MICROPLASTIC CONCENTRATIONS IN CAT FISH (*Clarias gariepinus*) AND NILE TILAPIA FISH (*Oreochromis niloticus*) SPECIES FROM OGBA RIVER, BENIN CITY, NIGERIA

BY

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BENIN CITY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF AGRICULTURE (OPTION IN AQUACULTURE AND FISHERIES MANAGEMENT)

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CERTIFICATION

This is to certify that this project was carried out by Emmanuel Iriobosa OKONOBOH in the Department of Aquaculture and Fisheries Management, Faculty of Agriculture, University of Benin, Benin City, Nigeria.

DR. O.M. WANGBOJE **PROJECT SUPERVISOR**	**DATE**
DR. O.M. WANGBOJE **HEAD OF DEPARTMENT**	**DATE**

DEDICATION

This project is dedicated to my King for my existence in this universe. I also dedicate it to my parents Prince and Mrs. OKONOBOH for their endless and relentless love towards my education, their drive on me, provision at all cost, care and encouragement always just to see me grow and better in all areas. Special regards and love to my sisters and brother (Rita, Joan, Kevin) for the bond of love and support.

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My heartfelt gratitude goes to my Maker (God Almighty), the Creator of the universe and Himself the “Author and Finisher of my faith”. All was possible because He lives and loves.

This research work was conceived and further implemented under the guidance of my supervisor. He has been supportive in many ways, not only because he was my coordinator at the time of writing this project but also because he always gave the care and guidance as a father.

With utmost gratitude and unspoken words, I sincerely want to appreciate my Project Supervisor who happens to be the Head of Department (HOD), of Aquaculture and Fisheries Management, Dr. O.M. Wangboje. Sir, your advice, guidance, moral and academic investment in my life has grown me so well and has brought me this far. Thank you very much Sir.

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ABSTRACT

Microplastics have been recognized worldwide for their toxic effects in fish, man and wildlife. The Ogba River in Benin City, Nigeria, has been reported to be contaminated to varied levels on Microplastic concentrations as a result of anthropogenic impact. This preliminary study was conducted to determine the levels of Microplastic concentrations in Cat fish *(Clarias gariepinus)* and Tilapia fish (*Oreochromis niloticus)* from Ogba river, Benin city, Nigeria. Microplastic is a minute particle of chemical pollutant in marine environment and classified as less than 5 mm size. The microplastics could not degrade for long years and they are ingested, incorporated, and accumulated in tissues of living organisms. The existence of microplastics in living organisms is influenced by the interaction of biological and non-biological factors and ecological security, although the exact mechanism is unclear. Fish samples was collected using a fishing net or scoop net while operating a dug-out canoe with the assistance of local fishermen, samples was placed in labelled zip-lock bags and conveyed to the laboratory in an ice box. Samples was rinsed thoroughly with running water. The cleaned fish sample was placed in 10% KOH solution and the beaker was covered. The beaker was safely stored away for a period of 14-21 days, the samples was left to digest in closed vials overnight at 60°C in an oven. The digested sample was purified using wet 30% H_2O_2. and sieved through two sieves (1 mm and 38 mm), or 5um to ensure the capturing of microplastics of the smallest sizes. The filter was rinsed into a glass petri-dish using pure water and was subjected to a temperature of a 100°C for 12hrs in an oven.

CHAPTER ONE

1.0 INTRODUCTION

Plastics have made our lives much easier by providing an economical and convenient option, but at the cost of major ecological risks (Fok and Cheung, 2015). Due to their excellent versatility and durability, they are widely used, and the demand has increased markedly. Since the beginning of manufacture in 1950 (15 MT), the cumulative production of plastic is predicted to reach 34 B T in 2050 (Geyer, 2020). Plastics are long-chain polymers composed of carbon, oxygen, hydrogen, silicon, and chloride, and are made from natural gas, oil, and coal (Shah *et al*., 2008). Currently, plastics have been used as an excellent material in today's day-to-day life. They are used in almost all applications such as packaging, automotive, aquaculture, fisheries, biomedical, shipping, agriculture, building and construction, telecommunications, furniture, transportation, plumbing, personal care products, textiles, clothing, etc. (Ogunola *et al*., 2018).

Plastics have even replaced more conventional materials such as glass and metals because of their lightweight nature, malleability, durability, flexibility, low cost, persistency, thermal and electrical insulation, corrosion resistance, high strength-to-weight ratio, and waterproof properties (Pellini *et al*., 2018; Zhang *et al*., 2021a; Ram and Kumar, 2020). The global plastic production reached 359 million tonnes in 2018, an increase of 46.5% compared to 2008 and 3.2% compared to 2017 (Plastics Europe 2019; Mao *et al*., 2020). Among all the countries, China generates the most (30%), followed by Canada, Mexico, USA (18%), and Europe (17%) (Tiwari *et al*., 2020). However, plastics are emerging, persistent, and ubiquitous contaminants that could harm the growth and development of organisms, induce

oxidative stress, weaken the immune system, reduce lifespan, and impact fertility (Chen *et al*., 2020).

Microplastics are formed when plastics degrade or break down into smaller fractions under physical, chemical, mechanical, and biological actions (Plastics Europe 2019; Lestari *et al*., 2020). These plastics are microscopic and pervasive particles and they have been continuously increasing in the environment due to their continuous production, non-biodegradable, persistent, and long-life span in the environment (Chamas *et al*., 2020). Thus, they have been declared as one of the ten emerging contaminants in the United Nation Environmental Programme (UNEP) Year Book 2014 that could potentially threaten human health and other organisms in all biomes (Constant *et al*., 2020). Therefore, the accumulation of microplastics in environmental components is gaining attention and becoming a major concern among global researchers and scientists. The abundance of microplastics in lakes, rivers, estuaries, oceans, and beaches worldwide has been documented in highly populated areas or areas with intensive anthropogenic activities (He *et al*., 2020). Because of the small size, microplastics can enter the human food chain through the consumption of seafood as well as other terrestrial food items, and subsequently can have impact on human health (Bondelind *et al*., 2020; Rist *et al*., 2018; Chatterjee and Sharma, 2017; Barboza and Gimenez, 2015). Secondly, plastic waste disposal in municipal waste disposal systems produces poisonous leachate, which can contaminate water and soil (Rajmohan *et al*., 2019; Kataria *et al*., 2022). Unprecedented use of plastic products and improper waste management techniques will continue to increase plastic waste (Geyer *et al*., 2017).

The irresponsible behaviour of people regarding the use of plastics, dumping plastic products, improper management systems, and associated harmful impacts have turned the planet into a

plastic planet (Chatterjee and Sharma, 2019). Microplastics are synthetic, long-chain, and organic polymers that can be found in various ecosystems such as soils, subsurface systems, rivers, lakes, wetlands, oceans, and atmosphere (Kumar *et al*., 2021a). These polymers come in a wide range of particle sizes and densities that can be harmful to aquatic ecosystems, animals, and human beings (Razeghi *et al*., 2021). Microplastics are typically defined as plastic particles with at least one dimension under 5 mm (Au, 2017; Rillig *et al*., 2017) or as any polymer with the largest dimension smaller than 5 mm or within a size smaller than 5mm (Eerkes-Medrano *et al*., 2015; Anderson *et al*., 2016; Lestari *et al*., 2020). Microplastics can be large microplastic particles (L-MPP, 1–5 mm) or small microplastic particles (S-MPP, <1 mm), as well as microbeads, fragments, fibres, pellets, flakes, sheets, or foams. Plastics are also classified into different categories based on their size viz. giant (>1 m), large (<1 m), medium (<2.5 cm), micro (<5 mm), and nano (<0.1 μm) (Elgarahy *et al*., 2021). They are derived from natural and organic materials such as coal, natural gas, and crude oil by polymerization or polycondensation processes (Phuong *et al*., 2016).

The plastics especially the polystyrene and polyethylene are basic and are indispensable for industries such as agriculture, food packaging, garments, pharmacy, shipping, automotive industry etc. (Momen Doust *et al*., 2017; Ma *et al*., 2019). Microplastics (MPS), can easily consumed by aquatic and terrestrial animals, including humans, and this raises concerns about accumulation in the body as well as toxicity (Paderv and Eric Lichtfouse Didier Robert and Wang, 2020; Conkle *et al*., 2018; Wright and Kelley, 2017).

1.1 Justification of the Study

Microplastics (MPS) can easily be consumed by aquatic and terrestrial animals, including humans, and this raises concerns about accumulation in the body as well as toxicity (Wright

and Kelley, 2017). Plastics have made our lives much easier by providing an economical and convenient option, but at the cost of major ecological risks (Fok and Cheung, 2015). Due to their excellent versatility and durability, they are widely used, and the demand has increased markedly. Since the beginning of manufacture in 1950 (15 MT), the cumulative production of plastic is predicted to reach 34 B T in 2050 (Geyer, 2020). From available literature, there are studies on microplastic in some selected fishes from Ogba River, Benin City, Nigeria, although there are several studies on contaminants which have been carried out in Ogba River in the past including, heavy metals in water and fish species (Wangboje and Oronsaye, 2001), bioaccumulation studies (Obasohan, 2008), physiochemical studies (Emeka, 2011) and heavy metals in selected tissues of *Clarias gariepinus* (Wangboje *et al.,* 2013). There is dearth of information on Microplastic, ensuring the provision of essential information for risk assessment that could be used to make more informed decisions relating to our natural aquatic resources.

1.2 Aim and Objectives of the Study

The aim of the study is to determine the levels of microplastic concentrations in *Clarias gariepinus* and *Oreochromis niloticus* from Ogba River, Benin City, Nigeria.

The specific objectives of the study are to:

1. determine the level of microplastics in fish in Ogba River;
2. classify the microplastics found in fish in Ogba River; and
3. Identify the type of Microplastic in physical and chemical composition found in fish in Ogba river.

CHAPTER TWO

2.0 LITERATURE REVIEW

The presence of microplastics has become a serious ecological concern in several environmental compartments on the Earth; even karst groundwater cannot avoid microplastic pollution (Panno *et al*., 2019). Also, scientific studies confirmed the existence of microplastics even in the deepest oceans, tallest mountains, and poles (Walkinshaw *et al*., 2020). The worldwide presence of microplastics in the environment is regarded as an ecological hazard and a significant concern by scientists, governments, and policymakers (Li *et al*., 2016; Vaughan *et al*., 2017; Bergmann *et al*., 2019; Chen *et al*., 2020). The existence of microplastics in living organisms is influenced by the interaction of biological and non-biological factors and ecological security, although the exact mechanism is unclear. When compared to the rural environment, the urban environment contains a large amount of microplastics, and common practices such as domestic waste disposal, street washing, and rain runoff, transport microplastics into the sewer system, where they end up in municipal wastewater treatment plants (WWTPs) (Bilgin *et al*., 2020). The removal and/or recovery of microplastics from WWTPs can significantly reduce their amount to be discharged into the natural environment such as water bodies.

Plastic debris is an important environmental problem and is defined as a contaminant of emerging concern that may affect biodiversity and human health (Huerta Lwanga *et al*., 2017). Furthermore, its accumulation in the ocean is unavoidable due to the lack of effective methods

to remove it from marine ecosystems. Microplastics (MPs), one size fraction of plastic debris, can be easily transferred to the seas in many ways, including wastewater discharges, aquaculture, fishing, and harbor activities, rivers, agricultural runoff, streams, drainage systems, and atmospheric deposition (Jiang *et al*., 2020; Klein and Fischer, 2019; Piñon-Colin *et al*., 2020). To date, most of the studies on MP pollution have concluded that urbanized coastal areas have higher MP abundances than open seas and are closely related to anthropogenic factors such as population density, land use, and point source pollution such as wastewater treatment plant (WWTP) discharges (Barrows *et al*., 2018; Hendrickson *et al*., 2018; Su *et al*., 2020).

2.1 Source of Microplastics

Microplastics are heterogeneous substances with varying shapes, sizes, morphology, polymer compositions, and density (Duis and Coors, 2016; Auta *et al*., 2017a; Wang *et al*., 2019). Microplastics are classified into two types based on their source: primary and secondary.

2.1.1 Primary Microplastics (PMP)

They are small plastic particles released directly into the environment by domestic and industrial effluents, spills, and sewage discharge or indirectly by runoff. For example, scrubbing agents used in cosmetics and biomedical uses, plastic pellets accidently lost during production or handling (OECD, 2022). They are also manufactured as microbeads in industries and used in personal care products, sandblasting media, or raw materials for fabricating other products (Andrady, 2017; Schessl *et al*., 2019).

2.1.2 Secondary Microplastics (SMP)

They are formed in the environment as a result of the breakdown of larger plastic particles via several degradation mechanisms such as chemical and physical ageing, UV radiation (photo-

oxidation), mechanical transformation (via waves abrasion), and biological degradation by microorganisms (De Sá *et al*., 2018; Ogunola *et al*., 2018).

They are further divided into two categories:

(1) Those formed during the use of products, such as from tyre abrasion and synthetic microfibers from clothing and other textile products.

(2) Those formed by the degradation and fragmentation of macroplastics that have been released into the environment (OECD, 2022).

One of the primary causes of the global increase in microplastic pollution is the difficulty in removing them from environmental matrices due to their tiny size and low visibility (Auta *et al*., 2017a).

The statistics reveal that as much as 51 trillion microplastic particles litter the sea, which is approximately 500 times more than stars in the cosmic galaxy. Estimates shows, about 80 percent of litter in oceans are made of plastics and annually, about 8 million tonnes of plastic find way to oceans causing threats to tourism, marine ecosystem, coastal life, marine fishery resources and wildlife resulting in damage of 8 billion dollars (UN 2017) (Guo and Wang, 2019; Desforges *et al.,* 2014; WormLotze *et al.,* 2017). It is used in almost every industry including, food, agriculture, medicine, automotive, electronics, clothing, construction, and civil works are just a few of the industries that use it (Parades *et al*., 2019; Dilkes-Hoffman *et al*., 2018).

Plastic pollution is an emerging issue in recent days. Persistent plastic particles reach the atmosphere, land and water by multiple pathways. Research has confirmed that the existence

of plastic particles is found surprisingly everywhere, from the Artic to the Antarctic region. The probability of ingestion of plastic by all living forms is quite natural, as the whole planet's environment is polluted with microplastic particles. The bioaccumulation of microplastics is a threat and the consequences for living beings are yet to be explored. Microplastics present in different drinking water sources like rivers, lakes, treatment units etc. are studied by several researchers, covering various aspects. Microplastic pollution is an ongoing environmental concern, it must be addressed and research should be expanded. Microplastics provide a potential transfer pathway for contaminants, plastic additives, and microorganisms to enter living organisms, thereby causing health threats to organisms. Microplastics in the environment can be ingested by organisms through the food chain, thereby causing toxic effects, such as intestinal damage, neurotoxicity, immunotoxicity, reproductive toxicity and cardiotoxicity (da Costa Araujo and Malafaia, 2021; Deng *et al*., 2017; Hou *et al*., 2021a, b; Lim *et al*., 2021; Wei *et al*., 2021; Zheng *et al*., 2021). Microplastics can be enriched in organisms and produce biological amplification. MPs have been detected in food products, such as seafood, salt, honey, sugar, tap water, bottled water6, beer, etc. (Banerjee and Shelver, 2021). As the top of the food chain, humans are inevitably exposed to health threats from Microplastics. Notably, microplastics have been detected in human feces, colonic tissue, lung and placenta, which indicate a potential impact of MPs on human health (Ibrahim *et al*., 2021; Ragusa *et al*., 2021; Schwabl *et al*., 2019; Yan *et al*., 2020b).

Since the mass production and use of microplastics (MPs) in the 1950s, they have been widely used in packaging, construction building, electric power, industrial machinery and other industries (Geyer *et al*., 2017). Global plastic production is growing exponentially due to low manufacturing costs and a wide range of applications. In 2017, the cumulative

production all worldwide of plastics was approximately 8.3 billion tons and is expected to increase to 34 billion tons in 2050 (Petersen and Hubbart, 2021). Plastic waste entering the environment can fragment into MPs after weathering, photo-oxidation, crushing and biological decomposition (van-Wezel *et al*., 2016). Alternatively, MPs are manufactured specially in a small size range for various commercial uses (Banerjee and Shelver, 2021). In general, MPs are plastic fibers, particles and films with particle size < 5 mm, including nanoplastics (NPs) with diameter < 0.1 μm (Banerjee and Shelver, 2021; Hartmann *et al*., 2019). The chemical composition of MPs mainly includes polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyamide (PA), polyester polyethylene terephthalate (PET) and polystyrene (PS) (Alimi *et al*., 2018; Browne *et al*., 2011). MPs mostly originate from plastic beads used for exfoliation or cleaning in cosmetics and personal care products, breakage of plastic pellets in factories and during transportation, tire wear and loss of fibers during textile washing (He *et al*., 2019; Khalid *et al*., 2021). In addition, studies have proven that landfills are also an important source of MPs, which have also been identified in the leachates from both active and closed landfills (He *et al*., 2019). MPs can be transferred over long distances through rivers and wind due to their chemical stability, small particle size and light density, and are widely detected in air, aquatic and terrestrial ecosystems (Amato-Lourenco *et al*., 2020; Auta *et al*., 2017; Horton *et al*., 2017; Shan *et al*., 2018).

