TYPES AND ABUNDANCE OF MICROPLASTICS IN MACRO-INVERTEBRATES ALONG THE KENYAN COAST

BY

ONYANGO WINNIE AWUOR

I56/89321/2016

THESIS SUBMITTED TO THE SCHOOL OF BIOLOGICAL SCIENCES IN PARTIAL FULFILMENT OF REQUIREMENT FOR THE AWARD OF A MASTER OF SCIENCE DEGREE IN HYDROBIOLOGY: FISHERIES RESOURCE MANAGEMENT

UNIVERSITY OF NAIROBI

AUGUST 2020

DECLARATION

I hereby declare that this thesis is my original work and has not been submitted to any other university or college for the award of a degree or diploma.

Name	Signature	Date
Onyango Winnie Awuor	_____	_____

This thesis has been submitted to the university by my approval as the supervisor.

Name	Signature	Date
Prof. Agnes W.N. Muthumbi School of Biological Sciences, University of Nairobi, P.O. Box 30197-00100, Nairobi-Kenya.	_____	_____
Dr. Deborah V. Robertson-Andersson School of Life Sciences University of KwaZulu-Natal, Private Bag X 54001, Westville, Durban	_____	_____

ACKNOWLEDGEMENT

I would like to thank The National Research Fund (NRF Kenya-South Africa Collaborative Research) who provided research funds for this project and the University of KwaZulu-Natal for hosting me during part of my study period. My sincere gratitude goes to my supervisors, Prof. Agnes Muthumbi and Dr. Deborah V. Robertson-Andersson for their effort towards completion of this report. Much thanks to Dr. George Ong'amo, University of Nairobi for guiding me through the data analysis. I would also like to thank The Kenya Marine and Fisheries Research Institute (KEMFRI) together with The University of Nairobi technical staff for providing all the necessary equipment for the study and also offering technical assistance. I am equally grateful to my parents and all my siblings for their patience with me during my research. Above all, I would like to thank God for granting me good health and safety throughout the research period.

DEDICATION

I dedicate this work to God who has been my guide, my strength and my protector throughout the research period, my parents, Mr and Mrs. Tobias Onyango, for their financial support and constant prayers, my siblings; Ken, Jacky, Maureen, Chris and Rachael, my fiancé, Dedan, my most cherished nephews; Biko, Xander and Tanner, and to the memory of my beloved grandmother, Mama Herinah Oloo whose prayer, guidance and love for education have been my hope and driving force to succeed in my studies.

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LIST OF ABBREVIATIONS

DO	Dissolved Oxygen
FT-IR	Fourier-Transform Infrared
GC	Gas Chromatography
HCL	Hydrochloric acid
HNO_3	Nitric Acid
H_2SO_4	Sulphuric (IV) acid
KEMFRI	Kenya Marine and Fisheries Research institute
KMC	Kenya Meat Commission
KOH	Potassium Hydroxide
MPs	Microplastics
NaCl	Sodium Chloride
NaI	Sodium Iodide
NaOH	Sodium Hydroxide
NRF	National Research Fund
OCs	Organochlorine Pesticides
PA	Polyamides
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated byphenyls
PE	Polyethylene
PET	Polyethylene terephthalate
POM	Particulate Organic Matter
POPs	Persistent Organic Pollutants
PP	Polypropylene
PS	Polystyrene
PSU	Practical Salinity Units

PVC	Polyvinylchloride
SGR	Standard Gauge Railway

ABSTRACT

Microplastics (MPs) are plastics less than 5 mm in diameter. Due to their small size, they are easily mistaken for food by sea fauna, particularly the filter and deposit feeders. Ingestion of microplastics may cause poisoning, infertility, gene disruption, gut blockage or damage of the digestive tracts of organisms resulting to reduced feeding. The aim of this study was to determine the type and abundance of microplastics in macro-invertebrates (oysters, jellyfish, and crabs) along the Kenyan Coast (Tudor, Port Reitz and Mida Creeks). Sampling was done during low spring tide in January/February 2018. A total of n = 285 individuals comprising crabs, oysters and crabs were collected from eight stations. Identified species were: *Uca* dussumieri (Milne Edwars, 1882), *Uca inversa* (Hoffman, 1874) and *Uca vocans* (Linnaeus, 1758) for crabs, *Saccostrea cucullata* (Born, 1758) for oysters while jellyfish belonged to the genus *Crambionella*. Crabs occurred in seven stations while oysters and jellyfish were encountered in three stations only. *U. dussumieri* was the dominant species of crabs occurring in six out of the stations. Samples were digested using 10 % KOH at 60 ºC for 24hrs. Digested samples were sieved using 38 µm sieves then filtered through Whatman filter membranes (0.8µ). The filters were viewed under a dissecting microscope and suspected microplastics isolated and tested using a hot needle test. All the samples contained microplastics, mainly fibres that were of different lengths and colours. Colourless fibres were the dominant fibres accounting for 60% of all the fibres. Mean (± SE) lengths of microplastics ranged from 0.1 mm to 4.2 mm. Blue fibres ingested by crabs were the longest at 4.2 mm. Mean (± SE) concentration of microplastics per gram of tissue for the three organisms were: 0.65 ± 0.131 in crabs, 3.36 ± 0.53 in oysters and 0.03 ± 0.01 in jellyfishes. These means were compared using a repeated independent t-test, and were found to be statistically different: crabs and oysters (t= 5.61, df =14, p = 0.01), jellyfish and oysters (t = 5.28, df = 10, p = 0.01) crabs and jellyfish (t= -3.45, df = 12, p = 0.002). Oysters had the highest concentration of microplastics which was attributed to their filter feeding habits which generates a lot of currents and concentrates more particles in the water including microplastics. This study provides evidence of microplastics pollution in waters along the Kenyan coast and their ingestion by filter and deposit feeding fauna which are important as food for humans or fish of economic importance. Consumption of these organisms therefore, could lead to the transfer of microplastics in their tissues into human diet with implications on human health. This study hence, recommends proper plastic waste management to reduce their accumulation in the marine environment and eliminate any possible threat to the economically important sea fauna that ingest them.

CHAPTER ONE

1. INTRODUCTION

Plastics are synthetic and semi-synthetic polymers made out of materials such as crude oil, crude oil, natural gas, and cellulose (Plastic Europe, 2015). According to EFSA (2016), Plastic additives comprising both organic and inorganic material make up 4 % of the total weight of plastics. Organic additives include phthalates and alkyl phenols while inorganic additives include substances such as Zinc, Sulfur and Barium. The main polymers constituting plastics include Polystyrene (PS), Polyethylene (PE), and Polypropylene (PP) (EFSA, 2016).

The production of plastics in large scale started in the 1950s (Kershaw *et al*., 2015) due to the increased demand of tasks that require use of plastics and their production has steadily increased over time (Dehaut *et al*., 2016). This growth or increase has realised an increase of 38 % between 2004 and 2014, as reported by various environmentally inclined outlets (Forster, 2016). Due to the increasing population growth, plastic production is projected to double by 2025 (Lusher *et al*., 2017). China is the leading producer accounting for 28 % of the world plastic production (Plastic Europe, 2015).

Plastics are used for different purposes, including clothing, personal cleaning products, electronics, packaging, footwear, building and construction (Boucher and Friot, 2017). The wide application is due to their durability, excellent thermal and electrical insulation, as well as their ability to be moulded into various shapes (Dris *et al*., 2015). The most widely used plastics are: Polyethylene terephthalate (PET), Polyvinyl Chloride (PVC), PP, PS and PE, representing about 90% of the entire global production (Do Sul and Costa 2014).

Plastics are ubiquitous in both marine and coastal ecosystems (Dris *et al*., 2015) comprising approximately 80% of marine wastes (Wainainah, 2018). On approximation, eight million metric tons of plastics gain entry to oceans on a yearly basis. Land-based sources such as urban centres and landfills account for 80% of the plastic litter in the marine environment. These plastics enter the seas via rivers, storm water run-off, wind, industrial discharge or wastewater outflows (Smith *et al*., 2018). In Kenya, plastic pollution is a menace in the coastal towns such as Mombasa, Kwale, Lamu and Kilifi (Tan, 2012). As a result, a ban on plastic carrier bags was issued in 2017 by the government to eradicate further degradation of the environment by plastic wastes (Kimani *et al*., 2018).

Plastics are found in almost all the sea habitats including, at the water surface, in the water column, on the beaches, as well as in the deep seas (Lusher, 2015). Distribution of plastics in the water columns largely depends on their density (Smith *et al*., 2018). Plastics vary in density ranging from highly dense polymers, including PVC, PE and Polyamide (PAs) that sink to the sea floor, to lighter polymers such as PS, PE and PP that often float on the sea surface (Smith *et al*., 2018). Approximately five trillion plastic particles are floating on the surface of the ocean while several other pieces have been recorded on the seafloor (Cole and Galloway, 2015). Plastics are a big threat to the environment. Of particular concern are the microplastics, which refer to plastics less than 5 mm in diameter (Watts *et al*., 2014).

Depending on origin, there are two classes of microplastics, namely primary and secondary microplastics (Wright *et al*., 2013; EFSA, 2016: Smith *et al*., 2018). Primary microplastics are those plastics that are manufactured specifically to be microscopic, and include materials such as scrubbers, microbeads, resin and plastic powders (EFSA, 2016). On the other hand, secondary microplastics tend to emanate from fragmentation of the larger plastics through processes of photodegradation, wind action, and abrasion (Eriksen *et al*., 2014; Brennecke *et al.,* 2015; EFSA, 2016;Avio *et al*., 2017; Boucher and Friot, 2017; Nelms *et al*., 2018; Smith *et al*., 2018). Degradation of plastics draws influence from various aspects like pH, irradiation, temperature, age and type of polymer (Smith *et al*., 2018). The process of degradation, however, takes a long period of time because of the chemical composition of plastics and the additives incorporated in them during manufacture. This therefore leads to persistence and accumulation of plastics within the environment (Waite, 2017).

In the marine environment, microplastics may be found in various shapes such as fragments, pellets, fibres, and microbeads (EFSA, 2016; Lusher *et al*.; 2017; Waite, 2017; Shahul *et al*.; 2018). The presence of microplastics in the ocean is a worldwide concern. This is because of their small size that renders them invisible particularly to filter, deposit and detritic feeders such as oysters and crabs (Lusher *et al*., 2017). Most studies of microplastic ingestion are based on laboratory experiments and only a few studies have investigated ingestion of microplastics by organisms in the wild (Avio *et al*., 2017).

Studies indicate that the implications of ingestion of microplastics could be adverse on organisms; the likely outcomes include blockage or damage of the digestive tracts of animals that, in turn, reduces their feeding and assimilation capacity (Hoss and Settle, 1990). Due to their large surface area to volume ratio, microplastics may adsorb and accumulate

contaminants such as POPs, which are then leached into the digestive fluid and transferred to other body tissues upon ingestion (Hammer, 2012; Galloway, 2015; Naidoo, 2017). Potential impacts of these chemicals include; poisoning, infertility and disruption of the genetic makeup of organisms (Forster, 2016). In addition, microplastics ingested by organisms at the lower trophic levels, either zoo- or phytoplankton, are likely to be incorporated into the food chain leading to a cascade of effects (Katija *et al*., 2017). Several studies are currently being conducted to investigate the pollution of marine and freshwater bodies by plastics (Shahul *et al*., 2018). A recent study by Kimani *et al*. (2018) realized pollution of the Kenyan Coast by microplastics and their subsequent ingestion by the zoolanktons. The aim of this study was to establish the presence and concentration of microplastics in the sea oysters, crabs, and jelly fish that are either filter feeders or detritic feeders and also play a vital role as food for fish that are of commercial importance to humans.

1.1. Justification

Plastic waste pollution is one of the key issues affecting the Kenyan Coast. Currently, plastic pollution has been reported in several towns such as Mombasa, Kwale, Lamu and Kilifi (Aradi, 2018). Since most of these plastics are often washed into the sea either via run off or rivers, they may affect the sea fauna through entanglement, by-catch, ingestion or even death. So far, only a few studies have attempted to investigate the presence of microplastics in the Kenyan Marine environment with particular interests in ingestion by the zooplanktons. This therefore, represents the first study to investigate the presence of microplastics in economically important marine macro-invertebrates along the Kenyan Coast. As stated by Smith *et al*. (2018), consumption of sea foods is one of the pathways through which humans are exposed to microplastics. Therefore, presence of microplastics in oysters, crabs and jellyfish, that are economically important either as sea food or food for fish of economic importance, could lead to the transfer of microplastics in their tissue to human diet.

As aforementioned, consumption of microplastics by humans has implications on human health through the release of adsorbed chemicals such as POPs Organochlorine Pesticides (OCs), Polychlorinated biphenyls (PCBs), Polycyclic aromatic hydrocarbons (PAHs) (EFSA, 2016; Waite, 2017; Nelms *et al*., 2018). When these chemicals are leached into the digestive fluids, they may be transferred to other tissues (Hammer, 2012; Galloway 2015; Naidoo, 2017) leading to poisoning, infertility and disruption of their genetic makeup (Forster, 2016). It is possible that consumption of sea food filled with microplastics can indirectly result to life-threatening incidences such as death.

This research is therefore important as it will generate an understanding on the types and abundance of microplastics ingested by sea fauna along the coast of Kenya. It is worth to note that some of the fauna such as crabs and oysters are highly valued seafood or act as food source for fish of commercial value. Kenya has put in place measures to reduce pollution of the environment by plastics. Recently, the Kenyan government issued a ban on the manufacture and use of single use plastic bags in the country to curb their accumulation in the environment (Ochieng', 2019). This initiative earned the country recognition amongst other countries in tackling the problem of plastic pollution. The findings of this study will thus help strengthen the case for the ban on single use plastic bags in the country. In addition, the study will provide data and information that will enable policy makers and key stakeholders to make informed decisions in order to address the problem of plastic waste pollution.

1.2. Research objectives

1.21. General objective

The general objective of this study was to determine the types, abundance and concentration of microplastics in selected filter and detritic feeding macro-invertebrates along the Kenyan Coast.

