7
1/5/2018	Mwa-T	500	34.5	56669	28.5	9797.4
2/2/2018	Mwa-SGR	500	33.7	54356	28.0	8983.2
3/2/2018	Kirepwe	500	34.1	54893	27.9	10746.1
31/01/2018	Mikindani	250	33.7	55559	29.1	249.7
31/01/2018	Nyali- B	250	35.9	53139	29.9	233.0
31/01/2018	K. M. C.	250	33.4	54734	28.7	276.3
1/2/2018	Makupa	250	33.4	55291	28.8	401.5
1/5/2018	Maw-T	250	34.5	56669	28.5	452.0
2/2/2018	Mwa-SGR	250	33.7	54356	28.0	397.4
3/2/2018	Kirepwe	250	34.1	54893	27.9	264.1
31/01/2018	Mikindani	20	33.7	55559	29.1	0.05
31/01/2018	Nyali -B	20	35.9	53139	29.9	0.05
31/01/2018	K.M.C	20	33.4	54734	28.7	0.05
1/2/2018	Makupa	20	33.4	55291	28.8	0.05
1/5/2018	Mwa-T	20	34.5	56669	28.5	0.05
2/2/2018	Mwa-SGR	20	33.7	54356	28.0	0.05
3/2/2018	Kirepwe	20	34.1	54893	27.9	0.05
(Second Sam	pling (Sept. 2	2018)				
13/9/2018	Mikindani	500	35	56503	19	865.4
13/9/2018	Nyali-B	500	35	56505	18.4	570.7
13/9/2018	K.M.C	500	35	56505	18	551.3
14/9/2018	Makupa	500	35	56503	18	871.3
14/9/2018	Mwa-T	500	35	56503	18.5	652.9
14/9/2018	Mwa-SGR	500	35	56503	17	636.8
15/9/2018	Kirepwe	500	35	56502	17	619.6
15/9/2019	Mayonda	500	35	56502	17.5	747.5
15/9/2022	Dabaso	500	35	56502	17.5	876.5
13/9/2018	Mikindani	250	35	56503	19	201.8
13/9/2018	Nyali-B	250	35	56505	18.4	194.7
13/9/2018	K.M.C	250	35	56505	18	182.5
14/9/2018	Makupa	250	35	56503	18	195.4
14/9/2018	Mwa-T	250	35	56503	18.5	198.9

14/9/2018	Mwa-SGR	250	35	56503	17	193.9
15/9/2018	Kirepwe	250	35	56502	17	201.2
15/9/2018	Mayonda	250	35	56502	17.5	199.5
15/9/2018	Dabaso	250	35	56502	17.5	194.8
13/9/2018	Mikindani	20	35	56503	19	0.05
13/9/2018	Nyali-B	20	35	56505	18.4	0.05
13/9/2018	K.M.C	20	35	56505	18	0.05
14/9/2018	Makupa	20	35	56503	18	0.05
14/9/2018	Mwa-T	20	35	56503	18.5	0.05
14/9/2018	Mwa-SGR	20	35	56503	17	0.05
15/9/2018	Kirepwe	20	35	56502	17	0.05
15/9/2018	Mayonda	20	35	56502	17.5	0.05
15/9/2018	Dabaso	20	35	56502	17.5	0.05