2.2 Microplastics Contamination of Inland and Drinking Water

Microplastics are widely spread worldwide (O Connor *et al*., 2019; Fu *et al*., 2020) and have been found in a large number of environmental matrices, including biological ones (Jamieson *et al*., 2019; Corami *et al*., 2021; Leslie *et al*., 2022,) and also unconventional as placenta and faeces (Ragusa *et al*., 2021; Zhang *et al*., 2021). Aquatic environments can be considered an

important reservoir for these contaminants as microplastics from different sources continuously flow into them. Due to the noticeable abundance of floating plastic waste, the marine ecosystem was first investigated for the presence of microplastics. Sea water environment is also the one on which research has focused more in the last decade, because of possible direct effects on aquatic organisms even if other aquatic environments are gradually gaining importance in microplastics analysis, especially in second half of 2010s. Primary microplastics mainly reach sea water through atmospheric deposition and/or via inland waters, while secondary microplastics are the direct consequence of degradation phenomena affection floating plastic waste in the sea (Boucher *et al*., 2017; Alimi *et al*., 2018). In the last years, given the concerns about the effects on human health, research interest has partially moved on the ways through which microplastics reach inland waters and effects they may have on drinking water supply (Alimi *et al*., 2018). The water sources most involved in microplastic contamination of inland water are civil or industrial sewage, combined sewer overflows and urban or agricultural run-off. Wastewater accumulates different microplastics used in domestic and civil areas (textile fibres released during washing, considered the most important source of primary plastics (Corami *et al*., 2020a), plastic wear products, gaskets, paints and microbeads used in cosmetics) but also industrial ones (drilling fluids, cementing pastes containing PET microbeads, rust removal products and paints containing polyester scrubbers). The effectiveness of wastewater treatment plants in removing microplastics is controversial (The Water Environment & Reuse Foundation, 2017; Tang *et al*., 2020; Elkhatib *et al*., 2020).

2.3 Effect of Microplastic in Man

Plastic cutting boards are a potentially significant source of microplastics in human food. Thus, we investigated the impact of chopping styles and board materials on microplastics released during chopping. As chopping progressed, the effects of chopping styles on microplastic release became evident. The mass and number of microplastics released from polypropylene chopping boards were greater than polyethylene by 5–60% and 14–71%, respectively. Chopping on polyethylene boards was associated with a greater release of microplastics with a vegetable (i.e., carrots) than chopping without carrots. (Himani Yadav, Md Rakib Hasan Khan, Mohiuddin Quadir, Kelly A Rusch, Partho Pritom Mondal, Megan Orr, Elvis Genbo Xu, Syeed Md Iskander, Environmental Science and Technology, 2023). Based on our assumptions, we estimated a per-person annual exposure of 7.4–50.7 g of microplastics from a polyethylene chopping board and 49.5 g of microplastics from a polypropylene chopping board. We further estimated that a person could be exposed to 14.5 to 71.9 million polyethylene microplastics annually, compared to 79.4 million polypropylene microplastics from chopping boards. The preliminary toxicity study of the polyethylene microplastics did not show adverse effects on the viability of mouse fibroblast cells for 72 h. This study identifies plastic chopping boards as a substantial source of microplastics in human food, which requires careful attention.

Determining the amount of microplastics (MPs) in food is key to clarifying their potential toxicity to humans. Here, we collected canned, instant, and salt-dried sea cucumbers *Apostichopus japonicus*, the most valued sea cucumbers, from Chinese markets to determine their content of MPs. Accordingly, consuming 3 g of sea cucumbers could result in an exposure risk of an average of 0.51 MPs, 0.135 MPs, and 0.078 MPs day-1 for canned, instant, and salt-dried sea cucumbers, respectively. MPs were in size range of 12–575 μm, and fibrous

shape was dominant. Furthermore, among the five polymers identified, polypropylene showed the highest energy binding with two catalysts engaged in organic chemical oxidation. This study extends the knowledge regarding MPs occurrence in food and provides a theoretical basis for MPs toxicity in humans. (Mohamed Mohsen, Chenggang Lin, Mohnad Abdalla, Shilin Liu, Hongsheng Yang and 2023).

2.4 Effect of Microplastic in Soils

When microplastics enter soils, they can change soils' physical and chemical properties, such as soil structure, porosity, pH, and nutrient availability (Wang *et al*., 2022a), and destroy soil microbial community (Liu *et al*., 2019b). In addition, microplastics can adsorb pollutants through hydrogen bonding and electrostatic interactions (Wang *et al*., 2022b), such as heavy metals, organic pollutants, pathogens and resistance genes (Ren *et al*., 2021). The combination of two or more pollutants causes serious degredation of soil health. Moreover, microplastics in soil can inhibit seed germination and be transported to stems and leaves from the roots, thereby interfering with plant growth (de-Souza Machado *et al*., 2019), affecting photosynthesis and nutrient metabolism (Zhou *et al*., 2021), inducing oxidative damage (Li *et al*., 2021a) and altering genotoxicity (Zhou *et al*., 2021). It was reported that nanoplastics (50 nm) can enter plant cells, pass through the food chain and cause harm to the human body (Sun *et al*., 2022). The amount of microplastic in soil varies greatly with its use. Since the environment is complex, separation and extraction are needed before soil microplastic detection. The authors retrieved 882 articles on microplastics in soils through the Web of Science, of which 156 are reviews (March 15, 2023, and the keywords were microplastics and soil). Among these reviews, some focused on extraction techniques (Koyuncuoğlu and Erden, 2021; Li *et al*., 2020a; Yang *et al*., 2022), and others briefly described the extraction and

detection techniques of microplastics in soil (Dioses-Salinas *et al*., 2020; Fojt *et al*., 2020a; Junhao *et al*., 2021) or the detection technology (Junhao *et al*., 2021; Kumar *et al*., 2020; Möller *et al*., 2020; Okoffo *et al*., 2021; Ren *et al*., 2022; Wang *et al*., 2022b; Yang *et al*., 2021). Importantly, some new extraction and detection techniques, such as pressurized fluid extraction, laser direct infrared and matrix-assisted laser desorption ionization time-of-flight mass spectrometry, are rarely mentioned. There is a lack of systematic and comprehensive reviews on the extraction and detection technology of microplastics in soils. Therefore, it is necessary to clarify the sources and distribution of soil microplastics, and propose effective detection technique to protect soil and environmental health.

2.5 Effect of Microplastic in fish

Microplastics exposure has been studied in respect of particular physical or biological reactions. So far, most investigations on the effects of MPs on fish have been undertaken in the laboratory. The fish used in the MPs exposure tests came from a variety of environments, with the bulk coming from the sea. MPs may build up in the gastrointestinal system of fish after consumption, producing obstructions across the digestive tract and limiting feeding owing to appetite (Lusher *et al.,* 2013; Wright *et al.,* 2013). Intake of MPs could also induce anatomical and functional changes in the digestive tracts, causing dietary and development issues in fish (Huang *et al.,* 2022; Jabeen *et al.,* 2018; Borrelle *et al*., 2017; Peda *et al.,* 2016). Many pieces of research have been carried to demonstrate that MPs pose a threat to fish, with mortality occurring frequently before they reach maturity owing to MPs intake.

Many researchers have studied the negative consequences of microplastics on species, which can vary from interruption of biological functions to death. Microplastics poisoning is categorized as follows depending on the nature of MPs after intake:

1) build-up in the gastrointestinal tract, producing physical harm such as blockage and damage.

2) release as pseudofeces, disrupting organisms' energy transfer.

3) transfer inside the body, exposing inner organs and tissues to MPs.

Microplastics caused detrimental effects on species were outlined to provide a solid research foundation for sustainable Microplastics toxicological investigations and to evaluate the potential for huge ecological disruption.

Microplastic-induced impairments in species ranged from minimal biological systems disturbance to substantial unfavorable consequences that resulted in mortality (Mallik *et al*., 2021). Fish is being contaminated with MPs worldwide and it finds its way to human body through food (Sequeira *et al*., 2020). Many scientists have recently focused on the effects of MPs in fish and on human health (Chae and An, 2018). Despite the fact that the number of publications is growing, the possible consequences are still largely unknown (Kutralam-Muniasamy *et al*., 2020).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Ogba river is a forth order (4°) river, located at the Southwest region of the outskirt of Benin City in Edo State, Nigeria between Latitude 6.20°N and Longitude 5.34°E. The river is about 42 km long and takes its source at Ekewan and flows in a South East direction through Ogba village and empties to Osiomo River, into Benin River, which in turn empties into the Atlantic Ocean. It is situated within the rainforest belt of Edo State, southern Nigeria; flowing in a south-westerly direction in a steeply incised valley and through sandy areas before passing through Benin City and joining the Osiomo River (Atuanya *et al.,* 2012; Odigie, 2015). Local communities have access to a variety of resources in the Ogba River riparian area, including fisheries and domestic water supply. The bamboo trees (*Bambusa vulgaris*), which make up the riparian vegetation in the study sections, are a dominant indigenous plant species in the study sections. In the neighbourhood of the River, is a Zoological garden, a prison, a Bridge, farm lands, a master drainage system and the Edo state office of the Agricultural Development Programme (ADP). However, a significant portion of natural vegetation has been lost as a result of anthropogenic activity such as widespread deforestation for agricultural purposes. The River also serves as an inflow point for effluents from Benin City via a master drainage system (Wangboje *et al.* 2016).

3.2 Sampling

The following stations was established on observed anthropogenic activities. Three sampling stations (Figure1) namely Agricultural Development Programme (upstream), Ogba Zoo (intermediate) and Ogba Bridge (downstream), were established along the stretch of the River

based on anthropogenic activities and characteristics of effluents. At the Ogba Zoo, (Zoological garden) the activities carried out here include recreation, fishing, washing and discharge of solid waste. At the Ogba Bridge station, bathing, fishing, traditional worshiping, swimming, laundry and sand excavation are observable while at the Agricultural Development Programme station, fishing, bathing, laundry, car wash and swimming take place.

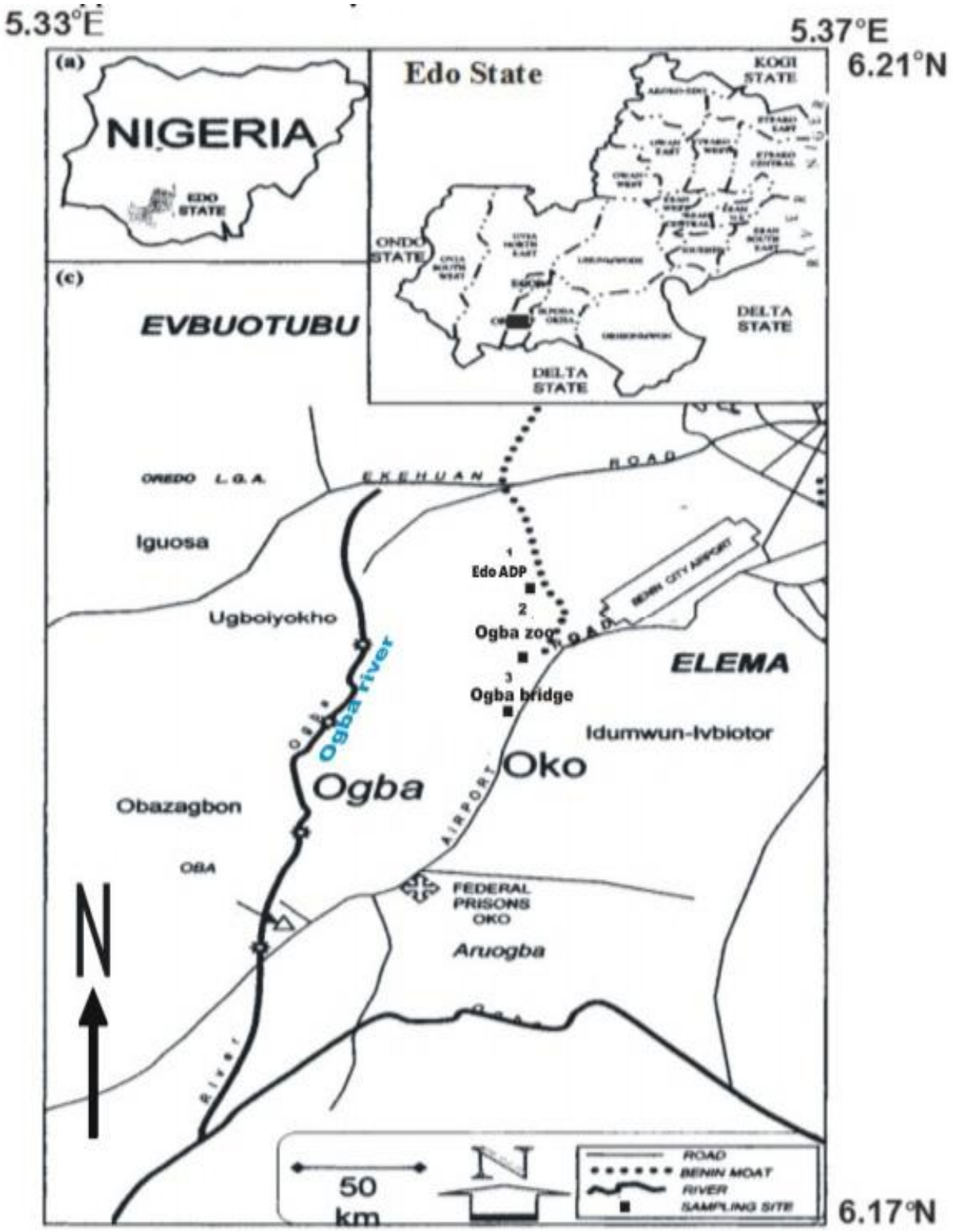


Figure 1: Map of Ogba River and position of stations.
Source: Google map, 2017.

Station 1: This station, Agricultural Development Program (ADP) is located at latitude 6.29° and Longitude 5.58°. The activities carried out here include fishing, bathing, laundry, car wash and swimming take place.

Station 2: This station, Ogba Zoo is located at Latitude 6° 17¹443" N and Longitude; 005° 35¹045" E with an average elevation of 46m above sea-level. Areas within the Ogba Zoo area currently enclosed within a perimeter wire mesh fence were considered as undisturbed, while the areas outside the enclosement are assumed to be disturbed. The activities carried out here include recreation, fishing, washing and discharge of solid waste.

Station 3: This station, Ogba bridge the activities carried out here include bathing, fishing, traditional worshiping, swimming, laundry and sand excavation are observable.

3.3 Collection of Samples

Fish samples was collected (regardless of sample stations) between 7:00am and 10:00am. The collection of samples was carried out using a fishing net or scoop net while operating a dug-out canoe with the assistance of local fishermen. After the landing of the catch, samples was placed in labelled zip-lock bags and conveyed to the laboratory in an ice box within 24 hours.

3.4 Preparation of sample for analysis

3.4.1 Cleaning the sample

Samples was rinsed thoroughly with running water. This is to remove any debris adhering to the body of the fish (Desforges *et al*., 2015; Davidson and Dudas, 2016).

3.4.2 Preparing the digesting solution

The digesting solution consist of 10% KOH. The required amount of pure water was added to a beaker. An amount of KOH amounting to 10% of the solutions total volume was measured and added to the beaker. The solution was stirred lightly to ensure homogenization (Karami *et al.,* 2017).

3.4.3 Digestion of sample

The gills, muscle or intestine may be used for this procedure. The cleaned fish sample was placed in the 10% KOH solution ((Foekema *et al*., 2013; Rochman *et al*., 2015). The beaker was covered. The beaker was safely stored away for a period of 14-21 days during which time digestion will take place. Alternatively, the samples was left to digest in closed vials overnight at 60°C in an oven. The digestion method using 10% KOH has been documented as the best method to extract microplastics with the highest isolation efficiency (Dehaut *et al*., 2016; Lusher *et al*., 2017; Thiele *et al*., 2019).

