UNIVERSITY OF CAPE COAST

MICROPLASTIC OCCURRENCE AND DISTRIBUTION IN THE WATER, SEDIMENT AND FISH OF THE PRA ESTUARY, GHANA

ANDOH KWAKU AMPONSAH

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ANDOH KWAKU AMPONSAH

A thesis submitted to the Department of Fisheries and Aquatic Sciences of the School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of a Master of Philosophy (M.Phil.) degree in Oceanography and Limnology

NOVEMBER 2021

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature…… Date ……

Name: ANDOH KWAKU AMPONSAH

Supervisor's Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision laid down by the University of Cape Coast

Principal Supervisor' Signature……. Date……..……

Name: .. DR. ERNEST AMANKWA AFRIFA

Co-Supervisor's Signature…… Date……

Name: …. DR. PAUL KWAME ESSANDOH

ABSTRACT

The study investigated the occurrence and distribution of microplastics in the water, sediment and fishes from the Pra estuary. Samples were collected using a manta trawl and Ekman grab within sixteen sampling points of 1km apart. Microplastics (MPs) were categorized into shapes, colour, size and polymer types using a stereomicroscope and ATR-FTIR spectrometer. A total of 12 species, *Gobionellus occidentalis, Ethmalosa fimbriata*, *Chrysichthys nigrodigitalus*, *Elops lacerta, Mugil bananesis*, *Cynoglossus senegalensis, Galeoides decadactylus, Sarotherodon melanotheron, Apsilus fuscus, Pseudotolithus senegalensis Callinectis aminicola,* and *Penaeus penaeus* were identified to accumulate microplastics. Occurrence of MPs in the gastrointestinal tract exceeded that of the gills. Microplastics were present in the vesical tissues of all the shellfishes sampled. The overall mean (± s.d) density of microplastics in the surface water and sediment were 196,259.84 ± 60168.72 items.km^{-2} and 116.44 ± 11.31 items.kg^{-1}d.w respectively. The occurrence of microplastics was significantly higher at S-N (a tributary passing the Anlo community) along the Pra estuary. Temporally, microplastic abundance was observed to increase during the early raining season (April) compared to the other months. Further, the flow velocity had a significant influence on microplastic distribution in the water column. Transparent and black fibres, less than 0.5 mm were found to dominate the surface water, sediment and fish. Polyethylene and Polypropylene were found to be the most occurring polymer type in the Pra estuary. Hence, there is the need for proper plastic waste management strategies to reduce the amount of plastics waste entering into the aquatic environment.

KEY WORDS

Microplastics

Spatial – temporal

Hydrographic conditions

Fish

ATR-FTIR

Pra Estuary

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DEDICATION

To my family:

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LIST OF ACRONYMS

ATR – FTIR – Attenuated Total Reflectance – Fourier Transform Infrared

BW – Body weight

CF – Condition Factor

CW – Carapace width

d.w – Dry weight of sediment

DO – Dissolved Oxygen

ppt – Part per thousand

FV – Flow velocity

GIT – Gastrointestinal Tract

MPs – Microplastics

NTU – Nephelometric Turbidity Unit

pH – Hydrogen ions

TL – Total Length

VM – Visceral mass

CHAPTER ONE

INTRODUCTION

The proliferation of microplastics within the aquatic environment has become a serious issue of concern in recent years for both the scientific and legislative communities. Several studies have reported the deleterious effects of microplastics on vital ecosystems including their potential indirect transfer to humans through aquatic resources (Abbasi et al., 2018; Carbery, O'Connor & Palanisami, 2018; Cook & Halden, 2020; Curren et al., 2020; Fernández Severini et al., 2020; Pan et al., 2021; Smith et al., 2018; Su et al., 2019; Wright & Kelly, 2017). Aside these threats, the uneven distribution and dynamic transfer of microplastics within different aquatic media have necessitated the regional and sub-regional documentation of microplastic occurrence, abundance, and distribution globally. However, in Africa and particularly Ghana, studies on microplastics are largely focused on the occurrence in the marine and lagoon environment (Adika et al., 2020; Chico-Ortiz et al., 2020; Gbogbo et al., 2020). There are limited research work on the spatio-temporal assessment of microplastics in the water, sediment and fish in the Pra estuary, despite its high richness in biodiversity and support to livelihoods (Okyere, 2018). The Pra estuary also presents a unique medium with a heavy silt load, owing to the continuous illegal mining activities (galamsey) occurring within and upstream of the river. Thus, undertaking this current study on the occurrence of microplastics in a highly turbid system, provides insightful information on the spatio-temporal distribution of microplastics in the Pra estuary to inform policy decision.

Background of the Study

Plastics, since its invention in 1907, has grown to become a household commodity with their use expanding from basic domestic wear to sophisticated equipment (Baekeland, 1909). Despite being toxic to the environment, humans have become over-reliant on these synthetic organic polymers (North & Halden, 2013; Wright & Kelly, 2017). Thus, causing the global plastic production to rise exponentially from 15 million metric tons (mmt) in the 1950s to 368 mmt in 2019, and projected to hit about 1,600 mmt by the year 2050 (Chateau, Bibas, Lanzi, Mavroedi, & Valriberas, 2020; PlasticsEurope, 2020). This raises a serious concern for plastic waste managers and other stakeholders. Plastic pollution is seen as a global environmental issue, with occurrence spanning from air, land, water to biota (Allen et al., 2019; Hurley et al., 2018; Jambeck et al., 2015; Savoca et al., 2019). The strong molecular stability and density enhance the resistance of plastics to several degrading elements such as strong acids and microbial action, causing them to have longer environmental shelf-lives (Andrady, 2011; Hourston, 2010; Webb et al., 2013). Within the aquatic environment, plastics are a major threat to organisms; with issues of entanglement from ghost gears (Angiolillo, 2019), suffocation and gut blockage being widely reported for species such as fish (Rochman et al., 2014; Rummel et al., 2015), turtles (Digka et al., 2020), and seabirds (Cartraud et al., 2019). Lately, the most pervasive form of aquatic plastic debris is microplastics (Hui et al., 2020; Thompson et al., 2004).

Microplastics (MPs) are synthetic organic polymer groups with a diameter size between 1µm to 5 mm (Andrady, 2011) with occurrence in two forms; primary (manufactured) and secondary (fragmentation). Microplastics such as

microbeads, fibre, and pellets are industrially utilized in cosmetic and pharmaceutical products while the others such as fragments, films, filament generated through fragmentation occur from the exposure to Ultraviolet (UV) light or abrasions (Andrady, 2011; Webb et al., 2013). Microplastics are ubiquitous, transcending all aquatic niches and found in the most remotest of places like the polar ice sections (Bessa et al., 2018; Obbard et al., 2014). Studies on microplastics within the marine environment are well documented compared to the freshwater environment. However, freshwater serves as the main conduit of MPs from inland to the ocean (Tibbetts et al., 2018). This makes the freshwater environment a suitable field to understand the types, distribution, fate, and effects of microplastics within the aquatic medium. In water, MPs tend to float vertically within the water column or sink to the bottom depending on the structural densities of the polymers (Erni-Cassola et al., 2019; Fischer et al., 2016). A meta-analysis of polymer groups predominantly sampled from the marine environment reveals the dominance of groups such as low-density polymers (polypropylene and polyethylene) and high-density polymers (polyester, polyamide, and acrylics) within the water column (Erni-Cassola et al., 2019). However, it will be improper to ascribe a similar occurrence for the freshwater medium due to the geospatial variation in the type of terrestrial plastic waste intruding the water at various points in time.

Currently, full knowledge of the toxicity of microplastic in the aquatic environment is still being explored. Some studies have given evidence of the bioaccumulation (Bessa et al., 2018) and trophic transfer (Batel et al., 2016) of micro and nano-sized plastic particles in several aquatic organisms through ingestion, filtration, or dermal absorption (Cole et al., 2013; Pomeren et al.,

2017). In the organism, microplastics possess a serious health risk to the species which includes internal abrasions, gut blockage, oxidative stress, reproductive toxicity, and mortality (Kim, Yu & Choi, 2021; Ogonowski et al., 2016; Rosenkranz et al., 2009). Aside the direct effect of MPs exposure on aquatic species, MPs double as binders of some persistent organic pollutants (e.g. polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, Polycyclic Aromatic Hydrocarbons, bisphenol A, Dichlorodiphenyltrichloroethane), metals (e.g. Cu, Hg, Pb) and microbes (e.g. *Aeromonas* spp*., Vibrio* spp.) that are absorbed from the environment or as associate additives during manufacturing and could induce complications such as; liver toxicity, endocrine disruption, cancer, and pathogenic infections (Akhbarizadeh et al., 2017; Avio et al., 2015; Kirstein et al., 2016; Rios et al., 2007; Rochman, Kurobe et al., 2014). Attention has been drawn to the potential of MP toxicity on humans since humans are at the apex on the trophic chain. Some studies have linked possible exposure complications like reproductive defects (e.g., low sperm count, hormonal disruption), neurological (e.g., toxin response), psychological (e.g., mental retardation) and gut microbiome disruptions to the advent of microplastics in the body on an acute observatory line (Cook & Halden, 2020; Smith et al., 2018; Wright & Kelly, 2017).

Microplastics holdup in the aquatic environment is facilitated by their hydrophobic nature in the medium and complemented by the variation in the hydrodynamic parameters and type of substratum of the aquatic bed (Enders et al., 2019; Liu et al., 2019). Some studies suggest that, the occurrence and abundance of microplastics in streams are influenced by the flow patterns (i.e., sinking induced by low turbulence) and soil texture of the water (Hoellein et al.,

2019; Ixora et al., 2019). Thus, understanding the interplay that exists between these two factors will help to project the transport pathways and potential hotspots of MPs within riverine and estuarine systems. The microplastics transport mechanism within the riverine section especially the estuarine zone is still being expanded. The estuary serves as a transit point for loads from land to sea. The load on arrival is released into the ocean after exceeding the absorption capacity of the estuary; which makes it a reservoir for accumulated load including MPs (Liu et al., 2019). Also, influxes from the freshwater and seawater into the estuary contribute to the rich productive ecosystem within this medium (Douglas et al., 2019); which makes it essential for the study of the fate of MPs in the aquatic environment.

Statement of the Problem

In Ghana, the menace of plastic pollution in the environment continues to be a serious issue of concern for both solid waste and water resource managers. Large volumes of plastic wastes end up in the water bodies through periodic washing of waste from fringe communities and direct disposal of waste from individuals and private waste management companies. In recent years the explosion of plastic contamination within the aquatic environment has become very alarming, raising the interest of research in this area mostly due to the threats and stress it imposes on biodiversity, ecological functioning; and ultimately health implication to humans (Galloway et al., 2017; Worm et al., 2017). A greater percentage of the studies on microplastics have been conducted in developed countries such as Germany, United Kingdom (UK), South Korea, Netherlands and Belgium that have relatively better waste management practices (Ying et al., 2020). However, little work has been done

in Africa, where waste management practice is scarce resulting in the paucity of data within the African sub-region especially, Ghana.

In Ghana, data on microplastics assessment and their resultant complications on fish is limited. However, plastic pollution continues to be one of the major challenges confronting the nation. The River Pra has a broad watershed, having its aligning tributaries passing through several urban (e.g., Kumasi) and peri-urban communities (e.g Kade and Twifo-Praso) which could be potential sources of plastics entering the river (WRC, 2012). The Pra estuary has a highly diverse ecosystem with several consumed fish species which are bottom feeders hence highly susceptible to microplastics ingestions. The estuary receives tidal influx from the Gulf of Guinea, and freshwater inflow from River Pra. Studies by Adika et al. (2020) and Adu-Boahen et al. (2020) in Ghana indicates the presence of microplastics in the ocean and rivers. This suggest that the Pra estuary could be potential hotspot for microplastics accumulation. Therefore, there is the need for this research to be conducted to serve as a baseline study which outlines the abundance and distribution of microplastics in the Pra estuary.

Purpose of the Study

The study seeks to provide a baseline information on the spatio-temporal occurrence, distribution and type of microplastics within the subsurface water column, sediment, and fish in the Pra Estuary. This study equally seeks to establish the relationship between the physicochemical properties of the estuary to the distribution of microplastics in the same medium.

Objectives of the Study

The main objective is to assess the occurrence and distribution of microplastics in the water, sediment, and fish species in the Pra estuary.

The specific objectives of this research are to:

1. Quantify the abundance of microplastics within selected sections of the water, sediment, and fish in the Pra Estuary.
2. Identify the types of microplastic polymers occurring within the water, sediment, and fish in the Pra Estuary.
3. Assess the spatio-temporal distribution of microplastics within sections of the water and sediment in the Pra Estuary.
4. Monitor some physicochemical parameters and possible influence on microplastics distribution in the water and sediment in the Pra Estuary.

Research Questions

1. Are there microplastics occurring within the surface water, sediment and fishes in the Pra estuary?
2. Are microplastics evenly distributed within the water and sediment in the Pra estuary?
3. How are microplastics distributed spatio-temporally within the water and sediment of the Pra estuary?
4. Which organs of the fish (gills and gut) have the greatest concentration of microplastics?
5. What type of microplastics are occurring within the surface water, sediment and fishes in the Pra estuary?

Research Hypotheses

1. **Ho –** Microplastics are evenly distributed spatially in the surface water and sediment from the mouth of the Pra Estuary towards the upstream section.
2. **Ho –** Microplastics abundance along the Pra Estuary are uniformly distributed over a temporal scale.
3. **Ho –** Microplastics in the Pra Estuary is dominated by a single class category in the water and sediment along all sampling stations.
4. **Ho –** There is no relationship between the in-situ physicochemical properties of the Pra Estuary and the microplastics abundance.
5. **Ho –** There is no difference in microplastics abundance occurring within the gills and the gut of fishes within the Pra Estuary.

Significance of the Study

The quantification of microplastics abundance in the water, sediment and fish from the Pra estuary provides a vital information on the extent of microplastic accumulation and vulnerable species within the study area. Such information is necessary in driving strategic policy actions needed to combat the menace of microplastics within the aquatic system. The study provides a preliminary assessment of microplastics in the Pra estuary which could serves as an essential foundation for future research that seeks to widen the understanding of microplastics in the area. Importantly, the analytical description of microplastic polymer types occurring within the study is needed to support the development of effective management strategies that targets the reduction of the dominant plastics occurring within the Pra estuary. Although not conclusive enough, the identification of MP polymer type which are

environmentally present in this research could offer valuable knowledge on the potential sources of microplastics in the estuary. Also, evaluating the relationship between MPs abundance and the physicochemical parameters such as salinity, turbidity, flow velocity, pH, dissolved oxygen (DO) and temperature provides a broader insight on the principal elements that influence MP transport and distribution within the Pra estuary.

Limitations

Preliminary design of the study to sample within a 16 km distance from the mouth of the estuary to the upstream was reduced to 15 km due to inaccessibility to the last sampling station. Accessibility was hampered due to widely distributed boulders limiting the sampling vessel and equipment usage. The sampling duration was limited to assessment of MPs in the dry season from December 2020 – April 2021, due to the limited sampling coverage of 6 months assigned to this research by the research committee. The filtrating time per sample increased significantly during sediment and fish gut processing; this was due to the high colloidal particles and fat within the samples respectively. The presence of high organic load within all sampling stations increased the times and impeded smooth visualization and enumeration of the microplastics within the samples. The dark filter background generated from dissolved digested organic and dust particles made microplastics with dim colours difficult to differentiate.

Delimitations

The study was carried out on the Pra estuary, the second largest estuary in Ghana. This area was chosen because the riverine section passes through several

urban and peri-urban communities that contribute directly or indirectly to plastics entering the river before emptying into the Atlantic Ocean. In addition, the Pra estuary has rich biodiversity which makes data collection on microplastics occurrence within the fish possible. Data on the occurrence and distribution of microplastics in water, sediment and fishes in the estuary are essential baseline information for evaluating the number of plastics accumulated within the system. This study uses for the first-time ATR – FTIR analysis in identifying the unique spectral signatures of the suspected microplastics detected in Ghana. The study area provides a unique opportunity for studies on the relationship between physicochemical parameters and microplastic abundance.

Definition of Terms

Microplastics: "Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water" (Frias & Nash, 2019).

Bioaccumulation: The accumulation of harmful substances or contaminants in a living organism.

Siltation: The process of suspended silt deposition and/or accumulation within a water system.

Polymer: A chemical structure composed of chain (s) or ring (s) of interconnected units of similar molecules (monomers).

Spectral Signature: A unique reflectance or emittance produced by a material within a particular wavelength.

Organization of the Study

This research is organized into six comprehensive chapters (1-6). Chapter One (Introduction) outlines the general background of the study, emphasising on the definition, nature, causes and fate of microplastics within the aquatic environment. The chapter highlights the statement of the problem, which looks into the threat microplastic pollution possesses within the aquatic ecosystem and possible MP consumption by humans. This chapter establishes the purpose of the study and gives the preambles for the research objectives, questions and hypothesis. The significance of the study, limitation, delimitation, definition of terms, and the organisation of the study are addressed in chapter one. Chapter Two presents the literature review. In this chapter the review encompasses global trends in plastic and microplastic distribution, physicochemical parameters influencing transport, microplastics trophic transfer potential, threat to ecosystem stability, health risk to humans, standard analytical methods in assessment, categorization groups, and microplastics studies in Ghana. The materials and methods are presented in Chapter Three. This describes the study area, techniques in data collection, and analyses of data. Chapter Four is the results section, which shows the results of study in tables, charts, plates and figures. The statistical reports in the study are also presented with comments on graphical information in this section. Chapter Five presents the discussion of the findings. The last Chapter Six outlines the conclusions and recommendations of the study in a summarized format.

CHAPTER TWO

LITERATURE REVIEW

This section of the study reviewed trends of plastic production globally and also examined the plastic waste crisis within the aquatic environment. It assessed the accumulation and characteristics of microplastics in estuarine fishes; and the emerging concern of microplastic contamination of Ghana`s coastal watersheds.

Plastics and trends in Global Production

Plastic is one material that revolutionized the world in the 19th centuries; due to its high plasticity and durability properties setting it for multiple functions (Laskar & Kumar, 2019). Plastics are synthetic organic polymer that are made from fossil or cellulose products through polymerization or polycondensation process (Abioye et al., 2018; Maryanty et al., 2021). In 1907, Bakelite was the first ever fully synthetic plastic to be made by Leo Baekeland (Baekeland, 1909). Overtime, different types of plastics such as Polyethylene (PE), Polyethylene terephthalate (PET), Polytetrafluoroethylene (PTFE), Polyvinyl chloride (PVC), Polymethyl methacrylate (PMMA), Polypropylene (PP), Polystyrene (PS), Phenol-formaldehyde (PF), Epoxide (EP), and Polyurethane (PUR) have emerged, thus increasing their usage globally. Increased plastic consumption in an ever-growing population continue to cause global production of plastics to rise exponentially from 1.5 million metric tons in the 1950s to about 368 million metric tons in 2019 (Bibas et al., 2020; Statista, 2021) as shown in Figure 1. Net regional distribution of plastic production indicates; Northern Asia particularly China accounts for a quarter of the total world

production, followed by USA, Australia, Korea, Europe. According to Bibas et al. (2020), global production is projected to hit 1600 million metric tons by 2050. This forecast is highly dependent on the ever-growing demand on plastics and plastic based products especially within the packaging, construction, consume goods, automotive, agriculture, and medical sectors.

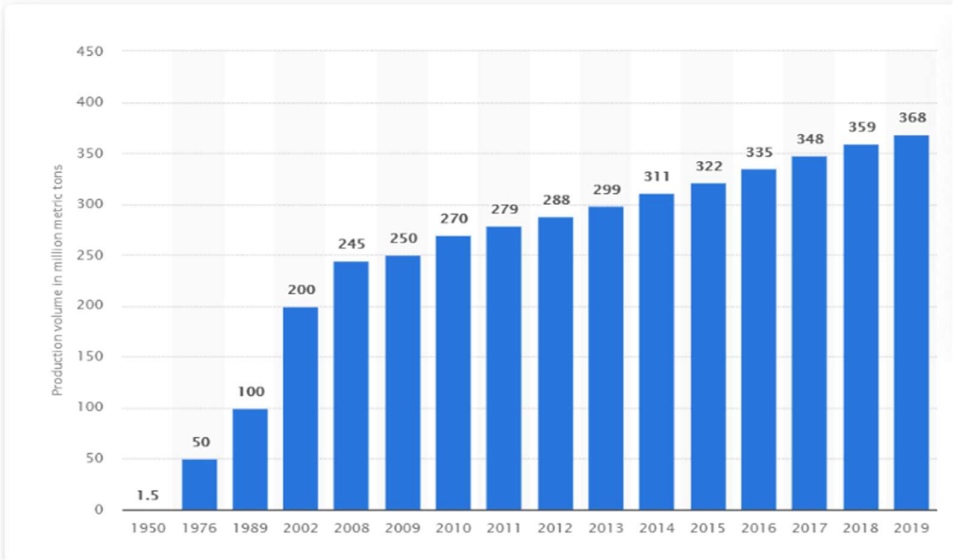

Figure 1: Global plastics production from 1950 to 2019. Source: Statista (2016)

The high market value of plastics is another key factor that continue to cause a rise in global production. In 2020, the global plastic market was valued at USD 579.7 billion and is projected to reach USD 750.1 billion within a seven-year span from 2021 (Statista, 2021). Although, the global per capita consumption of plastics for 2015 was stated at 45 kg per person, Statista (2016) reported that, countries within the North American Free Trade Agreement (NAFTA) were the world largest consumers of plastics with a per capita consumption of plastics of 139 kg per person. Reports suggest plastic consumption rate is directly proportional to the Gross Domestic Product (GDP) of countries indicating the

clear disproportionality in regional plastic consumption (Babayemi et al., 2019). The consumption of plastics which is highly tilted to the packaging sector drives the market of plastics; and is largely moved by the global shift from reusable plastics to single-use products such as plastic bags, containers, water sachet and cutleries. According to World Economic Forum report WEF (2016), the packaging sector accounted for 50% the total amount of plastic waste generated in 2015. This substantive development promotes the need to intensify a global paradigm shift from single-use products, to ensure a more sustainable plastic waste management for the future. Increase in the production of single-use plastics is predicted to impact significantly on the petrochemical industry. Plastics production is expected to consume about 20% of total crude oil production by 2050 (WEF, 2016). The crude oil sector which stands to benefit from the increasing plastic production is highly aligned towards the developed countries such as Australia and the United State of America.

A review by EUROMAP (2016) for the 2009 - 2015 period showed that plastic production in Africa occurred in 10 member countries. A total of 16.3 Mt plastics were produced within the period with the following distribution: South Africa (9.0 Mt, 55%), Egypt (4.0 Mt, 24%), Nigeria (2.3 Mt, 14%), Libya (0.4 Mt, 3%), Morocco (0.4 Mt, 2.4%), Algeria (0.2 Mt, 1.2%), Tunisia (0.01 Mt, 0.09%), Ghana (0.01 Mt, 0.07%), Kenya (0.006 Mt, 0.04%) and Tanzania (0.003 Mt, 0.02%). The general per capita consumption of plastics in Africa (population of about 1.216 billion) which was 16 kg/ person in 2015, represented 36% of the global average (Babayemi et al., 2019; Statista, 2016). However, between 2009 and 2015, the cumulative level of plastic importation by these producing countries was recorded at 28 Mt with Egypt importing the

largest share at 7.3Mt. Generally, information on plastics circulation within Africa is limited due to scarcity of reliable plastic assessment database platforms. However, Africa's plastic contribution has been centred on imports (Babayemi et al., 2019). The market share of plastics imported into Africa within 2019 stood at USD 323.27 million representing 9% of the global plastic import value (WITS, 2019). This value captured the combined 2019 import value for the joint Middle East and North Africa and Sub-Saharan African countries representing USD 252.40 million and 70.87 million respectively (WITS, 2019). According to projection by Babayemi et al. (2019), about 235.3 Mt of plastics are to be utilized by 33 African countries by 2030 if there is no major policy on plastics restriction.

Within Sub-Saharan Africa particularly Ghana, both primary plastic and plastic-based products production is estimated at over 52,000 tonnes per annum. This is collectively generated by about 120 manufacturing companies according to Ghana's NPMP (2020). Currently, most of the plastics circulating are mostly imported. Ghana is estimated to be importing over 2.58 million tonnes virgin plastics each year of which only 19% is reusable (Oppong-Ansah, 2020). This gives an indication of the largest share of circulating plastics ending up as waste on landfills. The consumption pattern of plastics in Ghana on a sectorial level aligns directly with global trends show the largest proportion within the packaging sector. As an emerging economy within the sub-region, plastics production and consumption presents a wider income potential that cannot be side-lined. According to Ampofo (2013) and GEF (2019), there are about 50 different plastic groups that are circulating within the country. Interestingly, only four namely; polyethylene (PE), polystyrene (PS), polyvinyl chloride

(PVC) and polypropylene (PP) enter the recycling stream. Most of the plastic products manufactured within Ghana include the commonest black polyethylene bags for shopping, thin-film 'pure water' sachet, 'Ghana must Go' strip bags, cutlery, mats, plastic bottles and woven sacks. However, the greater portion of this product can be categorized as single-use products. Packaging plastic-based products in Ghana are extremely affordable and easily accessible, thus compounding concerns associated with proper management. Report estimates the daily per capita plastic waste generated in Ghana as 0.45 kg and a per daily national plastic waste production of 3000MT; which undoubtably pose serious ecological risk to the environment (GEF, 2019; NPMP, 2020).

Plastics Waste Crisis Within the Aquatic Environment

Plastic waste or plastic litter represents a group of discarded plastic materials that ends up largely on landfills or into the environment either on purpose or by accident. Kaza et al. (2018), in a World Bank report, stated that about 242 million tonnes of plastic waste were produced globally in 2016 with figures expected to go even higher due to the increase in population and the emergence of the pandemic (COVID-19). Plastics enter the aquatic environment through indiscriminate dumping, runoffs and/or atmospheric deposition (van Emmerik & Schwarz, 2020). Within the aquatic medium, plastics have the potential to obstructs waterways, clog drains when concentrated, leading to perennial flooding events and widespread of harmful diseases such as malaria and cholera (Abota, 2012; Williams et al., 2019). Aside the impact on health, plastic pollution reduces the aesthetic value of water bodies and watersheds which negatively affects returns within the tourism sectors (van Emmerik & Schwarz, 2020). Plastic pollution is considered a major threat to livelihood among fishing

communities; damaging vessel propellers and clogging fishing nets (Hong, Lee & Lim, 2017; Lartey, 2015; van Emmerik & Schwarz, 2020). In Ghana, Nyabor (2020) reported, the ordeal of local fishermen having to be catching more plastics than fish on a daily basis. This attest to the popular phrase of the likelihood of having more plastic in the ocean by 2050 than fish if no stringent measures on plastic waste management are put in place globally (Defruyt, 2019).