## **APPENDIX 2: Sediment grain size proportions**

			Very				Very		
	<b>•</b> ••••	Constant all states to	Coarse	Course	Medium	Fine	fine	<b></b>	
DATE	Station	Sample Label	sand	sand	sand	sand	sand		Total
31/01/2018	Mikindani	JAN-TUR-MIK-A	23.6	25.3	8.3	25.5	15.3	2.1	100
31/01/2018	Mikindani	JAN-TUR-MIK-B	29.8	22.5	7.4	24.7	13.2	2.5	100
31/2/2018	Mikindani	JAN-TUR-MIK-C	22.5	25.1	8.5	27.1	15.7	1.1	100
31/01/2018	КМС	JAN-TUR-KMC-A	23.8	14.8	6.5	27.9	24.9	2.1	100
31/01/2018	КМС	JAN-TUR-KMC-B	23.0	13.5	6.6	32.4	21.1	3.5	100
31/01/2018	KMC	JAN-TUR-KMC-C	25.3	12.7	5.9	26.2	25.7	4.3	100
31/01/2018	Nyali-B	JAN-TUR CGN-A	13.8	27.6	11.9	27.9	14.1	4.7	100
31/01/2018	Nyali-B	JAN-TUR CGN-B	13.3	25.0	13.5	31.2	15.1	2.0	100
31/01/2018	Nyali-B	JAN-TUR CGN-C	14.6	31.1	12.5	24.7	14.4	2.7	100
1/2/2018	Makupa	JAN-MAKUPA-A	16.7	27.1	14.0	32.3	8.7	1.2	100
1/3/2018	Makupa	JAN-MAKUPA-B	14.9	27.8	14.3	33.8	7.6	1.6	100
1/4/2018	Makupa	JAN-MAKUPA-C	12.3	9.9	4.9	21.3	49.4	2.3	100
1/5/2018	Mwache-T	JAN-PR- MWA- T-A	0.4	7.3	3.8	10.5	72.6	5.3	100
1/6/2018	Mwache-T	JAN-PR- MWA-T- B	2.0	5.9	3.1	10.0	68.9	10.0	100
1/7/2018	Mwache-T	JAN-PR- MWA- T-C	2.4	6.4	4.0	11.0	68.4	7.8	100
2/2/2018	Mwache-SGR	JAN-PR-MWA- SGR-A	10.1	12.0	15.1	54.2	5.5	3.2	100
2/3/2018	Mwache-SGR	JAN-PR-MWA- SGR-B	10.2	10.3	5.6	39.4	31.6	3.0	100
2/4/2018	Mwache-SGR	JAN-PR-MWA- SGR-C	8.6	10.5	4.7	12.1	60.4	3.7	100
3/2/2018	Dabaso	JAN-Mida Dab-A	4.9	10.4	7.0	49.9	18.5	9.3	100
3/3/2018	Dabaso	JAN-Mida Dab-B	14.9	12.4	7.9	42.0	13.7	9.1	100
3/4/2018	Dabaso	JAN-Mida Dab-C	12.6	12.2	7.2	41.2	16.3	10.5	100
31/01/2018		Second sampling							
31/01/2018	Mikindani	SEP-TUR-MIK-A	0.00	1.62	11.34	22.33	34.35	30.36	5 100
31/01/2018	Mikindani	SEP-TUR-MIK-B	0.00	4.17	15.47	23.77	32.46	24.13	100
31/01/2018	Mikindani	SEP-TUR-MIK-C	7.69	3.27	5.59	21.16	32.63	29.65	5 100
31/01/2018	КМС	SEP-TUR-KMC-A	0.00	0.00	0.03	21.19	51.78	27.00	100
31/01/2018	КМС	SEP-TUR-KMC-B	0.00	0.14	8.03	26.65	38.92	26.27	100
31/01/2018	КМС	SEP-TUR-KMC-C	2.25	7.58	3 7.33	30.23	37.06	15.55	5 100
31/01/2018	Nyali-B	SEP-TUR CGN-A	0.79	7.72	2 16.56	25.51	30.83	18.58	3 100
31/01/2018	Nyali-B	SEP-TUR CGN-B	0.00	1.99	9 12.69	26.67	35.89	22.76	5 100
1/2/2018	Nyali-B	SEP-TUR CGN-C	0.00	0.00	) 5.24	22.05	25.41	47.30	) 100
1/3/2018	Mwache-T	SEP-PR- MWA-A	0.00	0.00	0.00	27.69	56.11	16.20	) 100
1/4/2018	Mwache-T	SEP-PR- MWA- B	0.00	0.00	0.00	11.13	57.59	31.28	3 100
1/5/2018	Mwache-T	SEP-PR- MWAC	0.68	3.19	9 0.91	23.27	51.44	20.50	) 100
1/6/2018	Mwache-SGR	SEP-PR-MWA- SGR-A	0.00	0.22	L 3.92	22.29	43.54	30.03	3 100
1/7/2018	Mwache-SGR	SEP-PR-MWA- SGR-B	0.00	0.00	0.00	0.00	1.55	98.45	5 100
2/2/2018	Mwache-SGR	SEP-PR-MWA- SGR-C	19.07			13.37		15.10	