3.4.4 Purification of digested sample

To ensure the complete removal of all residual organic matter, the digested sample was purified using wet 30% H_2O_2 (Nuelle *et al*., 2015; Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2015).

3.4.5 Sieving the digested sample

The resultant liquified samples would be sieved through two sieves (1 mm and 38 mm), or 5um cellulose nitrate filters to ensure the capturing of microplastics of the smallest sizes. The filter will be rinsed into a glass petri-dish using pure water or 95% methanol (Cauwenberghe and Janssen, 2014).

3.5 Identification of microplastics

The petri-dishes was subjected to a temperature of a 100^0C for 12hrs in an oven. The resultant dried filtrate was visually identified under a microscope or transferred to a slide before visual identification.

3.5.1 Verification of microplastic polymers

The need for further study of the collected microplastics is informed by the fact that visual identification is prone to error. This is because, even for the most experienced professional in this field, it is very difficult, if not impossible to differentiate microplastics from natural fibres visually. While there are several methods of verifying microplastics, the choice of what methods to use is highly influenced by the requirement of the study, availability of analytical equipment as well as cost. The method to be used will be the tagging method. This is because it is fast and cheap, while producing relatively accurate results.

3.5.2 Tagging method

The tagging method of microplastic verification is cheap, fast and relatively reliable. To verify by tagging, a sub-sample of the microplastics filtrate was stained using Nile red dye; preferably the water-based Nile red. The stained filtrate is then viewed under the influence of blue light with the aid of a microscope (Shim *et al.,* 2016).

3.5.3 Hot needle method

The suspected microplastics was further verified using the hot needle method, where possible, to see if the MPs will melt when in contact with the heated end of a needle.

3.6 Measurement of microplastic indices

The indices to be evaluated include:

1. Plastic load.
2. Frequency of occurrence of plastic ingestion.

3.7 Analysis of data

3.7.1 Plastic load (PL)

The average amount of microplastic per fish is referred to as the plastic load (PL). This value was included in all fish sampled, even those which are found to have no plastic present; hence the average PL can be a value less than one (Van-Cauwenberghe and Janssen, 2014; Desforges *et al.,* 2015; Avio *et al.*, 2015).

$$\text{Plastic load (PL)} = \frac{\text{Total number of microplastic particles}}{\text{Total number of fish sampled}}$$

3.7.2 Frequency of occurrence of microplastic ingestion (FO)

Frequency occurrence is a value that refers to the percentage of fish with at least one piece of microplastic.

FO = Number of fish with at least one microplastic particle.

3.8 Contamination controls

All work surfaces, vials and utensils was cleaned beforehand with 70% ethanol solution before use and in-between individual samples to prevent cross-contamination. Throughout all processing and analysis, strict protocols will be undertaken to ensure that contamination risk is minimized (Mathalon and Hill 2014; Masura *et al*. 2015; Lusher *et al*., 2017; Provencher *et al*., 2017; Hermsen *et al*., 2018). The laboratory work area will be cleaned methodically before any work is carried out and between each fish. The outer part of the fish will be rinsed

twice with ultra-pure water and once with ethanol to eliminate any potential particles attached to fish body surface (Karami *et al*., 2017). Both procedural and environmental blank samples will be prepared during every stage of the methodology (open vials during dissection, polypropylene jars with 10% KOH during digestion, and open Petri dishes during sieving and microscope analysis) (Hermsen *et al*., 2017; Kroon F. *et al*., 2018). Blank sample will be placed directly alongside the work area and processed, filtered and analyzed using the same methods used for the samples.

3.9 Experimental design

This was a factorial experiment in a Completely Randomized Design (CRD) with three stations by 4 months by 4 microplastic type by (2 common commercial important fish species (*Clarias gariepinus* and *Oreochromis niloticus*) replicated 3 times). A Genstat software (version 12.1) was used for statistical analysis. ANOVA (one-way) was used to determine the difference between mean values of microplastics while significant means ($p<2.25$) was separated with the New Duncan multiple range test.

CHAPTER FOUR

4.0 Result

The result of microplastic pollutants in *Clarias gariepinus* and *Oreochromis niloticus* from Ogba river, Benin city, Nigeria.

4.1 Data and Time of Sample Collection

The Table below shows the data and time of collection of samples.

Table 1. Data and Time of collection of samples

Data of collection	Station 1	Station 2	Station 3
June 17th 2023	8 : 05 am	8 : 35 am	9 : 00 am
June 24th 2023	8 : 10am	8 : 32 am	9 : 24 am
July 17th 2023	8 : 32 am	9 : 01 am	9 : 30 am
July 24th 2023	8 : 15 am	8 : 35 am	9 : 15 am
August 17th 2023	8 : 25 am	9 : 14am	9 : 40am
August 24th 2023	8 : 07 am	8 : 25 am	9 : 02am

4.2 Level of Microplastics

The level of plastic in *Clarias gariepinus* informs the abundance of microplastic in fish from the stations. The values range from 0.06 to 1.40. There is no significant difference between all stations in the month of June. There is no significant difference between station one, two and station three. There is no significant difference station one, two and three in the month of June. Also, there is no significant difference between station one, two and three in the month of august.

There is no significant difference between the means in June, July and August. There is no significant difference in station two across all months. The level of plastic in *Oreochromis niloticus* informs the abundance of microplastic in fish from the stations. The values range from 0.04 to 0.86. There is no significant difference between all stations in the month of June and August except from July. There is significant difference between station one, two and station three. There is no significant difference station one, two and three in the month of June. Also, there is no significant difference between station one, two and three in the month of august. There is significant difference in station two across all months.

Table 2: Mean level of microplastics (*Clarias gariepinus*) from harvested fish according to stations

Month	Station 1 (ADP)	Station 2 (Ogba Zoo)	Station 3 (Ogba Bridge)
June	0.77 ± 0.60^{a}	0.96 ± 0.06^{a}	1.37 ± 0.57^{a}
July	0.60 ± 0.50^{a}	0.92 ± 0.08^{a}	0.95 ± 0.06^{a}
August	0.60 ± 0.50^{a}	1.40 ± 0.53^{a}	1.10 ± 0.17^{a}

Table 3: Mean level of microplastics (*Oreochromis niloticus*) from harvested fish according to stations

Month	Station 1 (ADP)	Station 2 (Ogba Zoo)	Station 3 (Ogba Bridge)
June	0.04 ± 0.05^{a}	0.08 ± 0.12^{a}	0.20 ± 0.09^{a}
July	0.86 ± 0.15^{b}	0.48 ± 0.30^{ab}	0.62 ± 0.30^{b}
August	0.85 ± 0.20^{b}	0.74 ± 0.15^{b}	0.78 ± 0.16^{b}

4.3 Plastic Load

This is the number of plastic particles per fish sampled. In table 4 below, there is no significant difference in August across all stations. There is no significant difference between station two and three in August. However significant difference does exist between station two and three in June and July. In June, there is no significant difference between station one and three, while there is between station two and the other stations.

There is no significant difference between station one and three in July, however it does occur between station two and the other stations. There is significant difference between June and August in station one, however significant difference does not exist in June and July in station one. There is no significant difference in station two across all months, however there is significant difference in station three across all months. There is no significant difference in station three in July, however there is significant difference in station one and two in July.

In table 5 below, there is significant difference in August across all stations. There is no significant difference in June across all stations. There is significant difference in July between station one and two except from station three. However significant difference does exist between station one and three in across all the months except station two.

There is significant difference between station one and three in August, however it does not occur between station one and three in June. There is significant difference between June and August in station one. There is no significant difference in station two across all months, however there is significant difference in station one and three across all month.

Table 4: Plastic load (*Clarias gariepinus)* from harvested fish according to stations

Month	Station 1 (ADP)	Station 2 (Ogba Zoo)	Station 3 (Ogba Bridge)
June	0.70 ± 0.05^{b}	0.72 ± 0.09^{a}	1.03 ± 0.15^{b}
July	0.73 ± 0.07^{b}	0.92 ± 0.08^{a}	0.83 ± 0.10^{ab}
August	0.40 ± 0.09^{a}	$0.\ 66 \pm 0.31^{a}$	0.56 ± 0.24^{a}

Table 5: Plastic load (*Oreochromis niloticus)* from harvested fish according to stations

Month	Station 1 (ADP)	Station 2 (Ogba Zoo)	Station 3 (Ogba Bridge)
June	0.05 ± 0.04^{a}	0.40 ± 0.15^{a}	0.30 ± 0.08^{a}
July	0.49 ± 0.20^{b}	0.35 ± 0.08^{a}	0.55 ± 0.21^{ab}
August	0.74 ± 0.03^{c}	0.45 ± 0.12^{a}	0.63 ± 0.05^{b}

4.4 Frequency of Occurrence

In table 6, there is no significant difference across all stations in August. There is significant difference between August and the other months in station three.

There is no significant difference between station one and three in the month of June, while it exists between station two and the other stations. There is no significant difference between all stations in the month of August. There is no significant difference in station two across all the month. There is significant difference in station three across all the month. In the month of July, there is significant difference across all stations.

In table 7, there is no significant difference across all stations. There is no significant difference across all the months. There is no significant difference between station one, two and three in the month of June. There is no significant difference between station one, two and three in the month of July. There is no significant difference between station one, two and three in the month of August.

Table 6: Frequency of occurrence (*Clarias gariepinus)* from harvested fish according to stations

Month	Station 1 (ADP)	Station 2 (Ogba Zoo)	Station 3 (Ogba Bridge)
June	0.70 ± 0.05^{b}	0.72 ± 0.09^{a}	1.03 ± 0.15^{b}
July	0.73 ± 0.07^{b}	0.92 ± 0.08^{a}	0.83 ± 0.10^{ab}
August	0.40 ± 0.08^{a}	0.66 ± 0.31^{a}	0.56 ± 0.24^{a}

Table 7: Frequency of occurrence (*Oreochromis niloticus)* from harvested fish according to stations

Month	Station 1 (ADP)	Station 2 (Ogba Zoo)	Station 3 (Ogba Bridge)
June	0.37 ± 0.30^{a}	0.56 ± 0.11^{a}	0.55 ± 0.05^{a}
July	0.39 ± 0.06^{a}	0.43 ± 0.20^{a}	0.40 ± 0.06^{a}
August	0.37 ± 0.03^{a}	0.43 ± 0.05^{a}	0.36 ± 0.04^{a}

4.5 Classification of Microplastics

4.5.1 Physical Classification

Table 8: Types of Microplastic Particles in *Clarias gariepinus* Found Based on Physical Classification

	June			July			August	
	First Station	Second Station	Third Station	First Station	Second Station	Third Station	First Station	Second Station	Third Station
R1	A	A	A	B, C	A	A	B, D	A	A, C, F
R2	A	B, C	A	A	B, E	B, F	A	B, D	B, F
R3	B, D, E	A	A	A	A	B	B, C	A	B, C, F

A: plastic present, B: No plastic, C : Filament, D : Fragment, E : Foam, F : Pellet,G :Fibre

Table 9: Types of Microplastic Particles in *Oreochromis niloticus* Found Based on Physical Classification

	June			July			August		
	First Station	Second Station	Third Station	First Station	Second Station	Third Station	First Station	Second Station	Third Station
R1	B	B	B	A	B	B	A	B	B
R2	B, C	B	B	B	A	B	B	A	B
R3	B	A	B	B	B	A	B, C	B, C	B

A: plastic present, B: No plastic, C: Filament, D: Fragment, E: Foam, F: Pellet, G: Fibre

4.5.2 Polymer Classification

4.5.2.1 Characterization of microplastics extracted from Catfish (guts) sampled from different stations.

4.5.2.2 Station 1 (Agricultural Development Programme)

June

The FTIR spectrum of Microplastic obtained from station one in June sample can be seen in plate 1 below. The FTIR spectrum show absorbance band at different wave numbers. Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

There is a peak at 2925cm^{-1} is attributed to absorption of asymmetric CH_2 stretching. There is also, a peak at 2850cm^{-1} is attributed to symmetric CH_2 stretching. A peak at 1430cm^{-1} is attributed to CH_2 scissoring. There is a peak at 710cm^{-1} is attributed to CH_2 rocking. The peaks at 2925cm^{-1}, 1430cm^{-1} and 710cm^{-1} are absorbance wave numbers range used to identify polyethylene (PE) compound in FTIR spectrum. Therefore, microplastic of polyethylene identity was confirmed with these absorption wave numbers.

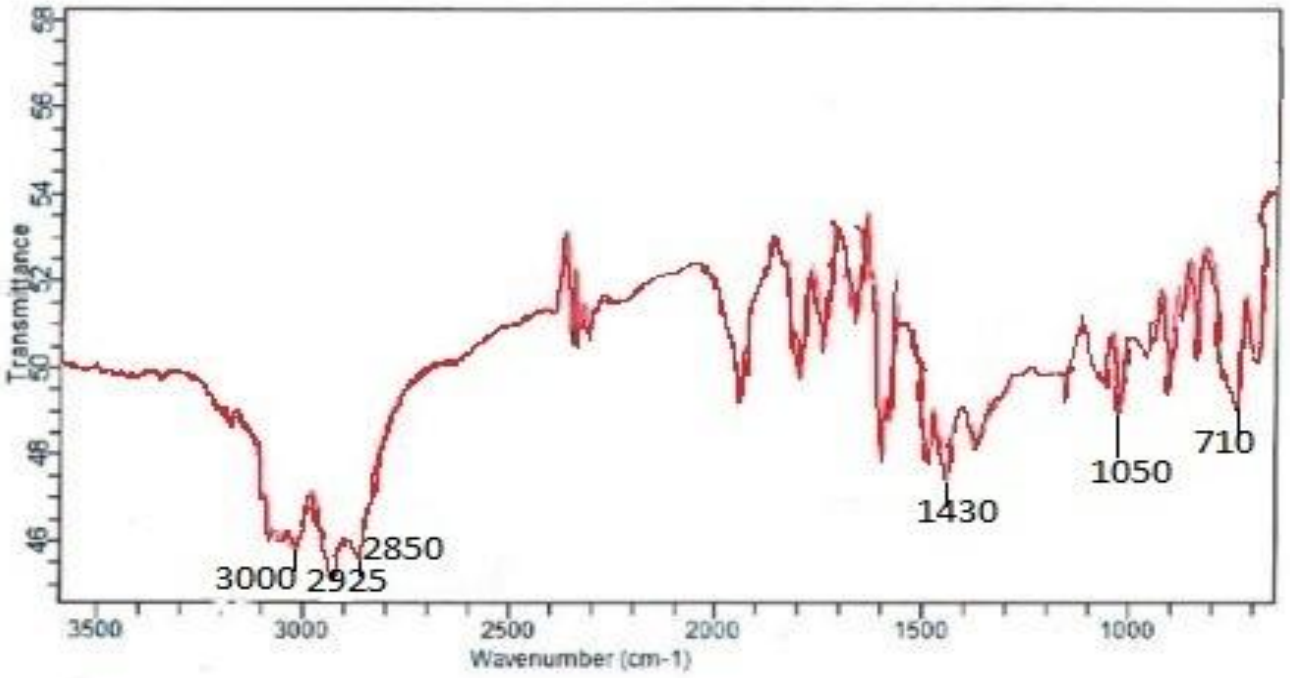
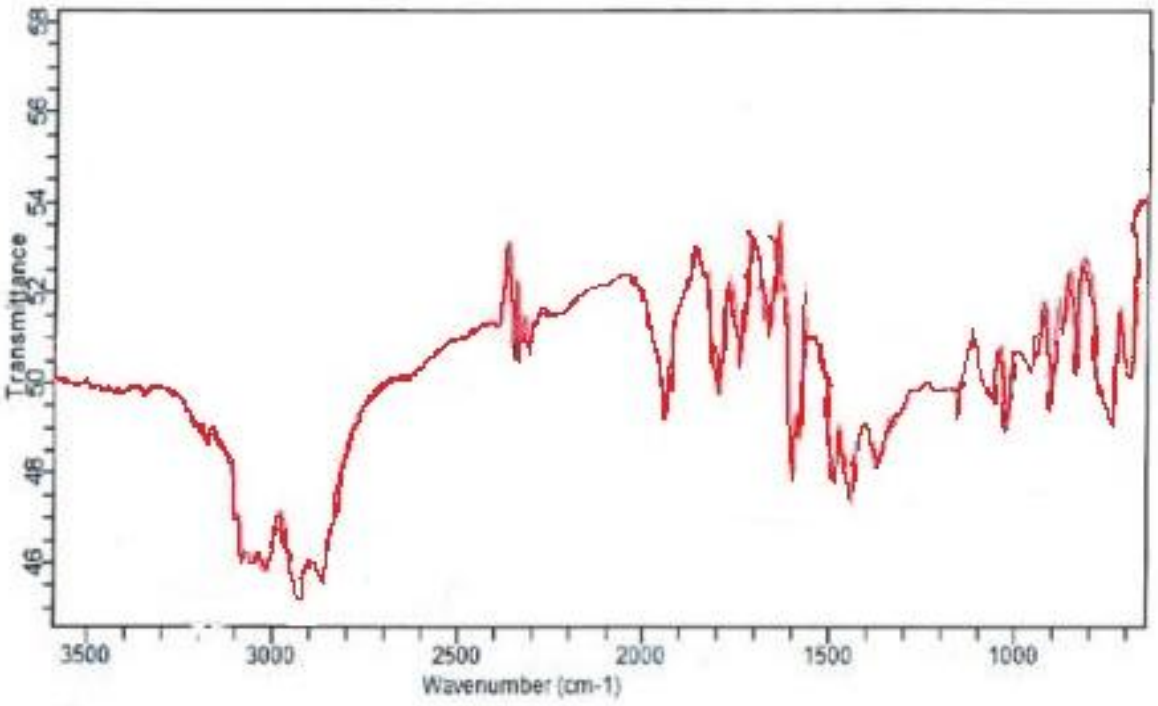


Fig. 2: FTIR spectrum for Microplastic particle obtained in station 1 in June, showing absorbance band at different wave numbers

Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.