Ecologically, plastic waste possesses an enormous health risk to vital aquatic ecosystems. There is wide literature on the clogging, suffocation, entanglement, ingestion, ghost trapping of fishes, marine mammals, sea birds, sea turtles by plastic pollution (Angiolillo, 2019; Cartraud et al., 2019; Digka et al., 2020; Rummel et al., 2015). Plastic debris suspending within the water column intercept light penetration limiting primary production which might affect species assemblage and structure within the system (Harris et al., 2010). Ingesting plastics induces a false sense of satiation leading to starvation among exposed aquatic organisms (van Emmerik & Schwarz, 2020). The greater challenge of plastic pollution is the long shelf life they have within the environment; a complete decomposition of plastics take more than 500 years (Barcelo, 2020). However, plastics fragment into smaller particles such as microplastics under exposure to intense UV light or mechanical abrasion which make it readily available for aquatic organism (Andrady, 2011; Webb et al., 2013). In a plastic exposure study on *Daphnia magna,* Rosenkranz et al. (2009) observed that ingested plastics were able to translocate, crossing the gut epithelia tissues into organisms, thus, raising concern of similar occurrence in larger aquatic organisms. The bioaccumulation of plastics in fish highlights

several physiological effects such as internal abrasions, gut blockage, oxidative stress, reproductive toxicity, and mortality (Kim et al., 2021; Ogonowski et al., 2016; Rosenkranz et al., 2009). Evidently, plastics have been explored to move through complex trophic levels (Batel et al., 2016). A study by Tosetto et al. (2017), observed the linear transfer of plastic particles from *Platorchestia smithi* to *Bathygobius krefftii* to ray-finned fish. Although no structural behavioural alteration was observed among trophic groups, the occurrence of plastics within the highest order presents the likelihood of plastic transfer to humans who consume fish.

Microplastics in the Estuarine Systems

The expansive spread of microplastics within aquatic environments have been well documented by several authors (Adu-Boahen et al., 2020; Jiang et al., 2020; Mani et al., 2015; Peng et al., 2018; Xu et al., 2020; Yan et al., 2019). According to GESAMP (2019) microplastic stock assessment provides information on the degree of pollutants in the system, prevailing hotspots and probably the source of the contaminates in the system. Microplastics are anthropogenically induced materials which are displaced within the environment. The estuary presents the final gateway that connects riverine flows to the ocean and in the opposite direction. Thus, the estuary signifies an important pathway for microplastics to enter the ocean (Xu et al., 2020). In a study by Yan et al. (2019), microplastics were found along the Guangzhou urban section and the Pearl estuary, at mean abundance of 19,860 items m^{-3} and 8902 items m^{-3} respectively. Within the water surface layer of the Minjiang, Jiaojiang and Oujiang estuaries in south-eastern China, Zhao et al. (2015) reported in their study mean densities of microplastics of 1245.8 ± 531.5 items

m^{-3}, 955.6 ± 848.7 items m^{-3}, and 680.0 ± 284.6 items m^{-3} respectively. Similarly in America, Yonkos et al. (2014) found microplastics in the surface water of the Chesapeake Bay, connected by four estuarine tributaries; Patapsco, Magothy, Rhode, and Corsica Rivers at average concentrations of 155374, 112590, 67469 and 40,852 pieces/km^2 over varying sampling periods. Aside microplastics transiting through the estuary into the sea as reported from detection within the surface water column (Yan et al., 2019; Yonkos et al., 2014; S. Zhao et al., 2015), some microplastics sink to the floor bed or get trapped in between sediments. According to a study by Peng et al. (2017), an average of 121 ± 9 items per kg d.w microplastics were found in the sediment of the Changjiang Estuary. Similar to several studies, microplastics were reported to accumulate within sediment in varying average concentrations such as 1674 ± 526 items kg^{-1} d.w in the Sanggou Bay (Sui et al., 2020), 252.80 ± 25.76 m^{-2} (Sruthy & Ramasamy, 2017), 851 ± 177 items kg^{-1} d.w in the Pearl River Estuary (Zuo et al., 2020), 120 ± 46 items kg^{-1} d.w in the Liaohe Estuary (Xu et al., 2020), and 963 ± 175.4 items/500 g d.w in the Jinjiang Estuarine Mangrove (Deng et al., 2020). These studies corroborate with the ubiquitous assertion of microplastics in the estuary.

Accumulation of Microplastics in the Estuarine Fish

The fate of microplastics in fish within the estuary proves a serious concern for the scientific community, since the estuarine environment is considered a preferred nesting and nursery ground for most marine and deltaic organisms such as salmon, herrings, and crabs (Wolanski & Elliott, 2016). Exposure to microplastics by estuarine inhabiting fish species are well documented by several authors (Abbasi et al., 2018; Jaafar et al., 2021; Pazos et al., 2017; Su et

al., 2019). Accumulation of microplastics within the affected fish were suggested to be through accidental ingestion or filtration through filtering apparatus. According to Su et al. (2019), the feeding type and habitat preference of aquatic species are important factors influencing exposure to microplastics in species. In the study, herbivory accumulated fewer microplastics compared to the other feeding types, which is associated with the narrow feed source and lower trophic level occupied by the species. Pazos et al. (2017), centred more on the environmental availability of microplastics as the major cause to the high presence of ingested microplastics by the investigated fish species from their study. In the study by Abbasi et al. (2018), microplastics were investigated via the skin, muscle, gut, gills and liver of fishes and prawns inhabiting the Musa estuary. Microplastics were predominately high within the gills and gut than the other organs to which Abbasi associated to the easiness for the particles to enter affected organs without restriction. Within the estuarine environment, Possatto et al. (2011), found microplastics in fishes that fed mostly within mangrove forest and tidal creeks which are hotspots for sedimentation of microplastics in the estuary.

Characteristics of Microplastics in the Estuary

The size, shape, and polymer type of microplastics are important aspects of the material that could influence their environmental behaviour within the situated systems (GESAMP, 2019). Within the aquatic environment, most precisely in the estuary the shape, size and polymer type affect the degree of further degradation, transport and sedimentation within the water (GESAMP, 2019). The colour of microplastics is another characteristic of the material that is widely considered when reporting MP assessment within aquatic

environment. Although there is no clear relationship between colour of microplastics and the transport of the material in water, MP colour has great relevance to the potential of bioaccumulating in the system (Wright et al., 2013).

Shapes

The shapes of microplastics originate from the weathering of larger plastics materials or moulded directly from the industry. Thus, reporting on the shapes of microplastics is crucial for predicting the source of the material such as fibrous MPs which are widely reported to originate from laundry water of textiles and fabrics or from weathered fishing ropes and nets within fishing areas (Peng et al., 2017; S. Zhao et al., 2014). The shapes of microplastics are documented in almost all aquatic MP assessment reports (Gallagher et al., 2016; Gray et al., 2018; Pan et al., 2021; Peng et al., 2017; Simon-Sánchez et al., 2019; Sruthy & Ramasamy, 2017; Wessel et al., 2016; Yonkos et al., 2014; Zhao et al., 2014). Although MP shape groupings differ among studies, due to the high subjectiveness during identification and the lack of standardized reporting protocols, majority of authors outlines their studies with the descriptions by Gago et al. (2019) and GESAMP (2019), namely; fibre, pellet, foam, line, film and sheets. According to Zhao et al. (2015), the shape of microplastics couple with the size and density have some influence on the distribution pattern of MPs in the estuary. Dominance of MP shapes in estuaries have been found to vary among studies such as; fibre and granules dominance in Yangtze Estuary (Zhao et al., 2015), foam in the Dongshan Bay (Pan et al., 2021), fibre in the Changjiang Estuary (Peng et al., 2017), pellets in the Mira Estuary (Duarte et al., 2020), and fragments in the South Carolina estuaries (Gray et al., 2018)

which indicate spatial heterogeneity in plastic use among the different study locations.

Size

The size of microplastics is the main element that defines and separate the material from other plastic types. Generally microplastics are continuum of weathered parts of large plastic materials with size ranging between 1µm to 5 mm (Andrady, 2011). According to Wright et al. (2013), the size of MPs play a significant role in the availability of the material to aquatic organisms; smaller sizes become readily available to lower feeders. Further, smaller sized plastic particles are easily trapped within filtering apparatus of species that occupy highly contaminated environs (Bakir et al., 2020; Su et al., 2019). This is possible because some aquatic species are non-selective to the size of their prey item (Andrady, 2017) while others mistaken size resemblance to their natural prey item (Zhu et al., 2019). Physically, the size of microplastics has influence on the nature of transport. Andrady (2017), in a review, documented that smaller sized MPs show high vertical transport due to their possession of lower rise velocities. Also, smaller sized MP particles are widely reported to present a larger surface area to size ratio that enables high susceptibility for biofouling to occur (Oberbeckmann et al., 2015). Similar to the categorization of MP shapes, there are no standardized protocols for reporting on size fractioning of detected MPs. According to GESAMP (2019), reporting on size fractions of MPs prevents ambiguity and enhances harmonization of samples. Pan et al. (2021), categorized MP sizes in class from 0.3 – 0.5, 0.5 – 1.0, 1.0 – 2.5, and 2.5 – 5.0 mm from the Dongshan Bay of China. The study limited inclusion of particles

below 0.5 mm. In the study by Yan et al. (2019), the most occurring MP sizes detected in the Pearl estuary was less than 0.5 mm.

Colour

The inclusion of colour differential in microplastic reporting continue to raise arguments within the scientific community due to the high subjectiveness during identification stemming from individual visual limitations (Frias & Nash, 2019; GESAMP, 2019). However, aquatic MP colour differentials provide important information on the ingestion preference by aquatic organisms. According to Wright et al. (2013), organisms that are visual feeders could mistake MP colour resemblance to prey item as food. A study by Bessa et al. (2018), also associated the presence of blue, black and transparent coloured MPs detected in the gastrointestinal tract of *Dicentrarchus labrax*, *Diplodus vulgaris* and *Platichthys flesus* to the resemblance of their pray item. In considering colour categorization, the use of distinctive or bright colours are widely documented such as blue, white, red, yellow, green, black and transparent (Gago et al., 2019). This provides common identifiable patterns that encourages comparability of findings to other studies.

Polymer Type

The study provides direct reference to the type of plastic material prevailing in the aquatic system that require policy action. According to GESAMP (2019), about 80% of plastics found in the world are composed of six different polymer types namely polypropylene (PP), polyethylene (PE), polystyrene (PS), polyethylene terephthalate (PET), polyurethane (PU) and polyvinyl chloride (PVC). The polymer type plays an important contribution to the transport and

distribution of the material in the water column (Zhao et al., 2015). Microplastics float or sink depending on the density variation among different polymer types. Erni-Cassola et al. (2019) categorized the predominant polymers found in the marine environment into two namely; low-density polymers (LDP) (polypropylene and polyethylene) and high-density polymers (HDP) (polyester, polyamide, and acrylics). High-density polymers naturally sink to the bottom floor when density exceed the density of the occupying medium. This is supported by the account of Haave et al. (2019) that found the high densities (greater than 1.2 kg dm^{-3}) of polyethylene terephthalate, polyamide and polyvinyl chloride as the main factor preventing their floating even in hyper saline mediums. However, the sedimentation of light-density polymers are related to biofouling events causing an increase in density (Anderson et al., 2018).

Source of Microplastics into the Estuary

Microplastics within aquatic environments are sourced from numerous points that need to be evaluated to inform policy. Studies suggest that marine litters originate from about 80% land-base and 20% marine-base sources (Andrady, 2011; Chico-Ortiz et al., 2020; Sruthy & Ramasamy, 2017; Wessel et al., 2016). Technically; contaminants such as plastics originate from anthropogenic activities; thus, tackling from the root reduces the environmental threat imposed by such materials (Yonkos et al., 2014). The estuary serves as a temporal sink and a transiting point for microplastics entering the ocean (Tibbetts et al., 2018; Wessel et al., 2016; Zhao et al., 2015). Several studies classify the sources of estuarine microplastics into two broad categories namely; primary sources which are manufactured or directly emitted MP particles and

secondary or indirect sources which are microplastics induced by the gradual weathering of larger plastics (Jambeck et al., 2015; Rodrigues et al., 2019; Yonkos et al., 2014; Zhao et al., 2015). Within the estuary the nature of MPs (shape, polymer type, and colour), anthropogenic activities characterizing the entire watershed and the direction of MP entry are key factors that enables source trackability (Gray et al., 2018; Lima et al., 2014; Pan et al., 2021; Peng et al., 2017; Sruthy & Ramasamy, 2017; Yonkos et al., 2014; Zhao et al., 2015). According to Yonkos et al. (2014), microplastics abundance in the Chesapeake Bay correlated positively to proximity to dense settlement and industrial areas. Microplastics found in proximity to populated areas mostly enter the estuary through channelized drainage systems, runoffs from dumpsites, sewage sludges, sewer overflows and littering (Adu-Boahen et al., 2022; Wessel et al., 2016; Yonkos et al., 2014). Around industrial setting, microplastics are reported to be released through accidental spillage (Bakir et al., 2014). However, direct release from wastewater treatment plants (WWTP) are commonly found in most estuarine MP studies (Bakir et al., 2014; Simon-Sánchez et al., 2019; Zhao et al., 2015). Also, fibrous MPs sourced inland into the estuary are wildly attributed to laundry water inflows and atmospheric deposition into the system (Lima et al., 2014; Peng et al., 2017; Sruthy & Ramasamy, 2017; van Emmerik & Schwarz, 2020; Zhao et al., 2014).

Considering sea-based sources of MP entrance in the estuary, Pan et al. (2021) attributed the occurrence of MP foams in the Dongshan Bay to the presence of Styrofoam buoys used in aquaculture activities on the waterbody. Zhao et al. (2014) reported that, the breakdown of discarded fishing net, ropes and lines from fishing activities were major contributors to fibrous MPs in the

Yangtze Estuary. Similar account was reported by Lima et al. (2014), suggesting fishery activities such mending of fishing nets, ropes and lines to be the source of MP fragments in the Goiana Estuary. Generally, sea-based activities that contribute to microplastics in the water include; heavy marine traffic, shipping activities, fishing, dumping, and gear maintenance resulting in the wear and tear of synthetic materials into the water column (Wessel et al., 2016; Zhao et al., 2014). Evidentially, direct trackability of MP sources within the estuary are limited due to constant movement of buoyant MPs and slow rate of plastic degradation resulting in a spatial shift from the point source to far reaching areas (Lima et al., 2014; Wessel et al., 2016).

Microplastics as an Emerging Contaminant of Concern within Ghana's Coastal Watersheds

The ubiquitous nature of microplastics coupled with the ecological health risk, microplastics presence within different aquatic systems (freshwater, estuary and marine) have been well documented globally (Eo et al., 2019; Lima et al., 2014; Mani et al., 2015; Rodrigues et al., 2018; Rodrigues et al., 2019; Sruthy & Ramasamy, 2017; Townsend, Lu, Sharley & Pettigrove, 2019; Yonkos et al., 2014). However, in Ghana only four field studies on microplastic occurrence (Adika et al., 2020; Adu-Boahen et al., 2022; Chico-Ortiz et al., 2020; Gbogbo et al., 2020) and one review (Acquah et al., 2021) have been conducted per literature search via google scholar, PubMed and web of science, using key words 'microplastics AND Ghana' from 1950 – 16 Aug, 2021. All four studies were conducted within the coastal regions of Ghana.

Adu-Boahen et al. (2020) reported a total of 85 microplastic items/m^3 (mean ± SD, 17 ± 4.90 items/m^3) within five sampling sites from the Akora River, a freshwater body sourced with several tributaries. Microplastics was noted to be accumulating within the inhabiting aquatic species such as fishes (30 items/21 individuals). The report demonstrated the importance of drainage channels (gutters) on microplastic entry into the Akora River. The connection of local sewage drains to streams and minor rivers within proximity in Ghana are common phenomenon in most coastal settlements or traversing communities upstream (Biney, 1982; Gbogbo et al., 2018). According to Zhang, Wang, Halden & Kannan (2019), sewage sludge presents a fine sink medium for microplastics sourced from domestic and industrial activities within populated settlement. The low concentration reported by Adu-Boahen et al. (2020) represented a snapshot of microplastics occupying the Akora River which was subjected to change over space and time. The dominant plastic types identified through visual identification and public survey in the Akora River were polyethylene, and polystyrene based materials such as pure water sachet and bottles (Adu-Boahen et al., 2022). Generally, plastics accounts for about 9% by weight in the waste stream of Ghana (Fobil, 2000). Plastic waste from bottles, delivery bags, pure water sachets and containers are the most common materials inundating drains and waste channels in Ghana, especially within the urban and peri urban settings which poses a major health challenge for waste managers (Abota, 2012; Fobil, 2000). According to Fobil (2000), about 70% of all municipal plastic waste in Ghana from the packaging sector were composed of polyethylene films mainly of low and high-density polyethylene (HDP); the remaining been polypropylene (PP), polyethylene terephthalate (PET),

polystyrene, and polyvinyl chloride (PVC). This corroborates with the plastics found by Adu-Boahen et al. (2020), although spectra validity should have been carried out in the study.

The study by Gbogbo et al. (2020) presented an analytical case in microplastics detection using Rose Bengal stain. Microplastics were detected in the water, sediment, crabs and faecal samples collected from the Coastal wetland of Sakumo II Lagoon (Table 1). The Rose Bengal stain discriminates against false positives in microplastics identification. This suggests a viable alternative for spectral validity; however, the stain is limited in defining the type of polymer present in the sample which hampers inferences on the source of the plastics. The Rose Bengal stain also presented a constrain in distinguishing among false positives that were red, which made reliability on this technique not conclusive without a complementary spectral confirmation. On the positive side, the use of staining dyes (e.g., Rose Bengal, Nile red, fluorophore and Propidium iodide) in microplastics detection presents a more affordable and readily available technique in environmental MP assessment (Bosker et al., 2019; Gbogbo et al., 2020; Lee et al., 2021; Prata et al., 2021). Microplastics in the Sakumo II Lagoon occurred most in the sediment which might correlate with the high MP occurrence identified in the crabs; indicating a high exposure threat to bottom filter feeders. The high prevalence of microplastics in the sediment of lagoons in Ghana was made evident in a report by Chico-Ortiz et al. (2020). The retention of microplastics in the Mukwei and Kpeshie Lagoon was identified to be sustained by the presence of mangroves which prevent the flow of particulate transported by current. The accumulation of microplastics in the sediment indicates a high probability of MP transfer into the food chain

which are susceptible for human consumption (Carbery, O'Connor, & Palanisami, 2018). The pathway for microplastic entry into humans has been considered globally (Galloway et al., 2017; Worm et al., 2017), with the most probable being through consumption. The study by Adika et al. (2020) showed the occurrence of microplastics in the gastrointestinal tracts of three commercial important landed fish species (*Sardinella maderensis*, *Dentex angolensis*, and *Sardinella aurita*) on the coast of Ghana (Table 1). Sardinella and small pelagic are processed traditionally and consumed whole in Ghana such as salted, fried, fermented, smoked, and dried (Nunoo, Asiedu, Kombat & Samey, 2015) which suggests the high threat of microplastic exposure to humans in Ghana.

Table 1: Microplastics studies in Ghana from 1950 – Aug, 2021

Location	Sampling type	Equipment	Method of ID	Mean density	Polymer type	Reference
Coastal wetland of Sakumo II Lagoon, Ghana	Water	Bottle	Rose Bengal stain	0.09 ml^{-1}	N/R	Gbogbo et al., 2020
	Sediment	Bottle		1.85 g^{-1}		
	Fish and Crab	Purchased		3 ± 2 and 8 ± 1		
	Faecal matter	Spatula scooping		0.35 g^{-1}		
River Akora, Ghana	fish	Net	Stereomicroscope	30 pieces /21 fishes	N/R	Adu-Boahen et al., 2020
	Water	neuston net (300 um)		< 5/10L	polyethylene, polystyrene	
Eastern Central Atlantic Ocean - Tema, Ghana	fish	Landings	Stereomicroscope	26.0 ± 1.6 - 40.0 ± 3.8	N/R	Adika et al., 2020
Mukwei and Kpeshie Lagoon, Ghana	Sediment	Cores	Fluorescent microscope	11.22 ± 2.69 - 25.94 ± 3.13 /10cm^3	N/R	Chico-Ortiz et al., 2020

Source: Literature search (2021), N/R – Not assessed

CHAPTER THREE

MATERIALS AND METHODS

This chapter provides a description of the study area, sampling design, techniques for measuring some physiochemical parameters and sampling microplastics in the surface water, sediment, fishes and shellfish within the Pra estuary. The section gives information on the tools used in sampling, extracting and identification of microplastics within the samples. Also, the information on the procedure for contamination control and statistical analysis are presented in this section.

Study Area

The study was conducted on the Pra estuary. The Pra estuary is located within the Shama District in the Western Region of Ghana, West Africa (5°01'00"N, 5°03'30"N and 1°36'30"W, 1°38'00"W). The estuary is sourced from the main Pra river; the largest river that drains from the south eastern section of the River Volta. Ranked the second largest estuary in Ghana, it joins the Gulf of Guinea east of Takoradi with an opening width of about 100m (Ghana Ministry of Fisheries and Aquaculture Development and Fisheries Commission, 2020). The vegetative landscape of the area is predominately thick mangrove ecosystems, swamps and salt marshes (Ghana Ministry of Fisheries and Aquaculture Development and Fisheries Commission, 2020). The rich fishery diversity of the estuary supports fishing activities for about 10 fringe communities (Okyere, 2018). The map of the study area is as shown in Figure 2.

Sampling Stations

Sampling was done monthly from December 2020 to April 2021, covering the dry season which is the most stable period for pollution load assessment due to limited new entries from surface runoff. A total of 16 sampling points; 1km apart from each other were used. The sampling sites encompassed S1 to S15 from the opening section of the estuary towards the inner-most riverine section and S-N located within the adjoining stream passing through the Anlo community (Figure 2). Further, three sampling stations were used; S1 - S4 categorized as the mouth of the estuary, S5 – S9 as the middle and S10 – S15 as the Head section of the estuary (Figure 2). The sampling stations were distributed to factor in all the fringe communities along the estuary (Appendix A).

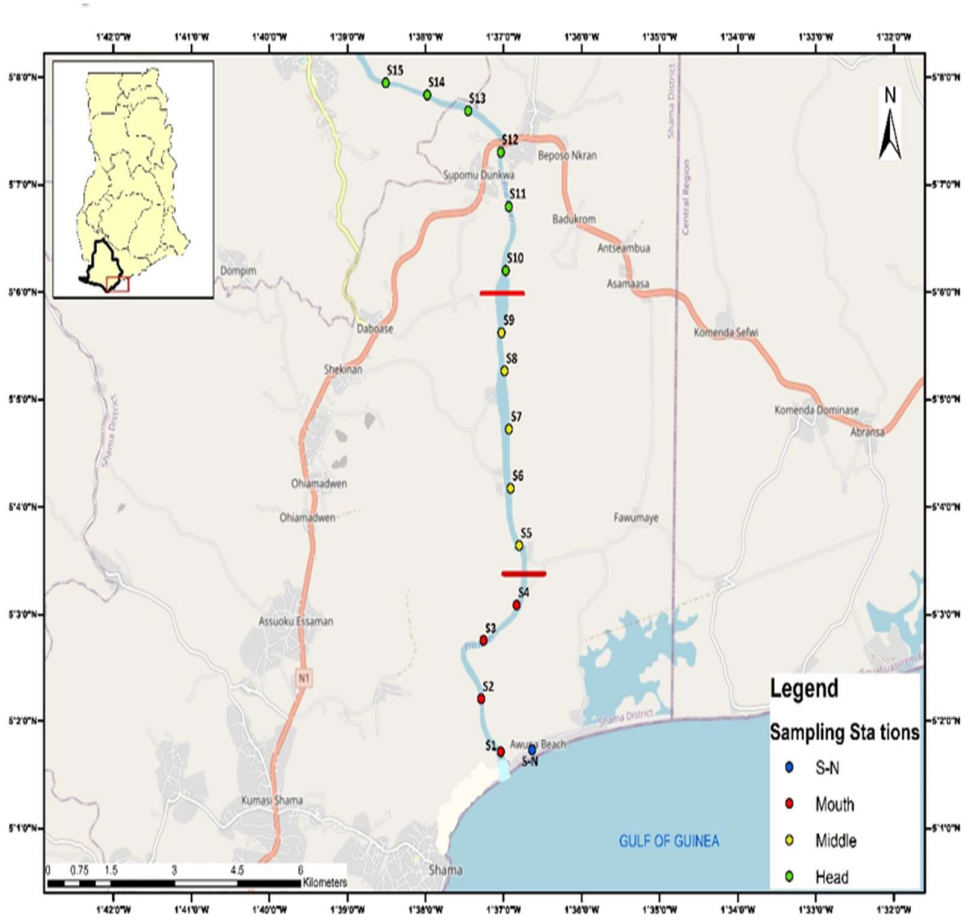

Figure 2: Map of Pra Estuary showing the sampling sites.

Sampling Design

Stratified random sampling was used during sediment sampling from each site. Samples were collected randomly in triplicates at each sampling point, 20 m apart using a grid spacing system. Sub-surface water samples were collected randomly at each sampling point. The selected physiochemical parameters of the water such as water temperature (^{o}C), dissolved oxygen (mgL^{-1}), pH, Salinity (ppt), turbidity (NTU) were taken *in situ.* Fish samples were collected randomly within the study area throughout the five months sampling period.

Sampling Techniques

Sampling Microplastics from Surface Water

The sampling of microplastics in the surface water was done following the protocol described by Viršek et al. (2016). Samples were collected using a 15 x 5-inch manta trawl with mesh size 333 µm (MSFD, 2013). The net was towed over a 1 km distance from a canoe.

The manta trawl was deployed 2 m away from the side of the canoe to prevent sampling within the wake zone (disturbed section) caused by canoe propellers. Before collecting samples, the initial GPS coordinates and time were recorded on a worksheet. Samples were collected by moving the canoe against the flow direction within a 1 km distance under the vessel speed of 2 knots. After sampling, the final GPS coordinates and time were recorded. The manta trawl was lifted and rinsed thoroughly from the outside of the net, from the mouth direction down to the cod end using fresh water from the river. The cod was carefully removed and rinsed thoroughly from the outside repeatedly into a 1 L glass jar with an aluminium lid. Collected samples were labelled and transferred to the laboratory for further analysis. The distance travelled were estimated using the GPS while the area (km^2) sampled was calculated by multiplying the distance by the width of the manta trawl.