2/3/2018	Makupa	SEP-MAKUPA-A	2.35	21.82	30.75	25.08	13.69	6.31	100
2/4/2018	Makupa	SEP-MAKUPA-B	5.17	26.36	29.99	22.04	10.53	5.91	100
3/2/2018	Makupa	SEP-MAKUPA-C	5.67	14.97	23.41	25.66	19.53	10.75	100
3/3/2018	Dabaso	SEP-Mida DabA	0.00	4.31	18.18	31.58	28.81	17.12	100
3/4/2018	Dabaso	SEP-Mida Dab-B	0.00	1.01	18.30	28.46	24.37	27.86	100
31/01/2018	Dabaso	SEP-Mida Dab-C	0.20	5.29	19.58	31.45	26.63	16.84	100
31/01/2018	Mayonda	SEP-Mida May-A	0.00	0.03	34.38	60.88	4.72	0.00	100
31/2/2018	Mayonda	SEP-Mida May-B	0.00	0.09	29.12	59.64	10.74	0.40	100
31/01/2018	Mayonda	SEP-Mida Mayo-C	2.17	0.80	33.21	54.57	8.74	0.51	100
31/01/2018	Kirepwe	EP-Mida Kirepwe-A	0.00	7.21	59.80	28.23	0.87	3.90	100
31/01/2018	Kirepwe	SEP-Mida Kirepwe-B	0.00	6.14	67.20	26.38	0.04	0.25	100
31/01/2018	Kirepwe	SEP-Mida Kirepwe-C	0.00	7.55	71.06	21.16	0.00	0.23	100

# **APPENDIX 3: Morphometrics of individual fish species**

## First sampling (Jan/Feb 2018)

First sampling	(Jan/Feb 2018)		<b>T</b> I		
	(TATION)		Total	<b>TI</b> ( )	
SITE	STATION	SPECIES	BW (g)	TL (cm)	SL (cm)
TUDOR	Mikindani	Geres oyena	34	13	10.5
TUDOR	Mikindani	Geres oyena	41	13.5	10.8
TUDOR	Mikindani	Geres oyena	34	12.9	10.6
TUDOR	Mikindani	Geres oyena	37	13	10.5
TUDOR	Mikindani	Geres oyena	29	12	9.7
TUDOR	Mikindani	Geres oyena	44	14	10.9
TUDOR	Mikindani	Geres oyena	36	13.2	10.7
TUDOR	Mikindani	Geres oyena	47	13.6	10.8
TUDOR	Mikindani	Geres oyena	32	12.2	10.3
TUDOR	Mikindani	Geres oyena	30	11.9	9.5
TUDOR	Mikindani	Geres oyena	43	13.5	10.6
TUDOR	Mikindani	Geres oyena	40	13.6	10.6
TUDOR	Mikindani	Geres oyena	34	12.7	10.4
TUDOR	Mikindani	Geres oyena	21	11.2	9.1
TUDOR	Mikindani	Geres oyena	32	12.5	10.3
TUDOR	Fort Jesus	Rastrelliger kanagurta	120.4	26.5	18.5
TUDOR	Fort Jesus	Rastrelliger kanagurta	121.3	22.7	18.4
TUDOR	Fort Jesus	Rastrelliger kanagurta	120.4	25.3	18.3
TUDOR	Fort Jesus	Rastrelliger kanagurta	121	22.6	18.1
TUDOR	Fort Jesus	Rastrelliger kanagurta	117.6	22.7	18.4
TUDOR	Fort Jesus	Rastrelliger kanagurta	129.4	22.7	18.6
TUDOR	Fort Jesus	Rastrelliger kanagurta	110.3	22.4	18
TUDOR	Fort Jesus	Rastrelliger kanagurta	110.3	22.5	18.2
TUDOR	Fort Jesus	Rastrelliger kanagurta	110.3	22.4	18
TUDOR	Fort Jesus	Rastrelliger kanagurta	107.2	21.1	17.9
TUDOR	Fort Jesus	Rastrelliger kanagurta	156.1	24.3	19.5
TUDOR	Fort Jesus	Rastrelliger kanagurta	154.1	24.3	19.4
TUDOR	Fort Jesus	Rastrelliger kanagurta	153.1	24.2	19.5
TUDOR	Fort Jesus	Rastrelliger kanagurta	107.4	22.1	17.4
TUDOR	Fort Jesus	Rastrelliger kanagurta	114.8	22.7	18.4
TUDOR	Fort Jesus	Rastrelliger kanagurta	98.9	21.7	17.5
TUDOR	Fort Jesus	Rastrelliger kanagurta	135.5	23	18.5
TUDOR	Fort Jesus	Rastrelliger kanagurta	117.7	22	18.3
TUDOR	Fort Jesus	Rastrelliger kanagurta	109.7	21.4	17.8
TUDOR	Fort Jesus	Rastrelliger kanagurta	108.7	21.4	17.8
TUDOR	Fort Jesus	Rastrelliger kanagurta	100.2	21.4	17.5
TUDOR	Fort Jesus	Rastrelliger kanagurta	113.1	21.6	17.8
TUDOR	Fort Jesus	Rastrelliger kanagurta	123.6	22.8	18.6