1.22. Specific objectives

i. To determine the composition of microplastics (Colour, size and abundance) from selected macro-invertebrates along the Kenyan Coast.
ii. To determine the concentration levels of microplastics in the selected macro-invertebrates and concentrations of microplastics in crabs, oysters and jelly fish.
iii. To compare the uptake of microplastics between filter and detritic feeders

1.3. Research Hypothesis

$H_{0:}$ There is no significant difference in microplastics uptake between filter feeders and detritic feeders along the Kenyan Coast.

$H_{a:}$ There is a significant difference in microplastics uptake between filter feeders and detritic feeders

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Occurrence of Plastics in the Environment

Plastic pollution is among the major concerns that have faced the global environment in the recent past such that some scholars have termed the current period *plasticene* (Boucher and Friot, 2017). Over the last six decades, plastic production has dramatically increased from about 0.5 million tonnes per year in 1960 to about 300 million tons in 2013. Europe is the second leading producer of plastics after China and accounts for 20 % of the total global plastic production (Avio *et al*., 2017).

Increased plastic pollution is attributed to their increased production and utilisation in various sectors (Ferris, 2017). More than 300 million tons of plastics are produced yearly for the manufacture of single use plastic items (Boucher and Friot, 2017). Plastics have several uses including packaging, construction, clothing, personal cleaning products, manufacturing of electrical and electronic materials, boat construction and agricultural production (Ferris, 2017).

According to Avio *et al*. (2017), plastic comprises about 60-80 % of the world's litters and approximately 10% of the annual products of plastics end up in oceans. The main mode of transport of plastics into the ocean include storm water runoff, discharges from wastewaters, rivers, transport of terrestrial litter by wind, commercial and recreational fisheries as well as direct dumping of plastic wastes by cargo and passenger ships and cruise ships (Avio *et al*., 2017). Occurrence of plastics in the marine environment is mostly attributed to the mismanagement of wastes from the coastal countries (Boucher and Friot, 2017 and Lusher *et al*., 2017).

Boucher and Friot (2017) report that microplastics being released from households into oceans throughout the world are higher (77%) than those from economic activities (23%) with most of the releases occurring during the use phase of the plastic products. In terms of land-based activities, microplastic losses via road runoff are the main pathway accounting for 66% of the losses while losses through wastewater treatment accounts for 25%. Terrestrial transport of microplastics through wind is the least source of microplastics into the ocean at only 7%. Globally, one third of the human population is linked to the wastewater treatment

system that translates to more than 71 % of microplastics that are released into oceans across the world (Boucher and Friot 2017) (Fig 2.1).

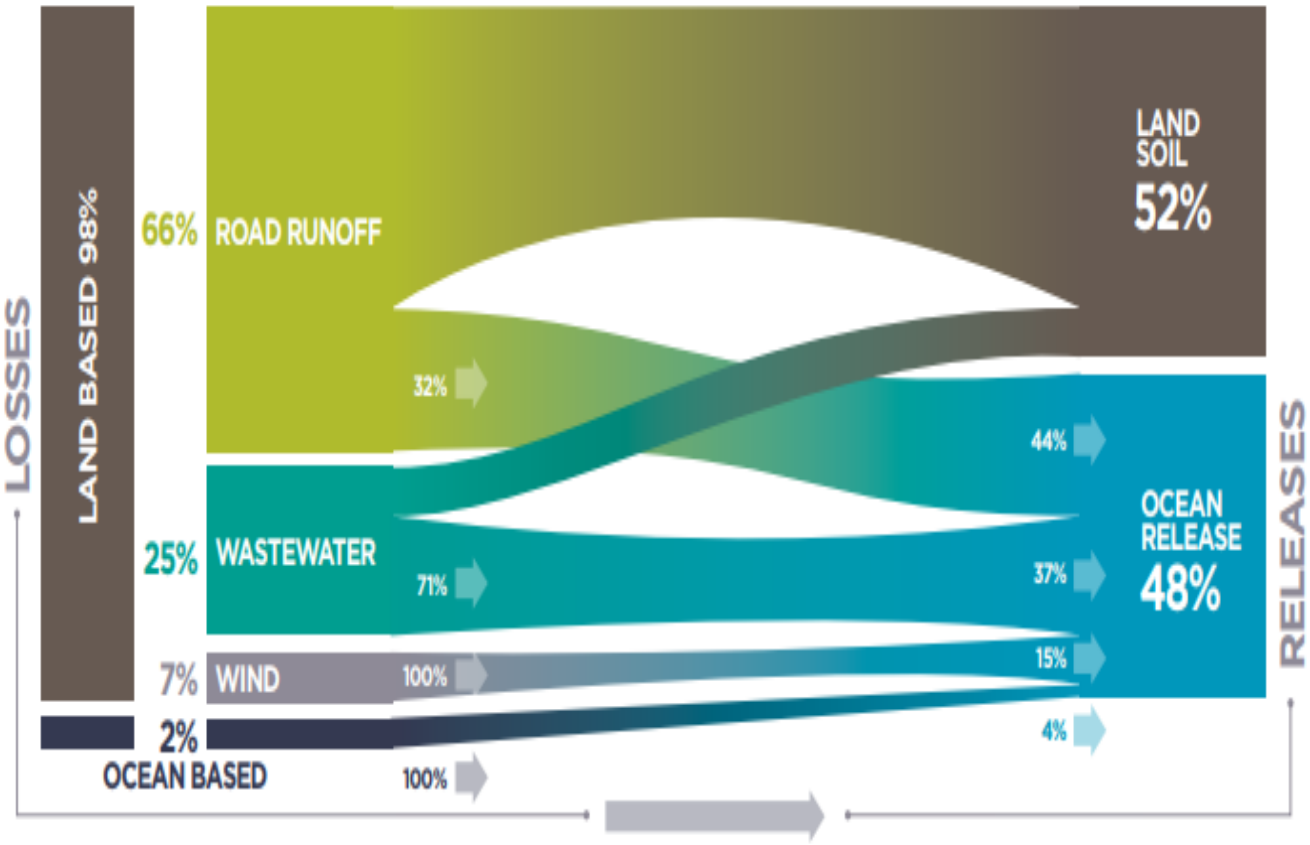

Figure 2. 1 Global Statistics of microplastic releases into the World Oceans (taken from Boucher *et al*., 2017)

In the recent years, the concentration of plastics in the oceans has risen to about 100 000 particles per m³ (Wright *et al*., 2013). On estimation, eight million tonnes of plastics gain entry into oceans every year due to production of single use plastics, and an estimated ratio of 1:1 of plastics to fish is expected by 2050 (Wearden, 2016). The distribution of plastics in the marine environment largely relies on their density (Loder and Gerdts, 2015): polymers such as Polyvinyl Chloride tend to sink in water due to their high density while others such as Polyethylene and Polypropylene stay afloat because they are less dense than seawater (Avio *et al*., 2017, Boucher and Friot, 2017).

The first report on floating plastic debris in the ocean surface was made by Carpenter *et al*., (1972). These floating plastic particles are usually dispersed across the ocean by ocean currents, ultimately ending up in the ocean gyres. In total, about 93 to 268 kilo tonnes of

microplastics float in the ocean. Dense plastics that sink to the bottom of the sea may accumulate in the sediments and become available to benthic feeders (EFSA, 2016; Boucher and Friot, 2017).

Plastics enter the marine environment in different sizes ranging from micrometer to several meters (Hidalgo-Ruz *et al*., 2012). Therefore, plastics can be classified as either macroplastics or microplastics (Brate *et al*., 2017). Macroplastics are the large visible plastic particles whereas microplastic refers to plastic particles less than five mm in size (Brate *et al*., 2017). These microplastics may be found on the sea surface, water column, estuaries, beaches, as well as in aquaculture systems (Lusher *et al*., 2017).

According to EFSA (2016), microplastics are classified either as primary microplastics or secondary microplastics. Primary microplastics are microplastics, which are specifically designed to be microscopic (Wright *et al*., 2013; EFSA, 2016). They include materials such as scrubbers, microbeads, pellets, plastic powders and resin (Shahul *et al*., 2018 and EFSA, 2016). Secondary microplastics are those that usually result from the fragmentation of the larger plastics through photo degradation, wave action or abrasion (Eriksen *et al*., 2014; Brennecke *et al* 2015; EFSA, 2016; Avio *et al*., 2017, Boucher and Friot, 2017; Nelms *et al*., 2018). Degradation of plastic material can last over 100 years and the rate depends on the type of polymer, availability of oxygen, temperature of the environment and presence of chemical additives. Degradation generally leads to a change in the chemical composition and the mean weight of the molecules (Avio *et al*., 2017).

Microplastics often originate from diverse sources, and as a result, they range in size, specific density, colour, chemical composition, and shape (Hidalgo-Ruz *et al*. 2012). Nonetheless, there is no general consensus regarding the minimum size of microplastics, however, most studies define microplastics as plastic particles < 5 mm in size (Nelms *et al*., 2018, Lusher *et al*., 2017). For pellets, the reference diameter size varies between 1-6 mm. In terms of shape, microplastics range from irregularly shaped particles to spherical and long thin fibres (Hidalgo-Ruz *et al*., 2012). Classification of microplastics based on shape includes films, microbeads, foams, microfibers and fragments (EFSA, 2016; Waite, 2017; Lusher *et al*., 2017; Shahul *et al*., 2018). Pellets are plastic particles that are often spherical with round ends whereas most fragments are fibres. Usually, the shape of microplastics depends on the fragmentation process that the plastic has undergone and the time of residence in the environment. Plastics with sharp edges, for instance, are those that have been recently

introduced into the sea while those with smooth edges often result from older fragments that have constantly been polished by other particles or sediments (Hidalgo-Ruz *et al*., 2012).

Hidalgo-Ruz *et al*. (2012) indicate that microplastics exist in different colours with white being the most common colour reported in majority of the studies. In addition, microplastic colour is a key characteristic in identifying the chemical composition of microplastics, for instance, materials made of PP are often clear or transparent while coloured plastics are associated with PE (Hidalgo-Ruz *et al*., 2012). Also, microplastics characteristics influence their distribution in the environment, that is, dense microplastics remain in contact for a longer time with abrasive sediments, colliding with them at greater force than the lighter plastic particles (Hidalgo-Ruz *et al*, 2012).

Occurrence of microplastics in the marine environment raises concerns due to their slow biodegradability leading to their persistence and accumulation in the environment (Hidalgo-Ruz *et al*., 2012). Also, microplastics are a threat in the sea due to their interaction with sea biota through ingestion, entanglement, introduction of alien species and suffocations of organisms (Boucher and Friot, 2017; Lusher *et al*., 2017). Smith *et al*., (2018) and Lusher *et al*., (2017) refer to ingestion as the most common interaction between sea animals and microplastics. This is influenced by the microscopic nature of microplastics that make them easily mistaken for food by organisms (Smith *et al*. 2018). A recent report by Wainainah (2018) indicates that over 100 million marine fauna including sea turtles die each year as a result of consuming plastics by mistaking them for jellyfish. The death is usually as a result of gut blockage (Lazar and Gračan, 2011).

Ingestion of microplastics can either occur directly or via predation. Direct ingestion of microplastics mostly occurs through indiscriminate feeding habits by sea fauna such as filter feeding or active selection as a result of mistaking plastics for food (Nelms *et al*., 2018). Several studies have revealed microplastic ingestion in many sea animals including fish, fish-eating birds, and sea invertebrates such as molluscs, annelids, cnidarians, echinoderms and amphipods (Smith et al., 2018, Avio *et al*., 2017 and Lusher *et al*., 2017). Most studies however, are based on controlled laboratory conditions and only a few studies have focussed on wild species (Avio *et al*., 2017). In most cases, the concentration of microplastics in the sea biota is determined in their stomach or the whole digestive tract (Hoogenboom, 2016; EFSA, 2016). This concentration is usually expressed in units such as; number of particles/marine organism or number of particles/g wet weight (EFSA, 2016).

2.2 Microplastic Ingestion by fish

Microplastics ingestion by fish has rarely been investigated as compared to bivalves and crustaceans. The earliest ingestion of microplastic by fish, as indicated by Lusher *et al*. (2017), was reported in Coastal species taken from the USA and UK. Recent studies have also reported evidences of ingestion of microplastics in the mesopelagic fish inhabiting the North Pacific Central Gyre and in the estuarine fish as a result of high input from the rivers. Microplastics have also been identified in the guts of wild fish larvae of commercially valuable fish inhabiting the English Channel waters (Lusher *et al*., 2017).

In North-eastern Brazil, microplastics made up of nylon fragments were observed in the stomachs of bottom feeding fish: it was hypothesized that the fish had either mistaken the fragments for prey or consumed fish that had ingested the microplastics (Avio *et al*., 2017).Microplastic exposure through predation has been observed in predatory fish such as Crucian carp; *Carassius carassius* (L., 1758). In Mwanza region located in Tanzania, microplastics have been indentified in commercially valuable fish species such as the Nile Perch: *Lates nilotica* (L., 1758) and the Nile Tilapia: *Oreochromis nilotica* (L., 1758) from the surrounding Lake Victoria (Shahul *et al*., 2018 and Lusher *et al*., 2017).

According to Lusher *et al*., (2017), the quantity of microplastics in the digestive tracts of fish is usually low, ranging between < 1 to 2 particles per individual. Studies of microplastic ingestion in the North Sea for instance, found only 2.6 % of the individuals to have ingested microplastics while in the Central Mediterranean, the proportion was 18 % (Lusher *et al*., 2017).

Although there is scarce information about the impacts of microplastics on the commercially important fish, upon their ingestion, the microplastics may be translocated into other tissues such as liver leading to inflammation, disruption of metabolic energy and oxidative stress (Lusher *et al*., 2017). Evidence of microplastic translocation has been recorded for fish species among them being Common goby (*Pomatoschistus microps*, Nordmann, 1840) and Seabass (Smith *et al*., 2018).