Mathematically;

$$Area\ Sampled\ (km^2) =$$

$$Distance\ covered\ (km)\ x\ width\ of\ Manta\ trawl\ (km) \quad \ldots\ldots\ldots\ldots (1)$$

Sampling Microplastics from Sediment

Sediment sampling was carried out using techniques described by Nel et al., (2018) to assess microplastics. At each sampling point, 3 replicate samples

were collected from the river bed. The GPS coordinate was recorded on the worksheet before the sediment was collected. Samples were collected using a 15 x15 cm Ekman Grab sampler, and stored in a labelled zip lock bag lined with aluminium foil and transported to the laboratory for analysis.

Fish Sampling (finfishes and shellfishes)

Fish were collected using set nets deployed randomly within the study area from S1 to S15. The nets were set over a 12-hrs period before removal. Finfishes collected were placed in ice slurry for preservation whiles shellfishes were purchased from fishermen within the Anlo community before transporting to the laboratory for further analysis. Specimen were grouped into various taxa and identified using identification manuals (Dankwa, 2000; Kwei and Ofori-Adu, 2005; Paugy, 2003; Rutherford, 1971; Schneider, 1990).

Hydrographic parameter determination

Multiparametric water quality checker (EUTECH - PCD650, Singapore) was used to take in-situ readings of the water temperature (^oC), dissolved oxygen (mgL^{-1}), pH and Salinity (ppt). Flow velocity (ms^{-1}) of the water at each sampling stations was taken using a stream flowmeter (GEOPACKS – MFP51, USA).

Flow velocity was calculated using the equation:

$$Water\ Velocity\ (V)\ m/s\ =\ (0.000854C)\ +\ 0.05. \ldots\ldots\ldots\ldots (2)$$

Where, C = number of counts per 60 sec.

The depth of each sampling point was recorded throughout the sampling period using a digital hand-held depth sounder (HONDEX PS-7, Japan). The sediment grain size was determined for all sampling points following procedure

stipulated by Dzakpasu (2012). Sediment samples were air-dried for 3 days. 150 g of the dried samples were placed in a Petri dish and weighed on an analytical electronic balance (OHAUS RANGER 7000, USA). The weighed samples were then dissolved in a prepared NaOH solution (100g NaOH dissolved in 1L water) in a 500 ml beaker, gently stirred for 5 mins to complete mixing and allowed to settle for 30 mins before the solution was drained. The sediment samples were transferred into aluminium cans and placed into an oven at 105 ºC until constant dried weight was attained. A 100 g of oven-dried sediments were weighed using the electronic balance and sieved through a set of different mesh size sieves in the order of 2 mm, 1 mm, 0.5 mm, 0.25 mm, 0.125 mm and 0.063 mm. In the sieve, samples were gently shaken to allow complete graduation of the particle sizes. After sieving, the graded sediments were gently emptied into Petri dishes and weighed on the analytical scale. The formula by Yankson (2000) was adopted to determine the mean particle size of the sediment. The results were then compared to the Wentworth scale (Appendix D).

According, to Yankson (2000),

$$\text{Mean Particle size } (MPS) = \frac{\sum x.Y}{100} \ldots\ldots\ldots (3)$$

where x = mean size of the soil separates (mm) and Y = corresponding percentage composition. However,

$$Y = \frac{Weigh\ \ of\ sieve\ content}{weight\ of\ dry\ sample}\ x\ 100 \ldots\ldots\ldots\ldots (4)$$

Pre-treatment Procedures

Microplastics Separation from Extracted Samples in Surface Water

At the laboratory, samples were separated through a sieve size of 63 µm and 5mm mesh net. Particulates > 5mm were sorted out by visual identification (naked eye), removed with tweezers, and rinsed thoroughly with distilled water to prevent losing attached microplastics. Larger particulates were placed in a separate Petri dish and air dried. The dried samples were counted, weighed, and categorized (Master List of Categories of Litter Items). Materials retained on the sieve (< 5 mm) were concentrated on one side of the sieve using a squirt bottle. Samples were poured into a glass container using Ultrapure deionized water for further microplastic extraction (using digestion technique).

Digestion of Water Samples

The digestion technique as outlined by Gago et al. (2019) were employed. Particulate matter was transferred into a 200 ml conical flask. The samples were chemically digested in potassium hydroxide (10% KOH) and hydrogen peroxide (30% H_2O_2) solution. The samples were covered with KOH at a 1:3 volume ratio (samples: solution respectively). The mixture was stirred for 1 min using a glass rod and placed in an oven (GEOTECH EN 932-5, USA) at 50°C for 24 - 72 hrs. After removing from the oven, H_2O_2 of volume ratio of 1:1 was added and stirred for 1 min using a glass rod. The mixture was allowed to settle in an oven at 50°C for 18 hrs to completely oxidize and digest the remaining organic matter that were not digested in the previous treatment. The samples were subjected to density separation before analysis were carried out.

Pre-treatment of Sediment samples

At the laboratory, the collected sediment samples were oven dried (GEOTECH EN 932-5, USA) at 105 ºC for 24 - 48 hrs until a constant weight was reached. The drying was controlled to prevent air borne contaminations. The collected samples were grinded and thoroughly mixed together to attain a homogenized composite sample for each sampling point. A 200 g of the sample was weighed using an electronic balance (OHAUS RANGER 7000, USA) and sieved through a 5 mm mesh sieve into 500mL beaker to separate large particles. The debris (> 5 mm) were visually inspected for larger plastic materials. Large non-plastic particles were categorized, counted, and weighed. Particulate samples less than 5 mm were weighed using an electronic balance allowing the number of microplastics particles of dry weight (d.w) to be determined before digesting for microplastics extraction.

Digestion of Sediment samples

The digestion followed technique described by Frias et al., (2018). The samples were poured into a 1 L glass beaker. A 100 ml volume of 10% H_2O_2 solution was added to the sediment. The mixture was carefully stirred with a glass rod for 1 min and allowed to settle for 18 hrs. The beaker was covered with aluminium foil and incubated at 50 °C for 24 - 72 hrs in an oven.

Fish gill and gut organ extraction

At the laboratory, the fish were defrosted and weighed using an (RANGER 7000, USA) electronic balance. Total Length (TL), Standard Length (SL), Head Length (HL) of fishes, carapace width (CW) of crabs and body length (BL) of shrimps were measured on a graduated measuring board. Width of

fishes were recorded using a Vernier calliper. Condition factor (CF) of fish were determined using the equation:

$$CF = \frac{Standard\ wei\quad (W)}{Standard\ Lengh\ \ (L)^3} \cdot \ldots\ldots\ldots\ldots (5)$$

Before dissecting the fishes, specimens were placed on an aluminium foil and wiped with tissue paper to remove any external material attached to the samples. The whole visceral mass and gills were removed using surgical scissors. This was done by making straight incisions from the anal port through to the mouth region exposing visceral contents whiles cuts were made from the neck and on the operculum to access the gills. The gastrointestinal tract (GIT) of specimens was identified and cut from the visceral content after weighing. The extraction of gastrointestinal tract was done following the techniques of Avio et al., (2015). Before digestion of the GIT and gills, the gastrointestinal tract were analysed under a dissecting microscope (40x magnification) for plastic debris as stipulated by Bessa et al., (2018) for effective assessment of microplastics in GIT in fish. During the visual analysis of GIT, all non-natural prey entities were removed using forceps onto a filter paper and placed in a Petri dish to be categorized.

Digestion of Fish Organ Samples

After visual analysis of the GIT, the remaining gut content and gills were each transferred into a 250 ml glass beaker and flooded with 200 ml of KOH (10% w/v) to digest organic matter. Internal tissues of crabs and total body of shrimps were digested wholly. The mixture was incubated at 40°C for 72 hrs for the gills of the finfishes and visceral mass of the shellfishes (Karami et al., 2017) and at 60°C for 24 hrs for the GIT (Kuśmierek and Popiołek, 2020) in

an oven (GEOTECH EN 932-5, USA). The digested samples were then subjected to density separation.

Separation of Microplastics

Density Separation of MPs from Water, Sediment and Fish samples

To be able to extract high-density microplastics, a 4.4M of Sodium iodide (NaI) was prepared by dissolving 80 g of salt in 100g of deionized water to conduct a density separation of samples in the range of 1.2-1.8 gcm^{-3}. For the separation in water samples, the NaI-solution was added to the mixture in a 1:3 ratio (sample: solution) and stirred with a glass rod for 1min. The samples were allowed to settle for 1hr with the lid of the beaker covered with aluminium foil to prevent cross-contamination from the atmosphere. The process was to ensure the suspension of microplastics. The supernatant from the mixture were poured and filtered through a 1.2 µm, GF/D 47 mm chm fiberglass filter (Cat No. GF3-047) using a single stand vacuum filtering system. During filtering, the filtration funnels were covered to prevent cross-contamination, whilst the walls of the funnel were thoroughly rinsed with deionized water to ensure full particle recovery. The filters were labelled and placed in a desiccator to dry for 24 hrs. The dry filters were weighed using an (OHAUS ADVENTURER, USA) analytical scale with accuracy 0.0001g, and placed in a Petri dish for visual identification and counting of microplastics.

For separation in sediment samples, sieved samples in the 500 mL beaker were flooded with NaI-solution (100 $g\,L^{-1}$). Stirring was done with a glass rod to disaggregate and suspend plastic particles. The suspension was poured into 15ml falcon tubes and centrifuged at 4000rmp for 5 min to separate the colloidal particles from the plastic materials. The supernatant was then filtered

through a 1.2 μm, GF/D 47mm chm fiberglass filter (Cat No. GF3-047) using a single stand vacuum filtering system. The filters were labelled and placed in a desiccator to dry for 24 hrs. The dry filters were weighed using an (OHAUS ADVENTURER, USA) analytical scale accuracy 0.0001g, then placed in a Petri dish for visual identification and counting of microplastics. The density separation of microplastics from fish organ samples followed the same procedure and reagent usage as done for water and sediment. The mixture after incubation were soaked with 10 - 15 ml of 4.4M of NaI-solution and stirred thoroughly with a glass rod for 1min before subjecting the supernatant to vacuum filtration for microplastics through a 1.2 μm, GF/D 47mm chm fiberglass filter (Cat No. GF3-047).

Visual Identification

Dry labelled filters of the samples were placed on a Petri dish and analysed for microplastics using a (OPTIKA LAB-10, Italy) dissecting microscope (magnification x40). Under the microscope, microplastics particles were sorted out using distinct colour isolation (Blue, Black, Yellow, Brown, White, Red, Green and Transparent). Sorted microplastic particles were categorized based on shapes (Fragment, Fibre, Pellet, Film, Foam and Sheet), colour, and size (GESAMP, 2019). The size of the microplastics were taken with the aid of an ocular rule calibrated into an image analysis software IMAGEJ (National Institutes of Health, USA) while visual images were taken with a camera. The weight of each categorized microplastic particle were taken using an (OHAUS ADVENTURER, USA) analytical scale accuracy 0.0001 g.

Chemical identification

In determining the polymer type of the microplastics for this study, chemical identification was conducted spectroscopically using ATR-FTIR spectrometer (BRUKER ALPHA PLATINUM ATR, USA) with a diamond crystal. The diamond crystal was wiped with isopropanol before the analysis. Microplastic particles were analysed directly on the glass filter under the device scanning table. The background spectrum was taken. The spectra signatures of samples were captured through 24 scans over a wavenumber range of 400–4000 cm^{-1} at resolution of 4 cm^{-1}. The spectra signatures were identified by comparing with an online polymer spectra database (EssentialFTIR, NICODOM IR Library Spectra. isl) considering acceptance metrices of 0.8.

Contamination Control

Prior to field samples collection, equipment was thoroughly cleaned. All unavoidable synthetic tools such as fish gear and clothing used during sampling were documented with respective colours outlined for any possible field contamination. At the laboratory, a cotton laboratory coat and nitrile gloves were worn at all times. The processing of the samples was carried out in a fume hood with limited access to the experimental environment. All the liquids used during the processing stage underwent filtration in a GF/D (2.7 µm) Whatman microfiber filter membrane. The instruments used during processing were washed once with liquid soup, rinsed with ultrapure deionized water, and finally with 70% ethanol. The workspace was covered with aluminium foil. To track and correct possible contamination, triplicate blanks were conducted at every stage of the treatment and separation stage and tested for microplastic

particles. The outlined contamination control protocol follows directions from (Gago *et al*., 2019; Karami *et al*., 2017).

Statistical Analysis

At the end of the study, data of microplastics density within the water and sediment, were normalised to item per km^{-2} and item per kg^{-1} respectively using the equation 6 and 7;

$$MP\ density(station) = \frac{MP\ items\ counted\ per\ site}{Area\ sampled\ per\ site\ (km^2)} \quad \ldots\ldots\ldots\ldots (6)$$

and $$\frac{MP\ items\ counted\ per\ site}{Weight\ of\ sediment\ (kg)} \ldots\ldots\ldots\ldots\ldots (7)$$

Microplastics in fish and shellfishes were presented as MP items per individual. The results were presented in mean ± Standard deviation (SD) in tables and charts. Graphs and statistical analysis were executed using SigmaPlot (Version 12.0) and Graphpad Prism (Version 5.01) respectively. All data were tested for normality using the Kolmogorov-Smimov test. A one-way analysis of variance (ANOVA) was used to determine the differences in the quantities of microplastics among individual sampling sites and the distribution of microplastics. The differences were considered significant at $p<0.05$ and differences in means were compared using Tukey multiple comparison test. A linear regression analysis was used to test significance in the relation among the abundance of microplastics in water, sediment, and fish, among varying sampling months. A linear regression analysis was used to test significance in microplastics tissue occurrence to biological indices (BW, TL, CF, Gill weight)**.** The Pearson correlation coefficient was used to determine the goodness of fit and the significance of the correlation. A multivariant analysis

was conducted to assess correlation between hydrographic parameters to microplastics occurrence during the study.

CHAPTER FOUR

RESULTS

The results from this study are presented in this chapter. Outlined in this section are tables and figures that contain analysed data that address the study's objectives in chronological order. Topics addressed in this section include the occurrence, density and spatial-temporal distribution of microplastics in the surface water and sediment in the Pra estuary, the relationship between sediment grain size and MP abundance and the characteristics of detected MPs (shapes, colour, size, polymer) in the surface water and sediment in the Pra estuary. The physiochemical parameters monitored over the study period and their relationships with microplastics abundance are presented in this section. The occurrence and abundance of microplastics accumulated in the gills and gastrointestinal tract of fishes and visceral tissue of sampled shellfishes (crabs and shrimps) are also highlighted in this section. The relation between the condition factors of the fish and the total MP abundance among the fish species and the characteristics of MPs in the fish are also presented.

Occurrence and Abundance of Microplastics

Surface Water

Results from the study indicated that plastic particles within the sizes range of 1 µm – 5 mm (microplastics) occurred in all water samples collected. Normalizing the concentrations of microplastics within the surface water to item per km^{-2} gave a total of 15,700,787 items.km^{-2} with a mean density of 196,259.84 ± 60168.72 item. km^{-2} over the 5-month sampling period. The highest concentration of particles (4606299 items. km^{-2}) was recorded in April

and the lowest (2000000 items.km^{-2}) in December as shown in Table 2. Among the sampling stations, the highest MP concentration was recorded at S-N in April (669291.3 items.km^{-2}) and lowest at S14 and S11 in December and January (62992.13 items.km^{-2}) respectively. The results are as presented in Table 2.

Table 2: Concentration of microplastics within the surface water (items.km^{-2}).

	Sampling Months				
Sites	**December**	**January**	**February**	**March**	**April**
S-N	196850.39	110236.20	314960.63	393700.79	669291.34
S1	125984.25	188976.38	307086.61	377952.76	590551.18
S2	70866.14	196850.39	212598.43	181102.36	299212.60
S3	70866.14	39370.08	220472.44	149606.30	291338.58
S4	118110.24	118110.24	141732.28	125984.25	259842.52
S5	118110.24	47244.09	165354.33	181102.36	299212.60
S6	173228.35	173228.35	188976.38	118110.24	307086.61
S7	102362.20	393700.79	283464.57	125984.25	236220.47
S8	70866.14	196850.39	228346.46	149606.30	236220.47
S9	157480.31	86614.17	204724.41	204724.41	204724.41
S10	188976.38	47244.09	149606.30	196850.39	251968.50
S11	236220.47	31496.06	141732.28	204724.41	204724.41
S12	70866.14	149606.30	141732.28	204724.41	133858.27
S13	165354.33	62992.13	173228.35	228346.46	173228.35
S14	62992.13	314960.63	188976.38	244094.49	220472.44
S15	70866.14	496062.99	94488.19	196850.39	228346.46
Totals	**2000000.00**	**2653543.29**	**3157480.31**	**3283465.57**	**4606299.21**

Source: Field study (2021)

The mean monthly variation in microplastic concentration within the surface water is illustrated in Figure 3. An increasing pattern was observed from 125000 ± 55221.1 items.km^{-2} (December) to 287893.7 ± 142342.5 items.km^{-2} (April) as indicated in Figure 3 and Table 2. Monthly concentrations ranged from 62992 - 236220 (December), 31496 - 496060 (January), 94488 - 314961 (February), 118110 - 393700 (March), and 669291 - 133858 items.km^{-2} (April). Statistically, the mean concentration of microplastics recorded in April was significantly higher than that of December and January ($p < 0.001$). However, no statistically significant differences existed among the mean particles recorded from December to March ($p < 0.05$).

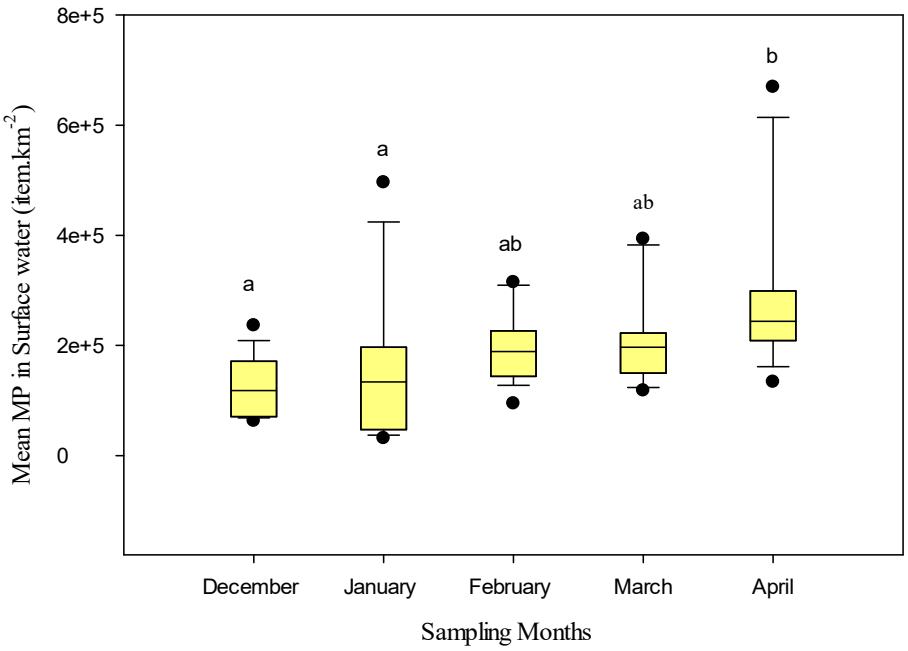

Figure 3: Mean monthly variation of microplastic in surface water. Where difference in alphabet represent mean significant differences at $p < 0.05$. The dotted point outside the boxplot indicate outliers.

The overall mean MP densities recorded for the various grouped stations were 330709 ± 223752 (S-N), 212598 ± 138613 (Mouth), 186142 ± 79909

(Middle), and 175853 ± 91787 items.km^{-2} (Head). A statistically significant difference existed between the means of S-N and the Head ($p = 0.0400$). The monthly mean levels of microplastics within the surface water is presented in Figure 4. The lowest mean MP concentration of 74803.14 items.km^{-2} was recorded within the mouth section during the month of December while the highest of 669291 items.km^{-2} was recorded at S-N in the month of April. Generally, the microplastics levels within S-N exceeded that of all locations for all months except January as shown in Figure 4. Statistically significant differences were found to exist among the means of the monthly MP levels ($p < 0.05$).

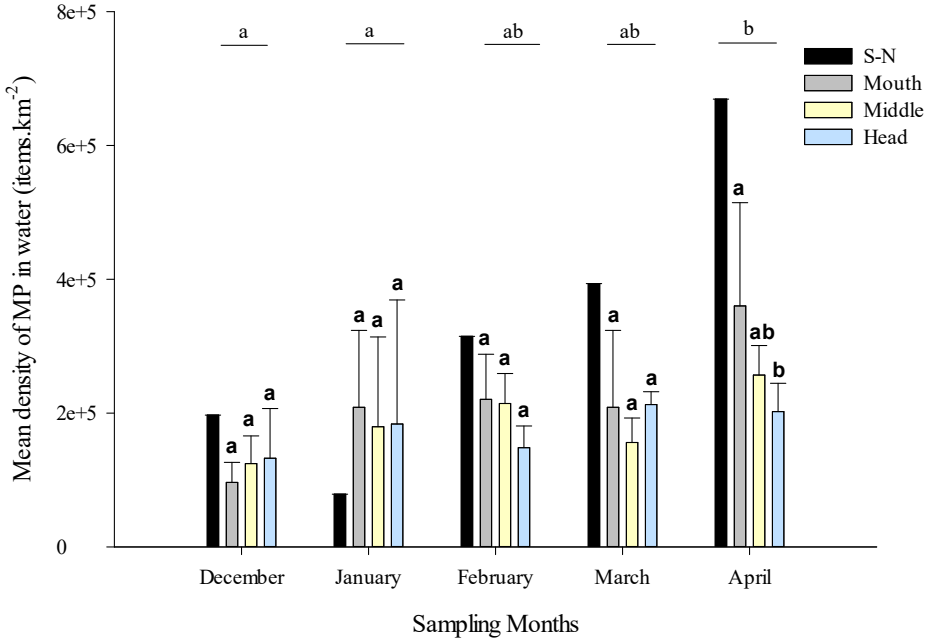

Figure 4: Mean spatio-temporal variation of microplastic in surface water. Where difference in alphabet represent mean significant differences at $p < 0.05$.

Sediment

Results from the study indicated the presence of microplastics in all sediment samples collected over the study period. Normalizing the data to item per kg^{-1} dry weight (d.w) yielded a total of 9,315 MP items kg^{-1}d. w with a mean density of 116.44 ± 11.31 items kg^{-1}d.w. The monthly concentrations of microplastics within the sediment is presented in Table 3. The lowest concentration of microplastics in the sediment was recorded in January (1,625 items.kg^{-1} d.w) and the highest in February (2,075 items.kg^{-1} d.w). The MP concentration ranges were December (45 - 200 items.kg^{-1}d. w), January (45 - 210 items.kg^{-1}d. w), February (80 - 210 items.kg^{-1}d. w), March (75 - 180 items.kg^{-1}d. w), and April (75 - 180 items.kg^{-1}d. w).

Sediment Grain Size and Microplastic Abundance

The particle size distribution of the sediment collected at each sampling site within the Pra Estuary is presented in Table 3. The recorded grain size range of 0.52 – 1.94 mm indicates a homogenous bed structure along all the sampling locations. According to the Wentworth scale (Wentworth, 1922), the particle size of the sediment fell in the category of sand. Spearman's correlation showed no significant relationship between sediment grain size and microplastics abundance ($p = 0.61$). The highest mean abundance of 210 items.kg^{-1}d. w was recorded at S-N in January whilst the lowest of 45 items.kg^{-1}d. w was recorded at S15 in December (Table 3).

Table 3: Concentration of microplastics within the sediment (items. kg^{-1}d. w).

Stations	Dp	R	Sampling Months				
			December	January	February	March	April
S-N	2.5±1.02	0.96	165	210	190	125	180
S1	4.12±0.55	0.59	200	150	165	155	120
S2	3.19±0.60	1.00	50	110	210	145	145
S3	2.98±0.24	1.04	200	105	155	130	110
S4	3.37±0.54	0.55	105	165	135	155	85
S5	2.80±0.88	0.87	65	95	90	105	140
S6	3.32±1.08	1.19	110	80	100	85	95
S7	2.75±0.81	0.84	90	100	130	145	125
S8	2.38±0.44	0.74	175	75	155	180	180
S9	3.40±1.15	0.97	60	140	100	100	100
S10	2.38±0.82	0.90	80	70	130	170	110
S11	3.31±1.74	0.56	90	65	95	95	90
S12	2.72±1.74	0.52	105	45	100	90	75
S13	1.66±0.44	0.55	140	95	120	75	85
S14	4.46±2.27	0.53	80	55	120	115	115
S15	6.36±1.32	1.94	45	65	80	130	100
Totals			**1760**	**1625**	**2075**	**2000**	**1855**

Dp – mean depth (m) ± standard deviation, R – sediment grain size (2 →1 and 1 → 0.5 = Very Coarse sand and Coarse sand respectively, Wentworth scale).
Source: Field study (2021)

The overall mean densities of microplastics in the sediment recorded for the various grouped stations were 174.00 ± 31.90 (S-N), 139.75 ± 18.78 (Mouth), 112.80 ± 13.44 (Middle) and 94.33 ± 18.28 (Head). The mean concentrations of sediment for the various grouped stations are illustrated in Figure 5. A statistically significant difference existed between the mean microplastics density for S-N and the various grouped stations ($p < 0.0001$). However, there were no significant differences observed among the monthly means for the other grouping ($p = 0.7389$).

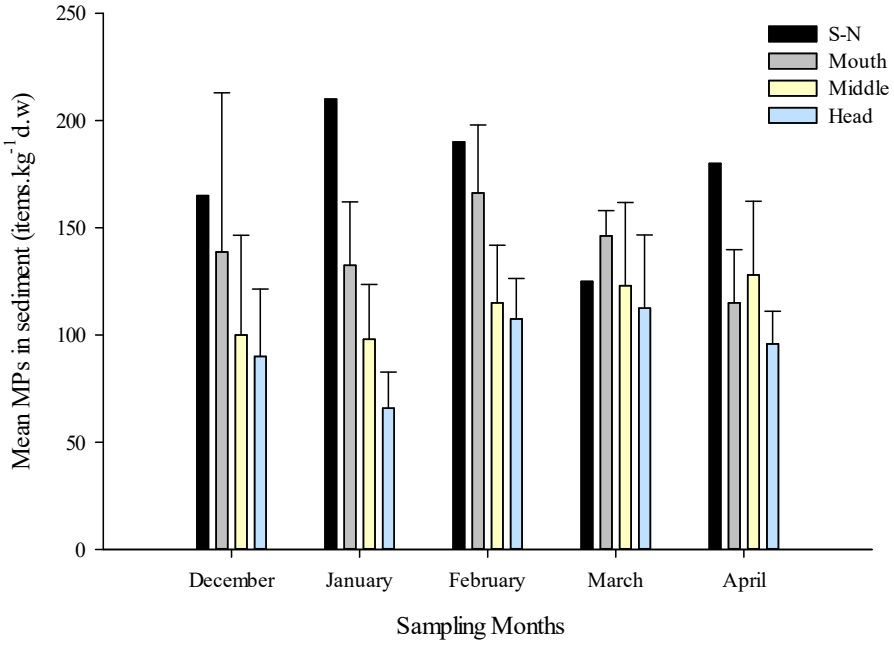

Figure 5: Mean spatio-temporal variation of microplastic in sediment.