TUDOD	Fourt Jacua	Destualling a lange south	122.0	22	10 C
TUDOR TUDOR	Fort Jesus Fort Jesus	Rastrelliger kanagurta Rastrelliger kanagurta	123.9 123.9	22 22	18.6 18.6
TUDOR	Fort Jesus	<b>a b</b>	123.9	22	18.6
TUDOR	Fort Jesus	Rastrelliger kanagurta Rastrelliger kanagurta	97.4	20.7	19
TUDOR	Fort Jesus	<b>a b</b>	97.4 96.4	20.7	17.3
		Rastrelliger kanagurta		20.5	17.3 19.7
TUDOR TUDOR	Fort Jesus Fort Jesus	Rastrelliger kanagurta	151		
		Rastrelliger kanagurta	112	21.7	17.6
TUDOR	Fort Jesus	Rastrelliger kanagurta	125.6	22.1	18.5
TUDOR	Fort Jesus	Rastrelliger kanagurta	118.7	21.5	18.3
TUDOR	Fort Jesus	Rastrelliger kanagurta	113.6	21.7	18
TUDOR	Fort Jesus	Rastrelliger kanagurta	112.5	21.6	18
TUDOR	Fort Jesus	Rastrelliger kanagurta	112.2	21.5	17.7
MIDA	Kirepwe	Gerres oyena	40	14.5	11.5
MIDA	Kirepwe	Gerres oyena	49	13.3	10.8
MIDA	Kirepwe	Gerres oyena	95	19	14.8
MIDA	Kirepwe	Gerres oyena	56	16	12.3
MIDA	Kirepwe	Gerres oyena	38	13.3	10
MIDA	Kirepwe	Gerres oyena	43	14.8	11.5
MIDA	Kirepwe	Gerres oyena	32	13.2	10.4
MIDA	Dabaso	Acanthopagrus berda	36	14	10.5
MIDA	Dabaso	Acanthopagrus berda	12	9.9	7.5
MIDA	Dabaso	Acanthopagrus berda	7	9	6.5
MIDA	Dabaso	Acanthopagrus berda	11	9.8	7.5
MIDA	Dabaso	Acanthopagrus berda	9	9	6.5
MIDA	Dabaso	Acanthopagrus berda	11	9.3	7
MIDA	Dabaso	Acanthopagrus berda	13	9.9	7.5
MIDA	Dabaso	Acanthopagrus berda	11	9.5	7.5
MIDA	Dabaso	Acanthopagrus berda	8	8.5	6.5
MIDA	Dabaso	Acanthopagrus berda	8	8.9	6.8
MIDA	Dabaso	Acanthopagrus berda	37	14.5	10.5
MIDA	Dabaso	Acanthopagrus berda	9	9.5	7
MIDA	Dabaso	Acanthopagrus berda	10	9.7	7.3
MIDA	Dabaso	Acanthopagrus berda	12	9.5	6.9
MIDA	Dabaso	Acanthopagrus berda	11	9.4	7.3
MIDA	Dabaso	Acanthopagrus berda	11	9.5	7.5
MIDA	Dabaso	Acanthopagrus berda	8	8.5	6.5
MIDA	Dabaso	Acanthopagrus berda	11	9.7	7.1
MIDA	Dabaso	Acanthopagrus berda	9	9.4	7.2
MIDA	Dabaso	Acanthopagrus berda	9	9	6.5
MIDA	Dabaso	Acanthopagrus berda	11	9.5	6.8
MIDA	Dabaso	Acanthopagrus berda	9	9.8	7.3
MIDA	Dabaso	Acanthopagrus berda	11	9.5	7.3
MIDA	Dabaso	Acanthopagrus berda	11	9.5	7
				2.0	-