2.3 Microplastic Ingestion by Invertebrates

Marine plankton and suspension feeders are a common pathway of microplastics into the food chain (Blastic, 2018) (Fig 2.1). Studies have demonstrated microplastic ingestion by several planktonic organisms including copepods, larval stages of decapods, molluscs and

echinoderms (Van Cauwenberghe and Janssen, 2014). Ingestion of microplastics by marine invertebrates mostly occurs as a result of microplastic small size which resembles planktons (EFSA, 2016, Hoogenboom, 2016; Lusher et al., 2017), and therefore suspension/filter feeders such oysters and mussels can easily mistake them for food (Lusher *et al*., 2017). Microplastics can also accumulate in sediments and become available to the benthic deposit feeders such as crabs, cucumbers, and annelids (Hoogenboom, 2016; Lusher *et al*., 2017).

2.3.1 Deposit Feeders

Deposit feeders usually burrow in sediments feeding mainly on microalgae, particulate organic matter and bacteria (Levington, 2010). They feed by sifting through the sediments and include; earthworms, polychaete worms and fiddler crabs (Anissimov, 2018). Studies on microplastic ingestion by crabs have also found particles of microplastics trapped in the gills and digestive tracts of these organisms (Akpan, 2014; Waite, 2017). These microplastics are likely to be passed on to the crab predators such as birds, sea otters, octopuses, other crabs and also humans (Akpan, 2014).

2.3.2 Filter and Suspension feeders

Suspension feeders are organisms with specialized structures to extract food particles in the environment (Riisgård and Larsen, 2010). These foods may either be zooplankton, phytoplankton, bacteria or detritus (Hentschel and Shimeta 2018). Suspension feeders employ several mechanisms to capture particles, including mucus-net filter-feeding, ciliary sieving, collar sieving, ciliary-spike suspension-feeding, cirri trapping, setal filter-feeding, colloblast prey-capture mechanism and tube-feet suspension feeding. The term filter feeders refers to a group of suspension feeders that use filter structures such as rakes, mucus and setae to capture their prey (Riisgård and Larsen, 2010). Filter feeders employ two modes of feeding: Active and passive feeding (Hentschel and Shimeta 2018). Active feeders such as sponges, ciliates, crustaceans and bivalves, create their own feeding current to acquire food or actively swim towards the prey. Passive feeders on the other hand, depend entirely on water to supply their food (Hentschel and Shimeta 2018), that is, they wait for potential prey to get trapped in their feeding structures such as tentacles as in jelly fish, corals and brittle stars (Hentschel and Shimeta 2018). Marine filter feeders have to sieve several cubic meters of water so as to obtain enough plankton, thus, they could end up ingesting some microplastics in the process (Tilley, 2018). Numerous laboratory studies have confirmed that bivalves such as *Mytilus edulis* L., 1758 and *Crassostrea gigas* Thunberg, 1793 ingest microplastics (Blastic, 2018).

The mean number of microplastics per individual bivalve often ranges between 0.2 to 4 particles per gram of the tissue. In terms of size, *M. edulis* are able to ingest microplastic in the range of 2 to 10 µm (EFSA, 2016; Shahul *et al*., 2018).

2.3.3 Trophic Transfers of microplastics

According to Lusher *et al*. (2017), trophic transfer of microplastics and their subsequent bioaccumulation has been recorded mostly in laboratory studies; for instance, green crabs feeding on mussels that have accumulated microplastics have been witnessed to accumulate microplastics in their guts under controlled laboratory conditions. Similarly, microplastics in green algae have been passed to water flea: *Daphnia magna* (Straus, 1820) and to predatory fish including Tench: *Tinca tinca* (L., *1758*), Northern pike: *Esox lucius* (L., 1758), Atlantic salmon: *Salmo salar* (L., 1758) and Crucian carp (*C. carassius*) (Lusher *et al*., 2017). Avio *et al*., (2017) also reveals the potential transfer of microplastics between organisms in the wild. Zooplankton species that undergo diurnal migrations for example, tend to transfer the microplastics to the deep waters either through predation or by producing pellets that sink to the sea bottom (Avio *et al*., 2017).Most bivalves and crabs are important sources of food for humans around the globe, therefore, have the potential to transfer the microplastics through the foodweb (Blastic, 2018).

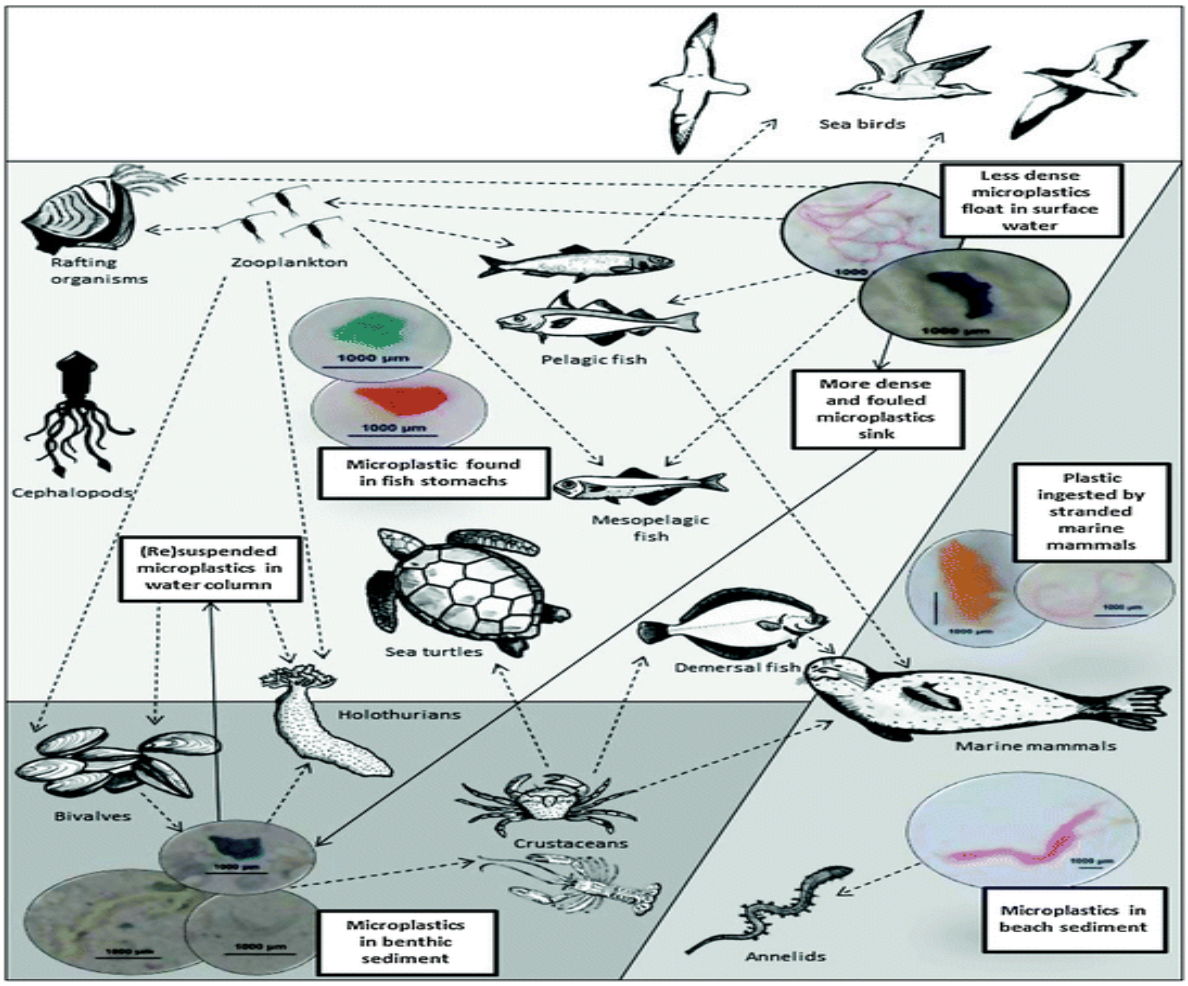

Figure 2. 1 An interaction of microplastics with sea biota (taken from Lusher *et al.*, 2015)

2.4 Biological Effects of Microplastics

Few studies have examined the implications of microplastics on organisms; this is because of limited costs as well as environmental and anthropogenic factors that subject both the wild and farmed animals to stress (*Avio et al.*, 2017). In the recent analysis by Avio *et al.*, (2017), microplastics were found to have caused adverse effects on 663 species of sea organisms interacting with them. Some of the risks associated with microplastic exposure include: changes in the chemical, biological, and physical behaviour of organisms (Shahul *et al.*, 2018). Such changes include decreased consumption of food hence weight loss, reduced immune response, decreased growth and fecundity rates, as well as depletion of energy (Lusher *et al.*, 2017), blockage or damaging of the digestive tracts thereby reducing the feeding capacity of the organisms (Hoss and Settle, 1990; Taylor *et al.*, 2016). Decreased food consumption for instance has been observed in *Idotea emerginata* (Fabricius, 1793) because of ingestion of PE microplastics (Shahul *et al.*, 2018). Also, exposure to PVC and PS

was observed to cause reduced feeding in *Arenicola marina* (L., 1758) (Cole and Galloway, 2015). As a result of their large surface area to volume ratio, microplastics may adsorb toxic chemicals such as Organochlorine Pesticides (OCs), Polychlorinated biphenyls (PCBs), Polycyclic aromatic hydrocarbons (PAHs) (EFSA, 2017; Nelms *et al*., 2018). When these chemicals are leached into the digestive fluids, they may be transferred to other tissues (Hammer 2012, Galloway 2015, Naidoo, 2017) leading to poisoning, infertility and disruption of their genetic makeup (Forster, 2016). EFSA (2016) indicate that, the translocation of microplastics into the tissues of animals is limited to particles of less than 150 µm and has been recorded in species of rodents, rabbits, dogs as well as humans. In humans, microplastics have been detected in the lymph measuring up to 150 µm in size. Such plastics could have adverse outcomes on the immune systems and lead to inflammation of the digestive tract (EFSA, 2016).

Experimental studies using marine species such as the calenoid copepods *Eurytemora affinis* (Nordquist, 1888) indicated their ability to excrete ingested microplastics thereby evading the harmful impacts of the plastics (Shahul *et al*., 2018). Microplastics are usually concentrated in the gut and eventually passed to their predators. Most fishes and fisheries resources such as Norwegian lobster, *Nephrops norvegicus* (L., 1758) and shrimps are usually gutted before consumption by humans and are therefore less likely to be sources of microplastics into human diet (EFSA, 2016). Other seafoods such as bivalves, echinoderms and small fish like sardines are however consumed together with their innards therefore high risk of microplastic exposure upon ingestion (Lusher *et al*., 2017). Microplastics may also be leached into edible tissues of the fish and end up in our diet (Coote, 2017). Approximately 11,000 plastic fragments are consumed by shellfish lovers in the European countries including Belgium in their sea food each year and less than 1 % of these plastics are absorbed by the body (Smillie, 2017). Depending on the consumption habits, it is approximated that human beings consume plastics from seafood at a rate of 1 to 30 particles per day (Lusher *et al*., 2017). Species that are economically important such as the Norway lobsters tend to retain microplastics in their systems for a long period of time, thus, escalating the health risks as well as a decreased profitability of the fishery (Lusher *et al*., 2017).

2.5 Extraction of Microplastics from Organisms

There exist several techniques for isolating and detecting of the microplastics from the tissues of animals. They include; dissection, depuration, visual inspection, density floatation and

digestion. Dissection involves the excision of tissues such as gills, livers and digestive tracts. For a research question that seeks to investigate the risks of microplastics on human health, excision can be performed on edible tissues such as tail muscles of shrimps. Identified microplastics are then isolated from the tissues through digestion, saline washes visual inspection or density floatation. Dissection is however relevant for microplastics measuring > 5 mm in size (Lusher *et al*., 2017).

According to Lusher *et al*., (2017), depuration is conducted when the interest is in the microplastics egested. In this method, the specimen is first cleaned to remove any microplastics on their surfaces and then placed on fresh water, sea water or sediments that contain no food and left for some time to enable complete gut evacuation. The media is replaced from time to time to avoid ingestion of the egested microplastics. Digestion of the faecal matter or visual inspection for microplastics under a dissecting microscope then follows (Dris *et al*., 2015). Since depuration varies between animals, minimum time should be taken between sample collection and preservation to avoid gut evacuation before analyzing the microplastics. During sampling, individuals with empty stomachs should be discarded from the sample as it is a sign of recent gut evacuation hence little or no plastics may be recovered from them (Lusher *et al*., 2017)

Digestion involves the use of solutions such as Hydrochloric acid(HCL), Sulphuric acid ($H_2SO_{4)}$, Sodium hydroxide (NaOH), Nitric acid ($HNO_{3)}$, Potassium hydroxide (KOH), or enzymes such as proteinase –k to digest the soft tissues of organisms (Cole *et al*., 2014). The digestive solution is added into the flask containing the specimen after which digestion is allowed to proceed at a specific temperature and time, followed by dilution with either hot or cold deionised water and then filtered (Claessens *et al*., 2013). The digested materials are washed in saline water then visualized under a dissecting microscope for microplastics (Lusher *et al*., 2017). Strong acids such as HNO_3 may destroy pH sensitive polymers such as Nylon and polyethylene, therefore the optimized alkaline digestion protocol is 10 M NaOH at 60 °C whereas the optimized enzyme digestion protocol is proteinase -K that is capable of digesting up to 97 % organic matter (Cole *et al*., 2014).

The density separation approach is based upon the differences in density between plastics and sediment particles (Shahul *et al*., 2018). Separation of the plastics is done by agitation of the sample of sediment in Sodium chloride (NaCl), Sodium iodide (NaI) or Zinc chloride (ZnCl) solutions (Lusher *et al*., 2017). During the process, plastic particles tend to float at the

surface as a result of their low density, hence extracted (Shahul *et al*., 2018). Density separation can also be applied after samples have been digested. NaCl is the most preferred solution because it is highly affordable and less hazardous. Nonetheless, it is not recommended for more dense particles (> 1.2 g cm $^{-3}$) such as PVC since it can lead to their underestimation. ZnCl and NaI are therefore better alternatives for separating dense plastic particles (Lusher *et al*., 2017).