The mean monthly concentration of microplastics in the sediment is presented in Figure 6. The highest microplastics recorded was 101.56 ± 44.71 items. kg^{-1}d. w in January and lowest 129.69 ± 37.17 items. kg^{-1}d. w in

February. Statistically, there were no significant differences among the means of particles in the sediments recorded for the various months (p = 0.145).

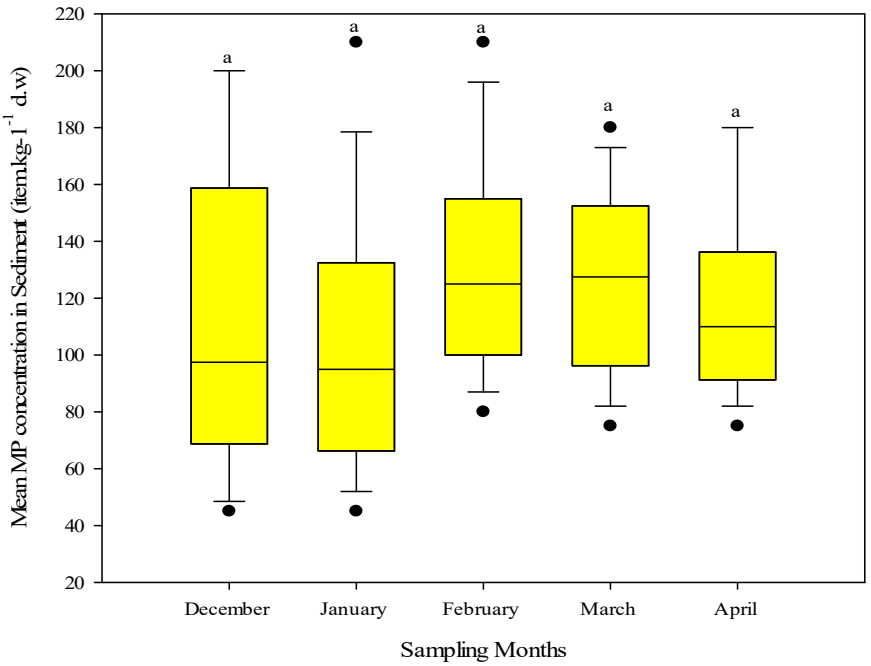

Figure 6: Mean monthly variation of microplastic in Sediment. Where difference in alphabet represent mean significant differences at $p < 0.05$. The dotted point outside the boxplot indicate outliers.

Shapes of Microplastics in Water and Sediment

The classification of isolated microplastics based on shapes is presented in Figure 7. Within the surface water column, fragments, fibre, pellets, film, foam, and sheet were the prominent shapes. The dominant shape over the study period was fibre, with a 65.72% occurrence. This was followed by fragments (23.61%), pellets (3.52%), film (3.03%), Sheet (2.28%), and foam (1.84%). The total count for the lowest shape (sheets), after normalizing the sorted particles to items.km^{-2}, was 291339 and the highest (fibre) was 10433071 items.km^{-2} as shown in Table 4. Statistically, the mean abundance of fibre was significantly higher than the other shapes ($H=19.513$, $p <0.001$).

For the sediment samples, fragment, fibre, pellet, and film were identified. The abundance was as observed in the water column with fibre being the highest (74.02 %), followed by fragment (10.59%), pellets (4.13%), and film (2.25%). A total of 6895 items.kg^{-1} was recorded for fibre whilst film recorded 210 items.kg^{-1} as indicated in Table 4. With the exception of pellet and film, statistically significant differences were observed among the isolated microplastics shapes ($p < 0.001$).

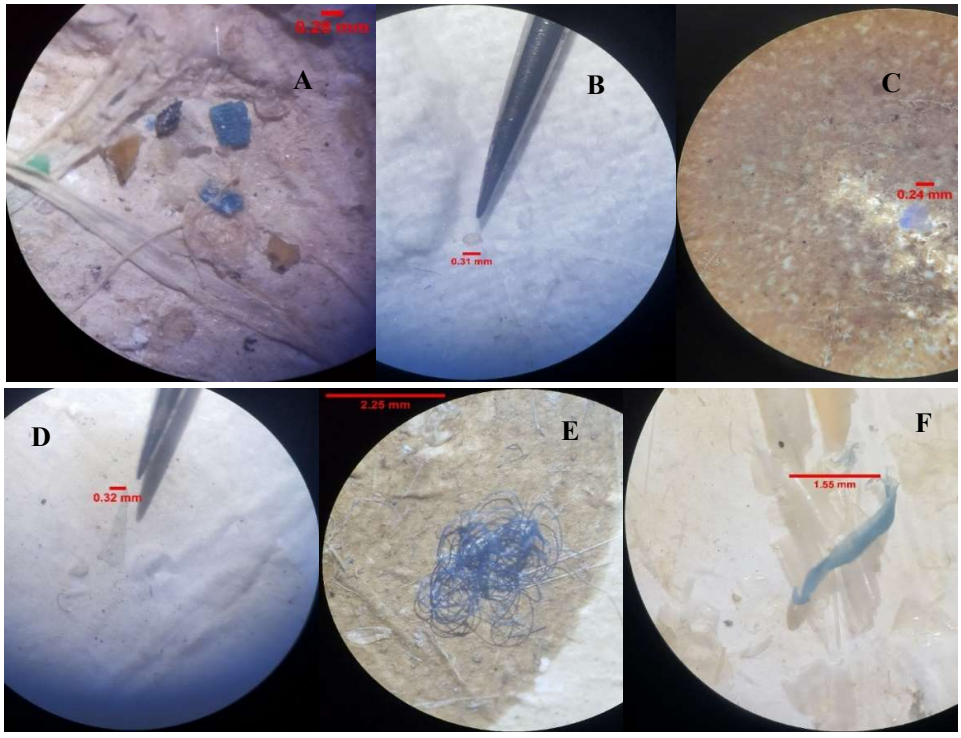

Figure 7**:** Microplastic shapes found in the Pra Estuary. A – Fragment, B – Pellet, C – Foam, D – Sheet, E – Fibre, F – Film. © field work 2021

Table 4: Composition of microplastic shapes in the surface water and sediment

Category	Abundance		Mean ± SD	
Shapes	**Surface Water (no.km^{-2})**	**Sediment (no.kg^{-1}d.w)**	**Surface Water (x.km^{-2})**	**Sediment (no.kg^{-1})**
Fragment	3748031	1825	749606±126402^{ab}	365±105.42^{a}
Fibre	10433071	6895	2053543±567268^{a}	1379±208.88^{b}
Pellet	559055	385	111811±62844^{ab}	77±11.51^{c}
Film	480315	210	96063±150165^{b}	42±14.40^{c}
Foam	291339		58268±104550^{b}	
Sheet	362205		72441±118294^{b}	

x - mean, **SD** – Standard deviation. Numbers with similar letters are not statistically significant ($p<0.05$). Source: Field study (2021)

As presented in Figure 8, the occurrence of microplastics shapes in the water and sediment among the sampled sites were observed to follow the same trend. Within the water, the range of MP shapes accounted was fibre (52.86 - 85.39%) > fragment (8.99-36.89%) > pellet (1.39 – 8.65%) > film (1.45 – 5.50%) > foam (0.72 – 4.81%) and sheet (0.89 – 5.34%). Similarly, the range of MP shapes in the sediment was fibre (65 – 90.29%) > fragment (8.74 – 26.43%) > pellet (0.85 – 9.20%) > film (0.63 – 8.00%).

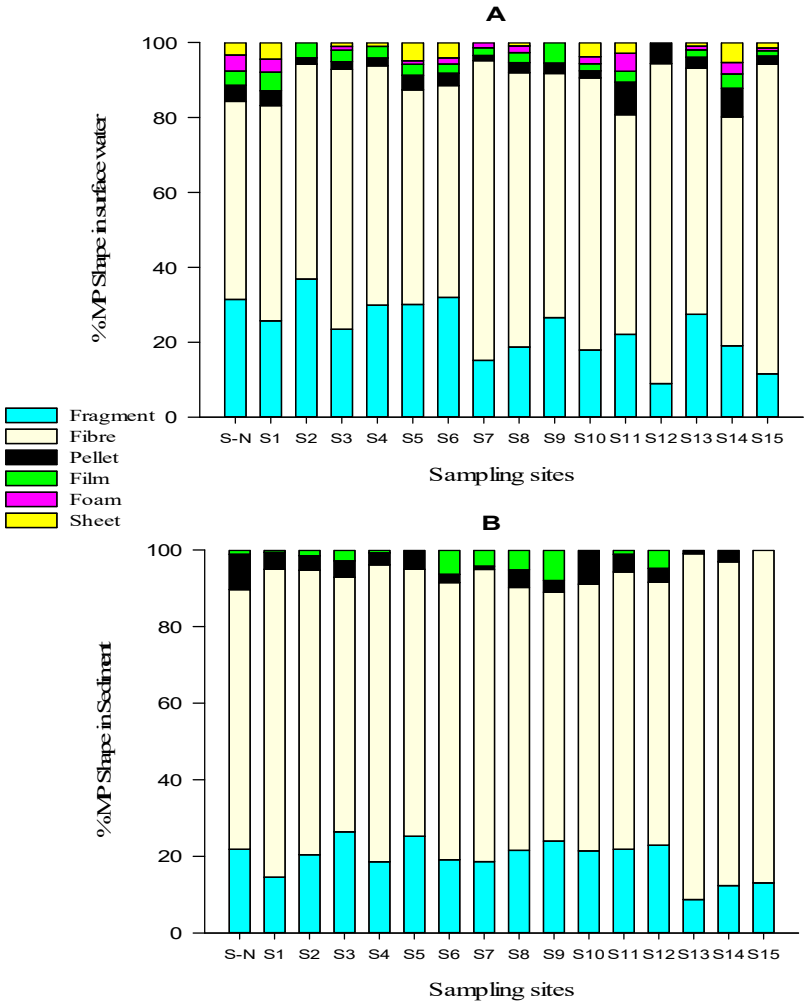

Figure 8: Composition of microplastic shapes in A – Surface water, B – Sediment.

Across the different sampling months, fibre-shaped MPs occurred the highest in the water and sediment accounting for 55.51 – 75.38% and 68.18 - 80.32% respectively (Figure 9). In the water, excluding fibre the order of occurrence for MP shapes was fragment (20.34 – 36.22%) > pellet (0.90 – 4.7%) > film (0.75 4.73%) > foam (0.72- 5.30%) and sheet (0.39 – 6.15). On the other hand, the occurrence of MP fragments within the sediment declined steadily over the sampling period. The order of MP shapes excluding fibre was

fragment (12.13 – 26.42%) > pellet (3.37 – 5.23%) and film (1. 45 – 3.00%).

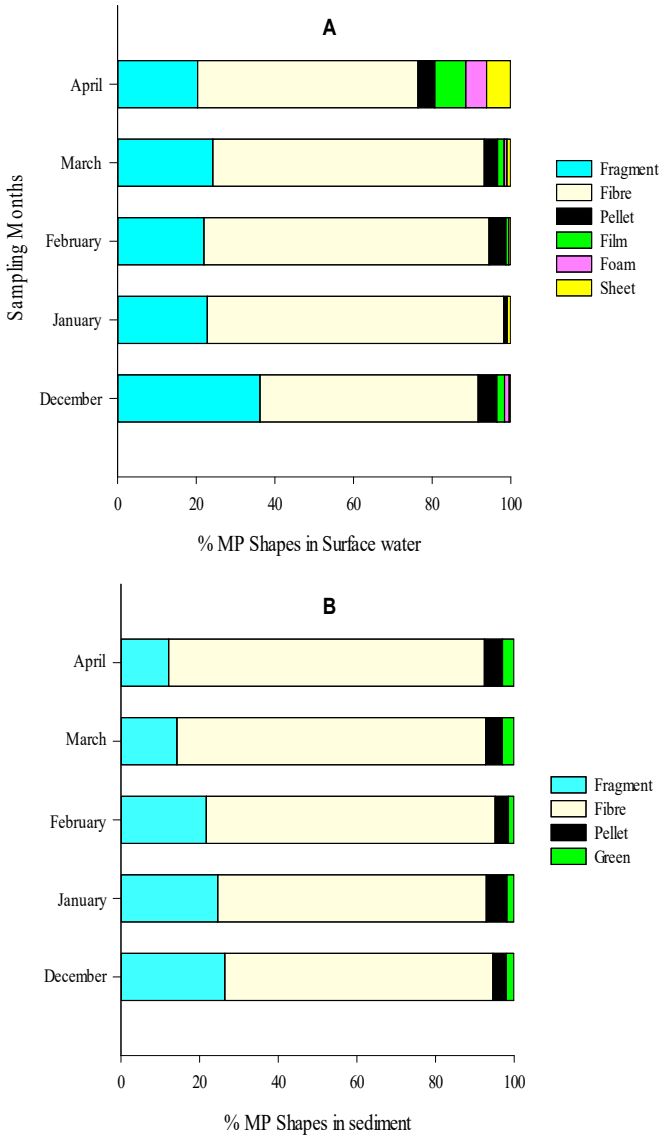

Figure 9: Monthly composition of microplastic shapes in A – Surface water, B – Sediment.

Colours of Microplastics in Water and Sediment

The isolated microplastics were distinctively classified into colours as presented in Table 5. Within the water samples, transparent particles occurred most (37.06%) whilst green particles were the least occurring (3.01%). The

colour present as normalisation to items per km^2 indicated the following concentrations: transparent (5818898) > black (4236220) > blue (2708661) > red (1173228) > yellow (692913) > white (598425) and green (472441). Statistically significant differences were recorded among the mean number of isolated MPs exhibiting the different colours ($H = 26.895$, $df=6$, $P < 0.001$). Sediment microplastics revealed the following colours: black (43.65%) > transparent particles (35.65%) > blue (10.44%) > red (4.23%) > green (2.46%) > white (2.14%) > and yellow (1.45%) in that order. Assessing colour proportionality by a normalized dry sediment weight (items.kg^{-1}d. w) as shown in Table 5, black was dominant with a total of 4075 items.kg^{-1} d.w while yellow recorded the lowest of 135 items.kg^{-1} d.w over the study period. Statistically significant differences in colour existed among the sediment MPs ($H=31.602$, $p < 0.001$).

Table 5**:** Composition of microplastic colours in the surface water and sediment

Category	**Abundance**		**Mean ± SD**		**% Frequency**	
Colour	**Surface Water (no.km^{-2})**	**Sediment (no.kg^{-1}d. w)**	**Surface Water (x.km^{-2})**	**Sediment (no.kg^{-1})**	**Surface Water**	**Sediment**
Blue	2708661	975	541732±101816^{ab}	195±57.23^{abc}	17.25	10.44
Black	4236220	4075	847244±215106^{ab}	815±103.14^{a}	26.98	43.65
Transparent	5818898	3325	1163780±397361^{a}	665±78.42^{ab}	37.06	35.62
Yellow	692913	135	138583±10989^{bcde}	27±14.83^{cd}	4.41	1.45
White	598425	200	119685±110993^{bcdef}	40±7.07^{cd}	3.81	2.14
Red	1173228	395	234646±162842^{abcd}	79±21.33^{abcd}	7.47	4.23
Green	472441	230	94488±124996^{cdef}	46±9.62^{bc}	3.01	2.46

x - mean, **SD** – Standard deviation. Numbers with similar letters are not statistically significant ($p<0.05$).
Source: Field study (2021)

In the water, transparent microplastics occurred highest (S15, 54.35%) followed by black (S2, 36.89%) > blue (S3, 21.78%) > yellow (S12, 11.24%) > white (S9, 8.26%) > red (S9, 12.84%) and green (S2, 6.56%) as presented in Figure 10. Whiles in the sediment, the MP colour composition followed: black (S10, 65.15%) > transparent (S8, 46.50%) > blue (S1, 15.29%) > white (S10, 8.04%) > red (S5, 7.14%) > green (S6, 6.45%) and yellow (S6, 4.30%).

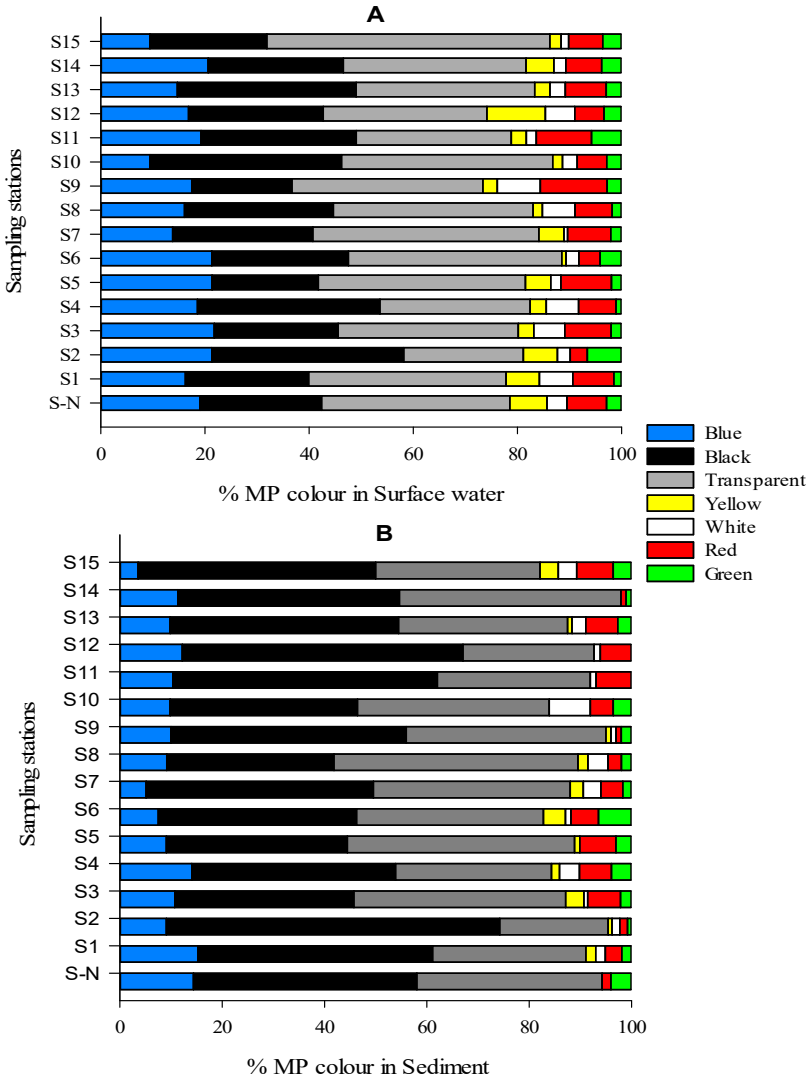

Figure 10: Composition of microplastic colours in A – Surface water, B – Sediment.

The monthly percentage distribution of colours of isolated MPs found in the water and sediment samples are illustrated in Figure 11. Within the sediment, blue-coloured MPs recorded the lowest occurrence of 8.13% in January and highest 13.50% in February. The minimum and maximum occurrences of the other colours were: black 40.24 – 49.19%, (February and April), transparent 33.06 - 36.87%, (April and February), yellow 0.54 - 2.85%, (April and December), white 1.88 - 2.42%, (January and April), red 2.96 - 6.27%, (April and December), and green 1.71 - 2.81%, (December and January) as shown in Figure 11A. Within the surface water, the colour composition ranged from blue 12.23 - 26.38%, (March and December), black 22.46 - 34.25%, (January and December), transparent 24.02 - 46.53%, (December and February), yellow 0.90 - 6.67%, (January and April), white 0.74 - 6.50%, (February and April), red 2.76 - 10.77%, (December and April), and green 0.25 - 6.67%, (February and April) as shown in Figure 11B.

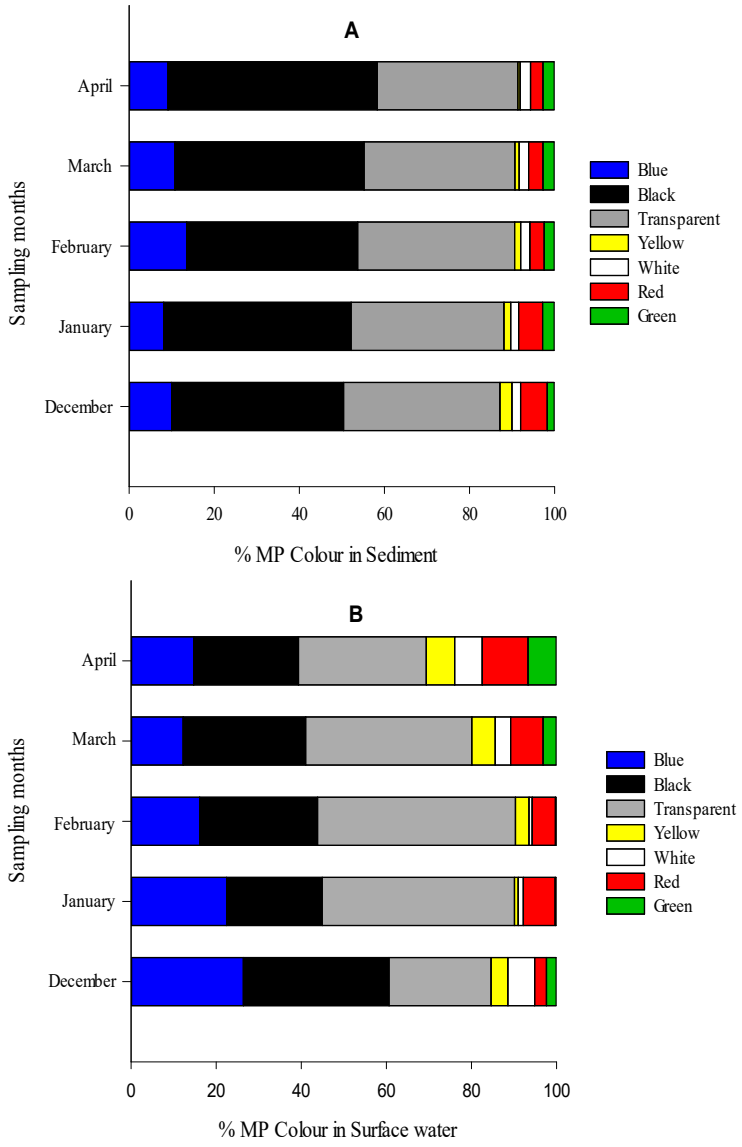

Figure 11: Monthly composition of Microplastic colours in A – Sediment, B – Surface water.

Size distribution of microplastics in Water and Sediment

The size ranges of the isolated MPs have been presented in Table 6. The dominant size range in both surface water and sediment was the MP particles less than 0.5 mm class, representing 46.10% and 54.71% respectively. The lowest size class for the microplastics in the surface water was 1.0 - 2.5 mm (14.81%) whilst that of the sediment was 2.5 - 5.0 mm (6.30%). Over the study period, a total of 5080 items.kg^{-1}dw were recorded for the highest size range (<

0.5 mm) in the sediment and 7204724 items.km^{-2} for particles within the same size range for the surface water. Statistically significant differences existed among the mean number of microplastics within the various size classes for both surface water and sediment ($p < 0.05$). Statistically, the mean concentration for the highest size class of the surface water (< 0.5 mm) was significantly higher than the other size classes ($H= 37.762$, $p < 0.001$) whereas in the sediment, the mean concentration of the < 0.5 mm size class was significantly higher than the 1.0-2.5 mm and 2.5-5.0 mm size classes ($H= 17.884$, $p < 0.001$).

Table 6**:** Composition of microplastic size in the surface water and sediment

Category	**Abundance**		**Mean ± SD**		**% Frequency**	
Size	**Surface Water (no.km^{-2})**	**Sediment (no.kg^{-1}d. w)**	**Surface Water (x.km^{-2})**	**Sediment (no.kg^{-1})**	**Surface Water**	**Sediment**
<0.5 mm	7204724	5080	1440945±330193^{a}	1016±124.42^{a}	46.10	54.71
0.5-1.0 mm	3157480	2315	631496±201550^{b}	463±48.04^{ab}	20.20	24.93
1.0-2.5 mm	2314961	1305	462992±223780^{b}	261±47.75^{b}	14.81	14.05
2.5-5.0 mm	2952756	585	590551±267659^{b}	117±32.13^{b}	18.89	6.30

x - mean, **SD** – Standard deviation. Numbers with similar letters are not statistically significant ($p<0.05$).
Source: Field study (2021)

The spatial occurrences of the microplastic size classes are presented in Figure 12. Within the surface water, the highest size class (< 0.5 mm) recorded a minimum of 37.62% and a maximum of 55.83% at S1 and S6 respectively. Similarly, the minimum (13.94%) and maximum (25.96%) occurrence of the 0.5 – 1.0 mm class occurred at S-N and S11 respectively. For the 1.0 – 2.5 mm class, S11 recorded the minimum of 8.65% whilst the maximum of 18.35% was recorded at S9. For the 2.5 – 5.0 mm the lowest size class, had a minimum of 10.20% recorded at S3 with the maximum of 25.96% at S-N. For the sediments, the highest size class (< 0.5 mm) recorded a minimum of 50.0% and a maximum of 61.016% at S2 and S13 respectively. Followed by 0.5 – 1.0 mm with a minimum of 20.0% recorded at S9 and a maximum of 29.41% recorded at S8, the size class 1.0 – 2.5 mm had minimum of 10.98% at S12 and maximum of 17.17% at S5. The lowest size class 2.5 – 5.0 mm, had a minimum of 3.85% at S15 and a maximum of 13.0% at S9 (Figure 12).