MIDA	Dabaso	Acanthopagrus berda	10	9.8	7
MIDA	Dabaso	Acanthopagrus berda	11	9.6	7
MIDA	Dabaso	Acanthopagrus berda	11	9.4	7
MIDA	Dabaso	Acanthopagrus berda	8	9	6.5
MIDA	Dabaso	Acanthopagrus berda	12	9.6	7
MIDA	Dabaso	Acanthopagrus berda	11	10	7.5
MIDA	Dabaso	Acanthopagrus berda	10	9.5	7
TUDOR	English Point	Leptoscarus vaigiensis	156.09	20.5	17
TUDOR	English Point	Leptoscarus vaigiensis	132.82	19.1	17
TUDOR	English Point	Leptoscarus vaigiensis	135.6	19.5	16.6
TUDOR	English Point	Leptoscarus vaigiensis	76.32	16.5	13.8
TUDOR	English Point	Leptoscarus vaigiensis	172.11	22	16.6
TUDOR	English Point	Leptoscarus vaigiensis	247.1	23.5	18.5
TUDOR	English Point	Leptoscarus vaigiensis	120.7	18.3	16
TUDOR	English Point	Leptoscarus vaigiensis	165.49	22.3	17.3
TUDOR	English Point	Leptoscarus vaigiensis	145.95	21.5	17
TUDOR	English Point	Leptoscarus vaigiensis	78.75	17	13
PORT-REITZ	Makupa	Gerres oyena	189.7	30.5	26
PORT-REITZ	Makupa	Gerres oyena	244.8	34.5	32.5
PORT-REITZ	Makupa	Gerres oyena	48.35	21.3	16.8
PORT-REITZ	Makupa	Gerres oyena	77.26	23.2	19.4
PORT-REITZ	Makupa	Gerres oyena	228.8	33.5	29
PORT-REITZ	Makupa	Gerres oyena	147.6	28.5	24
PORT-REITZ	Makupa	Gerres oyena	98.87	25.1	21.2
PORT-REITZ	Makupa	Gerres oyena	86.1	24.6	20.7
PORT-REITZ	Makupa	Gerres oyena	225	33.5	29
PORT-REITZ	Makupa	Gerres oyena	125.7	28.4	24
PORT-REITZ	Makupa	Gerres oyena	109.8	27.2	22.2
MIDA	Kirepwe	Gerres oyena	79	17.5	13
MIDA	Kirepwe	Gerres oyena	55	16	12.5
MIDA	Kirepwe	Gerres oyena	46	15	11.5
MIDA	Kirepwe	Gerres oyena	45	14.8	11.6
MIDA	Kirepwe	Gerres oyena	28	12.5	9.8
MIDA	Kirepwe	Geres oyena	48	15.5	11.5
MIDA	Kirepwe	Gerres oyena	59	15.5	12.5
MIDA	Kirepwe	Gerres oyena	43	14.8	11.5
MIDA	Dabaso	Terapon jarbua	18	10.2	7.3
MIDA	Dabaso	Terapon jarbua	23.6	12.5	9.5
MIDA	Dabaso	Terapon jarbua	21.2	12	9
MIDA	Dabaso	Terapon jarbua	13.5	10.5	8.1
MIDA	Dabaso	Terapon jarbua	15.9	10.7	8
MIDA	Dabaso	Terapon jarbua	15.8	10.5	8.2
MIDA	Dabaso	Terapon jarbua	15.5	10.5	7.8