ıowing

July

The FTIR spectrum of Microplastic obtained from station one in July sample can be seen in plate 3 below. The FTIR spectrum show absorbance band at different wave numbers. Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

There is a peak at 2925cm^{-1} is attributed to absorption of asymmetric CH_2 stretching. There is also, a peak at 2850cm^{-1} is attributed to symmetric CH_2 stretching. A peak at 1430cm^{-1} is attributed to CH_2 scissoring. There is a peak at 710cm^{-1} is attributed to CH_2 rocking. The peaks at 2925cm^{-1}, 1430cm^{-1} and 710cm^{-1} are absorbance wave numbers range used to identify polyethylene (PE) compound in FTIR spectrum. Therefore, microplastic of polyethylene identity was confirmed with these absorption wave numbers.

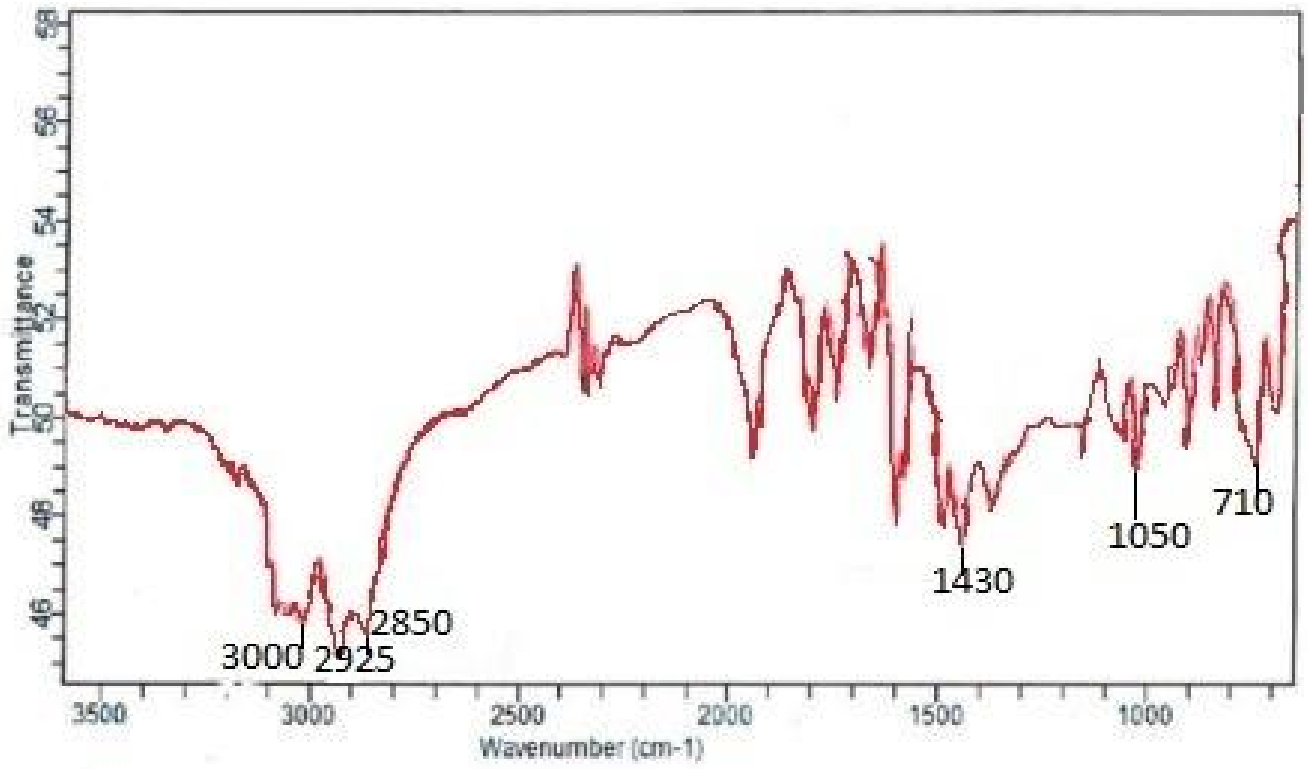


Fig. 4: FTIR spectrum for Microplastic particle obtained in station 1 in July, showing absorbance band at different wave numbers

Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

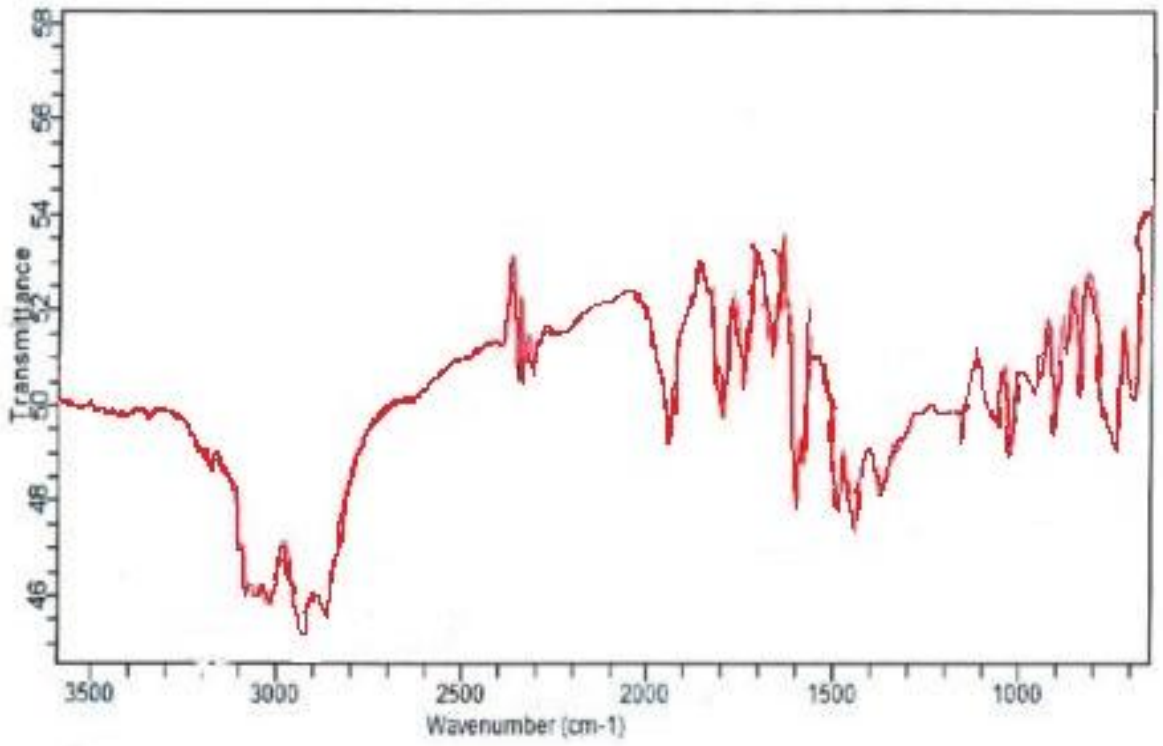


Fig. 5: FTIR spectrum for Microplastic particle obtained in station 1 in July, showing absorbance band at different wave numbers

August

The FTIR spectrum of Microplastic obtained from station one in August sample can be seen in plate 5 below. The FTIR spectrum show absorbance band at different wave numbers. Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

There is a peak at 2925cm^{-1} is attributed to absorption of asymmetric CH_2 stretching. There is also, a peak at 2850cm^{-1} is attributed to symmetric CH_2 stretching. A peak at 1430cm^{-1} is attributed to CH_2 scissoring. There is a peak at 710cm^{-1} is attributed to CH_2 rocking. The peaks at 2925cm^{-1}, 1430cm^{-1} and 710cm^{-1} are absorbance wave numbers range used to identify polyethylene (PE) compound in FTIR spectrum. Therefore, microplastic of polyethylene identity was confirmed with these absorption wave numbers.

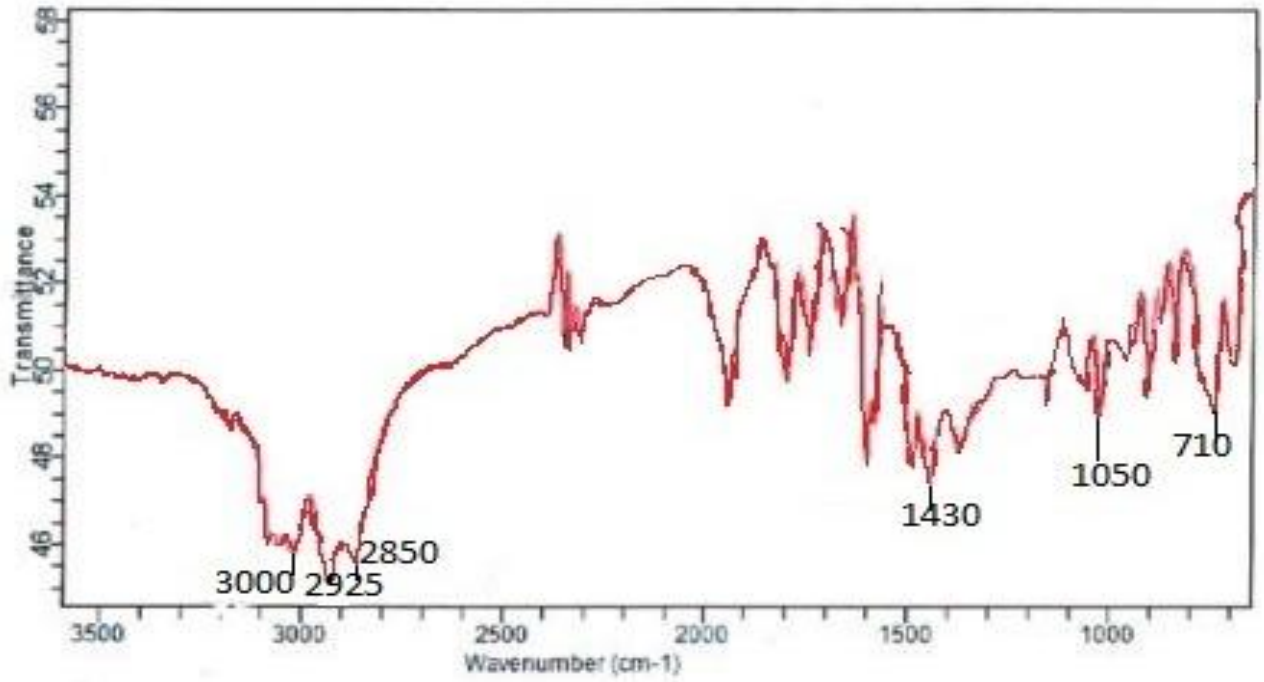


Fig. 6: FTIR spectrum for Microplastic particle obtained in station 1 in August, showing absorbance band at different wave numbers

Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

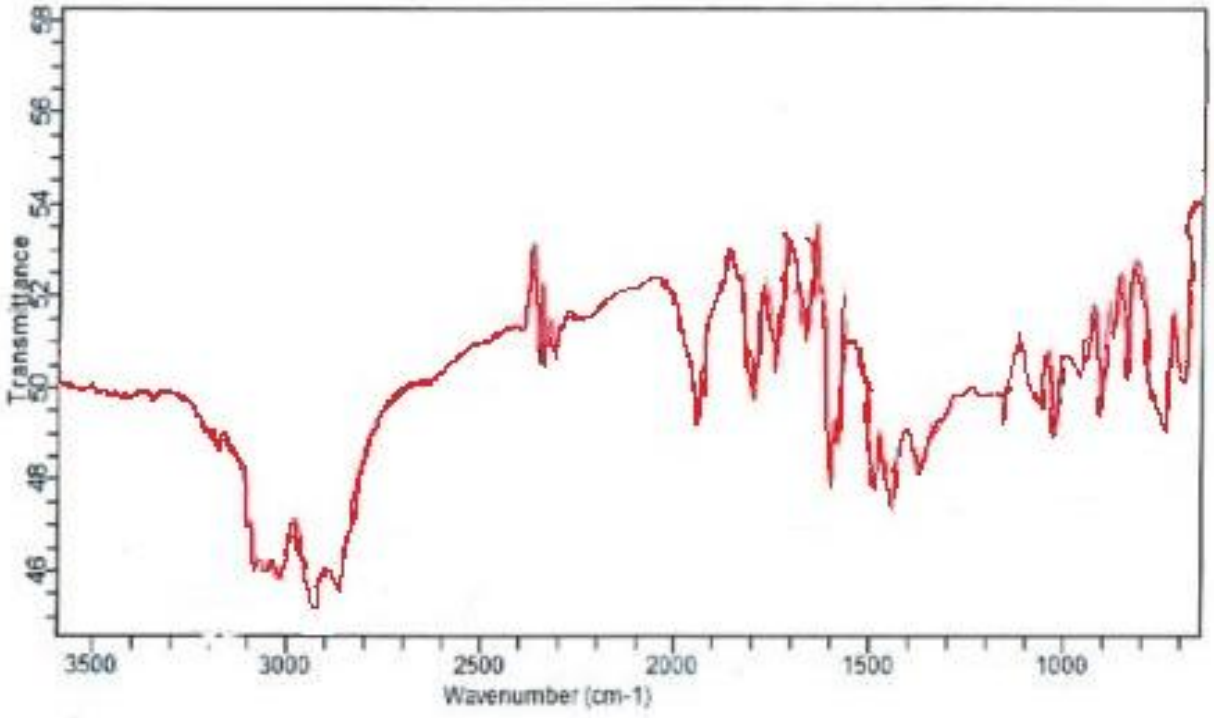


Fig. 7: FTIR spectrum for Microplastic particle obtained in station 1 in August, showing absorbance band at different wave numbers

4.5.2.3 station 2 (Ogba Zoo)

June

The FTIR spectrum of Microplastic obtained from station two in June sample can be seen in plate 7 below. The FTIR spectrum show absorbance band at different wave numbers. Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

There is a peak at 2925cm^{-1} is attributed to absorption of asymmetric CH_2 stretching. There is also, a peak at 2850cm^{-1} is attributed to symmetric CH_2 stretching. A peak at 1430cm^{-1} is attributed to CH_2 scissoring. There is a peak at 710cm^{-1} is attributed to CH_2 rocking. The peaks at 2925cm^{-1}, 1430cm^{-1} and 710cm^{-1} are absorbance wave numbers range used to identify polyethylene (PE) compound in FTIR spectrum. Therefore, microplastic of polyethylene identity was confirmed with these absorption wave numbers.