2.6 Identification of Microplastics

After microplastic extraction, identification follows to confirm quantities and types of microplastics. Identification is a very essential step in classifying microplastics depending on their colour, size, shape (Lusher *et al*., 2017), smoke produced during combustion, synthetic assays and density of the microplastics (Avio *et al*., 2017). Size measurements are based on the longest dimension of the plastic particles whereas classification based on shape relies on five major categories: films, fibres, foams, fragments and beads (Lusher *et al*., 2017). Polymer identification using specific density and colour however, can only be done on virgin pellets, otherwise for the plastic fragments, the technique is inappropriate as the colour and shape of the microplastics vary greatly and are less likely to be associated with a specific type of polymer (Avio *et al*., 2017).

Identification of plastics is usually done using several methods that are mainly based on Infrared (IR) spectroscopy such as infrared spectrophotometer, near-infrared spectrometer, Fourier transform infrared spectroscopy (FT–IR), and Raman spectroscopy. These methods have been utilised to recognise different polymers like Polyester, PE and PP. Raman spectroscopy and FT-IR are usually preferred because they are non-destructive and can complement each other in that, molecular vibration which may be inactive in Raman could be FT-IR active vice-versa (Lusher *et al*., 2017). Other strategies such as mass spectrometry (GC/MS) or pyrolysis-gas chromatography are also used to provide structural information of the microplastics (EFSA, 2016).

2.7 Quality Assurance Methods

When analysing organisms for microplastics, care should be taken to avoid contamination of the samples with microplastics in the clothes, air, reagents and equipment. Contamination can be minimized by covering the samples to avoid any contact with the air, use of filtered water

or deionized water and rinsing of equipment thoroughly with distilled water before use. Use of air flow cabinets can also help prevent contamination (EFSA, 2016).

CHAPTER THREE

3. STUDY AREAS, MATERIALS AND METHODS

3.1 Study Area

The study was conducted at Tudor and Port Reitz Creeks in Mombasa and Mida Creek in Kilifi was a control site (Fig 3.1). Sites around Mombasa were of main focus owing to the growing human population in the region and high solid wastes originating from the industrial and tourism sectors. Mida Creek, located in Watamu National Park was used as a control because of its distant location from Mombasa town, hence less influenced by human activities.

3.1.1 Tudor Creek

Tudor creek is located on the eastern side of Mombasa Island between latitude 03° 40' S and 04° 00' S (Nguli *et al*., 2006). It has a surface area of approximately 20 km^2 at mean sea level and extends 10 km inland (Wakwabi and Mees, 1999). The creek comprises of a long and deep inlet measuring up to 20 meters in length (Nguli *et al*., 2016).

Wakwabi and Mees (1999) state that, rainfall and tidal currents velocity of the region are affected by the Monsoon winds (The North East Monsoon winds: Nov-March and the South East Monsoon winds: April – Oct), the South East Equatorial Current, the East African Coastal Current, as well as the Equatorial Counter Current. The creek consists of three main parts: the marine mouth (30 m deep), the middle section (1 - 2 km wide and less than 5 m deep) and the upstream (< 1 m deep) that splits into different channels (Wakwabi and Mees, 1999). Three seasonal rivers namely: Kombeni, Tsatu and Mtsapuni empty into the creek. Approximately 0.9 m^3s^{-1} of fresh water is discharged into the creek annually with the highest discharge occurring between April and June (1.8 m^3s^{-1}) (Wakwabi and Mees, 1999).

Upstream, the creek is characterized by a seasonal, diurnal and tidal variation in the salinity and temperature of the surface waters (Wakwabi and Mees, 1999). Species found in this area range from brackish to freshwater species (Wakwabi and Mees, 1999). The ecosystem comprises of a mangrove forest containing species of *Rhizophora mucronata* Lamk, and *Avicennia marina* (Forssk.) vierh. (Mirriam, 2010). Compared to the upstream section, tidal influence in the middle section is lower. Salinity is between 34-36 Practical Salinity Units (PSU); surface temperature: 24–32 °C; turbidity: 1.5-2.5 m secchi disc depth and Dissolved

Oxygen (DO) concentration is 78-84% saturation (Wakwabi and Mees, 1999). At the mouth, salinity is 35 psu, surface temperature: 25-29 °C; turbidity: 3-6 m secchi disc depth and DO concentration 92-95% saturation (Wakwabi and Mees, 1999). These parameters are uniform throughout the year at the mouth, while at the upstream, they vary with tide, day and season (Mirriam, 2010).

3.1.2 Port Reitz Creek

The Port Reitz creek is found to the south of the Mombasa island (04°- 04'S & 39°- 39'E) with a catchment area of 1480 km² (Kamau, 2002). It was formed due to the drowning of previous rivers as a result of the rise in the sea level and is fed by water from three main rivers namely: Mwachi, Cha Shimba and Mombome (Kamau, 2002).The tidal pattern of this ecosystem is semi-diurnal with a tidal range of 1 m at neap tides and 2.5 m at spring tide (Kamau, 2002). Major pollutants include: waste materials, toxic organic and inorganic wastes, trace metals and oils from the atmosphere as well as inland anthropogenic activities leading to turbid and eutrophic waters (Oduor, 2017).

3.1.3 Mida Creek

Mida Creek is a tidal inlet located at the North Coast of Kenya in Kilifi District at longitude 39° 58' E and latitude 3° 20' S (Gang and Agatsiva, 1992). According to Osore *et al*. (2014), the creek occupies a total area of about 32 Km^2. The length of the creek is 11 km and it measures 500 m on the mouth and 1500-2000m in the middle (Osore *et al*., 2014). The depth of the shallow basin inland is 4 m and 7 m in middle section of the creek (Osore *et al*., 2014). Mida creek differs from other creeks in the region due to lack of river inflow, instead the creek receives fresh water through seepage from the ground and storm water runoff (Osore *et al*., 2014). In addition, the creek is a marine secured area, forming part of the Watamu Marine National Park and Reserve (Osore *et al*., 2014). The creek comprises a mangrove ecosystem with three dominant mangrove species: *Rhizophora mucronata*, *Ceriops tagal* (Perr.) C.B Robinson and *Avicennia marina* (Kairo *et al*., 2002). The forest provides important services such as food, honey, fuel, nutrient cycling, nursery and breeding grounds for fish, education and research, as well as recreation (Owuor *et al*., 2017). The creek experiences an average annual rainfall that is between 600-1000 mm with a rainy season that begins in May all the way to September (Frank *et al*., 2017).

Figure 3. 1 Locations of Sampling Points along the Kenyan Coast, a) Map of Kenya; b) Mida Creek (Dab) and c) Mombasa showing Tudor (Mik, KMC, Nyali Bd and Eng. Pt.) and Port Reitz Creeks (Maku, Mwa. SGR and Mwa Tsunza).

3.2 Field Sampling

3.2.1 Sampling Stations

A total of eight stations were sampled, four in Tudor Creek, three in Port Reitz and one in Mida Creek Table 3.1.

Table 3. 1 Codes and GPS locations of the sampling sites and locations in Mombasa and Mida Creek

Mida Creek

Site	Stations	Code	South	East
Tudor Creek	Mikindani	Mik	04° 49' 51s	E: 039° 21' 12s
	Kenya Meat Commission	KMC	04° 01' 34.7s	E: 039° 38' 47.5s
	Nyali Bridge	Nyali. Bd	04° 02' 48.1 s	E: 039° 40' 27.4 s
	English Point	Eng. Pt	04° 02' 35.1s	E: 039° 34' 54.1s
Port Reitz	Makupa	Maku	04° 02' 16.5s	E: 039° 38' 50.1s
	MwacheTsunza	Mwa. T	04° 02' 47s	E: 039° 40' 26.7s
	Railway Station	Mwa. Sgr	04° 01' 53.6s	E: 039° 38' 47.0s
Mida Creek	Dabaso	Dab.	04° 03' 23.0s	E: 039° 40' 58.9s

3.2.2 Physico-chemical parameters

At every station, physico-chemical parameters of the water such as temperature, salinity, pH, and conductivity were measured using respective meter probes: Temperature-thermometer (°C), conductivity meter, salinity meter, and pH meter. The meter probes were dipped in water and readings taken after ten minutes. This process was repeated three times in every station; thereafter, the values for each variable were averaged.

3.2.3 Macro-invertebrates sampling

Sampling was done during the spring low tide between 31st of January and 3rd of February 2018. Three replicate samples comprising 5 - 20 individuals of each species were collected

randomly in the selected stations of each study site. A chisel was used to remove oysters from the rocks and trees. Crabs were handpicked from the surface of the sediments and also scooped from their holes using a shovel. Jellyfish were caught by towing 500µm nets in the water for approximately 10 minutes. Maximum precautions were undertaken to ensure that only desired specimens were collected. During the sampling process, maximum caution was observed to avoid contamination of the samples by particles in the air. This was achieved by use of glass bottles for the crabs while oysters and jellyfish were wrapped in aluminium foils. The glass bottles were thoroughly rinsed with distilled water before putting in the crabs. Samples were carried in cooler boxes with ice to the laboratory and transferred to the freezer awaiting later analysis.

3.3 Laboratory Analysis

3.3.1 Digestion and Extraction of Microplastics

Digestion and extraction of microplastic from samples was done at the Kenya Marine and Fisheries Research Institute (KEMFRI) and the University of Nairobi Laboratories. Analysis of microplastics colour and length was performed at the University of KwaZulu Natal in South Africa, using FT-IR spectrometer.

3.3.2. Preparation of Organisms for Microplastics extraction

3.3.2.1 Crabs

Distilled water was used to rinse crab samples to remove any microplastics attached on the carapace. Species were identified using morphological identification guide by Mangale and Kulkarni (2013). To measure carapace length of the individual organisms (cm), Vernier callipers were used while weight was measured using a weighing balance. Individuals of every replicate were put together in a mortar and crushed.

3.3.2.2 Oysters

Oysters were rinsed with distilled water to remove biofilms on their surfaces as described by Lusher *et al*. (2017). Cleaned individuals were identified using oysters' identification guide by Watson (2018). The length (longest shell length) measurements were taken using a Vernier callipers while body weights were measured using a weighing balance. The samples were frozen for easy deshelling to extract tissues for digestion.

3.3.2.3 Jellyfish

Jelly fish samples were weighed using a weighing balance and the weights recorded. Distilled water was then used to rinse samples to remove any microplastics attached on the surface.

3.3.3 Microplastic Processing and identification

10 % KOH at 60˚C (Cole *et al*., 2014, Lusher *et al*., 2017) was used for the extraction of microplastics from the target invertebrates. As Lusher *et al*. (2017) describes it, this protocol is effective in dissolving tissues of invertebrates such as crabs and oysters as well as the gastrointestinal tracts of fish and mussels, thereby enabling plastic recovery.

The digestion and filtration processes were performed at the KEMFRI laboratories. Replicates of each sample were put in separate beakers in which 10 % KOH was added until the sample was completely submerged and then incubated at 60 °C for 24 hrs (Cole *et al*., 2014). Crabs samples did not completely digest after the 24 hrs therefore another 10 % of the KOH was added and incubation continued for another 12 hrs to ensure complete digestion. After digestion, samples were sieved using 38 µm sieve and filtered through Whatman filter membranes (0.8 µ). The membranes were dried in an oven for 12 hrs and visualized under a dissecting microscope for microplastics. Identification of the microplastics using a dissecting microscope was done both at the University of Nairobi and KEMFRI laboratories. Suspected microplastics were isolated into glass bottles for further analysis at the KwaZulu Natal University, South Africa. At KwaZulu Natal, the microplastics were tested using a hot needle tests and the materials that were made of plastics were transferred to an FT-IR for length determination and image capture. Microplastics were categorised in relation to their length, shape, and colour. Microplastics abundance in each sample was also determined and the concentration expressed as number of particles per gram tissues.

3.3.4 Laboratory Quality Control

The following means were applied to minimise contamination of the samples: working in a laboratory with minimum movement; wiping the working surface, wearing gloves and cotton lab coat; using glass equipment; testing distilled water for microplastics; rinsing all the equipment with deionised water before use. A control was set on the working table using a membrane filter in a petri dish. The filter was inspected for microplastics under a dissecting microscope and no microplastics were found, thus there was an assumption that the study was at minimum threat of plastic contamination.

3.3.5 Statistical Analysis

Analysis of data was done using Rcmdr package in R-console. 1-way ANOVA was used to compare the differences in the means of physico-chemical parameters between the stations. Variation in microplastic colour and size between the sampling stations was compared using a 2-way ANOVA. 1-way way ANOVA was used to compare the lengths of different colours of microplastics extracted from the tissues of target invertebrates. Means were separated using Tukey's *post-hoc* test where there were significant differences ($p < 0.05$). Interspecific differences in the concentration of microplastics was analysed using a repeated independent two sample t-test. Data was regarded significant at $p < 0.05$. Pearson's correlation coefficient was also performed to establish the association between the mean concentration of microplastics and the mean weight of the organisms.

CHAPTER FOUR

4. RESULTS

4.1 Physico-chemical parameters

The physicochemical parameters of water measured included: Temperature, pH, Salinity and Conductivity (Table 4.1). Mean (± SE) temperature of the waters ranged between 27.9 °C and 29.9 °C. Statistically, mean sea surface temperature varied significantly between the stations ($F_{6, 14}$ = 8.149; $p < 0.05$). Mean sea surface temperature was significantly higher in Nyali Bridge compared to Dabaso, KMC, Mwache Tsunza and Mwache SGR (Table 4.1.). Mean (± SE) pH of water ranged between 7.81 and 8.36. Mean pH values were significantly different between the stations ($F_{6, 14}$ = 131; $p < 0.05$). Pairwise comparison revealed a significant difference between all the groups except between KMC and Dabaso, Nyali and KMC, Nyali and Makupa, and Mwache SGR and Mikindani. Mean (± SE) water salinity ranged between 33.4 and 35.9 parts per thousand (ppt): these values were significantly different between the stations ($F_{6, 14}$ = 82.14; $P < 0.05$). Salinity was highest in Makupa compared to Dabaso, KMC, Mwache Tsunza and Mwache SGR. Additionally, mean conductivity varied significantly between all the stations ($F_{6, 14}$ = 38827273; $P < 0.05$). Highest mean conductivity of 56668.7 ± 1.27 µS/cm was recorded in Mwache Tsunza while samples from Nyali Bridge recorded the lowest mean of 53138.7 ± 0.9 µS/cm. (Table 4.1).