MIDA	Dabaso	Terapon jarbua	14.3	10.5	7.8
MIDA	Dabaso	Terapon jarbua	13.7	10.2	8.1
MIDA	Dabaso	Terapon jarbua	13.6	10.5	7.8
MIDA	Dabaso	Terapon jarbua	19.4	11.5	8.2
MIDA	Dabaso	Terapon jarbua	12.5	9.9	7.2
MIDA	Dabaso	Terapon jarbua	12.6	9.9	7.5
MIDA	Dabaso	Terapon jarbua	9.4	9.7	9.8
MIDA	Dabaso	Terapon jarbua	22.8	12.3	9.5
MIDA	Dabaso	Terapon jarbua	19.5	12.1	8.2
MIDA	Dabaso	Terapon jarbua	14.7	10.5	8.6
MIDA	Dabaso	Terapon jarbua	18.7	11.2	8.5
MIDA	Dabaso	Terapon jarbua	14.4	10.7	8.22
MIDA	Dabaso	Terapon jarbua	15.3	10.5	8.3
MIDA	Dabaso	Terapon jarbua	14.4	10.5	7.5
MIDA	Dabaso	Terapon jarbua	15.3	10.1	7.9
MIDA	Dabaso	Terapon jarbua	14	10.2	7.6
MIDA	Dabaso	Terapon jarbua	13.6	10.5	7.8
MIDA	Dabaso	Terapon jarbua	14	10.2	7.8
MIDA	Dabaso	Terapon jarbua	12.2	12.6	7.6
MIDA	Dabaso	Terapon jarbua	27.3	11.2	9.5
MIDA	Dabaso	Terapon jarbua	18.6	11.1	9
MIDA	Dabaso	Terapon jarbua	18.7	11.9	8.5
MIDA	Dabaso	Terapon jarbua	21.5	10.4	8.4
MIDA	Dabaso	Terapon jarbua	15.9	11.7	7.9
MIDA	Dabaso	Terapon jarbua	20.1	11.1	8.7
MIDA	Dabaso	Terapon jarbua	16.5	11.1	8.2
MIDA	Dabaso	Terapon jarbua	20.4	11.2	8.5
MIDA	Dabaso	Terapon jarbua	14.6	10.4	7.8
MIDA	Dabaso	Terapon jarbua	12.9	10.1	7.5
MIDA	Dabaso	Terapon jarbua	12.5	9.9	7.4
	Second sam	oling (Sept 2018)			
MIDA	Dabaso	Gerres oyena	149	13	10.5
MIDA	Dabaso	Gerres oyena	54.4	13.5	10.8
MIDA	Dabaso	Gerres oyena	39.5	12.9	10.6
MIDA	Dabaso	Gerres oyena	123.7	24.6	22.8
MIDA	Dabaso	Gerres oyena	92.4	12	9.7
MIDA	Dabaso	Gerres oyena	27.9	14	10.9
MIDA	Dabaso	Gerres oyena	170.7	30.2	24.2
MIDA	Dabaso	Gerres oyena	43.2	13.6	10.8
MIDA	Dabaso	Gerres oyena	64.4	12.2	10.3
MIDA	Dabaso	Gerres oyena	67.4	11.9	9.5
MIDA	Dabaso	Gerres oyena	54.4	13.5	10.6
MIDA	Dabaso	Gerres oyena	69.8	13.6	10.6