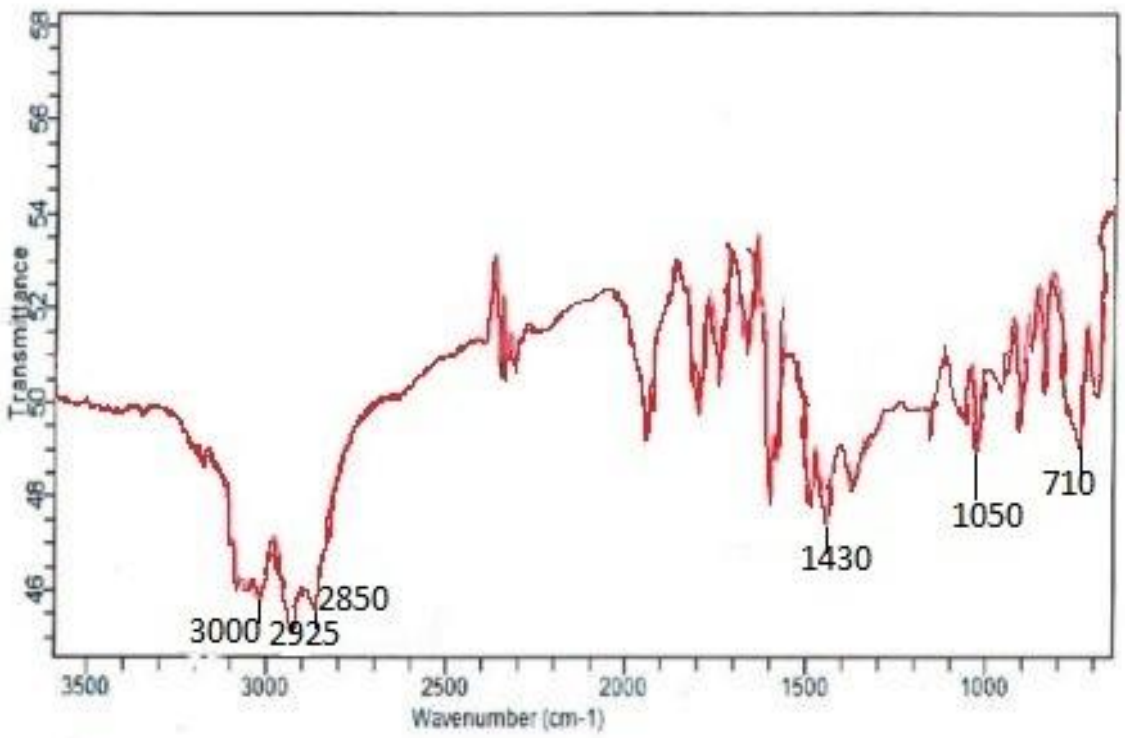


Fig. 8: FTIR spectrum for Microplastic particle obtained in station 2 in June, showing absorbance band at different wave numbers

Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

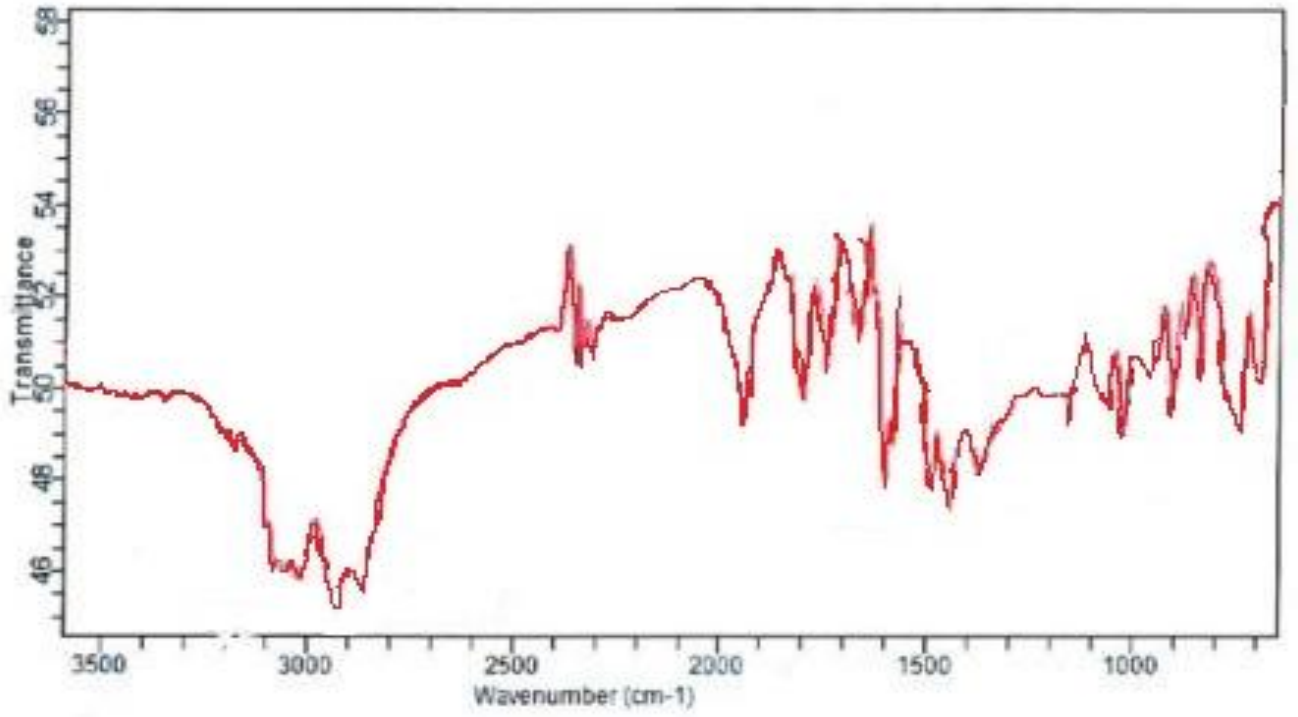


Fig. 9: FTIR spectrum for Microplastic particle obtained in station 2 in June, showing absorbance band at different wave numbers

July

The FTIR spectrum of Microplastic obtained from station two in July sample can be seen in plate 9 below. The FTIR spectrum show absorbance band at different wave numbers. Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

There is a peak at 2925cm^{-1} is attributed to absorption of asymmetric CH_2 stretching. There is also, a peak at 2850cm^{-1} is attributed to symmetric CH_2 stretching. A peak at 1430cm^{-1} is attributed to CH_2 scissoring. There is a peak at 710cm^{-1} is attributed to CH_2 rocking. The peaks at 2925cm^{-1}, 1430cm^{-1} and 710cm^{-1} are absorbance wave numbers range used to identify polyethylene (PE) compound in FTIR spectrum. Therefore, microplastic of polyethylene identity was confirmed with these absorption wave numbers.

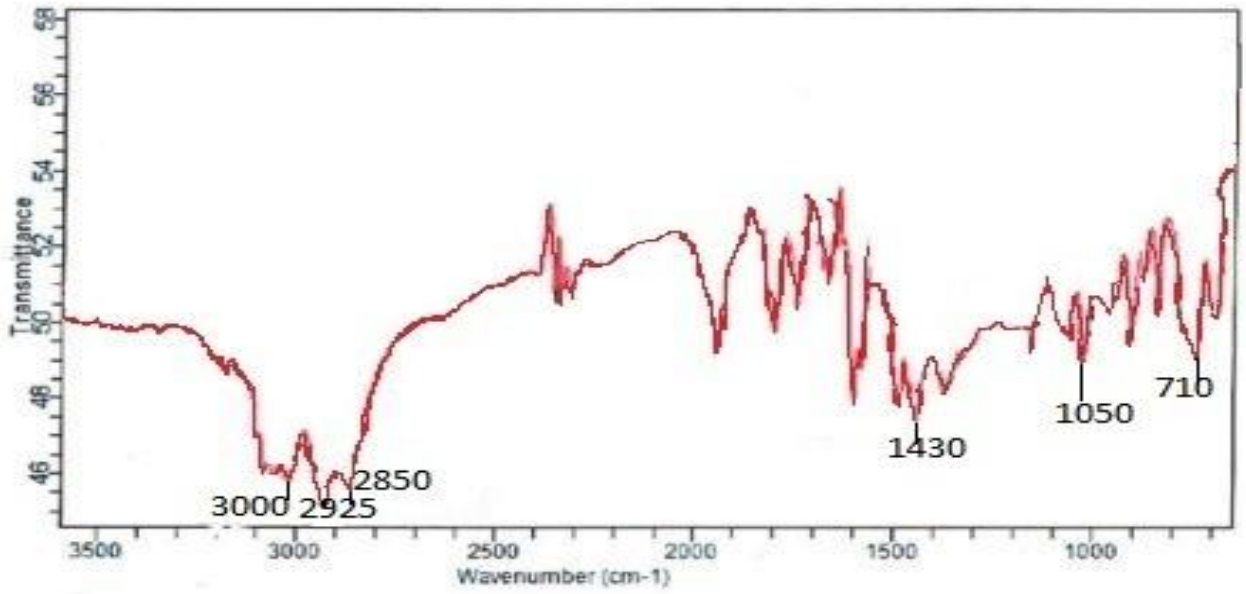


Fig. 10: FTIR spectrum for Microplastic particle obtained in station 2 in July, showing absorbance band at different wave numbers

Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

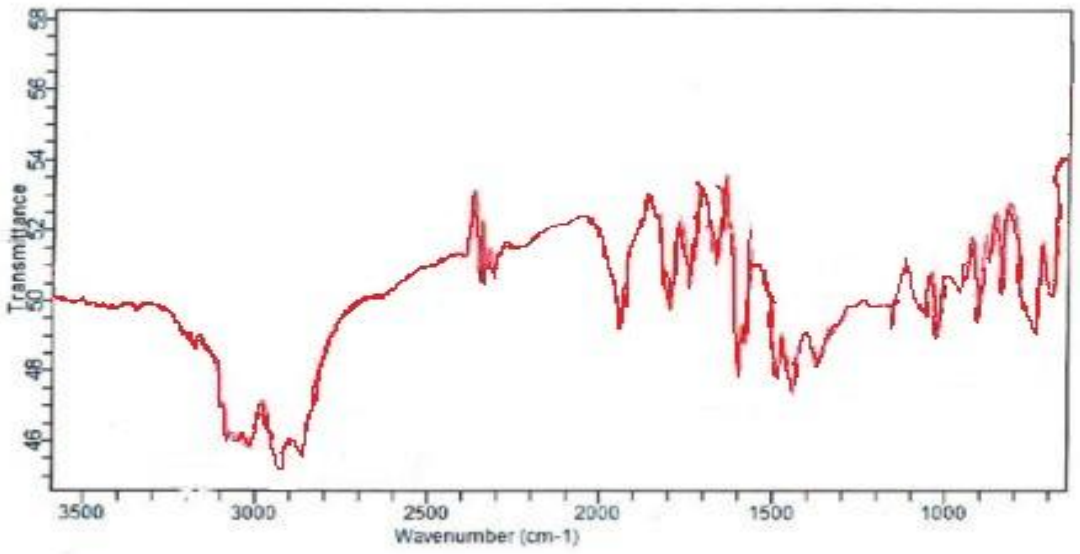


Fig. 11: FTIR spectrum for Microplastic particle obtained in station 2 in July, showing absorbance band at different wave numbers

August

The FTIR spectrum of Microplastic obtained from station two in August sample can be seen in plate 11 below. The FTIR spectrum show absorbance band at different wave numbers. Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

There is a peak at 2925cm^{-1} is attributed to absorption of asymmetric CH_2 stretching. There is also, a peak at 2850cm^{-1} is attributed to symmetric CH_2 stretching. A peak at 1430cm^{-1} is attributed to CH_2 scissoring. There is a peak at 710cm^{-1} is attributed to CH_2 rocking. The peaks at 2925cm^{-1}, 1430cm^{-1} and 710cm^{-1} are absorbance wave numbers range used to identify polyethylene (PE) compound in FTIR spectrum. Therefore, microplastic of polyethylene identity was confirmed with these absorption wave numbers.

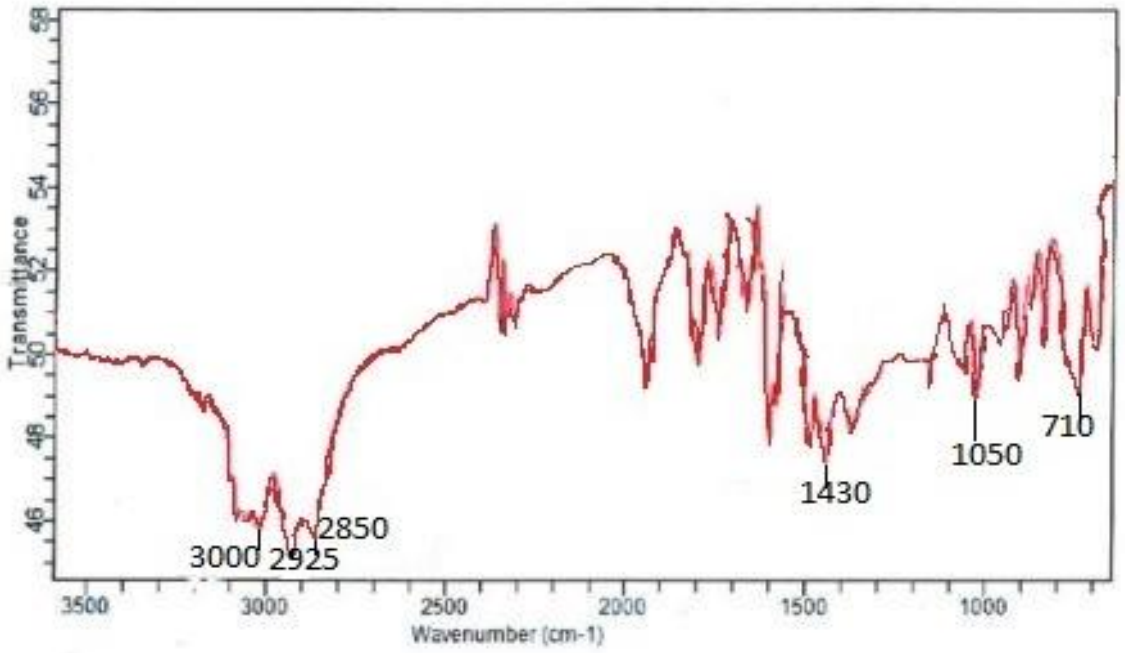


Fig. 12: FTIR spectrum for Microplastic particle obtained in station 2 in August, showing absorbance band at different wave numbers

Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

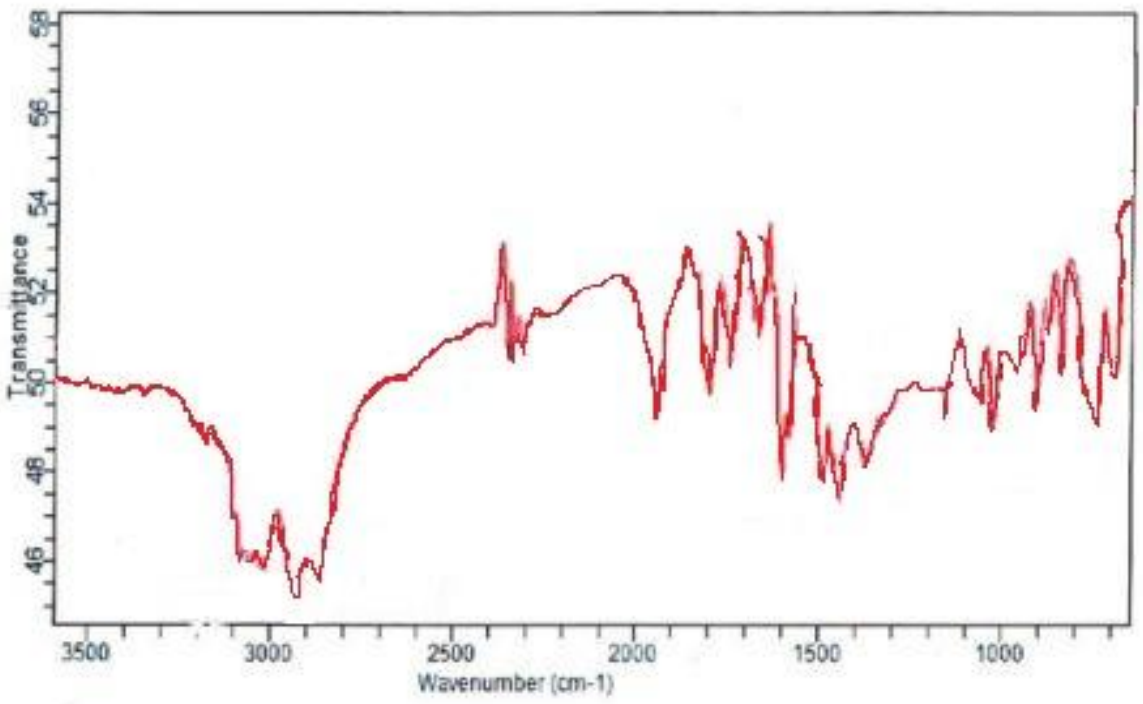


Fig 13: FTIR spectrum for Microplastic particle obtained in station 2 in August, showing absorbance band at different wave numbers

4.5.2.4 Station 3 (Ogba Bridge)

June

The FTIR spectrum of Microplastic obtained from station three in June sample can be seen in plate 13 below. The FTIR spectrum show absorbance band at different wave numbers. Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

There is a peak at 2925cm^{-1} is attributed to absorption of asymmetric CH_2 stretching. There is also, a peak at 2850cm^{-1} is attributed to symmetric CH_2 stretching. A peak at 1430cm^{-1} is attributed to CH_2 scissoring. There is a peak at 710cm^{-1} is attributed to CH_2 rocking. The peaks at 2925cm^{-1}, 1430cm^{-1} and 710cm^{-1} are absorbance wave numbers range used to identify polyethylene (PE) compound in FTIR spectrum. Therefore, microplastic of polyethylene identity was confirmed with these absorption wave numbers.