Table 4. 1 Mean (± SE) of the physico-chemical parameters from the sampling stations in Mombasa and Mida Creeks

Site	Temp (°C)	pH	Salinity (ppt)	Conductivity (µS/cm)
Mik	29.1 ± 0.17^{ab}	8.03 ± 0.04^{b}	33.8 ± 0.1^{ab}	55559.5 ± 1.71^{f}
KMC	28.7 ± 0.31^{a}	8.25 ± 0.08^{cd}	33.4 ± 0.15^{a}	54734.3 ± 2.1^{c}
Nyali Bd	29.9 ± 0.15^{b}	8.32 ± 0.01^{de}	35.9 ± 0.21^{d}	53138.7 ± 0.9^{a}
Mwa. T	28.5 ± 0.42^{a}	7.81 ± 0.01^{a}	34.9 ± 0.37^{c}	56668.7 ± 1.27^{g}
Mwa. Sgr	28.0 ± 0.29^{a}	7.96 ± 0.02^{b}	33.7 ± 0.1^{ab}	54355.7 ± 2.29^{b}
Maku	28.9 ± 0.06^{ab}	8.36 ± 0.03^{e}	33.5 ± 0.25^{a}	54904.5 ± 2.23^{e}
Dab	27.9 ± 0.21^{a}	8.18 ± 0.21^{c}	34.1 ± 0.15^{b}	54892.7 ± 0.61^{d}

4.2 Distribution of Macro-invertebrates Sampled

4.2.1 Crabs

During the study, a total of n = 206 crabs belonging to the genus *Uca* were sampled from seven stations namely: Mikindani, KMC, Nyali Bridge, Makupa, Mwache-Tsunza, Mwache-SGR and Dabaso. Three species of *Uca*; *U. dussumieri*, *U. inversa* and *U. vocans* were encountered from the collection. *U. dussumieri* were the most dominant crab species and accounted for 66% of the total crabs sampled (Fig 4.1). The species was observed in six out of the seven stations, that is, Mikindani, KMC, Nyali Bd., Makupa, Mwache T. and Dabaso. *Uca inversa* had a relative abundance of 9% and was encountered only in Makupa and Dabaso while *Uca vocans* had a relative abundance of 25% and was encountered in Mwache SGR only (Figure 4.1).

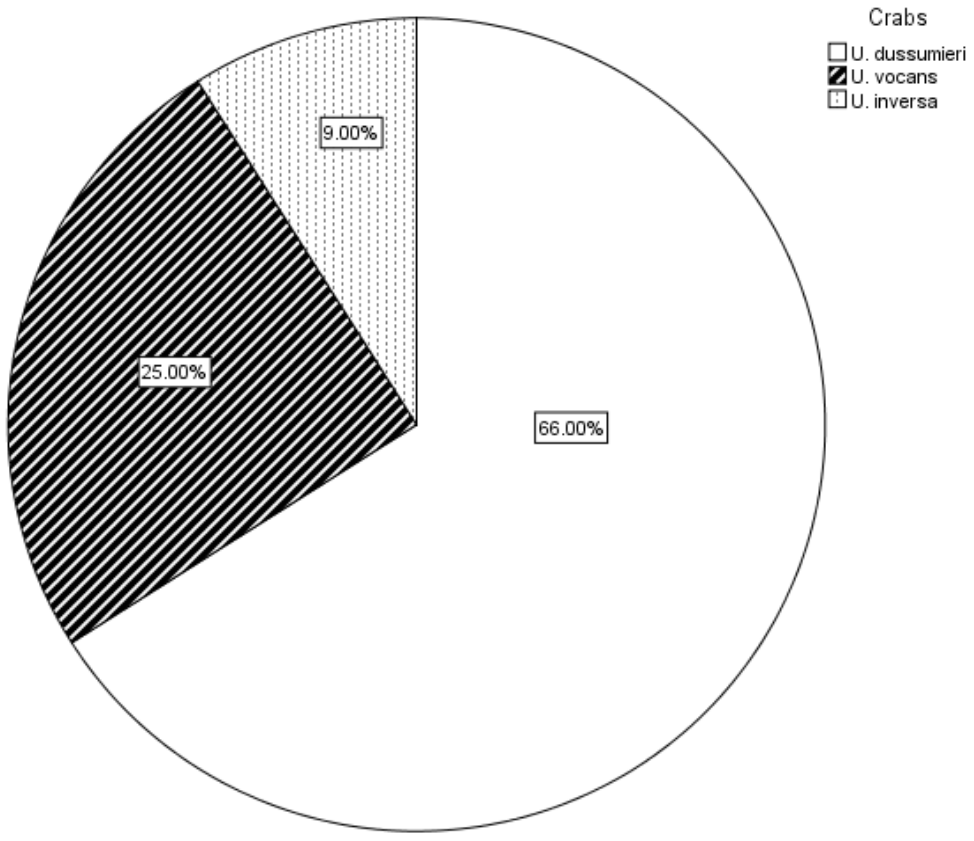

Figure 4. 1 Percentage proportion of crab species (*U. dussumieri, U. inversa* and *U. vocans*) encountered from different stations along the Kenyan Coast

The average (± SE) length of the crabs ranged between 1.3 and 1.8 cm for *U. dussumieri,* 1 and 1.2 cm for *U. inversa,* and 1.5cm for *U. Vocans.* Longest lengths were observed in the *U. dussumieri* species from Makupa (1.80 cm) while *U. inversa* from Mida Creek had a

relatively shorter length (1.05 cm) (Table 4.3). In *U. dussumieri* alone (dominant species), the mean (± SE) lengths varied between the stations ($F_{5, 9}$ = 6.56; p = 0.01) with species from Makupa recording the longest length (1.80 cm) while species from KMC had the shortest length (1.33 ± 0.02 cm). The variation was however insignificant between the Tudor stations, that is, Mikindani, KMC and Nyali Bridge ($F_{2, 6}$ = 3.81; p = 0.09). Weights of the crabs ranged between 2.7 and 9.9 g for *U. dussumieri,* 1.5 and 3.3 g for *U. inversa and 5 g* for *U. vocans*. *U. dussumieri* species from Makupa had the highest weight of 9.88g while the lowest weight was observed in *U. inversa* species from Mida Creek (1.5 g) (Table 4.2). Analysis of *U. dussumieri* revealed significant differences in the mean weights (± SE) of these species between stations in different sites ($F_{5, 9}$ = 4.96; p = 0.02) with species from Makupa having the highest mean weight (9.88 g), whereas those from KMC recorded the lowest mean weight (3.49 ± 0.2 g). In Tudor Creek, however, mean weight of the *U. dussumieri* from the different stations within the creek was not significantly different ($F_{2, 6}$ = 1.79; p = 0.25). Similarly, there was no significant difference in the mean weight of crabs in stations within Port Reitz ($F_{1, 2}$ = 3.04; p = 0.22).

Table 4. 2 Mean (± SE) Lengths (cm) and weights (g) of crabs of different species (*U. dussumieri, U. inversa* and *U. vocans*) from different stations along the Kenyan Coast

Site	Station	Ave L (cm)	Ave Wt(g)	No. of Indiv.
		Uca. Dussumieri		
Tudor	Mik	1.46 ± 0.10	4.73 ± 1.40	16
	KMC	1.33 ± 0.02	3.49 ± 0.20	47
	Nyali. Bd	1.56 ± 0.01	5.68 ± 0.21	22
Port Reitz	Mak	1.80	9.88	8
	Mwa. T	1.61 ± 0.02	7.31 ± 0.74	28
Mida	Dab	1.65 ± 0.06	5.75 ± 0.08	15
		Uca inversa		
Port Reitz	Mak	1.26	3.29	7
Mida	Dab	1.05	1.5	11
		Uca vocans		
Port Reitz	Mwa. Sgr	1.58	5.51	52

4.2.2 Oysters

A total of seventy individuals of oysters were collected from three stations, that is, English point (n = 28), Mwache Tsunza (n = 5) and Dabaso (n = 37), and all of them were identified as *Saccostrea cucullata*. Oysters from Dabaso and Mwache Tsunza were found attached on stems of the mangrove trees while at English Point, they were attached onto the rocks along the shores. In Mwache Tsunza, oysters were encountered only at a single point.

Mean (± SE) shell length of the oysters ranged between 4.31 and 6.2 cm and the de-shelled tissue weights ranged between 0.8g and 3.9g. Oysters from English Point had a relatively high mean length (6.20 ± 0.33 cm) than those from Mwache Tsunza and Dabaso. Statistically, this variation was significant between the stations ($F_{1, 4} = 16.87$; $p = 0.01$). Also, there was a noteworthy difference in the mean weight of the oysters ($F_{1, 4} = 35.29$; $p = 0.04$), with oysters from English Point having a higher weight value (3.39 ± 1.96 g) than the other sites.

4.2.3 Jellyfish

Jellyfish were encountered in Mikindani, Makupa and Dabaso and all of them belonged to the genus *Crambionella*. A total of n = 9 jellyfish were obtained during the study. In Mikindani and Dabaso, the number of jellyfish was n = 2, whereas in Makupa the number was n = 5. Weight of individual jellyfish ranged between 200g and 1000g. Jellyfish from Mikindani were heavier than those from Makupa and Dabaso and measured between 890 and 1000g. Each individual from Mikindani was thus considered a separate sample. Where individuals were small, that is,< 800 g, as in Makupa and Dabaso, more than one individual were put together to form a sample (Table 4.3).

Table 4. 3 Mean (± SE) Weights (g) of jellyfish from different stations along the Kenyan Coast

Stations/samples	No. of indiv	Total wt (g)	Ave wt (g)
Mikindani A	1	890	890
Mikindani B	1	1000	1000
Makupa A	4	897	224.3
Makupa B	1	831	831
Dabaso	2	596	298

4.3 Microplastics Composition and Abundance in Sampled Individuals

4.3.1 General Composition and Abundance of Microplastics.

All the digested samples were found to contain microplastics, particularly the fibres (Fig 4.2). Eight different colours of fibres were encountered, that is, colourless, black, blue, green, pink, purple, red and yellow. Colourless fibres were the most prevailing microplastics of all the fibres accounting for a proportion of 60%**.** Microplastics lengths ranged from 0.75 to11mm.

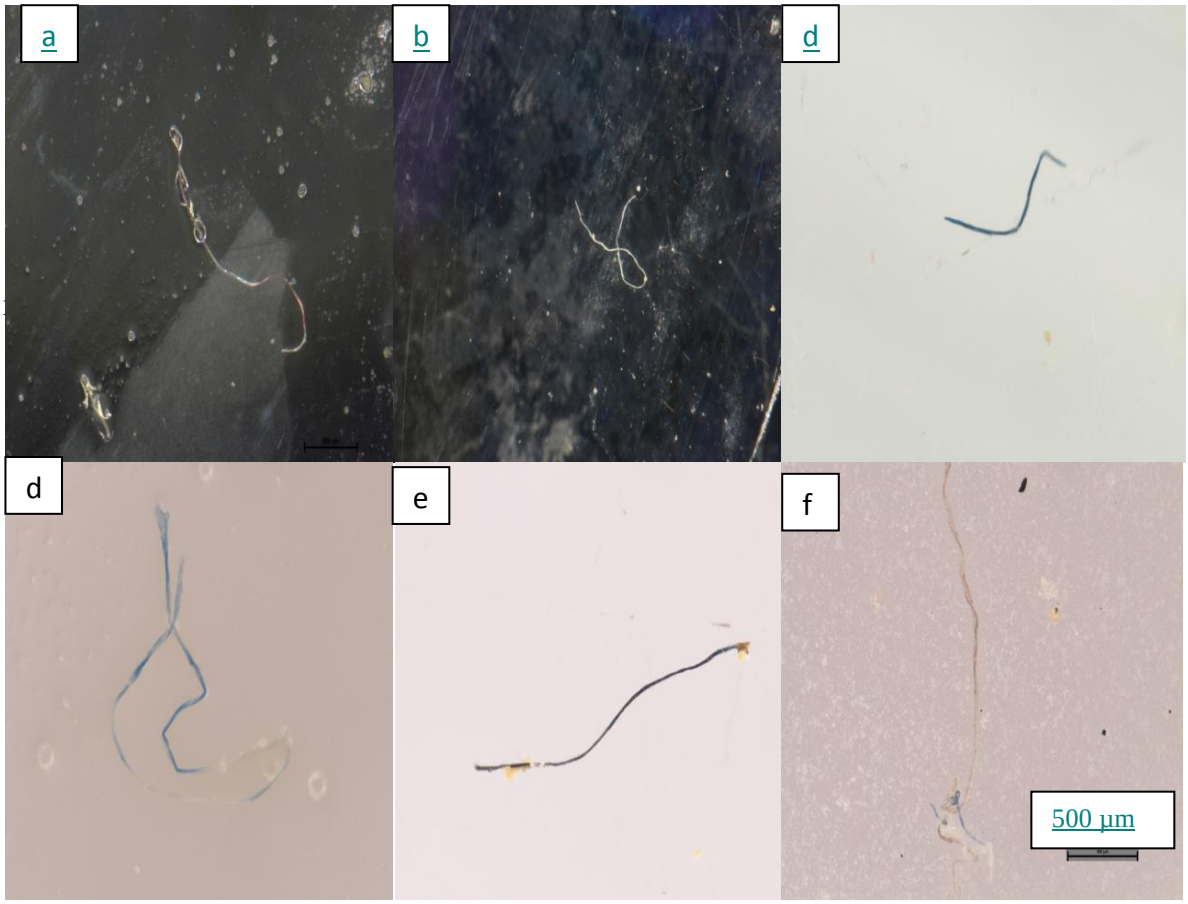

Figure 4. 2 Images of microplastics from FT-IR analyses in samples of crabs, oysters and jellyfish along the Kenyan Coast

4.3.2 Microplastics Composition, Abundance and Concentration in Crabs

Microplastics of seven different colours, including, red, purple, black, yellow, colourless, blue, and green were encountered in the crab samples. Purple fibres were obtained in crab samples from Nyali Bd and KMC only whereas yellow fibres occurred in samples from Nyali Bridge and Coast General. Like in the general occurrence of the microplastics, the colourless fibres had the highest proportion (59.6%), while purple and yellow had the lowest proportion (1.01% each). The red and black microplastics were of equal proportions (13.13%) (Fig 4.3). The different colours of microplastics were of varying lengths ranging from 0.45mm to 4.2

mm. Pair wise comparison revealed a significant difference in the mean (±) lengths of microplastics of different colours ($F_{6, 133} = 5.97$; $p < 0.05$) where black (2.3 ± 0.36 mm), blue (4.2 ± 1.06 mm), colourless (3.5 ± 0.46 mm), green (3.9 ± 0.83mm) and red (3.1 ± 0.58 mm) fibres were significantly longer than purple (0.45 ± 0.31mm) and yellow (0.6 ± 0.35mm) fibres (Table 4.4). On the other hand, lengths of fibres of the same colour did not vary significantly between the stations except for the green fibres that were significantly longer in Makupa than the other stations ($F_{6, 133} = 02.86$; $p = 0.53$) (Table 4.5).