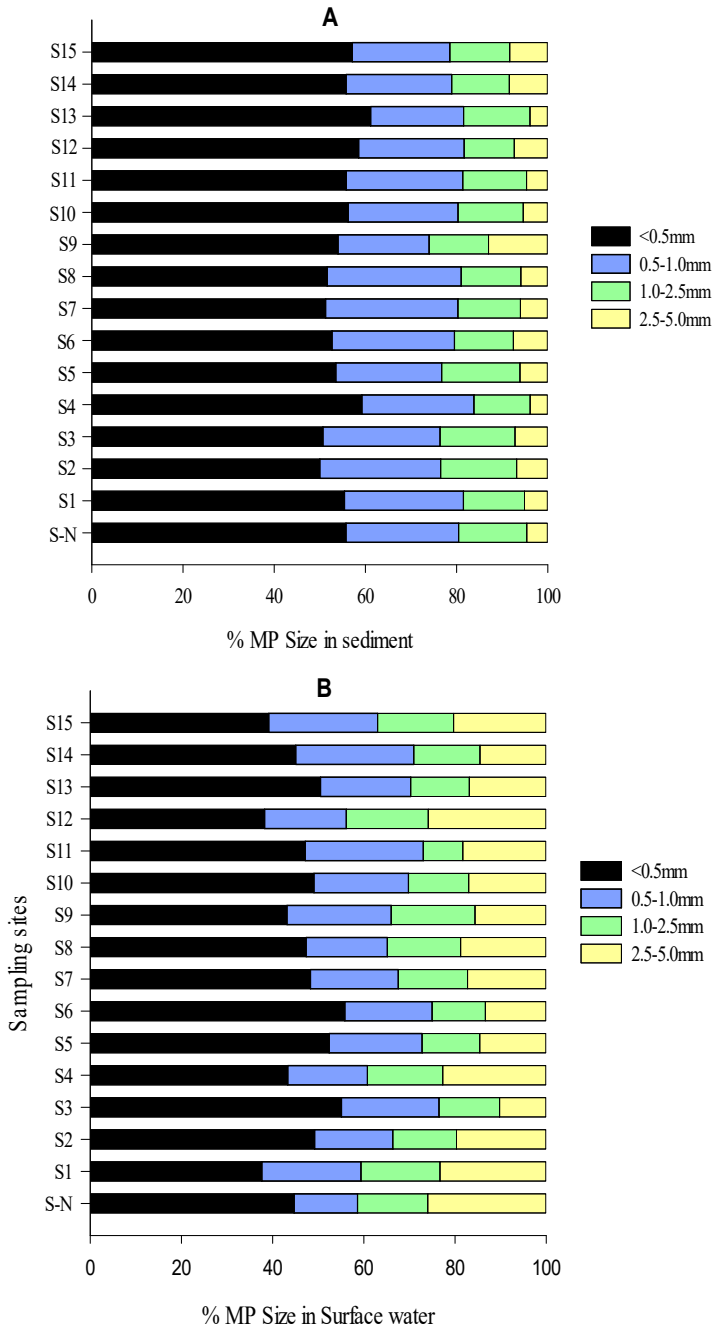

Figure 12: Composition of Microplastic size classes in A – Sediment, B – Surface water.

As shown in Figure 13, the highest MP size class (< 0.5 mm) had maximum occurrence of 54.72% recorded in December and minimum of 40% recorded in April in the surface water samples. This was followed by 0.5 – 1.0 mm MP size

class, with a minimum of 17.87% and a maximum occurrence of 22.06% recorded in March and February respectively. The size class of 1.0 – 2.5 mm had minimum of 9.84% accounted in December and a maximum of 17.61% in April. The lowest size class, 2.5 – 5.0 mm recorded 15.21% and 22.05% for lowest and highest occurrence in March and April respectively. In the sediment, the highest occurring size class (< 0.5 mm) had the minimum occurrence of 50.63% recorded in January and a maximum of 60.11% in December whiles the lowest size class 2.5 – 5.0 mm had minimum occurrence of 3.75% reported in December and a maximum of 7.71% reported in February (Figure 13).

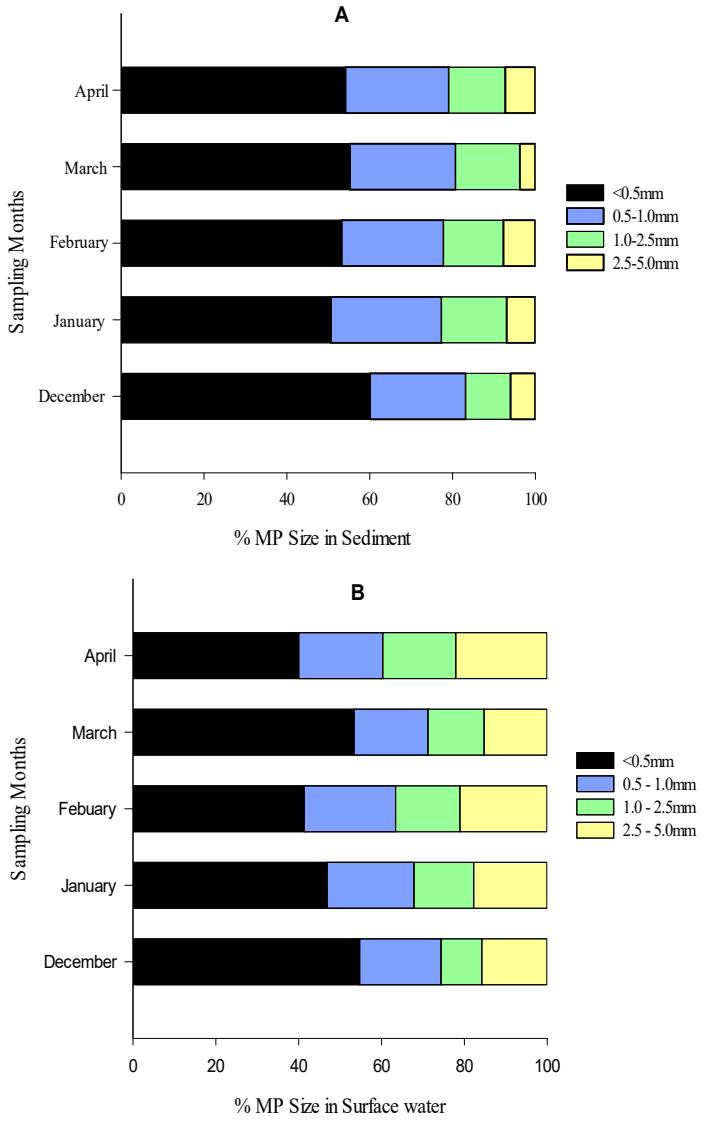

Figure 13: Monthly composition of Microplastic size classes in A – Sediment, B – Surface water.

Polymer of Microplastics in Water and Sediment

A total of 233 subsampled particles from both the surface water (122) and sediment (111) were randomly selected over the study period to be identified using an ATR-FTIR. All samples analysed were identified as plastic polymers at > 0.8 similarity Metrix after matching unique spectral signature in two spectral libraries as shown in the Appendix E.

The abundance and percentage composition of identified polymer types are presented in Table 7. Polyethylene was the dominant polymer occurring within the surface water and sediment. Polymers classified as others in Table 7 include Acrylonitrile butadiene styrene, polyesterurethane, and polyester epoxide which occurred once in the analysed samples. The order of occurrence within the surface water was Polyethylene (40.98%) > Polypropylene (23.77%) > Polyurethane (18.89%) > Polystrene (11.48%) > Polyester (9.02%) > Polyethylene terephthalate (7.38%) > and others (3.28%). Statistically significant differences were observed among the polymer numbers ($p<0.05$). Polyvinyl chloride was present only within sediment samples. The order of polymer occurrence within the sediment was Polyethylene (27.53%) > Polypropylene (17.33%) > Polystrene (15.26%) > Polyvinyl chloride (6.53%), Polyester (6.17%) > Others (3.80%) > Polyurethane (3.01%).

Table 7**:** Composition of microplastic size in the surface water and sediment

Category	Abundance		Mean ± SD		% Frequency	
Polymer	**Surface Water (no.km^{-2})**	**Sediment (no.kg^{-1})**	**Surface Water (x.km^{-2})**	**Sediment (no.kg^{-1})**	**Surface Water**	**Sediment**
Polypropylene (PP)	228346	96	45669±11679^{ab}	3.85±1.23^{abc}	23.77	17.33
Polyester (PES)	86614	34	17323±10266^{abcd}	1.37±1.12^{fg}	9.02	6.17
Polyethylene (PE)	393701	153	78740±26702^{a}	6.11±2.39^{a}	40.98	27.53
Polyurethane (PU)	39370	17	7874±5568^{cdef}	0.67±0.77^{efgh}	18.89	3.01
Polyethylene terephthalate (PET)	70866	113	14173±6588^{abcde}	4.52±2.32^{ab}	7.38	20.38
Polystrene (PS)	110236	85	22047±8626^{abc}	3.39±1.68^{abcd}	11.48	15.26
Polyvinyl chloride (PVC)	-	36	-	1.45±0.50^{def}	-	6.53
Others	31496	21	6299±6588^{cdef}	0.84±0.67^{fgh}	3.28	3.80

x - mean, **SD** – Standard deviation. Numbers with similar letters are not statistically significant ($p<0.05$).
Source: Field study (2021)

The monthly trend in polymer composition shows a uniform highest composition for polyethylene in the surface water and sediment except in January where polystyrene occurred highest in the sediment samples as shown in Figure 14. In the sediment, polyethylene which dominated the samples had the maximum occurrence of 36.36% recorded in February and minimum of 18.75% recorded in January. This was followed by polystyrene with highest occurrence of 31.25% accounted in January and lowest of 3.70% recorded in December. In the water, polyethylene was recorded highest at 48.0% in February and lowest at 35.0% in March, this was followed by polypropylene which recorded the highest at 30.0% in March and lowest at 20% in February.

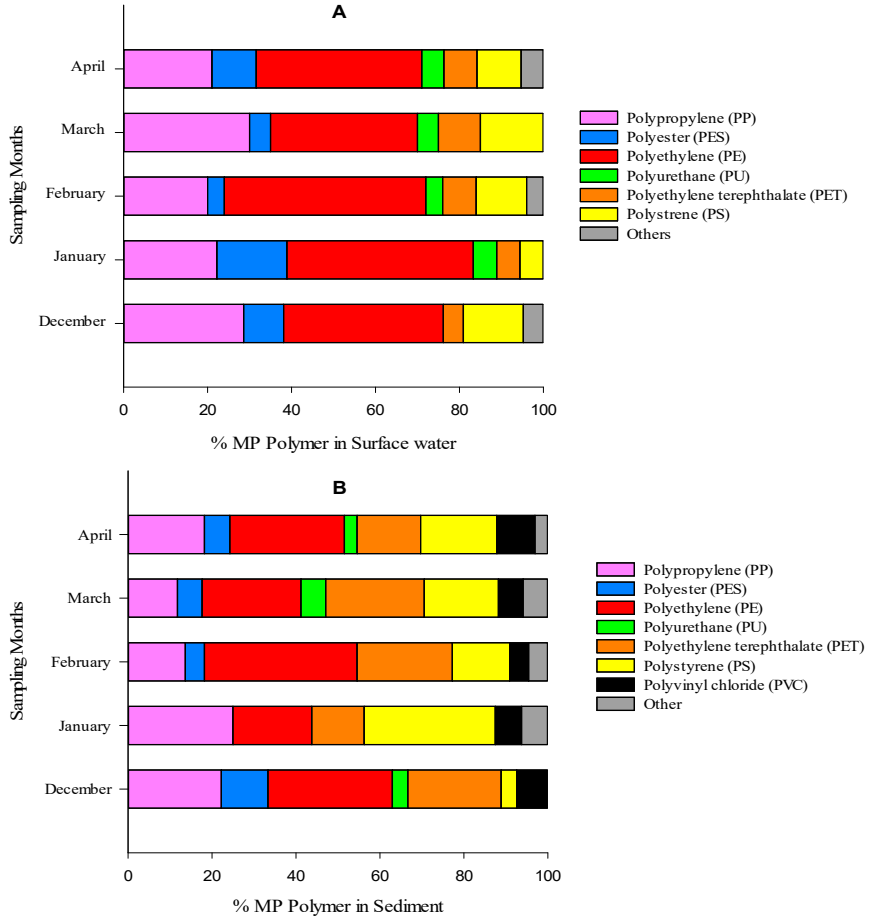

Figure 14: Monthly composition of MP polymer in A – Surface water, B – Sediment

Physiochemical parameters of the Pra Estuary

The monthly means of the physiochemical parameters (Dissolved oxygen (DO), Temperature, pH, Turbidity, Salinity, and Flow velocity) recorded at the sampling stations have been presented in Table 8. The mean dissolved oxygen (DO) measures over the sampling period were relatively constant in the range 5.51 ± 0.14 - 8.05 ± 0.27 mgL^{-1}. Among the grouped stations, the mean DO ranges were 5.51 ± 0.14 - 7.07 ± 0.17, 5.94 ± 0.29 - 7.67 ± 0.55, 5.88 ± 0.39 - 7.69 ± 0.23, and 6.14 ± 0.30 - 8.05 ± 0.27 representing S-N, Mouth, Middle, and Head respectively. The lowest recorded DO was 5.42 mgL^{-1} at S-N in February and highest of 8.46 mgL^{-1} was recorded at the Head in December. No statistically significant differences existed among the mean DO levels.

Temperature measured ranged from 26.15 ± 0.16 - 30.94 ± 0.71^{o}C. The station temperature ranges were 26.15 ± 0.16 - 29.73 ± 0.15, 28.41 ± 0.38 - 29.53 ± 0.32, 27.13 ± 0.52 - 30.93 ± 1.20, and 26.31 ± 0.48 - 30.94 ± 0.71 for S-N, Mouth, Middle, and Head respectively. The lowest temperature recorded was 26.2^{o}C at S-N in December and the highest of 32.3^{o}C was recorded at the Head in February. Statistically significant difference was observed to exist among the various group stations. S-N in December was significantly lower than the various grouped stations ($F=11.99$, $p < 0.0001$). Similarly, statistically significant difference was observed between the sampling months and temperature recorded ($F=11.99$, $df=4$, $p < 0.0001$).

The mean pH range recorded for the study period was 6.74 ± 0.11 to 7.77 ± 0.23. The pH ranges at the various stations were 6.77 ± 0.12 - 7.77 ± 0.23, 6.93 ± 0.13 - 7.48 ± 0.01, 6.74 ± 0.11 - 7.68 ± 0.18, and 6.92 ± 0.08 - 7.97 ± 0.04 representing S-N, Mouth, Middle, and Head respectively. The lowest pH level

of 6.6 was recorded in the Middle for April and the highest value was 7.9 recorded at S-N in March. There was no statistically significant difference occurring among the pH values recorded for each station at $p < 0.05$.

For turbidity, the mean values measured ranged from 9.92 ± 0.56 to 490.35 ± 10.28 NTU. Turbidity values for the middle and head sections exceeded 350 NTU throughout the sampling period. The highest turbidity of 508.22 NTU was recorded in the Middle in January and the lowest of 4.41 NTU recorded at S-N in February. Turbidity levels at the stations ranged from 8.43 ± 8.72 - 346.45 ± 20.48, 12.72 ± 6.73 - 400.66 ± 21.26, 337.74 ± 155.82 - 490.35 ± 10.28, and 411.50 ± 7.92 - 472.74 ± 17.86 NTU for S-N, Mouth, Middle, and Head respectively. Statistically significant differences, mean turbidity values existed among the sampling stations ($F = 34.24$, $p < 0.0001$) and sampling months ($F = 54.83$, $p < 0.0001$).

The mean salinity values ranged from 0.07 ± 0.02 - 24.50 ± 6.11 ppt over the study period. The highest salinity recorded was 31.53 ppt in February at S-N and the lowest was 0.04 ppt. The mean salinity ranges within the stations were 0.26 ± 0.11 - 24.50 ± 6.11 ppt, 0.16 ± 0.02 - 12.20 ± 5.57 ppt, 0.09 ± 0.03 - 2.25 ± 2.16 ppt, and 0.07 ± 0.02 - 0.13 ± 0.00 ppt representing S-N, Mouth, Middle, and Head respectively. Statistically significant difference was observed to exist among the various grouped stations ($F = 31.44$, $df=12$, $p < 0.0001$). Similarly, statistically significant difference was observed between the sampling months and salinity recorded ($F = 103.67$, $df=4$, $p < 0.0001$).

The mean flow velocity ranged from 0.18 ± 0.03 - 1.94 ± 0.17 ms^{-1}. The highest velocity recorded was 2.15424 ms^{-1} and the lowest at 0.148 ms^{-1}. Mean flow velocity ranges at the various stations were 0.18 ± 0.03 - 0.24 ± 0.03, 0.81

± 0.14 9 - 1.94 ± 0.17, 0.33 ± 0.10 - 1.24 ± 0.38, and 0.20 ± 0.07 - 0.47 ± 0.26 ms^{-1} for S-N, Mouth, Middle, and Head respectively. The lowest flow velocity was recorded at S-N in January whiles the highest was recorded at the mouth in April. Statistically significant difference was observed to exist among the various grouped stations ($F = 11.93$, $df=12$, $p < 0.0001$). Similarly, statistically significant difference was observed between the sampling months and salinity recorded ($F = 47.87$, $df=4$, $p < 0.0001$).

Table 8: Mean monthly variation of physicochemical parameters monitored

Parameter	Month	S-N	Lower	Middle	Upper	P-value
Flow velocity (m/s)	December	0.25±0.02	0.26±0.04a	0.34±0.10a	0.21±0.07a	0.054
	January	0.18±0.06	0.95±0.29a	0.56±0.13b	0.33±0.20b	0.002
	February	0.18±0.03	0.84±0.16a	0.49±0.12b	0.32±0.13b	<0.001
	March	0.24±0.03	1.71±0.13a	1.20±0.16b	0.41±0.27c	<0.001
	April	0.24±0.01	1.94±0.17a	1.24±0.38ab	0.47±0.26b	0.004
Salinity (ppt)	December	2.09±0.31	0.89±0.63a	0.11±0.01ab	0.10±0.01b	0.003
	January	14.53±1.10	6.85±4.88a	0.25±0.32ab	0.10±0.00b	0.003
	February	24.50±6.11	11.48±5.84a	1.58±1.04ab	0.11±0.00b	0.002
	March	14.00±2.06	0.19±0.02a	0.15±0.01ab	0.13±0.00b	0.003
	April	0.26±0.11	0.17±0.01a	0.08±0.02b	0.07±0.02b	<0.001
DO (mg/L)	December	6.31±0.08	7.67±0.55a	7.69±0.23a	8.05±0.27a	0.184
	January	6.56±0.32	6.74±0.08a	6.93±0.12ab	6.92±0.04b	0.030
	February	5.51±0.14	6.50±0.22a	6.10±0.32a	6.14±0.30a	0.120
	March	7.07±0.17	5.94±0.29a	5.88±0.39ab	6.48±0.31b	0.022
	April	6.42±0.11	5.97±0.04a	5.88±0.39ab	6.29±0.19b	0.029
pH	December	7.32±0.18	7.20±0.03a	7.20±0.03a	7.17±0.04a	0.363
	January	6.77±0.27	6.91±0.01a	6.90±0.03a	6.92±0.08a	0.918
	February	7.58±0.18	7.48±0.01a	7.68±0.18b	7.67±0.04b	0.002
	March	7.77±0.23	6.93±0.15a	7.58±0.22b	7.67±0.23b	<0.001
	April	6.77±0.12	6.93±0.13a	6.74±0.12ab	7.05±0.24b	0.047
Temperature (°C)	December	26.83±0.60	28.48±0.31a	28.26±0.47a	27.88±0.53a	0.163
	January	28.69±0.52	28.25±0.21a	28.56±0.24a	28.85±0.76a	0.239
	February	28.50±0.10	29.78±0.10a	31.04±1.40ab	30.98±0.73ab	0.014

	March	29.73±0.15	29.43±0.59a	29.16±0.89a	28.72±0.32a	0.233
	April	26.15±0.16	28.85±0.21a	30.42±0.42b	30.60±0.44b	<0.001
Turbidity (NTU)	December	326.17±5.54	384.17±14.22a	409.12±8.18b	434.51±5.88c	<0.001
	January	9.92±0.56	239.12±222.62a	487.06±11.30 ab	472.54±19.06b	0.014
	February	8.43±8.72	12.72±5.67a	364.31±160.53ab	470.32±4.97b	0.006
	March	61.83±0.70	387.02±8.62a	398.74±7.44a	412.69±6.33b	<0.001
	April	346.45±20.48	400.66±21.43a	437.61±6.90a	447.23±6.60b	<0.001

DO – Dissolved oxygen, Numbers with similar letters are not statistically significant ($p<0.05$). Source: Field study (2021)

The correlation between the physicochemical parameters (turbidity, temperature, dissolved oxygen, pH, salinity, flow velocity) and microplastics abundance in the surface water and sediment are presented in Tables 9 and Table 10 respectively. The dissolved oxygen showed a negative significant correlation with the microplastic abundance in the water ($r = - 0.135$, $p = 0.0004$) as seen in Table 9 from Figure 15 and Table 9, a significantly positive correlation was however observed between the flow velocity and microplastic abundance in surface water of the Pra Estuary ($r = 0.121$, $p = 0.0242$).

Table 9**:** Correlation of Surface Water MPs (Log item/km^2) with some physicochemical parameters in the Pra Estuary.

Parameters	**Coef**	**SE Coef**	**T-Value**	**P-Value**
Turbidity	-0.0003	0.000206	-1.71	0.0922
Temperature	0.0506	0.025961	1.95	0.0548
DO	-0.1346	0.036272	-3.71	**0.0004***
pH	0.0071	0.072816	0.10	0.9228
Salinity	0.0069	0.00494	1.39	0.1681
Flow velocity	0.1209	0.052578	2.30	**0.0242***

Where, Coef – Correlation coefficient, SE – Standard error, * = significate correlations. Source: Field study (2021)

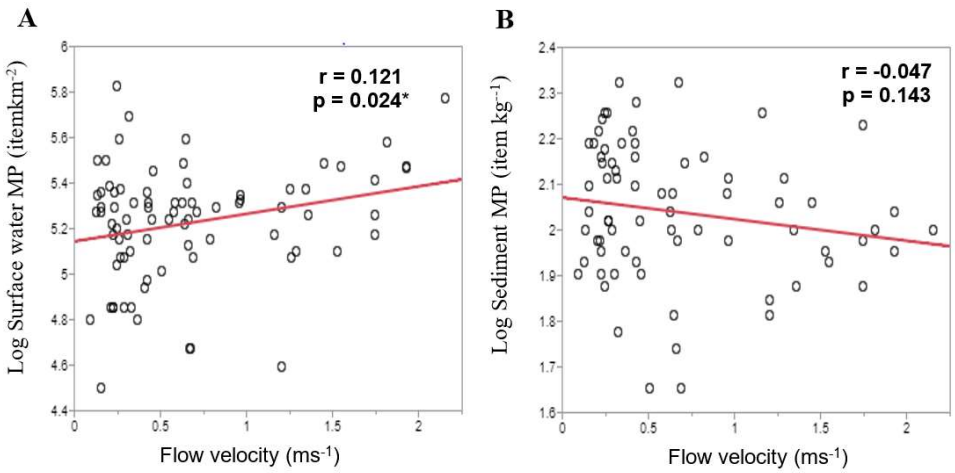

Figure 15**:** The relationship between flow velocity and MP abundance in surface water (A) and sediment (B).

The correlation between the physicochemical parameters investigated and the log transformed sediment microplastic abundance showed no significant relationship except pH which was positively correlated to sediment MP abundance (Table 10).

Table 10**:** Correlation of log Sediment MPs (item/kg) with some physicochemical parameters in the Pra Estuary

Parameters	**Coef**	**SE Coef**	**T-Value**	**P-Value**
Turbidity	$-1.749x10^{-5}$	0.0001	-0.14	0.8887
Temperature	0.0083	0.0157	0.53	0.6000
DO	-0.0242	0.0238	-1.02	0.3125
pH	0.1000	0.0429	2.33	**0.0228***
Salinity	0.0015	0.0029	0.51	0.6099
Flow velocity	-0.0474	0.0320	-1.48	0.1431

Where, Coef – Correlation coefficient, SE – Standard error, * = significate correlations. Source: Field study (2021)

Occurrence and Composition of Fish Sampled in the Pra Estuary

A total of 135 specimens, made up of finfishes (98) and shellfishes (38), were collected at the end of the study. Systematic identification of the specimens indicated that 10 species were finfishes while 2 were shellfish. The mean weight and total length of the species are presented in Table 11. Among the finfishes, *Sarotherodon melanotheron* (SM) was the most abundant (24 specimens), followed by *Pseudotolithus senegalensis* (PS) (21 specimens). *Gobionellus occidentalis* (GO) and *Ethmalosa fimbriata* (EF) had equal abundance (14 specimens), *Chrysichthys nigrodigitalus* (CN) (12 specimens), *Elops lacerta* (EL) (5 specimens), *Mugil bananesis* (MB), *Cynoglossus senegalensis* (CS), and *Apsilus fuscus* (AF) had abundance of 2 specimens each. The lowest abundant was *Galeoides decadactylus* (GD) (1 specimen). The highest mean weight recorded was 558.18 ± 147.9 g (*Chrysichthys nigrodigitalus)* and the lowest was 5.64 ± 0.46 g (*Gobionellus occidentalis)*. Specimen of *Sarotherodon melanotheron* had the shortest total length at 5.7 cm and the longest total length of 59.7 cm was recorded for *Pseudotolithus senegalensis.* Two shellfishes, *Callinectis aminicola* (CA) (12 specimens) and *Penaeus Penaeus* (PP) (26 specimens), were sampled during the study period. Four species habitats (benthopelagic, benthic, pelagic, and demersal) were identified to be occupied by the sampled specimen. The feeding type of the sampled species were Planktivorous, Omnivorous, and Carnivorous.

Table 11**:** The composition of fish species collected over the sampling period.