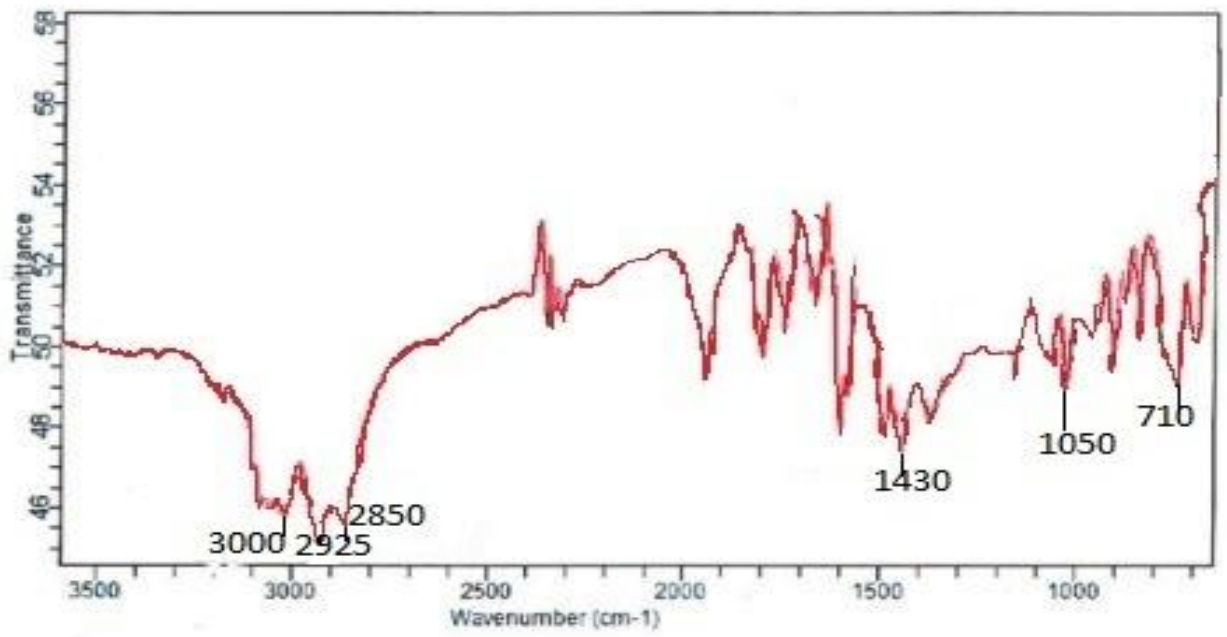


Fig. 14: FTIR spectrum for Microplastic particle obtained in station 3 in June, showing absorbance band at different wave numbers

Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

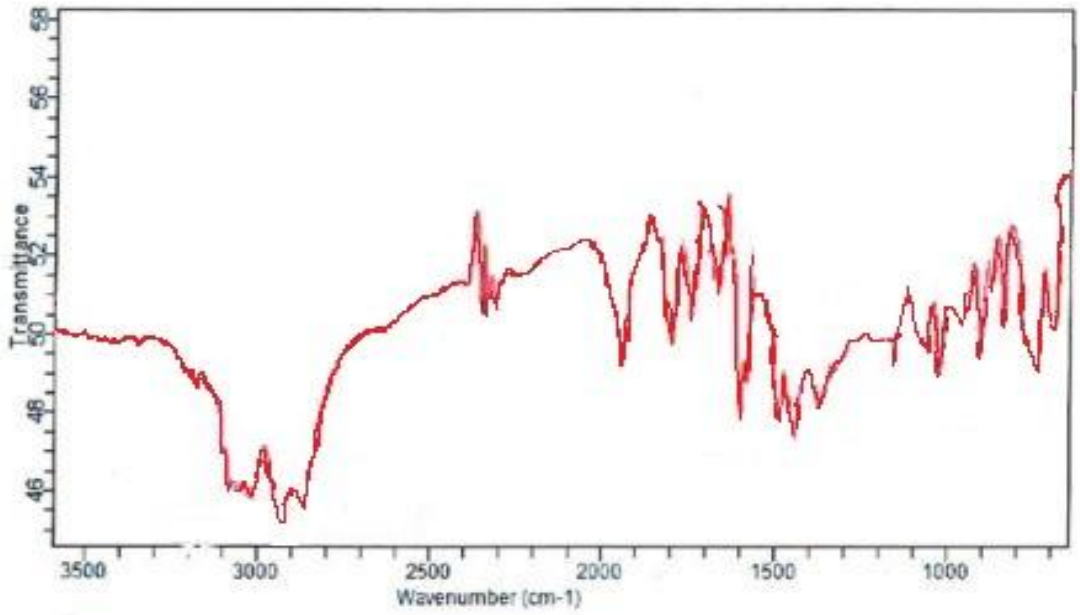


Fig. 15: FTIR spectrum for Microplastic particle obtained in station 3 in June, showing absorbance band at different wave numbers

July

The FTIR spectrum of Microplastic obtained from station three in July sample can be seen in plate 15 below. The FTIR spectrum show absorbance band at different wave numbers. Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

There is a peak at 2925cm^{-1} is attributed to absorption of asymmetric CH_2 stretching. There is also, a peak at 2850cm^{-1} is attributed to symmetric CH_2 stretching. A peak at 1430cm^{-1} is attributed to CH_2 scissoring. There is a peak at 710cm^{-1} is attributed to CH_2 rocking. The peaks at 2925cm^{-1}, 1430cm^{-1} and 710cm^{-1} are absorbance wave numbers range used to identify polyethylene (PE) compound in FTIR spectrum. Therefore, microplastic of polyethylene identity was confirmed with these absorption wave numbers.

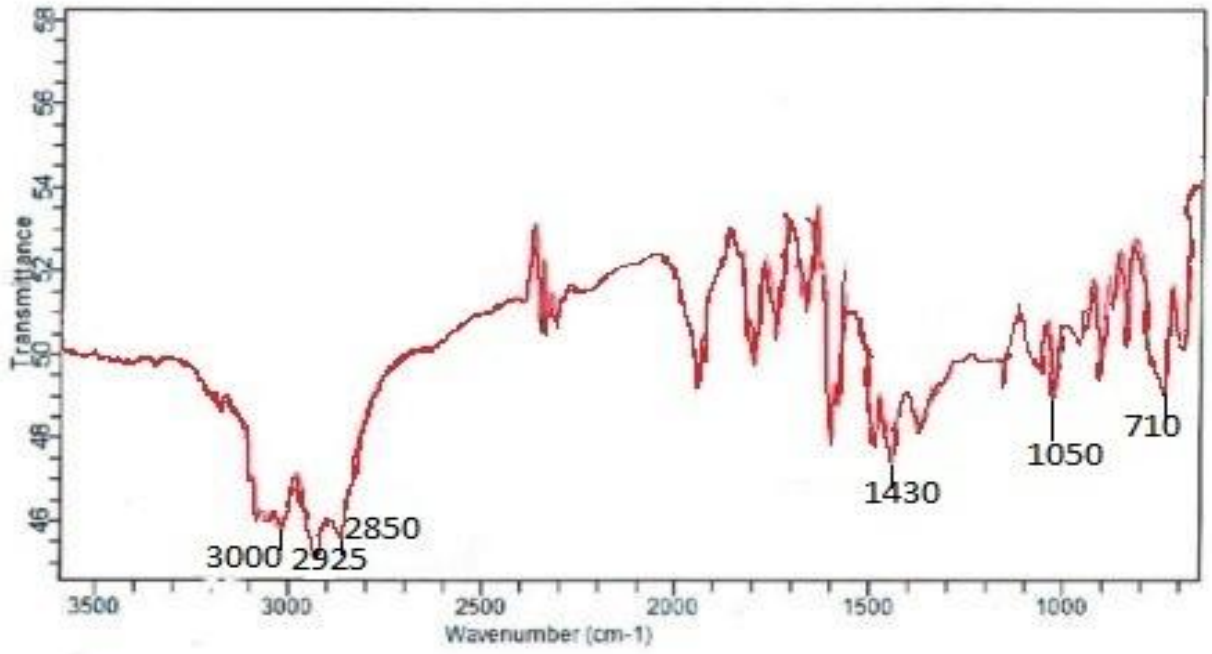


Fig. 16: FTIR spectrum for Microplastic particle obtained in station 3 in July, showing absorbance band at different wave numbers

Note that one of the fragments physically identified was not microplastics and therefore, show no FTIR spectrum result.

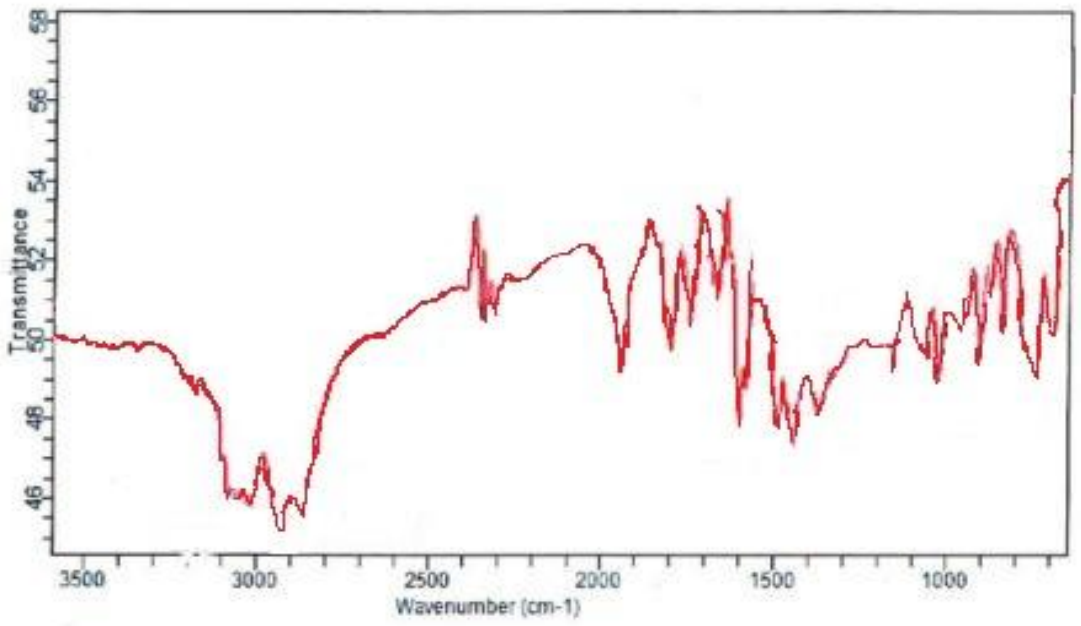


Fig. 17: FTIR spectrum for Microplastic particle obtained in station 3 in July, showing absorbance band at different wave numbers

August

The FTIR spectrum of Microplastic obtained from station three in August sample can be seen in plate 17 below.

There is a peak at 2925cm^{-1} is attributed to absorption of asymmetric CH_2 stretching. There is also, a peak at 2850cm^{-1} is attributed to symmetric CH_2 stretching. A peak at 1430cm^{-1} is attributed to CH_2 scissoring. There is a peak at 710cm^{-1} is attributed to CH_2 rocking. The peaks at 2925cm^{-1}, 1430cm^{-1} and 710cm^{-1} are absorbance wave numbers range used to identify polyethylene (PE) compound in FTIR spectrum. Therefore, microplastic of polyethylene identity was confirmed with these absorption wave numbers.

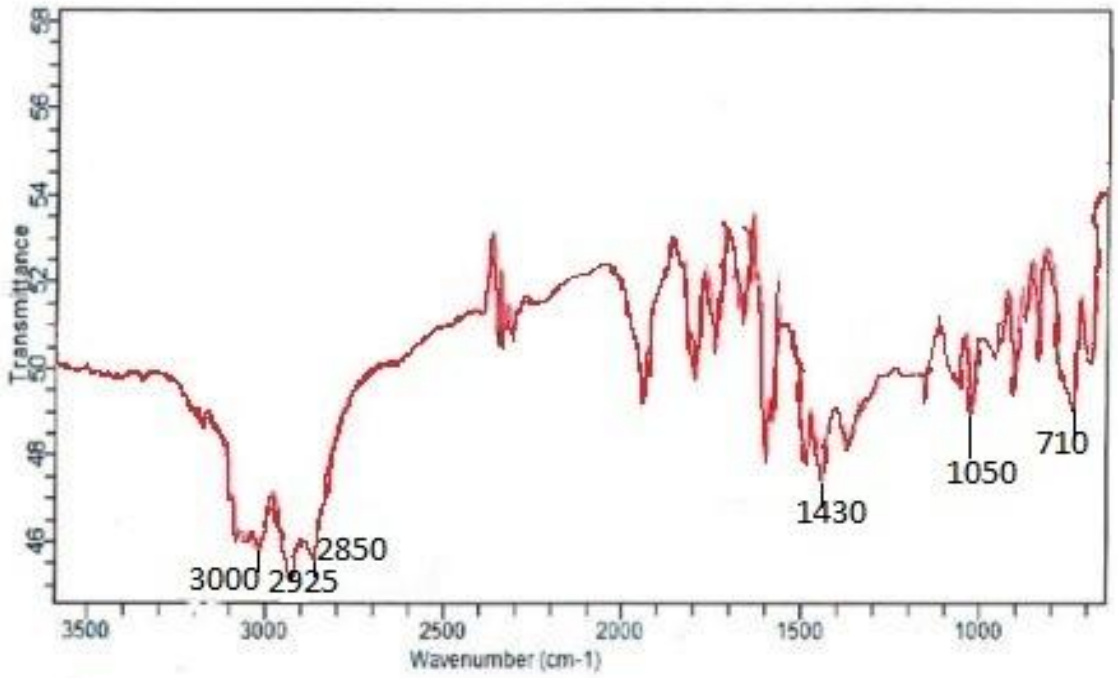


Fig. 18: FTIR spectrum for Microplastic particle obtained in station 3 in August, showing absorbance band at different wave numbers

The microplastics physically identified as filament in station 3 in August sample was not microplastic, therefore, no FTIR spectrum result.

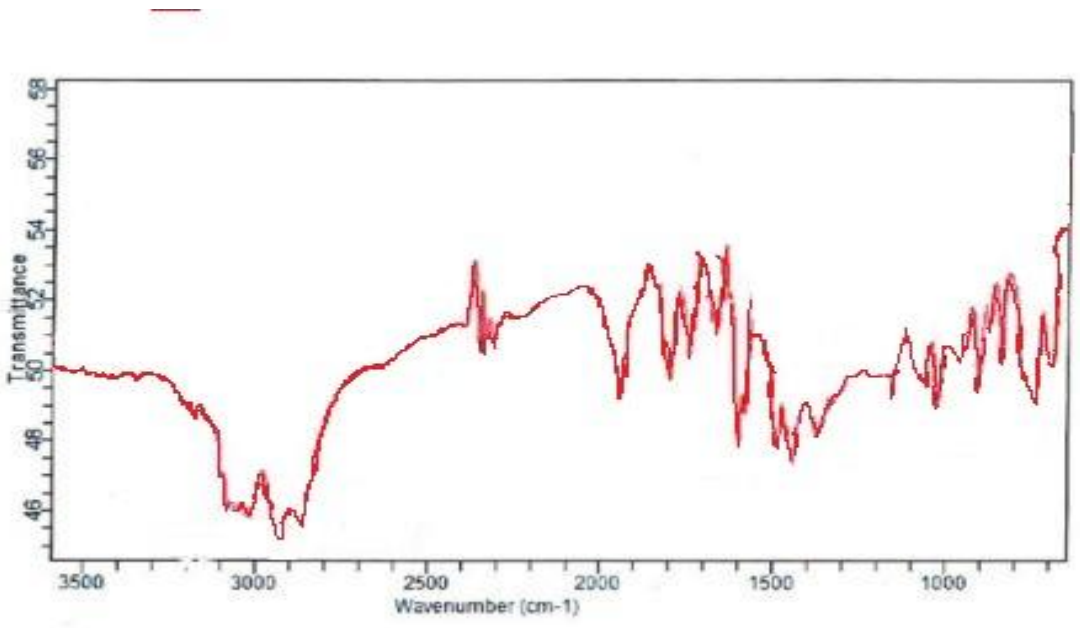


Fig. 19: FTIR spectrum for Microplastic particle obtained in station 3 in August, showing absorbance band at different wave numbers

4.5.2.5 Microplastic extracted from Tilapia fish (guts) sampled from different stations.

4.5.2.6 Station one (Agricultural Development Programme)

June

There is no microplastic particles in June sample, therefore no FTIR spectrum result.