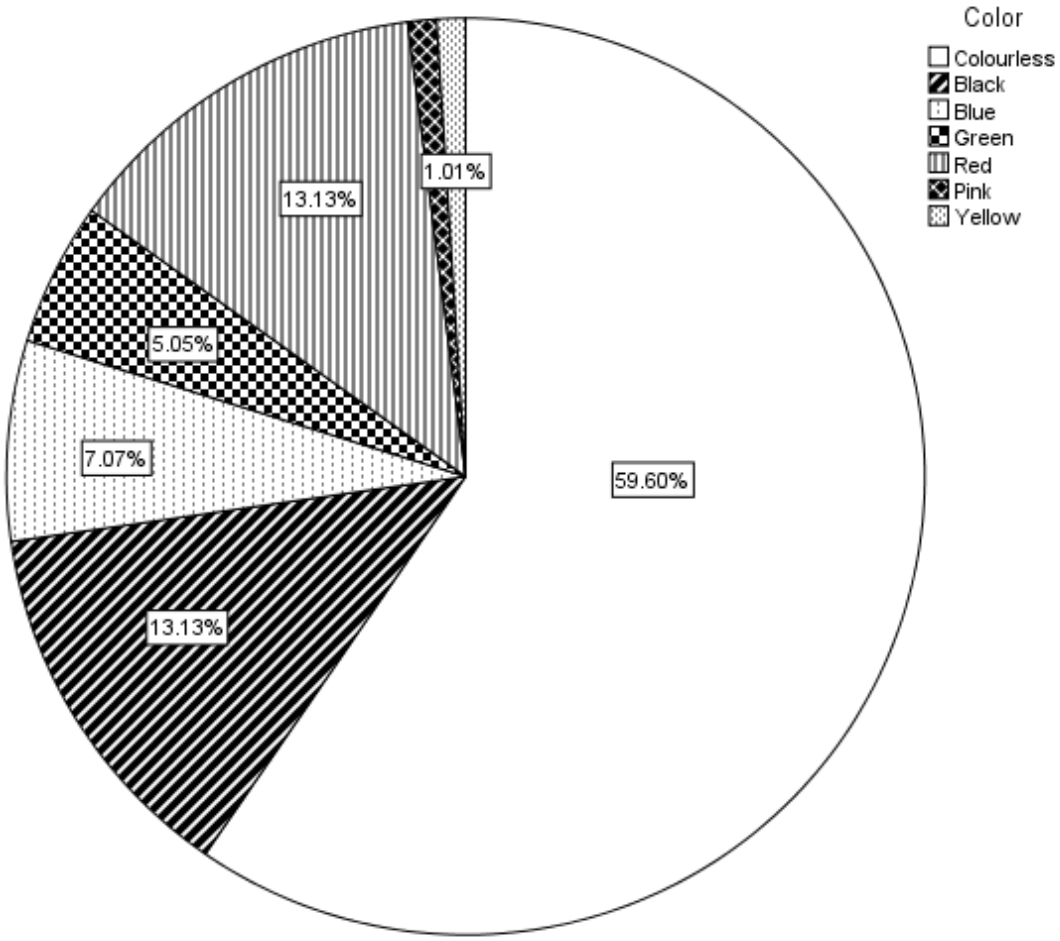

Figure 4. 3 Relative Abundance of Microplastics of different colours in crabs sampled along the Kenya Coast during the study period

Table 4. 4 Overall mean (± SE) length (mm) of microplastics of different colours found in crabs

Colours	Black	Blue	Colourless	Green	Purple	Red	Yellow	$F_{6, 133}$	*P*
	2.3 ± 0.36^{C}	4.2 ± 1.06^{C}	3.5 ± 0.46^{C}	3.9 ± 0.83^{C}	0.45 ± 0.31^{A}	3.1 ± 0.58^{C}	0.6 ± 0.35^{A}	5.971	< 0.05

Means (± SE) along rows followed by the same uppercase letters are not significantly different (Tukey pairwise comparisons of means $p \leq 0.05$).

Table 4. 5 Mean (± SE) lengths (mm) of microplastics of different colours in crabs per station

	Black	Blue	Colourless	Green	Red	Purple	Yellow	*F*	*F* value	*P*
Nyali Bd.	2.02 ± 0.30^{aA}	4.9 ± 2.95^{aA}	3.18 ± 0.41^{aA}	2.62 ± 1.51^{Aa}	2.25 ± 1.14^{aA}	2.0 ± 2.00^{aA}	2.00 ± 2.00^{aA}	$F_{6,14}$	0.38	0.88
Dabaso	1.5 ± 1.49^{aA}	1 ± 0.99^{Aa}	4.60 ± 2.34^{aA}	2.25 ± 1.88^{aA}	6.02 ± 2.53^{aA}	_	_	$F_{4,14}$	1.99	0.14
KMC	3.67 ± 1.58^{aA}	1.81 ± 1.81^{aA}	3.54 ± 0.61^{aA}	5.25 ± 1.14^{a}	3.08 ± 0.41^{aA}	0.50 ± 0.5^{aA}	_	$F_{5,14}$	2.68	0.07
Makupa	1.31 ± 0.19^{aA}	3.38 ± 0.37^{aA}	2.25 ± 0.25^{aA}	9.0 ± 3.00^{aB}	1.25 ± 1.25^{A}	_	_	$F_{4,5}$	0.057	0.01
Mikindani	2.83 ± 0.30^{aA}	8.0 ± 2.64^{aA}	5.17 ± 1.86^{aA}	7.17 ± 2.42^{aA}	3.5 ± 1.95^{aA}	_	_	$F_{4,10}$	1.22	0.36
Mwa.Sgr	3.01 ± 0.25^{aA}	6.9 ± 5.60^{aA}	2.57 ± 0.20^{aA}	1.00 ± 1.00^{aA}	3.43 ± 0.62^{aA}	_	2.00 ± 1.00^{aA}	$F_{5,12}$	0.72	0.62
Mwa.T	1.66 ± 0.91^{aA}	2.87 ± 0.33^{Aa}	2.45 ± 0.37^{aA}	2.0 ± 2.00^{aA}	2.37 ± 0.82^{aA}	_	_	$F_{4,12}$	1.67	0.20
$F_{6,13}$	0.82	0.848	0.79	2.86	1.39	0.92	1.21			
P-Value	0.58	0.56	0.59	0.053	0.29	0.51	0.36			

Mean (± SE) within columns followed by the same letters are not significantly different, means (± SE) along rows followed by the same uppercase letters are not significantly different (Tukey pairwise comparisons of means $p \leq 0.05$).

Mean (± SE) concentration of microplastics (number of MP per gram of tissue) in crabs ranged between 0.13 and 1.16mp/g tissue in *U. dussumieri*, 0.33 and 0.52 MP/g tissue in *U. inversa* and 0.79 MP/g tissue in *U. vocans* (Table 4.6). Higher concentrations of microplastics were observed in *U. dussumieri* found in Nyali Bd and Mikindani stations, that is, 1.16 ± 0.84 and 1.24 ± 0.32 MP/g tissue respectively, whereas *U. dussumieri* in Makupa had the lowest mean concentration of microplastics (0.13 MP/g tissue). The mean concentration of microplastics in *U. dussumieri* from different stations was however not significantly different ($F_{5, 9} = 0.88$; $p = 0.532$). Similarly, no significant difference was noted in the mean concentrations of microplastics between the three species of crabs ($F_{2, 17} = 0.226$; $p = 0.8$). There was no significant correlation between the mean weight of crabs and the concentration of microplastics in their tissues r (18) = 0.2; $p > 0.05$ (Fig 4.4).

Table 4. 6 Mean (± SE) concentration of microplastics (no. of microplastics/gram of tissue) in crabs from different localities

Site	Station	Ave MP. conc (MP/g)
	U. dussumieri	
Tudor	Mik	1.24 ± 0.32
	KMC	0.51 ± 0.11
	Nyali Bd	1.16 ± 0.84
Port Reitz	Mak	0.127
	Mwa. T	0.28 ± 0.18
Mida	Dab	0.9 ± 0.13
	U. inversa	
Tudor	Mak	0.52
Mida	Dab	0.33
	U. vocans	
Port Reitz	Mwa. Sgr	0.79

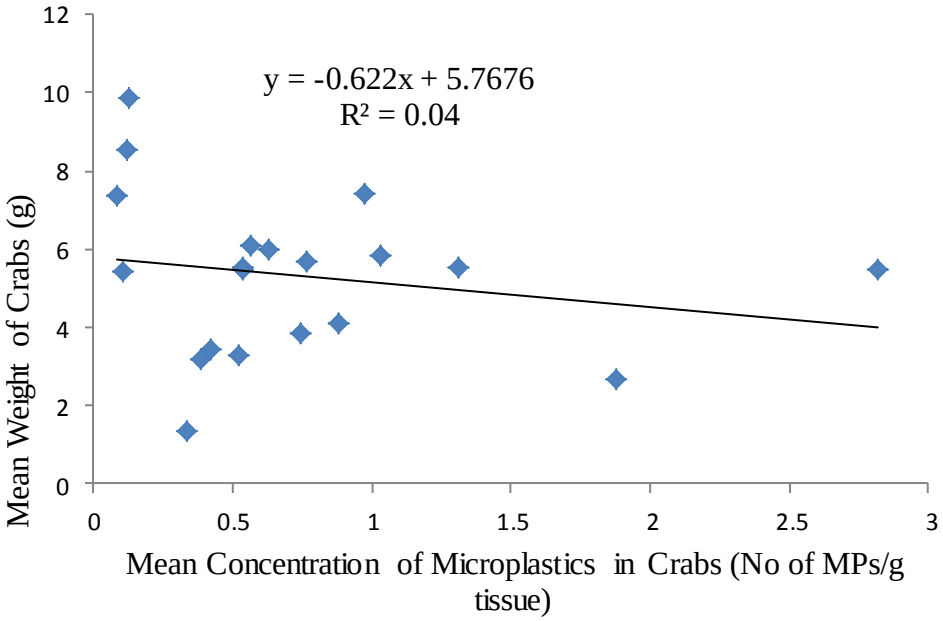

Figure 4. 4 Correlation between the Mean Weight of Crabs and MPs Concentration

4.3.3. Microplastics appearance and occurrence in oysters.

All the eight different colours of microplastics fibres were encountered in the oysters (black, colourless, blue, yellow, green, pink, purple and red). In Mwache T., only three colours of microplastics were obtained from the oyster samples, that is, black, blue and colourless. Generally, the colourless fibres were the majority making up 69% of the total amount of fibres. The occurrence of microplastics of other colours were: black 11%, blue 3%, green 3%, red 12%, pink 0.9%, purple 0.1% and yellow 1% (Fig 4.5). The mean (± SE) length of microplastics fibres ranged between 0.1 and 3 mm. The mean length was statistically different among the different colours of microplastics ($F_{7, 48} = 8.19$; $p < 0.05$) (Table 4.7) with black, blue, colourless and red fibres being significantly longer than pink and purple fibres. Stations however did not have any influence on the length of fibres of the same colour (Table 4.8).

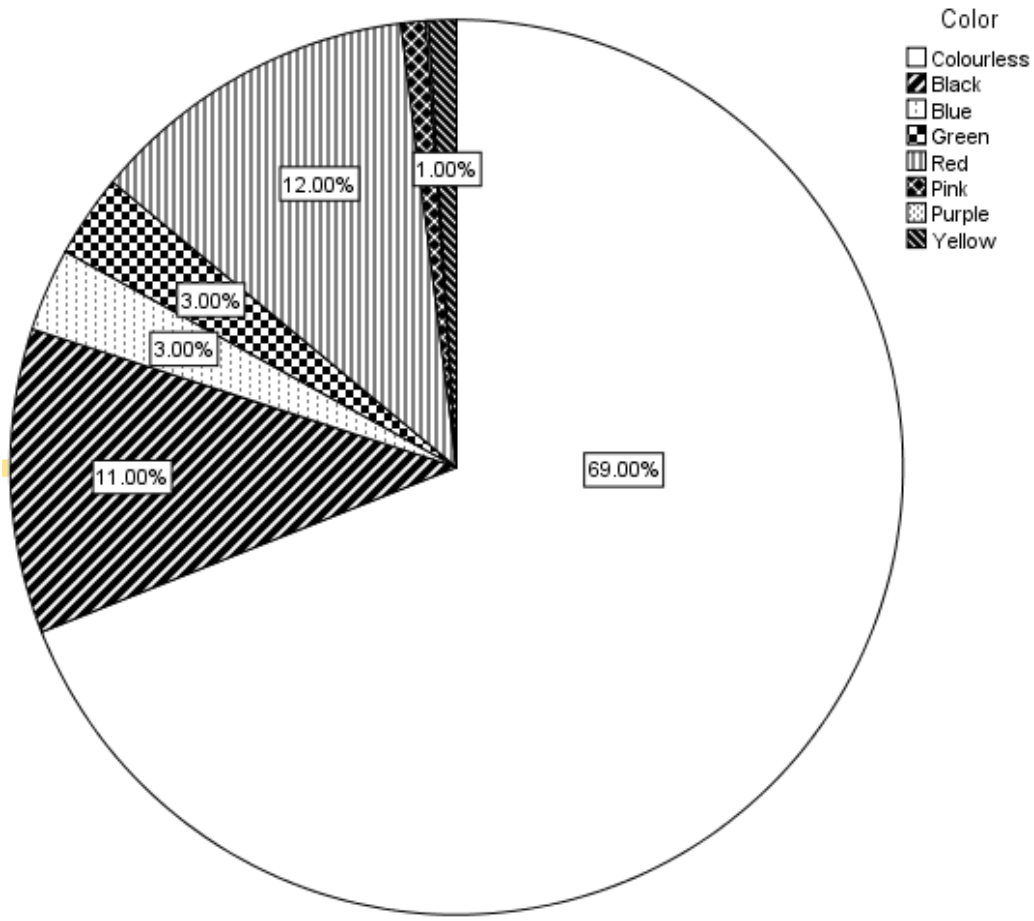

Figure 4. 5 Relative Abundance of Microplastics of different colours in Oysters sampled along the Kenya Coast during the study period

Table 4. 7 Overall mean (± SE) lengths (mm) of microplastics of different colours in oysters

Colour	Black	Blue	Colourless	Green	Pink	Purple	Red	Yellow	$F_{7, 48}$	*p*
	2.34 ± 0.18^{b}	2.26 ± 0.42^{b}	3.16 ± 0.47^{b}	1.82 ± 0.51^{Ab}	0.11 ± 0.08^{A}	0.21 ± 0.21^{A}	2.79 ± 0.55^{b}	1.11 ± 0.48^{Ab}	8.19	< 0.05

Means (± SE) along rows followed by the same uppercase letters are not significantly different (Tukey pairwise comparisons of means $p \leq 0.05$.