Family	Species	Habitat	Feeding Type	N	Mean weight (g)	TL Range (cm)	K	MP Gills	MP GIT	MP VM
	Finfishes							**Total (R)**	**Total (R)**	**Total (R)**
Cichlidae	*Sarotherodon melanotheron*	Benthopelagic	P	24	14.28±3.13	5.7-13.0	2.34±0.25	92 (1-9)	236 (2-19)	
Gobiidae	*Gobionellus occidentalis*	Benthic	O	14	5.64±0.46	9.6-13.0	0.38±0.08	34 (1-5)	93 (4-10)	
Elopidae	*Elops lacerta*	Pelagic	C	5	33.51±4.72	15.9-20.1	0.49±0.05	23 (3-6)	36 (5-9)	
Mugilidae	*Mugil bananesis*	Pelagic	O	2	8.70±4.90	7.5-12.0	0.84±0.08	3 (1-2)	5 (2-3)	
Claroteidae	*Chrysichthys nigrodigitalus*	Demersal	O	12	558.18±147.9	20.8-53.4	0.76±0.29	58 (2-9)	56 (3-9)	
Cynoglossidae	*Cynoglossus senegalensis*	Demersal	C	2	264.36±72.01	31.0-46.0	0.49±0.21	5 (1-4)	2 (1)	
Polynemidae	*Galeoides decadactylus*	Demersal	C	1	713.4	45.0	0.78	3	6	
Sciaenidae	*Pseudotolithus senegalensis*	Demersal	C	21	114.57±31.61	12.4-59.7	0.66±0.21	82 (2-10)	17 (1-2)	
Clupeidae	*Ethmalosa fimbriata*	Pelagic	P	14	84.66±7.47	17.6-26.3	0.91±0.14	18 (1-3)	23 (1-3)	
Lutjanidae	*Apsilus fuscus*	Demersal	C	2	230.25±8.96	40.0-42.0	0.34±0.05	0	7 (1-6)	
	Shellfishes									
Portunidae	*Callinectis aminicola*	Benthic	C	12	26.53±4.29	5.0 - 9.7*				32 (2-4)
Penaeidae	*Penaeus notialis*	Benthic	C	26	2.01±0.22	6.0-8.9				19 (1-3)

Where * = Carapace width, **N** = Number of specimens, P = Planktivorous, O = Omnivorous, C = Carnivorous, **TL** = Total Length, Mean ± Standard Error, **R** = Range, **K** = Condition factor. Source: Field study (2021)

Occurrence of Microplastics in the Fish

Microplastics were detected in all the species (Table 11) sampled within the Pra estuary over the study period. The gills and gastrointestinal tract of individual fish specimen were investigated separately. Among the organs assessed, MP occurrence was 100% in almost all species except *Sarotherodon melanotheron* (92% - gills and 100% - GIT), and *Pseudotolithus* senegalensis (76% - gills and 57% - GIT). Microplastics were not detected in the gills of *Apsilus fuscus*. For the two shellfishes, microplastics were present in the visceral tissues of the species sampled. However, among the shellfish specimens microplastics were detected in 31% of the *Penaeus penaeus* sampled and 91.67% of *Callinectis aminicola*.

Abundance of Microplastics in the Fish

A total of 850 microplastic particles were found in both the finfishes and shellfishes investigated. The particle load (item/individual) in the species sampled is presented in Figure 16. The total microplastics in the finfishes ranged from 5 – 332 items per fish with the lowest occurring in *G. decadactylus* and highest in *S. melanotheron*. *E. fimbriata* recorded the lowest microplastics of 1.29 ± 0.73 items/individual in the gills whiles *C. nigrodigitalus* recorded the highest microplastics of 4.83 ± 2.08 items/individual in the gills. Microplastics in the gastrointestinal tract of finfishes ranged from 0.81 ± 0.93 item/individual to 9.83 ± 4.63 items/ as seen in Figure. 18.

The order of microplastic abundance in the gills was *Chrysichthys nigrodigitalus* (mean ± sd) (4.83 ± 2.08) > *Elops lacerta* (4.6 ± 1.14) > *Sarotherodon melanotheron* (4.36 ± 2.52) > *Pseudotolithus senegalensis* (3.91

± 2.84) > *Galeoides decadactylus* (3) > *Cynoglossus* senegalensis (2.5 ± 1.12), *Gobionellus occidentalis* (2.43 ± 1.40) > *Mugil bananesis* (1.5 ± 0.71) > *Ethmalosa fimbriata* (1.29 ± 0.73 item/individual) as shown in Figure 16. However, the order of microplastics abundance in the GIT recorded was *Sarotherodon melanotheron* (9.83 ± 4.63) > *Elops lacerta* (7.2 ± 1.64) > *Gobionellus occidentalis* (6.64 ± 1.74) > *Galeoides decadactylus* (6) > *Chrysichthys nigrodigitalus* (4.67 ± 2.50) > *Apsilus fuscus* (3.5 ± 3.54) > *Mugil bananesis* (2.5 ± 0.71) > *Ethmalosa fimbriata* (1.64 ± 0.63) > *Cynoglossus senegalensis* (1.0 ± 0.00) > *Pseudotolithus senegalensis* (0.81 ± 0.93).

In this study, statistically significant differences existed between the microplastics levels in the gills and gastrointestinal tracts in almost all the fish species sampled with the exception of *Elops lacerta, Mugil bananesis, Chrysichthys nigrodigitalus, Cynoglossus senegalensis Ethmalosa fimbriata*. Within the shellfishes, microplastics load in the vesical mass (VM) recorded was (2.67 ± 1.44) and (1.64 ± 0.63) for *Callinectis aminicola* and *Penaeus penaeus* respectively.

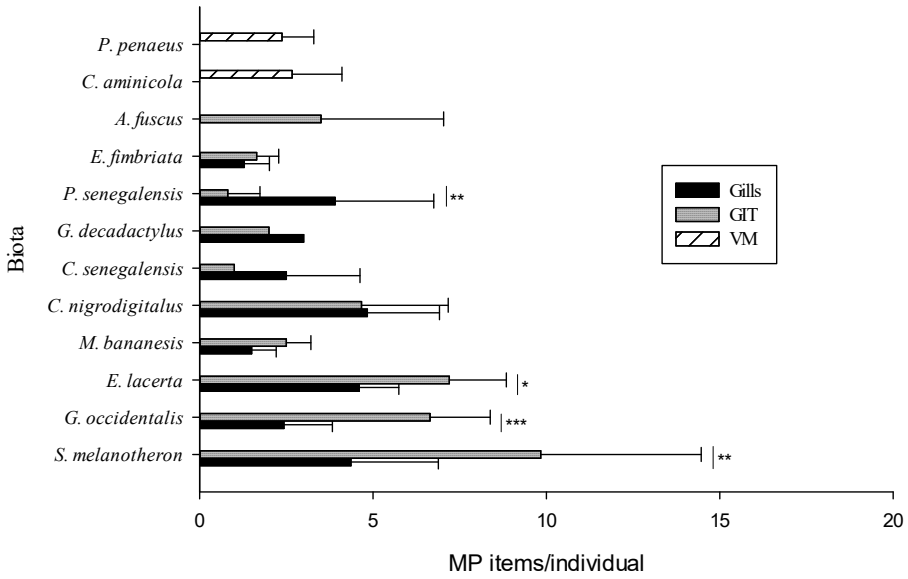

Figure 16**:** Mean Microplastics load (abundance) in the sampled fishes within the Pra Estuary. Asterisk (*) represent statistically significant ($p<0.05$).

A bivariant analysis of the microplastics in the gills and GIT of the sampled fish species showed a significant positive correlation ($r = 0.514$, $p < 0.0001$) between the accumulated microplastics in the gills and the GIT as shown in Figure 17.

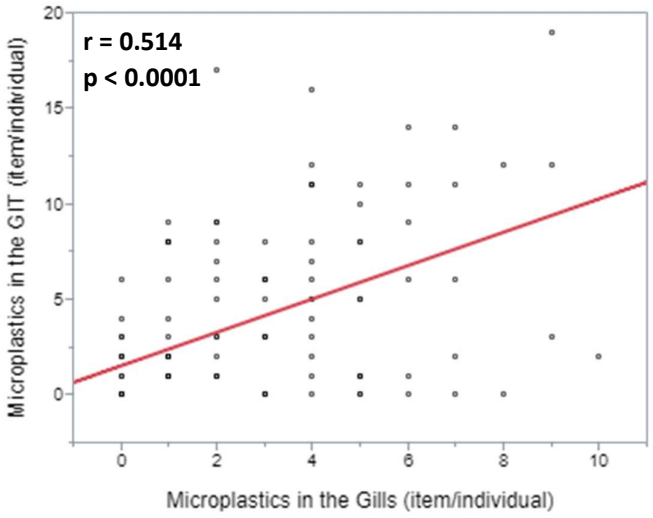

Figure 17**:** The relationship between the microplastics in the gills and GIT of the sampled fish species.

Total MP - length and weight relationship in finfishes

The relationship between the total MP and total length of fish is given in Figure 18. The analysis showed a significant negative correlation between the total MP (item/individual) and the total length (p = 0.0045). The correlation between the total MP (item/individual) and the wet body weight were found not to be statistically significant (r = - 0.030, p = 0.769).

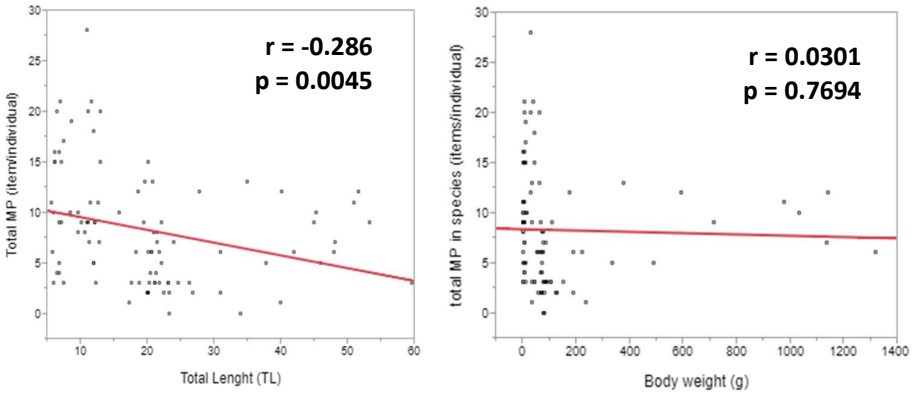

Figure 18**:** The relationship between the total MP (item/individual) and the total length(A) and Body weight (B).

The correlation between the total MPs found in the fish species and the condition factor is presented in Figure 19. A significant positive correlation was found between the total MPs per fish and the condition factor (p < 0.0001).

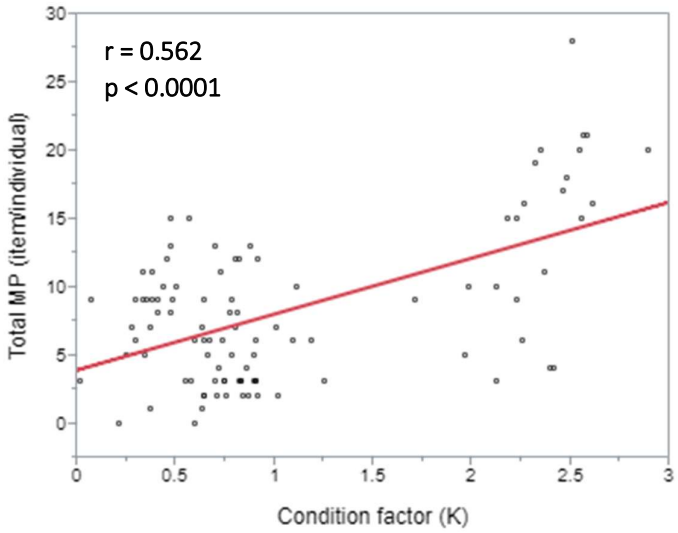

Figure 19**:** The relationship between the total MP (item/individual) and Condition factor

Occurrence of Microplastics and fish feeding preference

The mean microplastic abundance in the sampled fishes per the feeding type is presented in Figure 20. Planktivorous fish species recorded the highest microplastics levels of 3.845 ± 6.703, item/individual, followed by Omnivores at 2.567 ± 4.306 item/individual and the Carnivores at 1.814 ± 3.459, item/individual. Statistically, no significant differences were observed among the mean microplastic abundance in the sampled fishes per the feeding types recorded (H = 3.402, p = 0.182).

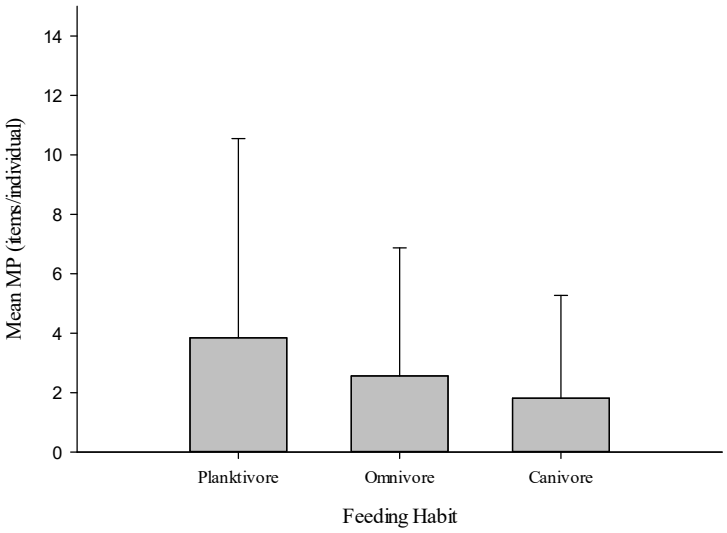

Figure 20**:** Mean microplastic abundance in the sampled fishes per the feeding type

Occurrence of Microplastics and Habitat Preference

The mean microplastic abundance in the sampled fishes per the habitat preference is presented in Figure 21. The highest amount of microplastics accumulated within the sampled fish species was observed in species inhabiting the Benthopelagic region (2.459 ± 5.982, item/individual), followed by the Demersal species (1.711 ± 3.363, item/individual), Benthic species (1.319 ± 2.926, item/individual), and the Pelagic species (0.800 ± 2.446, item/individual). Statistically, no significant differences were observed among the mean microplastic abundance in the sampled fishes per the species habitat recorded ($H = 6.066$, $p = 0.108$).

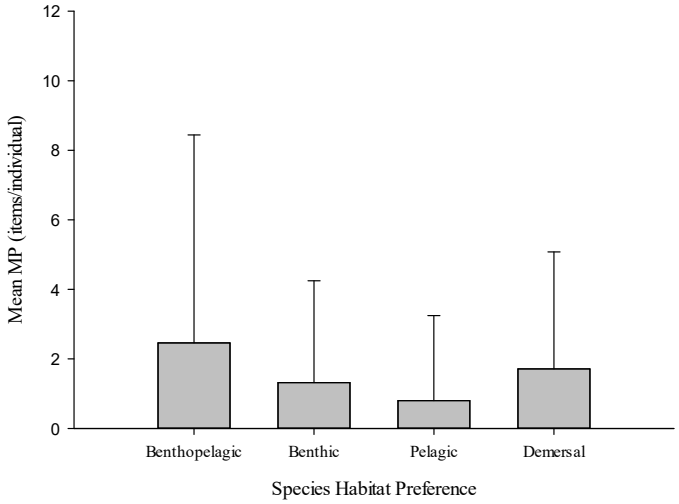

Figure 21**:** Mean microplastic abundance in the sampled fishes per species habitat preference.

Shape of Microplastics in the Fish

The percentage composition of the shapes recorded is presented in Figure 22. Fibre was the dominant MP shape occurring within the gills, gastrointestinal tract of fishes, and the visceral mass of shellfishes at 79.5%, 86.3%, and 84.3% respectively. As shown in Figure 22A, highest fragment occurred at 40% in MB, highest fibre was recorded at 100% in CS and highest pellet was recorded at 16.7% in GD. In Figure 22B, highest fragment was recorded at 33.3% in GD, highest fibre was recorded at 91.3% in EL, whiles the highest pellet was recorded at 33.3% in MB.

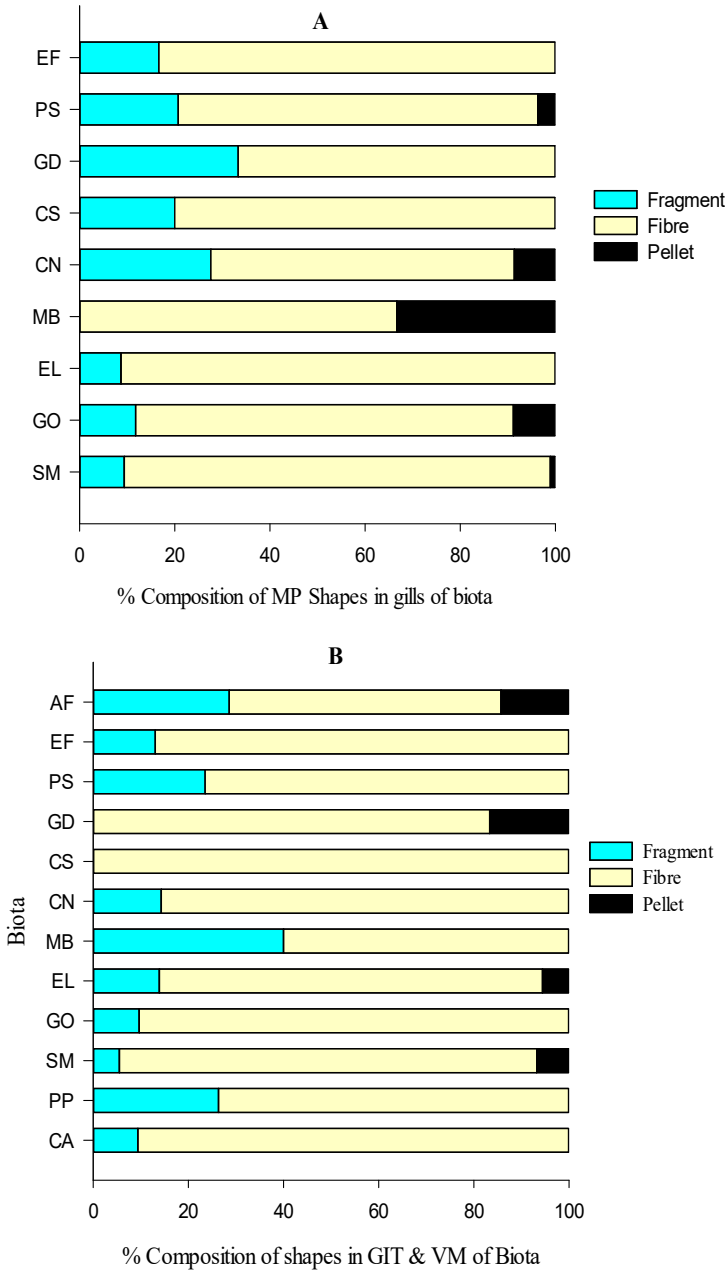

Figure 22: Composition of Microplastic shapes in A – GIT and VM, B – Gills of fish samples.

Colour of Microplastics in the Fish

Transparent MPs dominated the samples with 58.7%, 59%, and 60.8% in the gills, GIT, and the VM respectively. Within the gills, the blue coloured MP recorded highest of 33.3% in GD, black was highest 33.3% in EF, transparent

was highest 100% in MB, yellow was only present in SM at 2.1%, white was highest 2.1% in SM, and red was also recorded highest 6.3% in SM as shown in Figure 23A. Within the GIT, blue coloured MPs was highest 13.1% in SM, black was highest 33.3% in GD, transparent coloured MPs were 100% in CS, yellow was highest 1.8% in CN, red MPs were highest 8.3% in EL and green was found highest 8.7% in EF.

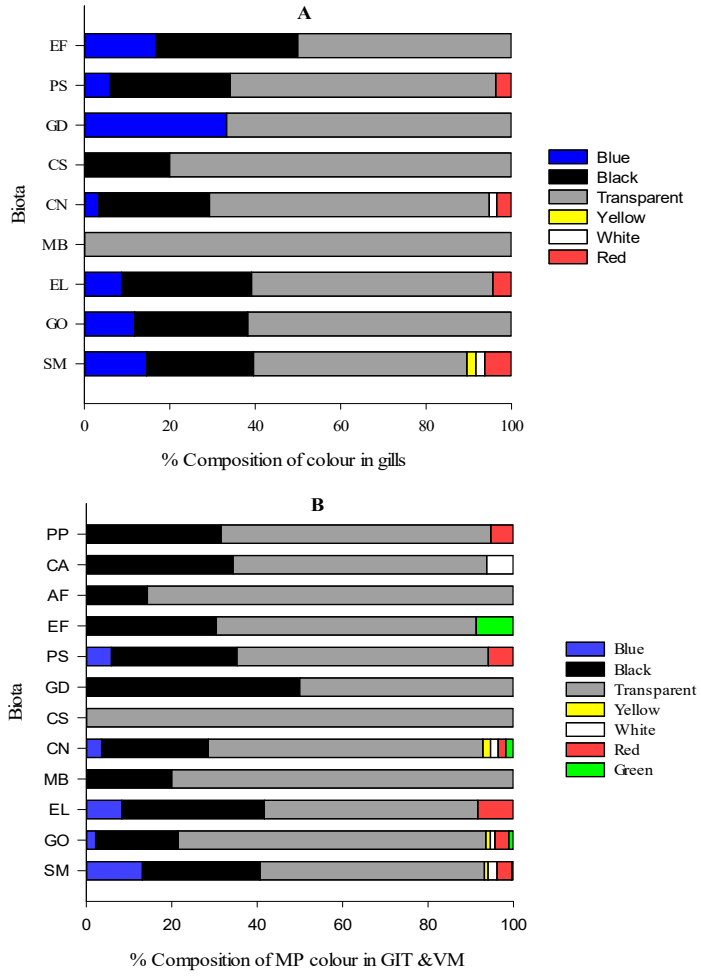

Figure 23: Composition of Microplastic colour in A – GIT and VM, B – Gills of fish samples.

Size of Microplastics in the Fish

The size range occurring within the fish follows similar pattern as recorded in the surface water and sediment with < 0.5 mm dominating the samples analysed. The < 0.5 mm classed MPs made up 54.3%, 43.5%, and 70.6% of total MPs in the gills, GIT and VM respectively. The size total composition ranges within the gills as illustrated in figure 24A was between 33.3 – 78.1% for < 0.5 mm, 0.5 – 1.0 mm (12.5 – 66.7%), 1.0 - 2.5 mm (6.3 – 33.3%), and 2.5 – 5.0 mm (1.7 – 3.7%). Within the GIT and VM inspected, the size ranges were between 35.6 – 78.9% for < 0.5mm, 0.5 – 1.0 mm (15.8 – 71.4%), 1.0 - 2.5 mm (4.3 – 28.6%), and 2.5 – 5.0 mm (3.6 0 - 33.3%) as shown Figure 24B.

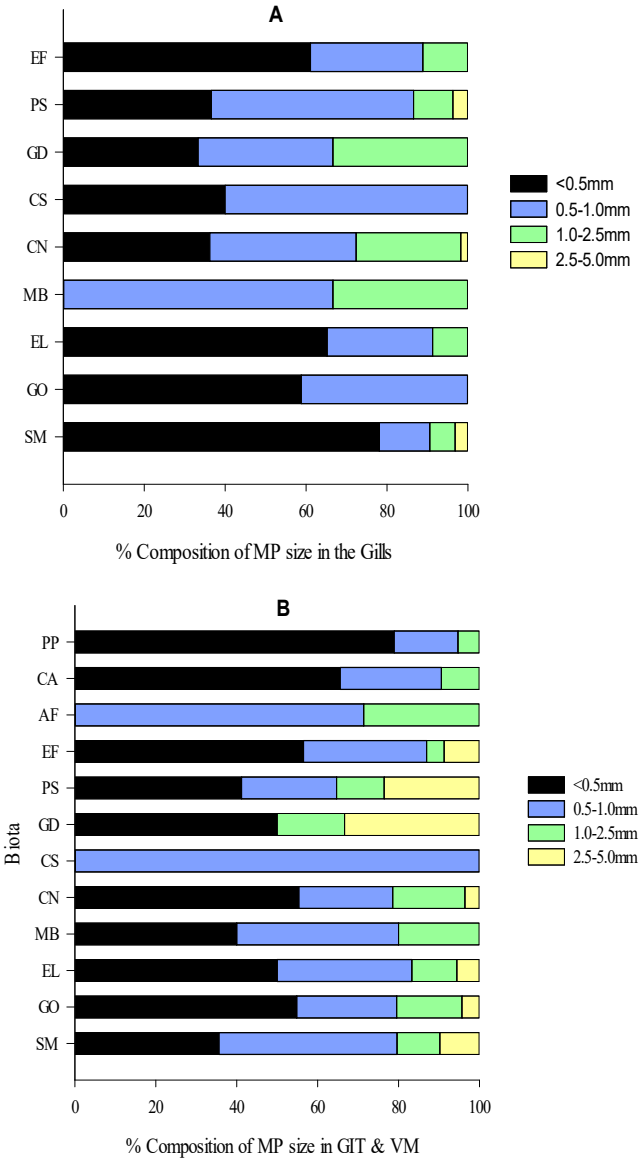

Figure 24: Composition of Microplastic size in A – GIT and VM, B – Gills of fish samples.

Polymer type of Microplastics in the Fish

A total of 236 microplastic items isolated from the fish were identified using an ATR-FTIR scanner. The gills accounted for 95 items while the GIT and VM accounted for 141 items. Polyethylene was the most occurring polymer type within the organs, with 50.5%, 52.6%, and 52% occurrence in the gills, GIT, and VM respectively. Within the gills, polyethylene occurred most (50.5%)

followed by polyester (31.6%), and polypropylene (17.9%). In the GIT, the order of occurrence was polyethylene (52.6%) > polyester (21.6%) > polypropylene (19.8%) > polystyrene (3.4%) > polyethylene terephthalate (2.6%). Within the visceral mass of the shellfishes, the occurrence of the polymers was polyethylene (52%) > polyester (28%) > polypropylene (16%) > polystyrene (4%). The percentage composition of polymer types within the fish is presented in Figure 25.

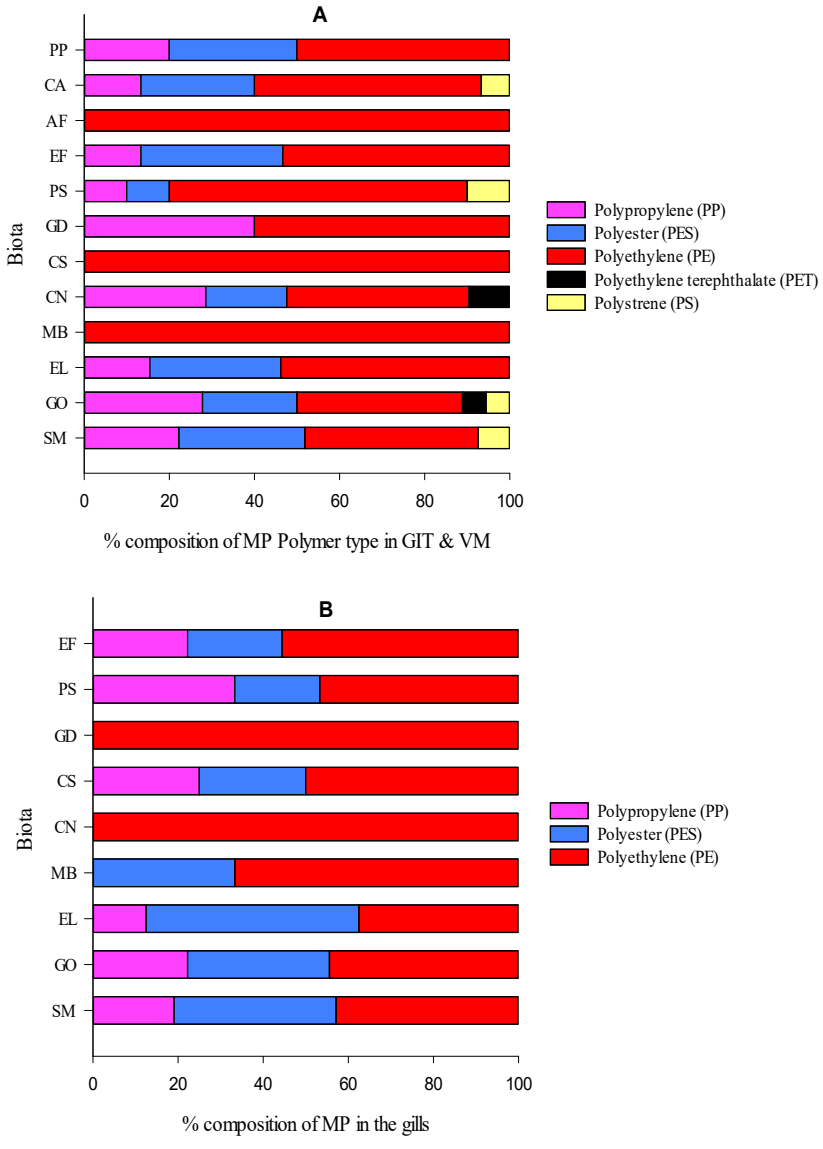

Figure 25: Composition of Microplastic polymer type in A – GIT and VM, B – Gills of fish samples.