July

The FTIR spectrum of Microplastic obtained from station one in July, sample can be seen in plate 19 below. There is a peak at 2825cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching. There is also, a peak at 1450cm^{-1} which is a characteristic of CH_2 scissoring. A peak at 1150cm^{-1} is attributed to the asymmetric stretching of oxygen atom. There is a peak at 850cm^{-1} is a characteristics of CH rocking. The peaks at 2825cm^{-1}, 1450cm^{-1} and 850cm^{-1} are absorbance wave numbers range used to identify polypropylene (PP) compound in FTIR spectrum. Therefore, microplastic of polypropylene identity was confirmed with these absorption wave numbers.

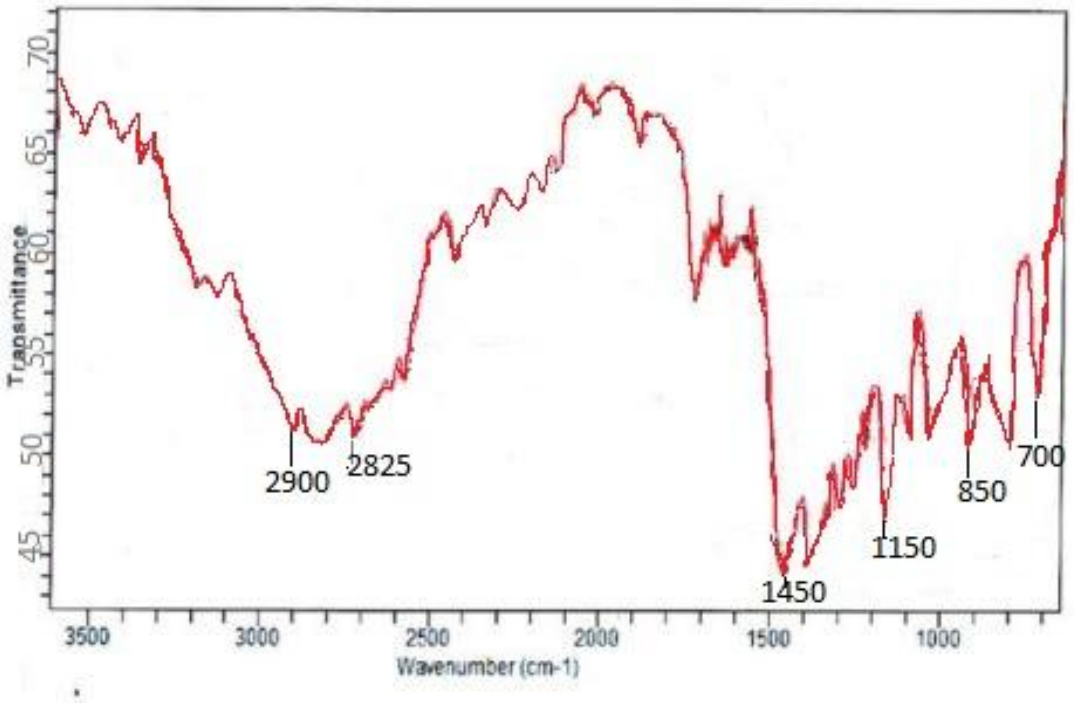


Fig. 20: FTIR spectrum for Microplastic particle obtained in station 1 in July, showing absorbance band at different wave numbers

The microplastics physically identified as filament in station 1 in July sample was not microplastic, therefore, no FTIR spectrum result.

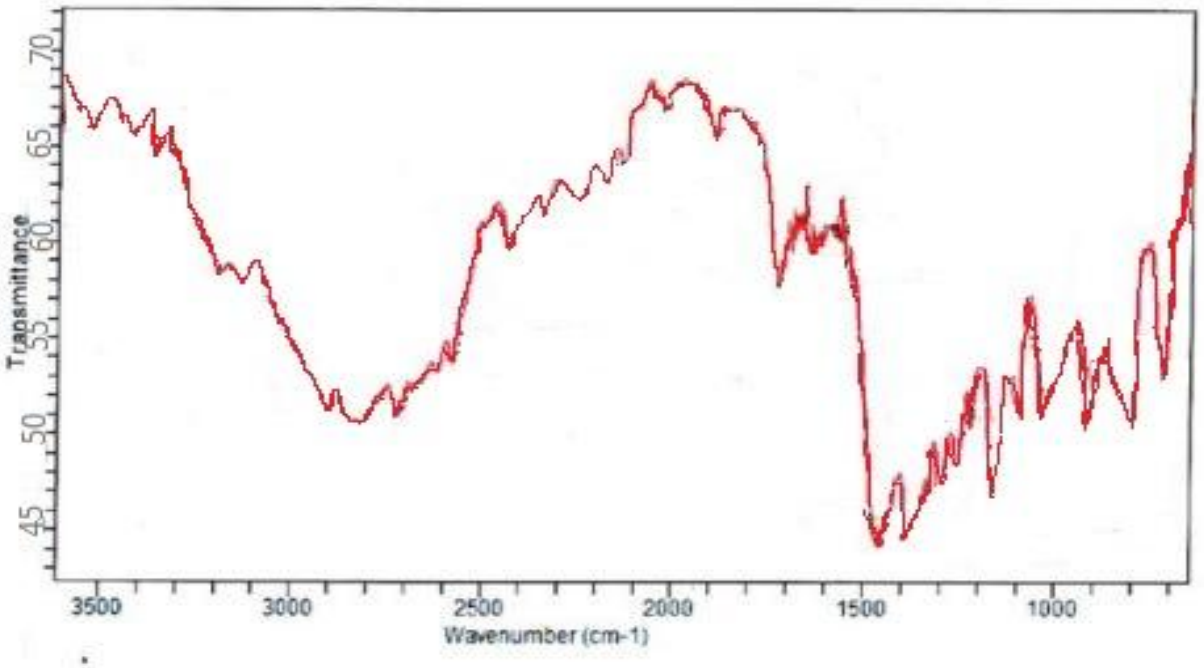


Fig. 21: FTIR spectrum for Microplastic particle obtained in station 1 in July, showing absorbance band at different wave numbers

August

The FTIR spectrum of Microplastic obtained from station one in August, sample can be seen in plate 21 below. There is a peak at 2825cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching. There is also, a peak at 1450cm^{-1} which is a characteristic of CH_2 scissoring. A peak at 1150cm^{-1} is attributed to the asymmetric stretching of oxygen atom. There is a peak at 850cm^{-1} is a characteristics of CH rocking. The peaks at 2825cm^{-1}, 1450cm^{-1} and 850cm^{-1} are absorbance wave numbers range used to identify polypropylene (PP) compound in FTIR spectrum. Therefore, microplastic of polypropylene identity was confirmed with these absorption wave numbers.

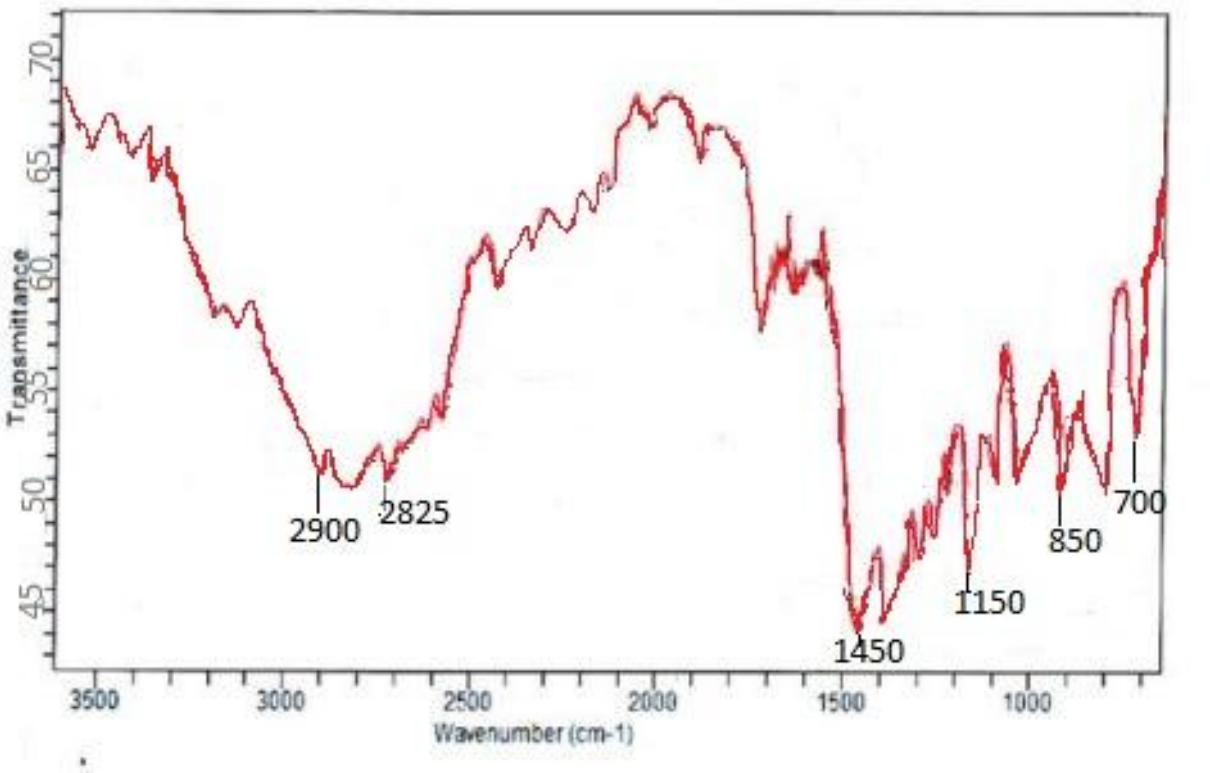


Fig. 22: FTIR spectrum for Microplastic particle obtained in station 1 in August, showing absorbance band at different wave numbers

The microplastics physically identified as filament in station 1 in August sample was not microplastic, therefore, no FTIR spectrum result.

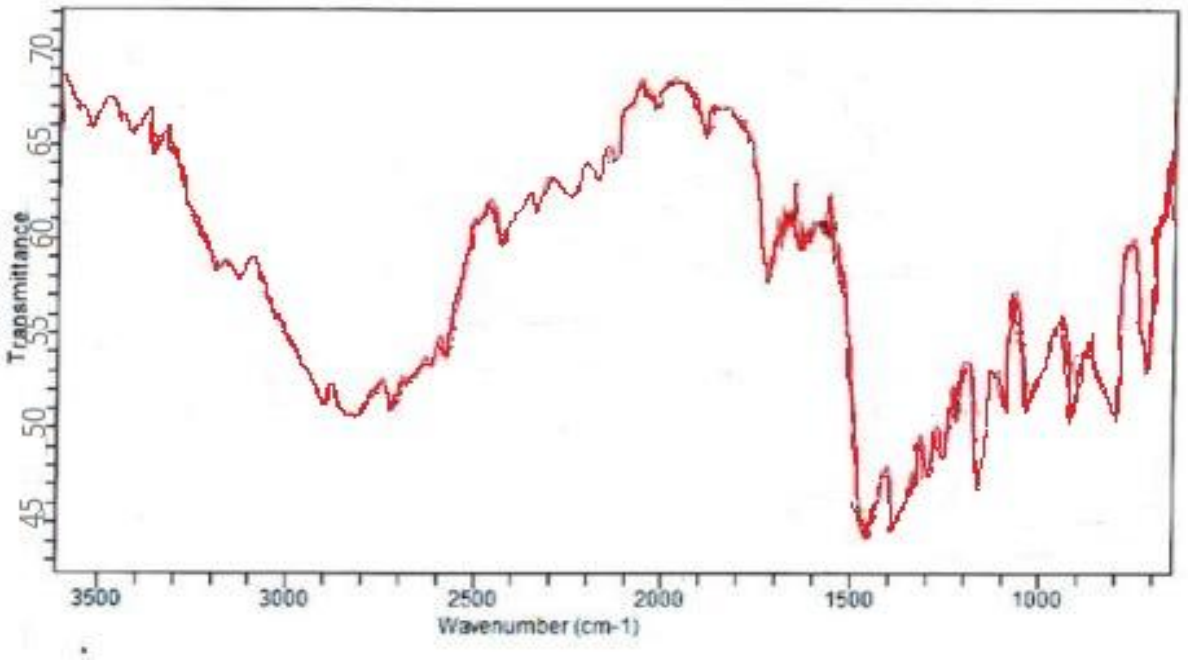


Fig. 23: FTIR spectrum for Microplastic particle obtained in station 1 in August, showing absorbance band at different wave numbers

4.5.2.7 Station two (Ogba Zoo)

June

The FTIR spectrum of Microplastic obtained from station two in June, sample can be seen in plate 23 below. There is a peak at 2825cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching. There is also, a peak at 1450cm^{-1} which is a characteristic of CH_2 scissoring. A peak at 1150cm^{-1} is attributed to the asymmetric stretching of oxygen atom. There is a peak at 850cm^{-1} is a characteristics of CH rocking. The peaks at 2825cm^{-1}, 1450cm^{-1} and 850cm^{-1} are absorbance wave numbers range used to identify polypropylene (PP) compound in FTIR spectrum. Therefore, microplastic of polypropylene identity was confirmed with these absorption wave numbers.

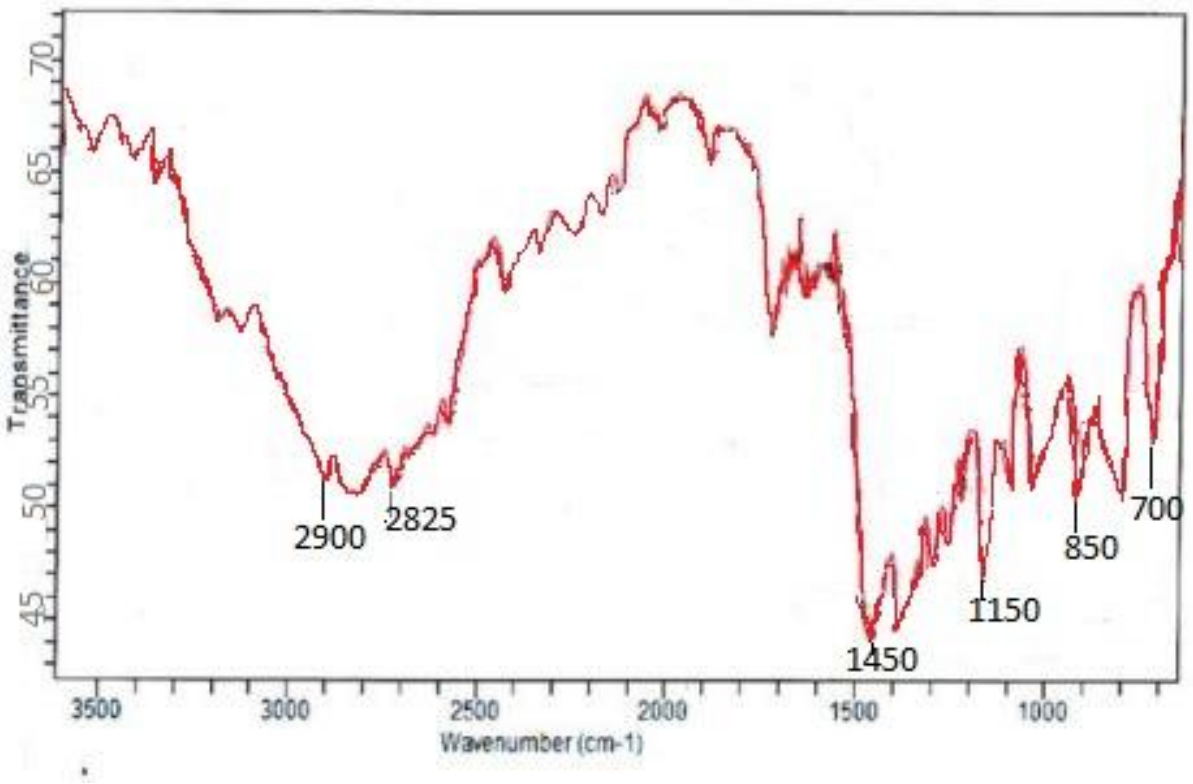


Fig. 24: FTIR spectrum for Microplastic particle obtained in station 2 in June, showing absorbance band at different wave numbers

The microplastics physically identified as filament in station 2 in June sample was not microplastic, therefore, no FTIR spectrum result.