Table 4. 8 Mean (± SE) lengths (mm) of microplastics of different colours ingested by oysters per station

	Black	Blue	Colourless	Green	Pink	Purple	Red	Yellow	$F_{7, 32}$	*p*
Dab	2.25 ± 0.12^{aB}	2.91 ± 0.08^{aB}	2.45 ± 0.19^{aBC}	1.62 ± 0.87^{aBC}	-	0.11 ± 0.21^{C}	3.80 ± 0.09^{aB}	1.11 ± 0.48^{aBC}	21.11	< 0.05
Eng. Pt	2.19 ± 0.35^{aB}	1.36 ± 0.71^{aBC}	3.91 ± 0.98^{aB}	2.62 ± 0.40^{aB}	0.43 ± 0.25^{C}		2.69 ± 0.54^{aBC}	2.60 ± 0.13^{aB}	4.47	< 0.05
Mwa.T	3	3	3	-	-		-			
$F_{2,4}$	1.22	2.76	1.09	1.93	-		12.1	1.81		
P-Value	0.39	0.18	0.42	0.26	-		0.20	0.26		

Means (± SE) within columns followed by the same letters are not significantly different, means (± SE) along rows followed by the same uppercase letters are not significantly different (Tukey pairwise comparisons of means $p \leq 0.05$).

Mean (± SE) concentration of microplastics in oysters was higher in samples from Mwache T. (5.75 MP/g of tissue) than those from Dabaso and Eng. Pt. which had a concentration of 2.99 ± 0.24 and 2.94 ± 0.88 MP/g of tissue respectively. During the comparative analysis of mean concentration of microplastics in oysters, Mwa.T was left out as oysters were only encountered at a single point: therefore they were not considered a replicate and using them would have meant pseudo replication. Results of the analysis revealed no significant difference in the mean concentration of microplastics between Dabaso and English Point oysters ($F_{1, 4} = 0.04$; $p = 0.95$) (Table 4.9). There was significant correlation between the mean weight of oysters and concentration of microplastics in their tissues, r (5) = 0.3; $p > 0.05$.

Table 4. 9 Mean (± SE) concentration (MP/g of tissue) of Microplastics in oysters from different stations along the Kenyan Coast

Site	Mean (± SE) MP Conc.
Dabaso	2.99 ± 0.24
English Point	2.94 ± 0.88
Mwache Tsunza	5.75
$F_{1,4}$	0.004
p – value	0.95

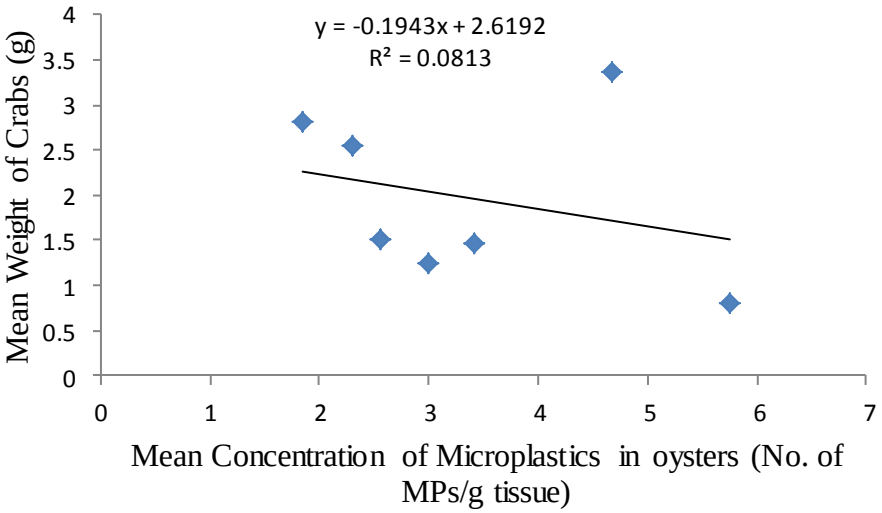

Figure 4. 6 Correlation between the Mean Weight of Oysters and MPs Concentration

4.3.4 Microplastics appearance and occurrence in Jelly fish

A total of seven different colours of microplastic fibres were encountered in jellyfish, that is, black, blue, green, colourless, purple, red and yellow. Out of these colours, only four:black, blue, colourless and red were obtained in Dabaso while in Makupa and Mikindani, only one out of the seven colours was missing in each station, that is, yellow in Makupa and purple in Mikindani. Colourless fibres were the majority accounting for 53% of the total number of fibres while purple fibres were the least at a percentage of 1% (Fig 4.7). Fibre lengths ranged from 0.3 to 2.23 mm where colourless fibres were slightly longer with a mean of 2.23±0.46 mm while purple fibres were the shortest at 0.30 ±0.30 mm. The means of these lengths were however not statistically different ($F_{6, 28}$ = 1.3; p = 0.29) (Table 4.10). Additionally, stations had no significant effect in the mean lengths of microplastics of different colours (Table 4.11). Mean concentration of microplastics in jellyfish was 0.05 MP/g tissue in Dab, 0.027 ± 0.01 MP/g tissue in Makupa and 0.03 ± 0.003 MP/g tissue in Mikindani (Table 4.12). Statistically, these concentrations were not significantly different among the three ($F_{1, 2}$ = 1.34; p = 0.43).

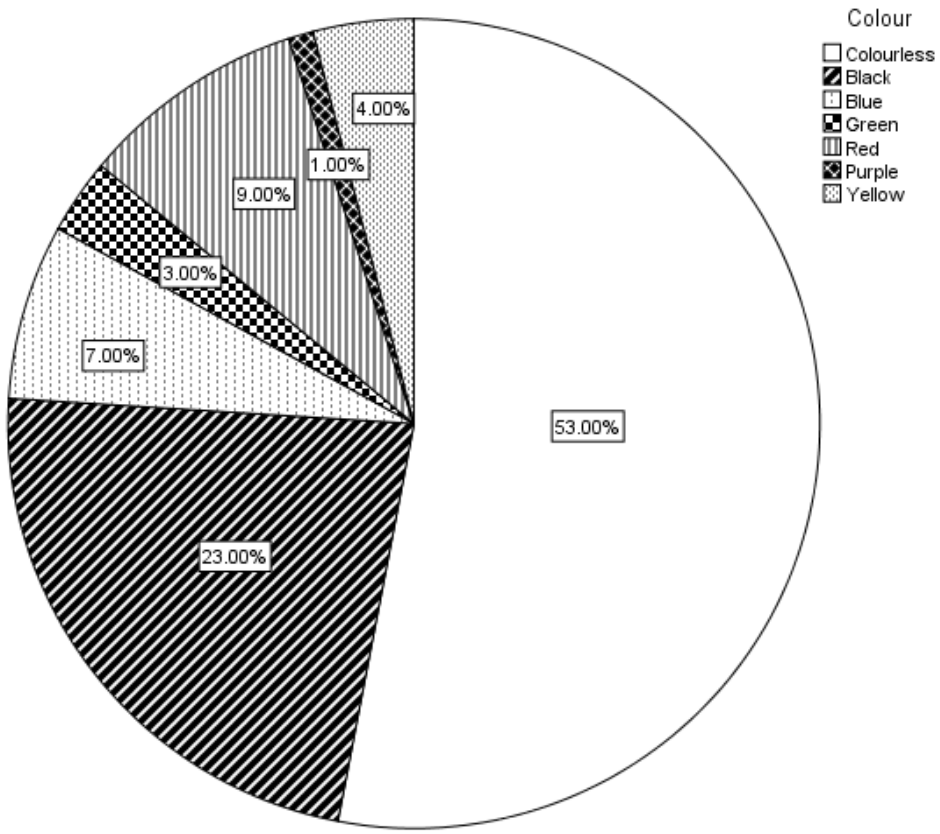

Figure 4. 7 Relative Abundance of Microplastic of different colours in Jellyfish sampled along the Kenya Coast during the study period

Table 4. 10 Overall mean (±) lengths of microplastics of different colours in jellyfish

Colour	Black	Blue	Colourless	Green	Purple	Red	Yellow	$F_{6, 28}$	*p*
	1.87 ± 1.2^{a}	2.14 ± 0.93^{a}	2.23 ± 0.46^{a}	0.53 ± 0.37^{a}	0.30 ± 0.30^{a}	1.81 ± 0.88^{a}	0.60 ± 0.60^{a}	1.282	0.3

Table 4. 11 Mean (± SE) lengths (mm) of microplastics of different colours in jellyfish per station

	Black	Blue	Colourless	Green	Red	Purple	Yellow	*F*	*p*
Dabaso	1.31	3.00	1.50	0.00	2.50	0.00	0.00		
Makupa	0.79 ± 0.79^{aA}	2.34 ± 2.34^{Aa}	3.21 ± 0.71^{aA}	0.94 ± 0.93^{aA}	2.34 ± 2.34^{aA}	0.75 ± 0.74^{Aa}	-	0.83	0.59
Mikindani	3.24 ± 3.24^{aA}	1.5 ± 1.49^{Aa}	1.62 ± 0.03^{aA}	0.37 ± 0.37^{aA}	0.94 ± 0.94^{aA}	-	1.5 ± 1.49^{aA}	0.58	0.76
$F_{2,2}$	0.29	0.11	3.17	0.33	0.2	0.6	0.6		
P-Value	0.78	0.9	0.24	0.76	0.83	0.63	0.63		

Means (± SE) within columns followed by the same letters are not significantly different, means (± SE) along rows followed by the same uppercase letters are not significantly different (Tukey pairwise comparisons of means $p \leq 0.05$).

Table 4. 12 Mean (± SE) concentration (Number of microplastics/gm of tissue) of microplastics in Jellyfish from different sites along the Kenyan Coast

Station	Mean (± SE) MP concentration
Mikindani	0.03 ± 0.003
Makupa	0.03 ± 0.014
Dabaso	0.05
$F_{1, 2}$	1.34
P	0.43

4.4 Interspecific Analysis of Microplastic Concentration

The results of the independent two sample test revealed a significant difference in the concentration of microplastics between crabs and oysters (t = 5.61; df = 14; p = 0.014), jellyfish and oysters (t = 5.28; df = 10; p = 0.01) and between crabs and jellyfish (t = -3.45; df = 12; p = 0.002). Generally, oysters were observed to concentrate more microplastics (3.36 ± 0.53/g of tissue) as compared to crabs (0.65 ± 0.13/g of tissue) and jellyfish (0.03 ± 0.01/g of tissue).

CHAPTER FIVE

5. DISCUSSION

Kenyan Coastal towns including Mombasa, Kwale, Lamu and Kilifi are constantly under threat of plastic pollution (Tan, 2012**).** A study by Kimani *et al*. (2018) clearly indicates that microplastics (plastics < 5 mm in diameter) are abundant in the Kenya's marine environment and are interacting with the zooplanktons therein through ingestion. However, no study has been conducted to determine the ingestion of microplastics by the macro-invertebrates in the region. This study, therefore, represents the first evidence of microplastics ingestion by marine macro-invertebrates along the Kenyan Coast, particularly within the creeks where subsistence fishery occurs. The study mainly focused on sites around Mombasa due to the growing human population in the region and high solid wastes originating from the industrial and tourism sectors (Okuku *et al*., 2019). Mida Creek, located in Watamu National Park was used as a control because of its distant location from Mombasa town, hence less influenced by human activities.

During the study, physico-chemical properties of water such as salinity, salinity, pH, temperature, and electrical conductivity were taken owing to their contribution to the breakdown of macroplastics to microplastics. At higher temperatures, salinity and pH, enzymatic action is favoured; this facilitates the rate of biodegradation of larger plastics increases resulting to the formation of many small plastic particles in the water (Klein *et al*., 2018). According to Hamza *et al.* (2016) and Saad *et al.* (2017), these physico-chemical parameters also help in determining quality of water as well as the survival and distribution of aquatic biota. Based on the results of this study, there was a slight variation in the physico-chemical parameters among the sites studied. Mean temperature varied between 27 °C and 29 °C, probably due to factors such as ground water inflows, setting of the surrounding, storm water runoff and turbidity (Hamzah *et al*., 2016). Water temperature was lowest at Dabaso; this is probably because Mida Creek experiences ground water seepage, which causes mixing of the warm waters and cold waters. The pH ranged from 7 to 8: this was within the acceptable range of 6.5–8.5 as set by USEPA (2012) and NEMA (2006) (Makokha, 2019). Nonetheless, pH values around Mwache area were slightly lower than in other areas. Hamza *et al*. (2016) attribute low water pH to increased acidity resulting from chemical contaminants in the water. Low pH in Mwache area therefore, could have been caused by leakage of combusted and un-combusted fuel as a result of heavy shipping activities in the nearby

Mombasa port (Okuku *et al*., 2019). In addition, the low pH in Mwache area may be due discharge of acidic water from the nearby agricultural and industrial sectors into rivers that empty into Port Reitz creek at Mwache area.