MIDA	Dabaso	Gerres oyena	87.8	18.3	12.8
MIDA	Dabaso	Gerres oyena	48.9	11.2	9.1
MIDA	Dabaso	Gerres oyena	46	12.5	10.3
MIDA	Dabaso	Gerres oyena	139	22.6	25.4
MIDA	Dabaso	Acanthopagrus berda	83.85	9	6.5
MIDA	Dabaso	Acanthopagrus berda	83.6	9.8	7.5
MIDA	Dabaso	Acanthopagrus berda	67.3	9	6.5
MIDA	Dabaso	Acanthopagrus berda	86.3	9.3	7
MIDA	Dabaso	Acanthopagrus berda	66.6	9.9	7.5
MIDA	Dabaso	Acanthopagrus berda	73.9	9.5	7.5
MIDA	Dabaso	Acanthopagrus berda	79	8.5	6.5
MIDA	Dabaso	Acanthopagrus berda	64.8	8.9	6.8
MIDA	Dabaso	Acanthopagrus berda	63.2	14.5	10.5
MIDA	Dabaso	Therapon jerbua	73.1	9.5	7
MIDA	Dabaso	Therapon jerbua	71	9.7	, 7.3
MIDA	Dabaso	Therapon jerbua	75.4	9.5	6.9
MIDA	Dabaso	Therapon jerbua	74.6	10.5	8.6
MIDA	Dabaso	Therapon jerbua	81	11.2	8.5
MIDA	Dabaso	Therapon jerbua	74.2	10.7	8.22
TUDOR	Fort Jesus	Rastrelliger kanerguta	51	10.5	8.3
TUDOR	Fort Jesus	Rastrelliger kanerguta	12.8	10.5	7.5
TUDOR	Fort Jesus	Rastrelliger kanerguta	38.5	10.1	7.9
TUDOR	Fort Jesus	Rastrelliger kanerguta	44.2	10.2	7.6
TUDOR	Fort Jesus	Rastrelliger kanerguta	33.7	10.5	7.8
TUDOR	Fort Jesus	Rastrelliger kanerguta	14.6	10.2	7.8
TUDOR	Mikindani	Gerres oyena	35.2	12.6	7.6
TUDOR	Mikindani	Gerres oyena	30.1	11.2	9.5
TUDOR	Mikindani	Gerres oyena	35.2	11.1	9
TUDOR	Mikindani	Gerres oyena	24.2	11.9	8.5
TUDOR	Mikindani	, Gerres oyena	30.1	10.4	8.4
TUDOR	Mikindani	, Gerres oyena	35.1	34.5	32.5
TUDOR	Mikindani	, Gerres oyena	16.3	21.3	16.8
TUDOR	Mikindani	Gerres oyena	24.2	23.2	19.4
TUDOR	Mikindani	Gerres oyena	24.2	33.5	29
TUDOR	Mikindani	Gerres oyena	30.1	28.5	24
TUDOR	Mikindani	Gerres oyena	35.2	25.1	21.2
TUDOR	Mikindani	Gerres oyena	24.2	24.6	20.7
TUDOR	Mikindani	Gerres oyena	30.1	33.5	29
TUDOR	Mikindani	Gerres oyena	35.1	28.4	24
TUDOR	Mikindani	Gerres oyena	16.3	27.2	22.2
TUDOR	Mikindani	Gerres oyena	24.2	17.5	13
TUDOR	Mikindani	Gerres oyena	24.2	14.8	11.6
TUDOR	Mikindani	Gerres oyena	35.2	12.5	9.8

TUDOR	Mikindani	Gerres oyena	24.2	15.5	11.5
TUDOR	Mikindani	Gerres oyena	30.1	15.5	12.5
TUDOR	Mikindani	Gerres oyena	35.2	14.8	11.5
TUDOR	Mikindani	Gerres oyena	24.2	14.5	11.5
TUDOR	Mikindani	Gerres oyena	30.1	13.3	10.8
TUDOR	Mikindani	Gerres oyena	35.1	19	14.8
TUDOR	Mikindani	Gerres oyena	26.3	16	12.3
TUDOR	Mikindani	Gerres oyena	24.2	13.3	10
TUDOR	Mikindani	Gerres oyena	24.2	14.8	11.5
TUDOR	Mikindani	Gerres oyena	35.2	16	12.3

APPENDIX 4: Average monthly rainfall and temperature in Kilifi County during the sampling period (January/February and September, 2018) (weather and climate) <u>https//</u>(<u>tcktcktck.org)</u> /kilifi/september-2018