CHAPTER FIVE

DISCUSSION

This chapter provides interpretation to the findings on the occurrence, spatial and temporal distribution of microplastics in the water and sediment in the Pra estuary. It also discusses the characteristics of microplastics isolated in this study. The influence of physiochemical parameters on microplastics distribution in the Pra estuary are addressed in this chapter. Further, the chapter covers the explanation for the presence of microplastics in the fishes sampled from the Pra estuary.

Occurrence and Abundance of Microplastics in the Surface Water

This study presents a first-time assessment of microplastics within the Pra Estuary. Microplastics occurred within all investigated surface water, sediment, and fish samples during the study, affirming global reports on the ubiquitous nature of the material within the estuarine environment (Townsend, Lu, Sharley, & Pettigrove, 2019; Yonkos et al., 2014). Overall, a mean concentration of 196,259.84 ± 60168.72 items. km^{-2} was determined for the surface water from December 2020 to April 2021. This high level of MPs is not surprising, taking into account the large volume of plastic wastes (greater than 250,000 tonnes) deposited into the ocean (Adika et al., 2020) annually from Ghana. Again, this observation can be supported by the fact that the Pra estuary, being Ghana's second-largest estuary, receives large inflows of water laden with plastics. The mean density of microplastics in the surface water of the Pra Estuary was higher by an order magnitude to the overall mean MP density (94,071 ± 50467.48 items.km^{-2}) of four estuarine rivers around the Chesapeake Bay of

USA (Yonkos et al., 2014). This could be attributed to the varying MP extraction efficiency among studies. In this study, NaI (1.8 gcm^{-3}) was selectively used during density separation to improve extraction. Also, sampling was conducted along the boundary line of S-N (Figure 2) which had short proximity to an adjacent community landfill, with a high microplastics release expectancy.

Spatial distribution of Microplastics in the Surface Water

The results of this study, as presented in Table 2, indicate a high heterogeneity in MP distribution within the Pra Estuary. The observed distribution and variability of microplastics could likely be influenced by several factors such as characteristics of the microplastics (e.g., shape, density, size, and polymer type), hydrographic parameters (e.g., turbulence, waves, tides, currents, salinity, and temperature), geomorphological features (e.g., land use), the discontinuity of upstream wastewater flashes and temporal weather variation (e.g., rainfall patterns, wind) (Lima et al., 2014; Mani et al., 2015; Sruthy & Ramasamy, 2017; Zhao et al., 2015). Samples were taken particularly in the dry season (late December to early April), under moderately low and stable hydrodynamic conditions that might have influenced an increase in the residence time of floatable materials within the estuary. The anthropogenic impact could not be considered null within the study area; the estuary is aligned with seven villages having fishing and agriculture as the major economic activities. Debris from fishing nets are widely denoted as a potential source of microplastics entry in the aquatic environment which can be traced in this context (Cole et al., 2011; Peng et al., 2017; Zhao et al., 2015). Sections within the estuary (Mouth – Head) showed a uniform microplastic distribution

indicating the possibility of multiple sources of microplastic entry. However, S-N was significantly higher than the Head section which indicates the varying concentration of MP load within the system. The constant tidal and residual current interaction within S-N, coupled with direct waste entry could account for this observation. The low concentrations of MPs within the Head section could also be attributed to a possible hedge effect promoting trapping of floating debris and also the continuous movement of particles downstream, subsequently emptying into the sea. The low density of MPs reported at the Head section in this study differs from the findings of Lima et al. (2014), where the lowest MP density was within the middle section, which was ascribed to reduced concentrations of marine and freshwater induced turbulence and stratification. The discrepancy in findings could be associated with differences in sampling periods and characteristics of the sampling stations.

Temporal distribution of Microplastics in the Surface Water

In this study, the highest concentration of microplastics within the surface water was recorded in April and the lowest in December. The sampling period coincided with the mid dry season (December) through to the early wet season (April). For this study, temporal mean differences in microplastics abundance were reported for the surface water, in agreement with earlier studies (Eo et al., 2019; Rodrigues et al., 2018; Rodrigues et al., 2019; Yonkos et al., 2014). This however deviates from the results of Castro et al. (2020) and Sui et al. (2020). An increase in MP concentration during the wet period affirms the hypothesis that majority of the microplastic items collected were sourced mainly from upstream influx (Lima et al., 2014; Rodrigues et al., 2019). Short rainfall flashes in April could have washed terrestrial microplastics into the Pra Estuary causing

concentrations to increase. Seemingly induced turbulence by light rainy events upstream causes resuspension of microplastics trapped within the sediments (Eo et al., 2019) and could have accounted for the increase in MPs density collected within the wet season. The presents of microplastics recorded during the mid-dry sampling period (Figure 3) was probably due to stable hydrodynamic conditions such as low flow velocity which might have increased the retention time of the plastic debris. During the dry season, marine intrusion into the estuary might have increased against freshwater entry. This phenomenon is expected to introduce more marine-based microplastics into the estuary while inducing an in-depth hypersaline vertical stratification that could cause the resuspension of highly dense microplastic particles trapped within the sediment (Li et al., 2020).

Occurrence and abundance of Microplastics in the Sediment

The presence of microplastics within all sediment samples collected indicates that the Pra Estuary was heavily polluted with microplastics which are widely distributed. The mean MPs concentration obtained for sediments in this study was similar to that obtained by Peng et al. (2017) for the Changjiang Estuary (121 ± 9 items.kg^{-1}d. w), On the other hand, it was 2 and 8 times higher than that of Haave et al. (2019) at Western Norway (48 ± 211 items.kg^{-1}d. w) and Nematollahi et al. (2020) at South of Caspian Sea (15 items.kg^{-1}d. w) respectively. However, the observed mean concentrations were substantially 8 times lower than that recorded for the Liaohe estuary, Daliao River, (237 ± 129 items kg^{-1}) the Shuangtaizi River (170 ± 96 items kg^{-1}), and the Pearl River Estuary (851 ± 177 items·kg^{-1}) (Jiang et al., 2018; Xu et al., 2020; Zuo et al., 2020). Variations among studies could largely be attributed to varying

extraction protocols, coupled with the difference in site characteristics. Concentration of MPs along the 16 sampling sites (Table 3) showed a high number of MPs accumulating in areas with anthropogenic influence such as populated areas as reported in several studies (Jiang et al., 2018; Peng et al., 2017) indicating varying microplastic sources into the Pra Estuary. Considerably, S-N recorded high values throughout the study period corroborating microplastic source to runoff entry from closest waste dump. Within the estuary, microplastic prevalence could be associated with riverine sedimentation generated by slow flow velocity and slow-moving bottom current and/or potential biofouling effect (Zhang et al., 2020).

Spatial distribution of Microplastics in the Sediment

The sediment MPs in S-N was significantly higher than that of the stations (Figure 5). This indicates the high contribution of microplastics load from S-N into the estuary. S-N is situated within dense salt marshes and mangrove ecosystems, the potential trappings, and retention of debris from tidal push and runoffs by the root (Chico-Ortiz et al., 2020) could account for the elevated MPs concentrations within the sediment. Even though only a single tributary was assessed for microplastic accumulation within this study, inflows from tributaries are generally considered potential sources for microplastics entry into the estuary (Zhao et al., 2018). However, within the estuary, the spatial evenness in MP distribution from the Head towards the mouth section suggests a high amount of microplastics transiting the estuary into the sea. Other factors to be considered are the nature of the plastic material such as (size, density, and polymer type), bed topography, and stable hydrographic parameters (Zhao et al., 2015). Slow bottom current coupled with tidal interactions within the Pra

estuary could probably introduce microplastics into the system. Also, there is a possibility that the high concentration of sediment microplastics within the estuary could be linked to the intense illegal alluvial gold mining (galamsey) occurring upstream. Sand washing cause siltation which could probably aid in the retention of microplastics transport within the micro surface layer of the sediment which needs further research. In this study, there was no significant relationship between microplastics concentration and the sediment grain size similar to accounts by Peng et al. (2017) and oppose to report by Haave et al. (2019). The finding in this work corroborates with the idea of the existence of complex interaction between hydrographic conditions within the estuary causing high retention of microplastics in the sediment rather than the relation with the sediment grain size.

Temporal distribution of microplastics in the Sediment

Results from the study indicated no statistically significant differences among the mean temporal concentrations of sediment microplastics over the study period (Figure 6). Concentration was homogenously high across the sampling months. Arguably, the intensity of precipitation in April needed to be taken into account. The likelihood of remobilization of MPs from the sediment could be linked to the episodic inflow of water from upstream during the early wet period. The moderately lower mean concentration of MPs in the sediment for April compared to that of February and March could indicate an early phase in the remobilization of the sediment. Since the study failed to account for sediment MPs within the mid-wet period where resuspension could be expected to be high, it was not clear how long higher concentrations of sediment MP

within the early phase of rainfall could have lasted to make inferences. However, factors associated with such shifts need to be well investigated.

Morphological characteristics and sources of microplastics in the surface water and sediment

Shapes

The dominance of fibre as the most occurring microplastic shape in the surface water (65.72%) and sediment (74.02%) corroborates the works of several authors (Zuo et al., 2020; Zhao et al., 2018; Zhang et al., 2019; Peng et al., 2017) who have also reported similar findings. The presence of fibre could be linked to several potential sources, such as fishing activities within the estuary or at sea, introducing fragments of worn-out fishing nets and ropes into the estuary. Shreds from textiles and old clothing introduced by washing activities and sewage entry have well been documented as fibre sources into the estuary (Peng et al., 2017). In Ghana, the direct entry of untreated greywater into waterways could be a major contributor to upstream microfiber in the estuary. Adu-Boahen et al. (2020) reported that gutters (wastewater channels) were the primary pathway for MPs into the Akora River in Ghana. High organic load entry into the estuary could induce biofouling events that might have increased the relative densities of microfibers to aid in the sinking or deposition of the microplastics in this case. Proceeding fibre as the prevalent MP were fragments, pellets, film, sheet, and foam in the estuary, except sediment where sheet and foam were absent (Table 4). These can be considered to originating from the weathering of macro-plastics entering the estuary. The packaging industry could account largely for the number of microplastics in this form. The

wide use and poor disposal of materials such as ‘polybags’ (polyethylene bags), ‘pure water’ Sackets, ‘take-away’ bowls (food deliverables) and plastic bottles are possible sources of the microplastic within the estuary. The absence of sheets and foams in the sediment for this study was similar to findings from Zhao et al. (2020) and Peng et al. (2017) and in contrast with Zhang et al. 2019. Foams are generally traced to Styrofoam used in fishing activities which is prevalent within the estuary. Temporally, this study reports similar MPs shape diversity across the sampling months (Fig.6). The increase in the levels of film, foam, and sheet in the surface water in April could be attributed to an influx of runoffs probably induced by early rains into the estuary.

Colours

Assessing colour could provide insight into the type of microplastics present and the ones that could be mistakenly ingested as prey items by inhabiting species within the estuary (Sui et al., 2020). The study identified seven colour categories from the microplastics in the estuary (Table 5). In contrast to the shapes where there was a similar dominance pattern occurring within the MPs in the surface water and sediment, the colour dominant MP colour in the surface water was transparent (37.06%) whiles black MPs dominated the sediment (43.65%). The discrepancy may be attributed to the differences in accumulating materials (e.g., density) and source. The black polyethylene bag is a common household packaging material in Ghana, its wide availability and affordability, parallel to its single-use consumptive nature could encourage it presence as microplastics in the estuary. Identification of the potential sources of the transparent materials was difficult due to the wide spectrum of materials that fall into this category in this study. The occurrence of black and transparent

microplastics in the estuary in the present study presents a high tendency for the materials to be ingested due to their close resemblance to floating organic matter. Statistically, there were significant variations in the overall mean composition of microplastic colours recorded within the estuary (Table 4). This variability could be associated with the wide range of pollution sources in the estuary. Comparatively, Sui et al. (2020) and Peng et al. (2017) found transparent microplastic items to be dominating the sediment of Sanggou Bay, China, and the Changjiang Estuary, China respectively in contrast to this study. Differences in findings could be linked to varying material preferences and lifestyle choices across the study areas. Along the sampling sites microplastic colour composition was uniformly distributed within the surface water and sediment (Figure 10) indicating the varying source of the pollutants.

Size

Within the aquatic environment, the bioavailability of microplastic is highly enhanced by its continuous weathering (Cole et al., 2011). Smaller sizes are readily ingested by aquatic organisms causing severe health complications which are well documented (Alomar et al., 2017; Ogonowski et al., 2016; Rosenkranz et al., 2009). In this study, the sizes of microplastics within the surface water and sediment are dominated by the < 0.5 mm class which could be easily ingested by aquatic organisms within the estuary. These findings were consistent with that of Sui et al. (2020) and Zhang et al. (2020). According to Pan et al. (2021), smaller-sized MPs (< 0.5 mm) present a relatively high surface area to volume ratio that intensifies absorption of organics and heavy metals. In this case, the dominance of < 0.5mm MPs in the Pra estuary coupled with the reported presence of heavy metals (Adokoh et al., 2011) elevates the health risks

of exposed organisms of which need necessary action. The size of microplastics in the estuary suggests that sewage entry could be the source of a significant amount of MP items presence in the estuary, notwithstanding the impact from airborne deposition. According to Lima et al. (2014), some domestic products such as facial cleansers and cosmetics have microplastics in them which are released into waterbodies through sewage transport. The presence of MPs within the size class of 1.0 – 2.5 mm showed higher occurrence in surface water of 22.05% compared to the sediment 7.71% (Figure 12) which demonstrates that degradation of plastics was occurring predominately within the surface water column.

Polymer type

This study revealed that Polyethylene (PE) MPs dominated the Pra estuary, corroborating the assertion that MP items originate from fragments of fishing nets, ropes, and polyethylene bags entering into the estuary. Fishing nets and ropes are principally composed of polyethylene (PE) or polypropylene (PP) (Zhang et al., 2020). This study correlates with findings by (Pan et al., 2021; Xu et al., 2020; Zhang et al., 2020; Zhao et al., 2018) who found polyethylene to predominate within their samples collected. The continuous presence of the PE within the estuary is purported to be tied to their weak photodegradability, suggesting a high ecological risk that needs attention (Xu et al., 2020). Occurring polymers identified for this study, which included PP, PET, PES, PS, PU are widely domestic and industrial use items. Materials such as plastic bottles, toys, cups, jelly cans, crates, bowls, containers, and bags are composed of PE, PP, PS, and PET. The textile industry utilizes more PES, PP, PU, and PET. PVCs which are highly dense (> 1.2 kg dm^{-3}) were identified to occur

within the sediment similar to Haave et al. (2019). The material is commonly used for pipes, cable covering, automobile linings, etc. The polymer composition indicates large inflows of terrestrial plastic wastes into the estuary. Over the months, the composition of PS increased significantly (31.25%) within the sediment in January (Figure 14A) which may probably be due to increased washing and enrichment of the material within the estuary.

Relationship between Physicochemical Parameters and MP Abundance and Distribution in the Surface Water and Sediment

The complex relationship between hydrographic conditions (i.e., salinity, temperature, dissolved oxygen, pH, turbidity and flow rate) and microplastics abundance, retention, fragmentation or transport are still been explored (Andrady, 2011; Castro et al., 2020; Feng et al., 2020; Jiang et al., 2020; Lima et al., 2014; Rodrigues et al., 2019). However, observing varying hydrographic parameters interactions under a short temporal scale (days, monthly) or seasonal pattern presents favourable inferences for consideration. In this study, dissolved oxygen and pH were the only parameters observed not to show any significant differences along the sampling period (Table 8). This might be more linked to uniform mixing events and the continuous intrusion of riverain inflows into the estuary throughout the study period (Dzakpasu & Yankson, 2015). The significant variability observed spatio-temporally for temperature, salinity, turbidity, and flow velocity were obviously due to river discharge, tidal fluxes, precipitation and surface wind (Dzakpasu & Yankson, 2015). The mean pH range for the Pra Estuary recorded was between 6.74 - 7.77, indicating a narrow range occupied by the estuary from a weak acidity to normal medium similar to

that observed by Okyere, (2019). This finding contradicts the narrow pH range (7.77 – 8.53) reported by Tufuor, Dodoo, Armah & Darpaah (2007). Estuarine acidity stems from the bedrock characteristics and influx of high organic matter into the system, a resultant from runoff intrusion (Dzakpasu & Yankson, 2015). This validates accounts of episodic riverain flashes into the Pra estuary within the period of study. The Pra estuary was generally turbid with turbidity ranging from 9.92 to 490.35 NTU. The higher NTU values recorded were due to silting events from alluvial gold mining happening upstream (Okyere, 2019). Increased suspended MP particles significantly correlates with lower saturated oxygen in the Pra estuary which could compounds stress for inhabiting organisms at are exposed (Dzakpasu & Yankson, 2015; Okyere, 2019).

The flow velocity showed a significant positive correlation with the surface water MP concentrations in the Pra Estuary (Table 9, Figure 15), suggesting strongly that the microplastic presents within the water column of the Pra Estuary may greatly be sourced from upstream. An increased riverain flow could probably be due to freshwater inflow into the system most likely due to a recent rainfall event or runoff entries (Lima et al., 2014). Thus, it was not surprising when highest surface MP abundance was recorded in April (early wet season), which is similar to the finding by Lima et al. (2014). Although, a significant negative correlation was observed between dissolved oxygen and the surface water MP abundance, lower DO at peak MP concentrations in the estuary could be still linked to high terrestrial inflows which could have introduced high organic matter that use up DO for decomposition (Dzakpasu & Yankson, 2015). The positive correlation between flow velocity and MP

abundance in the surface water corroborates the findings for MPs in the Douro estuary (Rodrigues et al., 2019). On the other hand, only pH was observed to be significantly correlated to MP density in the sediment (Table 10). Microplastics accumulation in the sediment could be more of sedimentation and biofouling events causing an increase in density which could induce sinking of floating debris (Anderson et al., 2018). The direct significant association of pH to the accumulation of MPs within the sediment remains unclear and needs further research.

Microplastics in the Fish within the Pra Estuary

Occurrence

Microplastics were found in all the sampled species identified to occupy a wide feeding and habitat region in the Pra Estuary (Table 11) confirming the widespread threat microplastics poses to the resident aquatic organisms. Similar account of 100% prevalence of MPs detected in the sampled fishes were reported by earlier works (Adika et al., 2020; Pazos et al., 2017; Sparks & Immelman, 2020). According to Abbasi et al. (2018) the gastrointestinal tract and gills of fishes represent significant hotspots for microplastic accumulation to other organs such as the liver and muscles because of the readiness of materials to enter into the system with little restriction.

Occurrence in the gastrointestinal tract of sampled fishes in the Pra estuary

In this study, microplastics were prevalent in the GIT of all the sampled species collected. However, occurrence was identified lowest in *Pseudotolithus senegalensis* (57%). This phenomenon could probably be highly dependent on

the feeding strategy and habit preference of the species (Adeogun et al., 2020; Bellas et al., 2016; Cole et al., 2011; Merga et al., 2020; Pazos et al., 2017).

Microplastics enters the gastrointestinal tract primarily through direct (mistaken for prey item or fed together with pray item) or indirect (ingestion of contaminated pray items) exposure to the material (Zhu et al., 2019). Herbivorous and planktivorous species (*Sarotherodon melanotheron* and *Ethmalosa fimbriata*) tend to mistakenly prey on small plankton-like materials that have visual resemblance to their diet, risking exposure to microplastics ingestion (Abbasi et al., 2018). Carnivorous species (*Elops lacerta*, *Cynoglossus senegalensis*, *Galeoides decadactylus, Pseudotolithus senegalensis* and *Apsilus fuscus*) on the other, are more prey selective, thus microplastics in GIT represent secondary exposure to the material, where prey items offload their MP burden upon being consumed by the predators (Zhu et al., 2019). The low occurrence of MPs in the GIT of *Pseudotolithus senegalensis* could probably reflect greatly on the low MP ingested by their pray item. *Pseudotolithus senegalensis* are vigorous predators that feed mainly on shrimps (Blay et al., 2006), which coincidentally recorded lowest MP occurrence in this study. The omnivorous species (*Gobionellus occidentalis, Mugil bananesis* and *Chrysichthys nigrodigitalus*) have a wider feeding range alternating between diets due to availability or nutritive selectivity suggesting a high susceptibility to MP exposure both direct and indirectly (Digka et al., 2020).

Considering the occurrence of MPs in fish species based on their habitat preference, the occurrence of microplastics in the GIT of species occupying the

benthic, demersal, benthopelagic and pelagic region is an indication of the vertical prevalence of microplastics within the Pra estuary, which agree to findings of MPs in the surface water and sediment in this study. The size, shape and buoyancy attributes of microplastics ensures the suspension and floating of the material in the water column making them readily available for the inhabiting fish species within those regions most especially pelagic and benthopelagic species (Rummel et al., 2015). Benthic species are well adapted to feeding within estuarine floors, preying on smaller invertebrates or detritus materials. Thus, exposure to microplastics by such species could be deliberate or accidental while foraging for food through contaminated sediments (Abbasi et al., 2018). Also, affinity to dietary exposure to microplastics could represent a more complex interaction than only size and colour deception of the material which have been widely reported (Bakir et al., 2020; Merga et al., 2020). According to Pazos et al. (2017) bio-filming of microplastic by microbial hitchhikers could present a favourable bait for fishes that consider them as more nutritious substance. Comparatively, microplastics occurrence in GIT of *Sarotherodon melanotheron* was reported at 12.9% by Adu-Boahen et al. (2020), and 13% in the stomach by Adeogun et al. (2020). 6% in the stomach of *Chrysichthys nigrodigitalus* by Adeogun et al. (2020), 22.92% in the GIT of *Pseudotolithus senegalensis* (Mboglen et al., 2019), and 14.41% in GIT of *Ethmalosa fimbriata* (Mboglen et al., 2019). The high occurrence in this study project the degree of MP contamination in the Pra estuary that needs considerable attention.

Occurrence in the Gills of sampled fishes in the Pra estuary

In this study, microplastics occurrence in the gills were evident in all of the sampled fishes except for *Apsilus fuscus*. The accumulation of microplastics in the gills of fishes has been described not to be deliberate but rather accidental or non-selective in nature (Su et al., 2019). However, the variability in particle sizes in the gills in many studies indicates dependency on the efficiency of the filtration apparatus, especially the gap in between gill rakers and filtration areas (Bakir et al., 2020; Lin et al., 2020). Naturally, large filtration areas coupled with closed gaps in between gill rakers found within plankton-feeders enhance trapping of dietary items (Abuzinadah, 1995) and possibly microplastics than predatory fish species, which suggests the occurrence on MPs in all planktivory and omnivory species in this study (Collard et al., 2017). However, Lin et al. (2020) points out that filtration area and gap size between gill rakers are mere complementary factors accounting to high MP accumulation in the gills of fish; suggesting habitat preference playing an important role too. This confirms the predominant occurrence of MPs in the gills of pelagic species compared to the occurrence in the gills of demersal species in this study.

Considering the two shellfishes that were investigated, occurrence of MPs in the whole visceral tissue was observed to be highest in the *Callinectis aminicola* (91.67%) and lowest in *Penaeus penaeus* (31%). *Callinectis aminicola* are bottom filter feeders which suggests high MP exposure from contaminated sediments. Similarly, shrimps are aggressive filter feeders that ingest any presumptive dietary item exposed to within their surrounding which might increase their susceptibility of accidently ingesting microplastics (Curren et al., 2020). Comparison to other studies, 35.9% of *Callinectes sapidus* (Waddell et

al., 2020) , 100% in *Callinectis aminicola* (Gbogbo et al., 2020), 89.34% in four wild crabs (*Charybdis japonica, Portunus trituberculatus, Matuta planipes, and Dorippe japonica*) (Zhang et al., 2021), 30.9 % in *Fenneropenaeus indicus* (Daniel et al., 2020), 90% in *Pleoticus muelleri* (Fernández Severini et al., 2020) were found to be contaminated.

Abundance

The order of microplastics abundance in the gills followed *Chrysichthys nigrodigitalus* (4.83 ± 2.08) > *Elops lacerta* (4.6 ± 1.14) > *Sarotherodon melanotheron* (4.36 ± 2.52) > *Pseudotolithus senegalensis* (3.91 ± 2.84) > *Galeoides decadactylus* (3) > *Cynoglossus* senegalensis (2.5 ± 1.12), *Gobionellus occidentalis* (2.43 ± 1.40) > *Mugil bananesis* (1.5 ± 0.71) > *Ethmalosa fimbriata* (1.29 ± 0.73 item/individual). In this study, microplastics showed high accumulation in the gills among the demersal species followed by the benthic species then the pelagic species. This might suggest a high MP concentration within the water column towards the bottom region. However, the high concentration of MPs in the gills of *Elops lacerta,* a pelagic species, indicates MP accumulation with the gills to be more of an interspecific interaction within the contaminated environment. Similarly, Su et al. (2019) found MPs in the gill not to relate with the total microplastics in the fish, indicating no relationship with feeding type. Also, the absence of MPs in the gills of some species (*Apsilus fuscus*) and individuals could be associated with constant flushing that could facilitate the removal of residual particles such as microplastics.

Within the gastrointestinal tract, MP accumulation was highest in *Sarotherodon melanotheron,* similar to the observation by Adeogun et al.