Salinity is a measure of the concentration of dissolved salts in water. Differences in sea surface salinity are usually caused by processes such as evaporation, ocean runoff, ice melt and ocean currents. In the open ocean, salinity range is approximately 30-40 parts per million (Talley, 2002). Results of this study show a variation in salinity among the sites studied with the highest salinity being recorded in Nyali Bridge and relatively low values in Makupa, Mwache SGR, Mikindani and Kenya Meat Commission. Low salinity in Mikindani, Kenya Meat Commission, and Mwache SGR was attributed to the dilution of the creek waters by fresh waters from the rivers that flow into the creeks. In Nyali Bridge, high salinity could be as a result of its nearness to the open sea hence regular interaction between the sea water and creek water. On the other hand, lower salinity values in Makupa were speculated to be as a result of dilution of the salty water by groundwater seeping into the creek. This is because Makupa is an enclosed creek with no inlet or outlet, and therefore dilution of salinity is likely to occur only through ground water seepage.

Electrical conductivity is influenced by ions in the water, whereby an increase in the concentrations of these ions results to a higher electrical conductivity, vice versa (Makokha 2019). Mwache Tsunza recorded a slightly higher conductivity than the other stations. This could have been caused by discharge of acidic water and fertilizers from the agricultural and domestic sectors into the Mwache river that empties into the creek. Similarly, Makokha (2019) indicates that activities such as geology, soil and land use can affect the water chemistry. Generally, the values recorded for the physico-chemical parameters were within the ideal limits except for electrical conductivity that was slightly higher than normal.

This study realized an uneven distribution of the target organisms along the Coast of Kenya. According Ravichandran *et al*. (2007), several factors including salinity, substrate suitability, mangrove distribution, and tidal inundation influence the distribution of intertidal organisms. Crabs were encountered in all the sites except at English Point. Three Uca crabs: *Uca dussumieri*, *Uca inversa* and *Uca vocans* were encountered with *Uca dussumieri* being the most abundant occurring in all the stations except Mwache SGR. The dominance of Uca dussumieri in the sampled stations shows that they are the most widespread species of Uca crabs, and also well adapted to survive in different environmental conditions. Uca vocans on

the other hand, were encountered only in Mwache SGR, a sign that they are either strong competitors with the ability to outcompete the other species in terms of food and other vital resources such as space, or they may just be better suited for that particular region. In terms of abundance, Uca inversa were the least: this may be attributed to risk of predation due to their small size. As illustrated by Mokhtari et al. (2015), the small sized crabs are at high risks of predation particularly by the small avian predators.

Jelly fish and oysters were encountered in three stations only, that is, Makupa, Mikindani and Dabaso for jellyfish, and English Point, Mwache Tsunza, and Dabaso for oysters. The number of jellyfishes encountered in Makupa was higher than in Dabaso and Mikindani while Port Reitz Creek had none. According to Purcell *et al*., (2007), the distribution of jellyfish is affected by factors such as water eutrophication, which leads to phytoplankton growth and zooplanktons abundance. Since zooplanktons are essential source of food to jellyfish, increase in their production leads to increase in jellyfish numbers (Purcell *et al*., 2007). The high number of jellyfish in Makupa therefore, could have been influenced by an increase in the growth of planktons due high levels of nutrients in this system which could have leached from the Kibarani dumpsite (Okuku *et al*., 2011). Moreover, since the creek is enclosed, the concentration of these nutrients tends to build up in the water, boosting further the plankton production.

The study also realized a variation in the mean shell length and weight of oysters belonging to the same species. Oysters from English point that were attached on rocks were slightly larger and heavier than those from Mwache Tsunza and Dabaso that were attached on mangrove stems. As indicated by McAfee *et al*. (2016), mangrove canopies tend to shade the substratum, resulting to reduced foraging efficiency hence slow growth of species underneath. Rocky shores on the other hand, form suitable habitats due to strong latitudinal gradient in humidity and temperature (McAfee *et al.*, 2016).

This study has established that macro-invertebrates such as crabs, oysters and jellyfish along the coast of Kenya were ingesting microplastics. Previous studies have also revealed ingestion of microplastics by crabs and oysters (Akpan 2014, Watts *et al*., 2014, 2015, Cole and Galloway 2015). Ingestion of microplastics by jellyfish however, is rarely studied. The first incident of microplastics ingestion by jellyfish was observed in *Pelagia noctiluca* (Forsskal 1775) by Macali *et al*. (2018) and is corroborated by the findings of this study.

According to EFSA (2016), Hoogenboom, (2016) and Lusher et al. (2017), ingestion of microplastics occurs as a result of their small size, which resembles planktons.

Microplastics obtained in this study were mainly fibres. This is consistent with other studies on microplastics such as those from, Nelms *et al*. (2018), Lusher *et al*., (2017), Naidoo & Glassom (2016), and Cole *et al*. (2014). The fibres were of different colours, that is, colourless, black, blue, green, red, yellow, pink and purple, an indication that they came from multiple sources. As reported in other studies, ingested fibres may have come from urban surface runoff, coastal tourism, fisheries, wastewater treatment plants, shipyards, rivers, synthetic textiles, and personal care products (Graca *et al*., 2017).

There was a variation in the occurrence of microplastics of different colours with colourless fibres being the dominant colour. This reveals the high contamination of the ocean with these types of microplastics. Possible sources of the colourless microplastics are the fishing nets mostly used by the local fishermen. In addition, the colourless fibres could have resulted from the bleaching of coloured plastics. The mean lengths of the microplastics ingested were below 5 mm for oysters and jellyfish while in crabs, microplastics measured up to 9 mm in length. It is possible that microplastics in the water column where the filter feeders live were broken into broken into smaller sizes by forces in the water column such as currents. Alternatively, filter feeders might just be having a high selectivity for shorter microplastics.

In regards to the concentration of microplastics, there was a variation within and among the species. Among the crab species, a higher concentration of microplastics was observed in *U. dussumieri* while *U. inversa* had the least microplastic concentration. This difference could be attributed to the fact that *Uca dussumieri* species were the most common species of crabs occurring in almost all the sites, therefore were at more risk of getting into contact with the microplastics. On the other hand, the low microplastic concentration among the *U. inversa* could probably be as a result of their small size hence a smaller stomach to contain many plastic particles. Also, it is possible that the *U. dussumieri* are fast feeders, and therefore able to ingest more plastic particles in the environment compared to *U. inversa* and *U. vocans*.

Stations also had an influence on the concentration of microplastics in the crabs. For instance, the mean concentration of microplastics was higher in *U. dussumieri* from Mikindani and Nyali Bridge than those from Port Reitz and Mida creeks. The high concentration of microplastics in Mikindani was attributed to increased solid wastes being released from the surrounding industrial and domestic sectors into the creek waters, whereas in Nyali Bridge,

high microplastics concentration could be as a result of increased input of pollutants from the nearby Coast General Hospital and the Technical University of Mombasa (TUM). Moreover, high microplastics concentration in Nyali Bridge may be due to a rise in human population in the area which is associated with increased in both municipal and industrial wastes (Okuku *et al*., 2019). Also, observed microplastics abundance in crabs from Mikindani and Nyali Bridge could have resulted from increased degradation rates of larger plastics into smaller plastics due to high water temperature, pH and salinity in these stations (Klein *et al*., 2018).

In oysters, the mean concentration of microplastics did not vary significantly between the stations except in samples from Mwache Tsunza which had a significantly higher concentration of microplastics than those from Dabaso and English Point. Elevated concentration of microplastics in Mwache Tsunza area could be as a result accidental releases or poor handling of cargo wastes comprising plastics, at the nearby Mombasa Port. This result is however not very conclusive as oyster samples from Mwache Tsunza were only encountered at a single point.

Interspecific comparison of microplastic concentration revealed a significant difference in microplastic concentration among the species. The variation was attributed mostly to the differences in their feeding mechanisms. Concentration of microplastics was higher in oysters than in crabs and jellyfish. This was attributed to the high water filtration capacity of these filter feeders (Zhou *et al*, 2014). Filter feeding bivalves often sieve several cubic meters of water in order to obtain enough plankton and Particulate Organic Matter (POM) (Tilley, 2018, Zhou *et al*, 2014). The filtration process generates a lot of currents, which brings forth suspended particles in the water including microplastics. Since filter feeding occurs in the water column, high concentration of microplastics in oysters could also mean that the microplastics in the Kenyan coastal waters are accumulating more in the water column hence readily available to the filter feeders than in the sediments. Influence of feeding modes on microplastic ingestion has also been highlighted by Cole and Galloway (2015) who discovered high microplastics consumption by marine zooplanktons including salps, sea urchin larvae, pelagic, and benthic copepods as a result of their suspension feeding mechanism.

Evidence of high microplastic ingestion by bivalves as demonstrated by this study, is also presented by EFSA (2016) in a research using bivalves *Mytillus tossulus* (Gould, 1850) and *Macoma balthica* (L., 1758), crustaceans and deposit feeding invertebrates. The high intake

of microplastics by the bivalves was attributed to their filter feeding mode (EFSA, 2016). Although jellyfish employ the same filter feeding mechanism to sieve zooplanktons in the water column, concentration of microplastics was much lower than in their oyster counterparts. A possible explanation to this observation is that, compared to oysters that actively filter water to obtain food, jellyfish are passive filter feeders, feeding only on particles they encounter. They are thus less exposed to microplastics than the oysters.

Understanding the concentration of microplastics in macro-invertebrates such as oysters and crabs is essential because they are economically important as source of food for humans around the globe (Blastic, 2018). Since these organisms are often consumed without gutting, microplastics retained in their digestive tracts are likely to be passed to human diet. Upon reaching the human gut, microplastics may be translocated into tissues such as the lymphatic systems with implications on human health. To date, there is little evidence regarding the toxicity of microplastics on humans; however, studies indicate that they may act as vectors of chemicals such as Polychlorinated Byphenyls (PCBs), which are often adsorbed, in the plastics during their manufacture (Van Cauwenberghe and Janssen, 2014). According to Naidoo (2017), Galloway (2015) and Hammer (2012), the chemicals adsorbed on the microplastics may be leached into the digestive fluids and transferred to other organs of the body. Forster (2016) states that consumption of food contaminated with microplastics could have implications on human health including poisoning, infertility and disruption of their genetic makeup. Microplastics may also cause blockage or damaging of the digestive tracts of animals, thereby reducing their feeding capacity (Hoss and Settle, 1990). In this study, it was impossible to determine the exact location of the microplastics in the organisms and, therefore, this should be an important consideration for future research. Nonetheless, effects of microplastics ingestion can only become clearer with increasing studies on the same.

This study has also revealed the transboundary nature of microplastics to an extent that even the nature reserves surrounded by the oceans are not spared. This is evident in Dabaso, which is a nature reserve located in Watamu Marine National Park. Since this is a protected zone with limited movement of people, it was expected that the number of microplastics in the samples would be few or none. Nonetheless, this was not the case as the number of microplastics extracted from the samples was equally high compared to the number of microplastics in samples taken from creeks surrounding Mombasa town. The source of these microplastics was speculated to be as a result of tourism activities around the reserve or could have been transported into the creeks from other sources via wind or ocean currents.

CHAPTER SIX

6. CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study has established the presence of microplastics in the filter and deposit feeding biota along the Kenyan coast and especially within the creeks where most subsistence fishery occurs. The study has also provided an outline of the various types of microplastics ingested by the macro-invertebrates in terms of colour and shape. As observed, microplastics obtained were mainly fibres with the colourless ones being the dominant. Variation in colour of fibres was an indication that these pollutants were from multiple sources. On the other hand, colourless fibres could have originated from the commonly used fishing nets.

The study has also established a variation in microplastics concentration among the different macro-invertebrates species studied with oysters having the highest mean concentration. This is attributed to their filter feeding habits which generates currents and brings forth food particles in the water, including microplastics. Moreover, filter feeders often filter large volumes of water in order to acquire food (plankton); therefore, they are more likely to be exposed to microplastics than deposit feeders. High concentration of microplastics in oysters could also mean that more microplastics at the Kenyan coast are accumulating in the water column than at the sea bottom, which makes them readily available to the filter feeders. In addition, the study has revealed the trans-boundary nature of microplastics to an extent that even nature reserves are not exempted. For instance, the concentration of microplastics in macro-invertebrates from Dabaso, which is a nature reserve, was as high as samples from the Mombasa creeks.

Besides the presence of microplastics in marine fauna, this study has established a spatial variation in physico-chemical parameters of water such as pH, salinity, temperature and water conductivity between different sites along the Kenyan Coast. These factors had an influence on microplastic abundance and the distribution of the target macro-invertebrates. For instance, microplastic concentration was higher in crabs from Mikindani and Nyali Bridge than the other station: this was attributed to high temperature, pH, and salinity which facilitated biodegradation of larger plastics into many small plastic particles.

6.2 Recommendation

- Regular beach clean-up should be conducted to reduce accumulation of plastic wastes on the beaches and prevent possible entry into the marine ecosystems.
- All sea foods should be thoroughly cleaned before consumption to remove any microplastics in their bodies.
- Impacts of microplastics on the organisms, particularly fisheries resources, still remain unclear and should be considered in future research work.
- This study also recommends further investigations to determine the possible types of polymers that the microplastics in the studied macro-invertebrates are made up of and in which body parts of the invertebrates the microplastics accumulated.
- Factors contributing to the distribution of benthic invertebrates along the Kenyan Coast should also be investigated to establish why organisms such as oysters and jellyfish are absent in some areas.
- This research also calls upon policy makers and key stakeholders to develop proper management strategy for plastic wastes to reduce their accumulation in the ocean ecosystem.

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