(2020). This could be attributed to the feeding habit of the species. Probably the high retention of microplastics in the gastrointestinal tract in herbivory or planktivory species could be attributed to the long digestive tract the species present for the digestion of complex fibrous plant matter (Montgomery, 1977), thus increasing the residence time of MPs in GIT. The abundance of MPs was lowest in the GIT of *Pseudotolithus senegalensis* in this study which was a carnivore indicating possible entry through ingestion of a contaminated prey.

A significant negative correlation observed between fish length and the total concentration of MPs in the fish indicate higher accumulation among smaller sized fishes. The high consumption of MPs among smaller fishes is still unclear, and could be widely not dependent on the size of the species. This is in agreement with a significant negative correlation found between MP in the GIT and total length of fishes in the Mondego estuary by Bessa et al. (2018). This is in contrast with findings from Adika et al. (2020), and Vendel et al. (2017). However, Pegado et al. (2018) found a significate positive correlation between MPs in the GIT and the standard length of fishes sampled within the Amazon river estuary. In this study the positive relationship between the condition factor and the total concentration of MPs in the fish could indicate higher feeding activity among healthier species within the estuary.

Characteristics of Microplastics in the Fish

Shapes

Among the detected microplastics that were found in the sampled fish and shellfishes, fibre dominated the shapes recovered in the gills, gastrointestinal tract of fishes, and the visceral mass of shellfishes with 79.5%, 86.3%, and 84.3% respectively. Similarly, fibrous MP dominance within gills, GIT and

visceral tissues have been found in several studies (Abbasi et al., 2018; Arias et al., 2019; Bessa et al., 2018; Daniel et al., 2020; Fernández Severini et al., 2020; Lin et al., 2020; Su et al., 2019; Waddell et al., 2020; Zhang et al., 2020). The prevalence of fibre in the fish is in conformity with fibrous MPs found in the surface water and sediment within the Pra Estuary making them readily available to the exposed species collected. Pellets were the least occurring MP shapes found in the gills, GIT and visceral tissues in the fishes and shellfishes sampled within the Pra estuary. This confirms the lower degree of occurrence of pellets in the Pra estuary.

Colour

The colour of microplastics within the environment plays an important role in the dietary exposure of the materials to the inhabiting species (Arias et al., 2019; Waddell et al., 2020). Species such as fish are inconsiderate to the ingestion of anthropogenic particles mimicking the colour of prey items (Bessa et al., 2018). In this study, transparent microplastics were the dominant colour found in all the organs investigated from the sampled species. Transparent materials are optically colourless which present an unseemly no resemblance to any distinctive dietary item, suggesting that accumulation was accidental, secondary or consumed together with prey items. The occurrence of transparent microplastics were predominant within the surface water column as evident in this study, probably indicating most fish activity occur within the pelagic regions in the estuary towards the bottom. Results from this study on the dominance of transparent MPs in the tracts of fishes supported the report by Arias et al. (2019) . It however contrasted the results reported by Zhang et al., (2020),. It also contrasted with the following: black MPs in the GIT and gills of

fishes in the Pearl River Estuary and Musa Estuary by Abbasi et al. (2018) and Lin et al. (2020), blue MPs in GIT by Bessa et al., (2018) and Savoca et al. (2019), and red MPs in shrimp from the coastal waters in Cochin, Kerala, India (Daniel et al., 2020).

Size

The most advantageous characteristics of microplastics that propels it availability and risk in species is the size (Abbasi et al., 2018; Bessa et al., 2018; Su et al., 2019). According to Su et al. (2019), smaller sizes plastics are readily consumed or filtered, easily translocatable into vital organs and deposition of associated chemicals into resident aquatic organisms. In this study, the most occurring size observed was < 0.5 mm in the gills, GIT and VM at 54.3%, 43.5%, and 70.6% respectively. The ingestion of smaller sized particles by species could be attributed to several factors such as mode of feeding, gill efficiency, gape size, biofouling of particles, easily digestible, energy conservation in feed ingestion (Abbasi et al., 2018; Bremigan & Stein, 2014; Collard et al., 2017). Herbivory and planktivory mistakenly consume or filter smaller sized particles that resembles planktons (Abbasi et al., 2018; Zhu et al., 2019). The closed gap between the gill rakes of some fish species ensures the seizure of smaller sized particles that are presumed dietary matter (Collard et al., 2017). The gap size of species plays an important role in feed size preference, where smaller gap size could influence consumption of smaller sized particles (Bremigan & Stein, 2014).

According to Oberbeckmann et al. (2015), small microplastics present a large surface area to size ratio which supports biofouling by microbes that could attract ingestion. Also, ingestion of small size materials could be more of

preference for easily digestible materials and materials that reduces the energy cost in trapping, handling and consuming prey items by some fishes, although not nutritionally rewarding (Gelwick & Mcintyre, 2017). Similarly, high exposure fishes and shellfishes to MPs within < 0.5 mm reflects highly of the dominate size range of MPs in the observed 90% dominance of < 0.1 mm MPs in the tract of fishes, 0.02–1 mm and 1–2 mm (34.81%) by (Lin et al., 2020).

Polymer Type in Fish

Of the 236 microplastics that were analysed with ATR-FTIR, Polyethylene was the most occurring MP item in the gills, GIT, and VM of the fishes and shellfishes in the Pra estuary at 50.5%, 52.6%, and 52% for respectively. This was not surprising since polyethylene prevalence in the organs of the sampled species directly corroborate with dominance of polyethylene found in the surface water and sediment from the Pra estuary. The concurrent presence of MPs within the fish and environment confirms that the Pra estuary could be considered a hotspot for microplastics which needed considerable attention. The identification of polyethylene in fish was in conformity with findings from several authors (Abbasi et al., 2018; Fernández Severini et al., 2020; Rummel et al., 2015; Savoca et al., 2019). Polyethylene MPs are lightly dense compared to polyester which suggest direct or indirect bioavailability via the water surface and the water columns for fishes. For the shellfishes, polyethylene MPs could be readily available in the sediment through sinks which are induced by intense biofouling events in the Pra estuary.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

This study was conducted to assess the occurrence and distribution of microplastics in the Pra estuary over a 5-month period. Microplastics were investigated in the surface water, sediment, gills and gastrointestinal tract of some sampled fishes in the estuary. The spatial and temporal distribution of microplastics in the water and sediment were determined. The characteristics of the MPs (shapes, sizes, colour, polymer metrics) were investigated with the use of a stereomicroscope and ATR-FTIR spectroscope. The study also explored the relationship between some selected hydrographic parameters and microplastic abundance in the water and sediment. The bioaccumulation of microplastics in the Pra estuary coupled with the relationship between microplastic abundance and the total length, condition factor, feeding habit, and habitat preferences of the sampled fish species were assessed.

Key findings to the study

- A total of 15,700,787 particles km^{-2} were detected in the surface water and 9,315 MP items kg^{-1}d. w in the sediment over the 5-month sampling period.
- Across the sampled stations microplastics was significantly higher at S – N than the other grouped stations. This was identified to be influenced by intrusion of waste from a localised dump site showing close proximity to the water body.

- Temporally, highest levels of microplastics were recorded in April (early wet season) within the surface water, which corroborates with the assertion of MP introduction through terrestrial inflows.
- Fibrous MPs were the dominant microplastics identified in the water, sediment and fishes in the Pra estuary.
- Transparent and black coloured MPs were found to dominate surface water and sediment respectively.
- The predominant MP sizes recovered from the study were sizes lesser than 0.5 mm in the Pra estuary.
- Polyethylene (PE) were found to be the most occurring polymer type in the Pra estuary.
- The flow velocity was identified to significantly influence microplastic distribution in the water column.
- Microplastics were 100% present in all the sampled species collected over the study period. Also, the characteristics of microplastics found in the fishes and shellfishes were found to reflect the environmental availability of the material in the Pra estuary.

Conclusions

This study provides a first-time report on the microplastic occurrence and distribution in an estuarine environment in Ghana. From the study, microplastics were observed not be evenly spatially distributed across the sampled stations on the Pra estuary. The abundance of microplastics in the water and sediment varied over the study period, depicting a non-uniform distribution on a temporal scale in the Pra estuary. Microplastics in the water, sediment and

fish were dominated by a single size class (< 0.5 mm). Also, the study showed that flow velocity had a significant influence on the distribution of microplastics in the Pra estuary. Finally, microplastics were identified in all the fishes sampled from the estuary, with occurrence in the gastrointestinal tract being higher than that found in the gills of the fish.

Recommendations

The study provides information on the occurrence and widespread of microplastics in the Pra estuary which support the proposal of the following recommendations;

- The refuse dumping site along the river passing through the Anlo community should be removed to prevent direct washing of plastics into the water body.
- A general public education and awareness creation on the proliferation of microplastics in the Pra estuary should be commissioned by the National Communication Authority and the Environmental Protection Agency of Ghana, to help foster consumer lifestyle changes on plastics.
- Proper plastic waste management strategies that incorporate effective household plastic waste management techniques should be promoted.
- The dominance of polyethylene particles in the estuary reinforces the need for government to regulate or ban the use of polyethylene materials such as packaging bags and other single use plastic materials in Ghana.
- The evidence of bioaccumulation of microplastics in the gills and gastrointestinal tract of sampled fishes in this study suggest the removal

of such parts during fish processing before consumption to reduce human expose to the materials.

Suggestions for Further Research

This study provides a baseline study of occurrence and distribution of microplastics in the Pra estuary which paves way for the following suggestions to be carried out as areas of potential research;

- A vertical assessment on microplastics in the water and sediment in the Pra estuary should be carried out to investigate the abundance of microplastics within the various deltaic strata.
- The optimum environmental factors that could influence the leaching of harmful associate chemicals into the estuary should be investigated under controlled laboratory conditions.
- The physiological effect of bioaccumulation of microplastics in the fishes and shellfishes should be carried out to further elucidate degrees of stress imposed by environmental exposure.
- Further research on potential hard or soft engineering to remove microplastics from the estuary should be encouraged.

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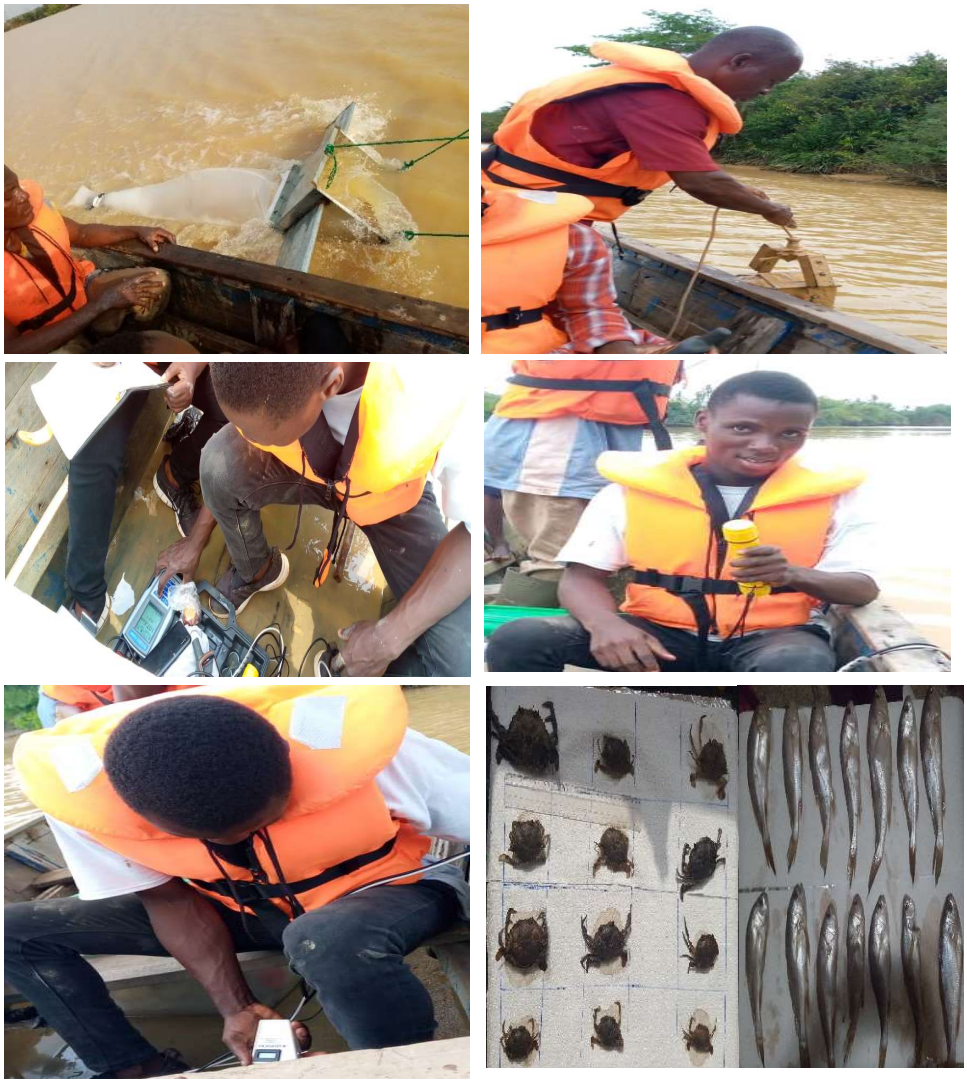
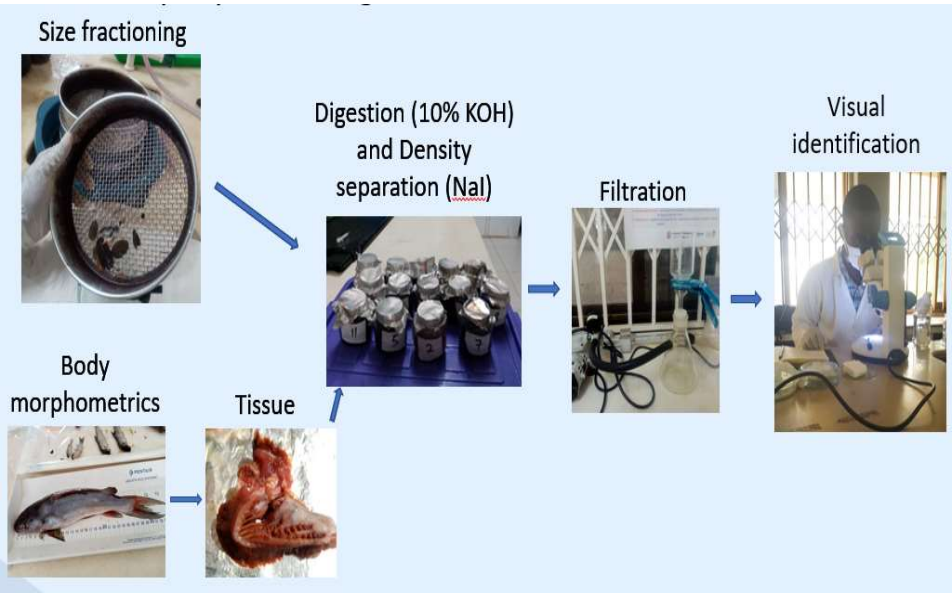
APPENDICES

Appendix A. Location of sampling sites with the fringe communities and land use activities

Sites	Communities	GPS coordinates	Land Use
S-N	Anlo	5° 1'41.15"N 1°36'48.99"W	Rural settlement
S1		5° 1'42.53"N 1°37'2.57"W	Agriculture
S2		5° 2'12.30"N 1°37'17.26"W	Agriculture
S3		5° 2'44.05"N 1°37'14.27"W	Agriculture
S4	Antotre	5° 3'5.08"N 1°36'48.74"W	Agriculture
S5	Krobo	5° 3'38.52"N 1°36'48.10"W	Rural settlement
S6	Bosomdo	5° 4'10.39"N 1°36'56.42"W	Rural settlement
S7	Borkorpe	5° 4'43.21"N 1°36'58.21"W	Agriculture
S8		5° 5'15.79"N 1°37'1.24"W	Agriculture
S9	Atwereboanda	5° 5'41.32"N 1°37'2.40"W	Peri-urban settlement
S10		5° 6'12.24"N 1°36'59.57"W	Agriculture
S11	Nomda	5° 6'47.41"N 1°36'55.33"W	Rural settlement
S12	Supom Dunkwa	5° 7'18.19"N 1°37'2.14"W	Peri-urban settlement
S13	Beposo	5° 7'40.49"N 1°37'23.60"W	Peri-urban settlement
S14		5° 7'49.55"N 1°37'58.55"W	Forested
S15		5° 7'56.93"N 1°38'30.62"W	Forested



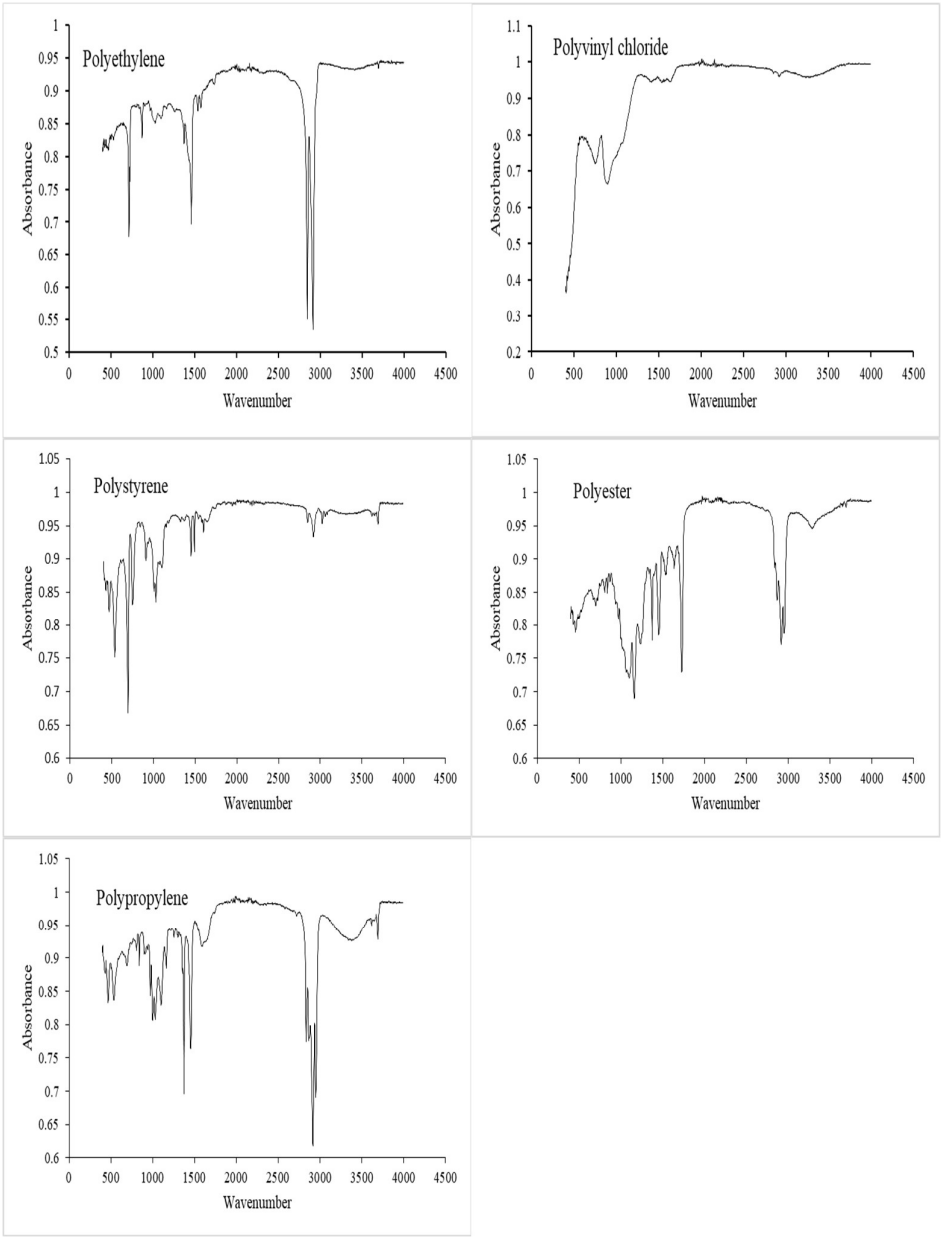
Appendix B: Photos of field sampling and sampled fish specimens.



Appendix C: Visual flow chart showing the extraction, processing and identification of microplastics from samples (water, sediment, and fish) collected.

Appendix D: Wentworth scale

Grade name	Particle size range (mm)
Boulder	> 256
Cobble	256 → 64
Pebble	64 → 4
Granule	4 → 2
Very coarse sand	2 →1
Coarse sand	1 → 0.5
Medium sand	0.5 → 0.25
Fine sand	0.25 → 0.125
Very fine sand	0.125 → 0.0625
Silt	0.0625 → 0.0039
Clay	< 0.0039



Appendix E: Types of microplastic polymers in the Pra Estuary.

Appendix F: Monthly Correlation coefficient for Microplastics within surface water, sediment and physiochemical parameters.

	MP_Sed_Dec	MP_Water_Dec	Turbidity_Dec	Temp_Dec	pH_Dec	DO_Dec	Salinity_Dec	FV_Dec
MP_Sed_December	-	0.0186	-0.4044	-0.147	0.4083	-0.4593	0.4558	0.1157
MP_Water_December	0.0186	-	-0.1397	-0.5719	0.4362	-0.2859	0.0779	-0.0382
Turbidity_Dec	-0.4044	-0.1397	-	-0.0569	-0.7323	0.7721	-0.8811	-0.1256
Temp_Dec	-0.147	-0.5719	-0.0569	-	-0.4269	0.281	-0.1176	0.2904
pH_Dec	0.4083	0.4362	-0.7323	-0.4269	-	-0.6704	0.6154	-0.0317
DO_Dec	-0.4593	-0.2859	0.7721	0.281	-0.6704	-	-0.7358	-0.1508
Salinity_Dec	0.4558	0.0779	-0.8811	-0.1176	0.6154	-0.7358	-	-0.0213
FV_Dec	0.1157	-0.0382	-0.1256	0.2904	-0.0317	-0.1508	-0.0213	-
	MP_Sed_Jan	MP_Water_Jan	Turbidity_Jan	Temp_Jan	pH_Jan	DO_Jan	Salinity_Jan	FV_Jan
MP_Sed_January	-	-0.2275	-0.6544	-0.3703	-0.5768	-0.3735	0.7869	0.1825
MP_Water_January	-0.2275	-	0.0114	0.2798	-0.0992	0.0771	-0.0995	-0.1702
Turbidity_Jan	-0.6544	0.0114	-	0.334	0.5121	0.4944	-0.9168	-0.2669
Temp_Jan	-0.3703	0.2798	0.334	-	0.1128	0.199	-0.3892	-0.3863
pH_Jan	-0.5768	-0.0992	0.5121	0.1128	-	-0.0026	-0.5694	0.1907
DO_Jan	-0.3735	0.0771	0.4944	0.199	-0.0026	-	-0.5969	-0.4155
Salinity_Jan	0.7869	-0.0995	-0.9168	-0.3892	-0.5694	-0.5969	-	0.1947
FV_Jan	0.1825	-0.1702	-0.2669	-0.3863	0.1907	-0.4155	0.1947	-
	MP_Sed_Feb	MP_Water_Feb	Turbidity_Feb	Temp_Feb	pH_Feb	DO_Feb	Salinity_Feb	FV_Feb
MP_Sed_Febuary	-	0.6747	-0.6221	-0.5695	-0.5638	0.1984	0.706	0.3471
MP_Water_Febuary	0.6747	-	-0.4161	-0.3504	-0.5173	-0.1126	0.686	0.0418
Turbidity_Feb	-0.6221	-0.4161	-	0.631	0.9084	-0.1879	-0.6919	-0.6654
Temp_Feb	-0.5695	-0.3504	0.631	-	0.6154	-0.0758	-0.6345	-0.2673
pH_Feb	-0.5638	-0.5173	0.9084	0.6154	-	-0.2011	-0.6029	-0.6729
DO_Feb	0.1984	-0.1126	-0.1879	-0.0758	-0.2011	-	-0.2894	0.6711
Salinity_Feb	0.706	0.686	-0.6919	-0.6345	-0.6029	-0.2894	-	0.0461
FV_Feb	0.3471	0.0418	-0.6654	-0.2673	-0.6729	0.6711	0.0461	-
	MP_Sed_Mar	MP_Water_Mar	Turbidity_Mar	Temp_Mar	pH_Mar	DO_Mar	Salinity_Mar	FV_Mar
MP_Sed_March	-	-0.0146	-0.067	0.176	-0.1981	-0.3167	0.0031	0.4328

Appendix F: Continued

	MP_Sed_Mar	MP_Water_Mar	Turbidity_Mar	Temp_Mar	pH_Mar	DO_Mar	Salinity_Mar	FV_Mar
MP_Water_March	-0.0146	-	-0.6472	0.2013	0.0471	0.5766	0.6335	-0.2848
Turbidity_Mar	-0.067	-0.6472	-	-0.2797	-0.22	-0.4886	-0.9907	0.1995
Temp_Mar	0.176	0.2013	-0.2797	-	-0.4686	-0.0315	0.216	0.4408
pH_Mar	-0.1981	0.0471	-0.22	-0.4686	-	0.3073	0.2941	-0.6595
DO_Mar	-0.3167	0.5766	-0.4886	-0.0315	0.3073	-	0.551	-0.6128
Salinity_Mar	0.0031	0.6335	-0.9907	0.216	0.2941	0.551	-	-0.313
FV_Mar	0.4328	-0.2848	0.1995	0.4408	-0.6595	-0.6128	-0.313	-
	MP_Sed_Apr	MP_Water_Apr	Turbidity_Apr	Temp_Apr	pH_Apr	DO_Apr	Salinity_Apr	FV_Apr
MP_Sed_April	-	0.5551	-0.5023	0.2926	-0.5102	-0.2451	0.2528	0.1145
MP_Water_April	0.5551	-	-0.9325	0.5254	-0.2257	0.1408	0.5813	0.2231
Turbidity_Apr	-0.5023	-0.9325	-	-0.5015	0.1253	-0.0537	-0.7462	-0.3543
Temp_Apr	0.2926	0.5254	-0.5015	-	-0.1565	0.1027	0.4782	0.4522
pH_Apr	-0.5102	-0.2257	0.1253	-0.1565	-	0.1971	-0.0504	-0.2283
DO_Apr	-0.2451	0.1408	-0.0537	0.1027	0.1971	-	-0.1577	-0.4569
Salinity_Apr	0.2528	0.5813	-0.7462	0.4782	-0.0504	-0.1577	-	0.6905
FV_Apr	0.1145	0.2231	-0.3543	0.4522	-0.2283	-0.4569	0.6905	-