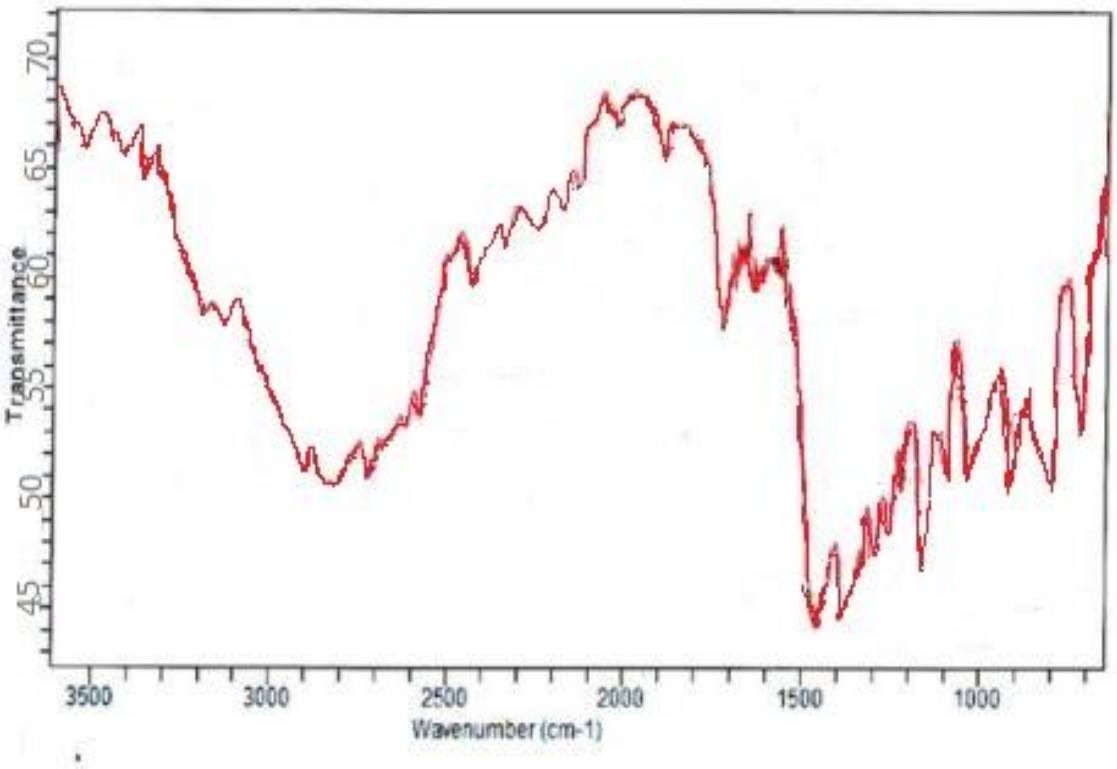


Fig. 25: FTIR spectrum for Microplastic particle obtained in station 2 in June, showing absorbance band at different wave numbers

July

The FTIR spectrum of Microplastic obtained from station two in July, sample can be seen in plate 25 below. There is a peak at 2825cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching. There is also, a peak at 1450cm^{-1} which is a characteristic of CH_2 scissoring. A peak at 1150cm^{-1} is attributed to the asymmetric stretching of oxygen atom. There is a peak at 850cm^{-1} is a characteristics of CH rocking. The peaks at 2825cm^{-1},1450cm^{-1} and 850cm^{-1} are absorbance wave numbers range used to identify polypropylene (PP) compound in FTIR spectrum. Therefore, microplastic of polypropylene identity was confirmed with these absorption wave numbers.

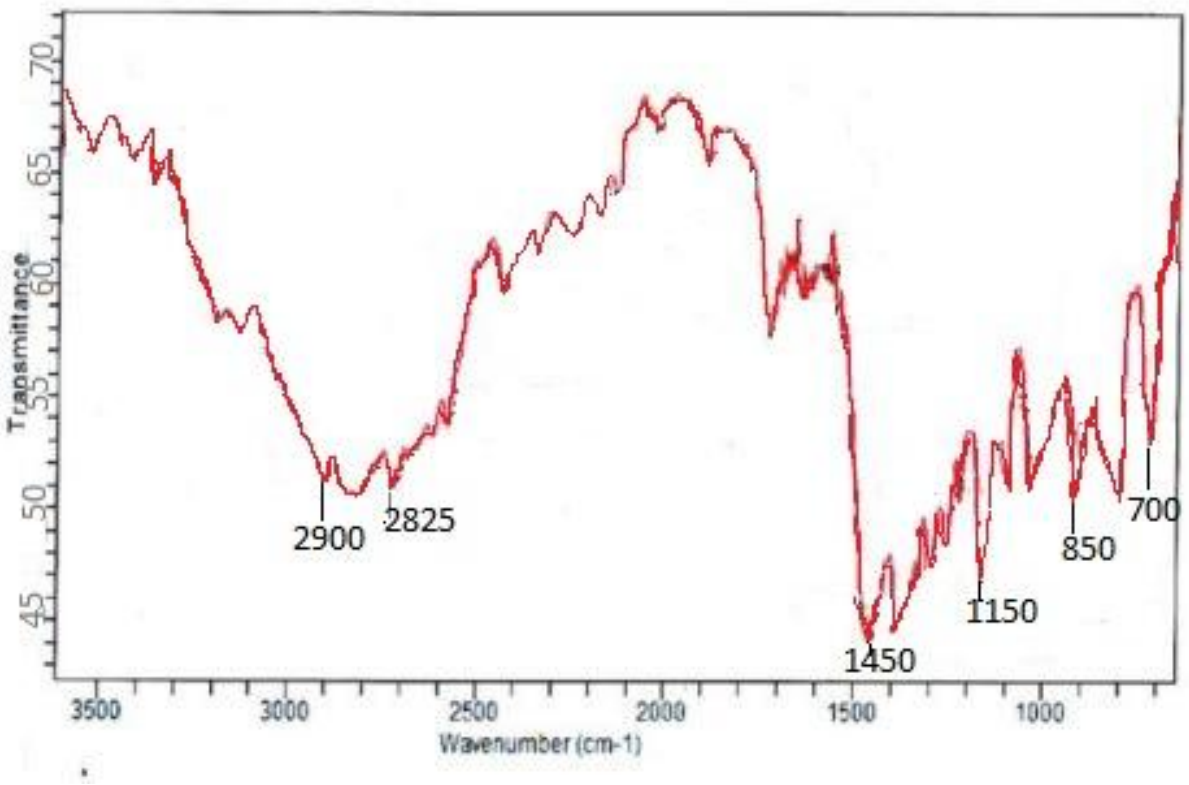


Fig. 26: FTIR spectrum for Microplastic particle obtained in station 2 in July, showing absorbance band at different wave numbers

The microplastics physically identified as filament in station 2 in July sample was not microplastic, therefore, no FTIR spectrum result.

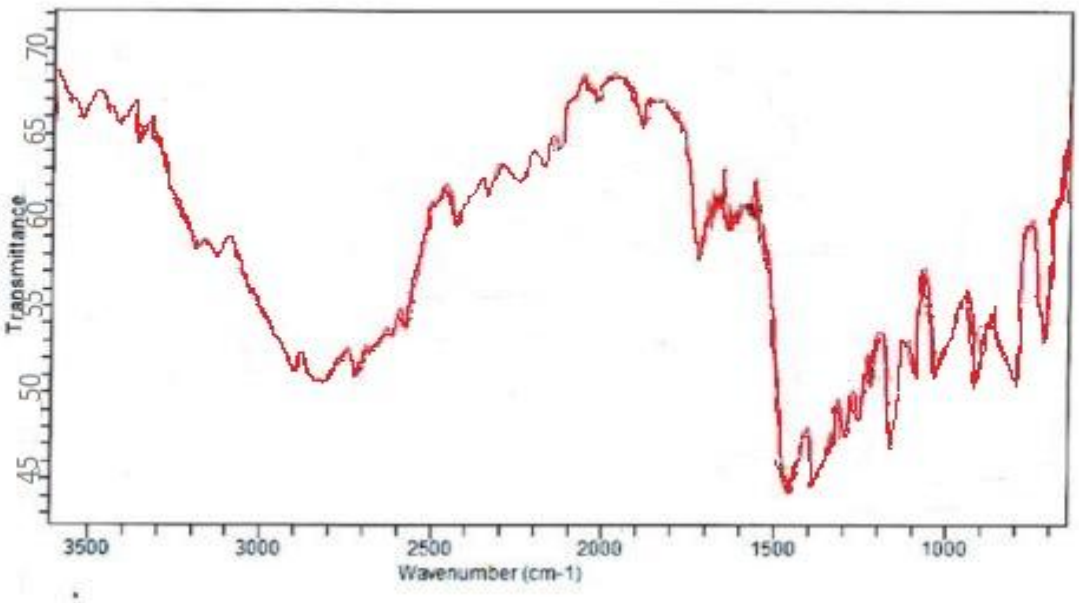


Fig. 27: FTIR spectrum for Microplastic particle obtained in station 2 in July, showing absorbance band at different wave numbers

August

The FTIR spectrum of Microplastic obtained from station two in August, sample can be seen in plate 27 below. There is a peak at 2825cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching. There is also, a peak at 1450cm^{-1} which is a characteristic of CH_2 scissoring. A peak at 1150cm^{-1} is attributed to the asymmetric stretching of oxygen atom. There is a peak at 850cm^{-1} is a characteristics of CH rocking. The peaks at 2825cm^{-1},1450cm^{-1} and 850cm^{-1} are absorbance wave numbers range used to identify polypropylene (PP) compound in FTIR spectrum. Therefore, microplastic of polypropylene identity was confirmed with these absorption wave numbers.

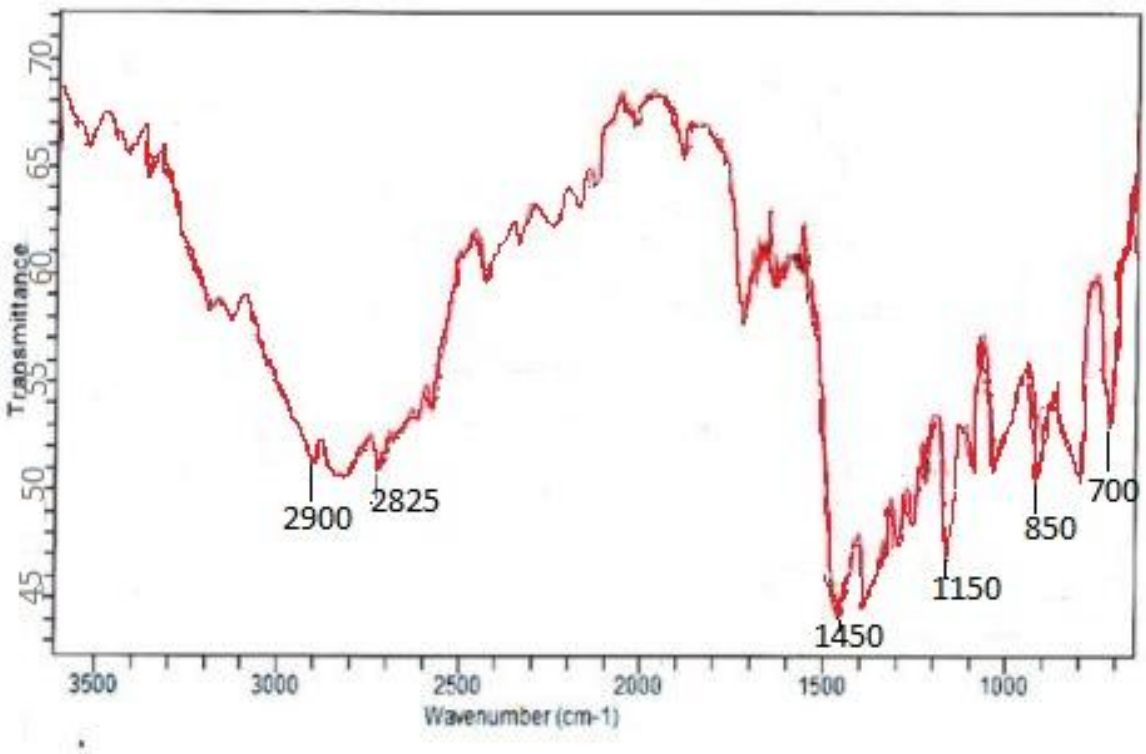


Fig. 28: FTIR spectrum for Microplastic particle obtained in station 2 in August, showing absorbance band at different wave numbers

The microplastics physically identified as filament in station 2 in August sample was not microplastic, therefore, no FTIR spectrum result.

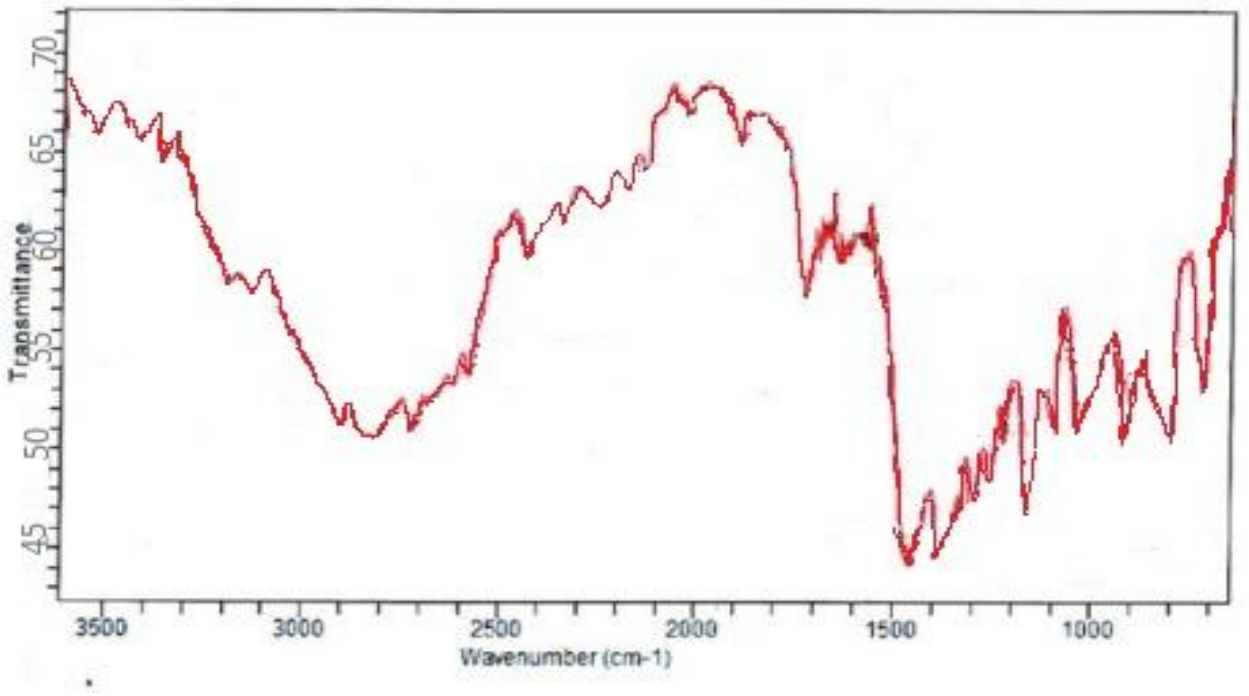


Fig. 29: FTIR spectrum for Microplastic particle obtained in station 2 in August, showing absorbance band at different wave numbers

4.5.2.8 Station three (Ogba Bridge)

June

There is no microplastic particles in June sample, therefore no FTIR spectrum result.

July

The FTIR spectrum of Microplastic obtained from station three in July, sample can be seen in plate 29 below. There is a peak at 2825cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching. There is also, a peak at 1450cm^{-1} which is a characteristic of CH_2 scissoring. A peak at 1150cm^{-1} is attributed to the asymmetric stretching of oxygen atom. There is a peak at 850cm^{-1} is a characteristics of CH rocking. The peaks at 2825cm^{-1}, 1450cm^{-1} and 850cm^{-1} are absorbance wave numbers range used to identify polypropylene (PP) compound in FTIR spectrum. Therefore, microplastic of polypropylene identity was confirmed with these absorption wave numbers.

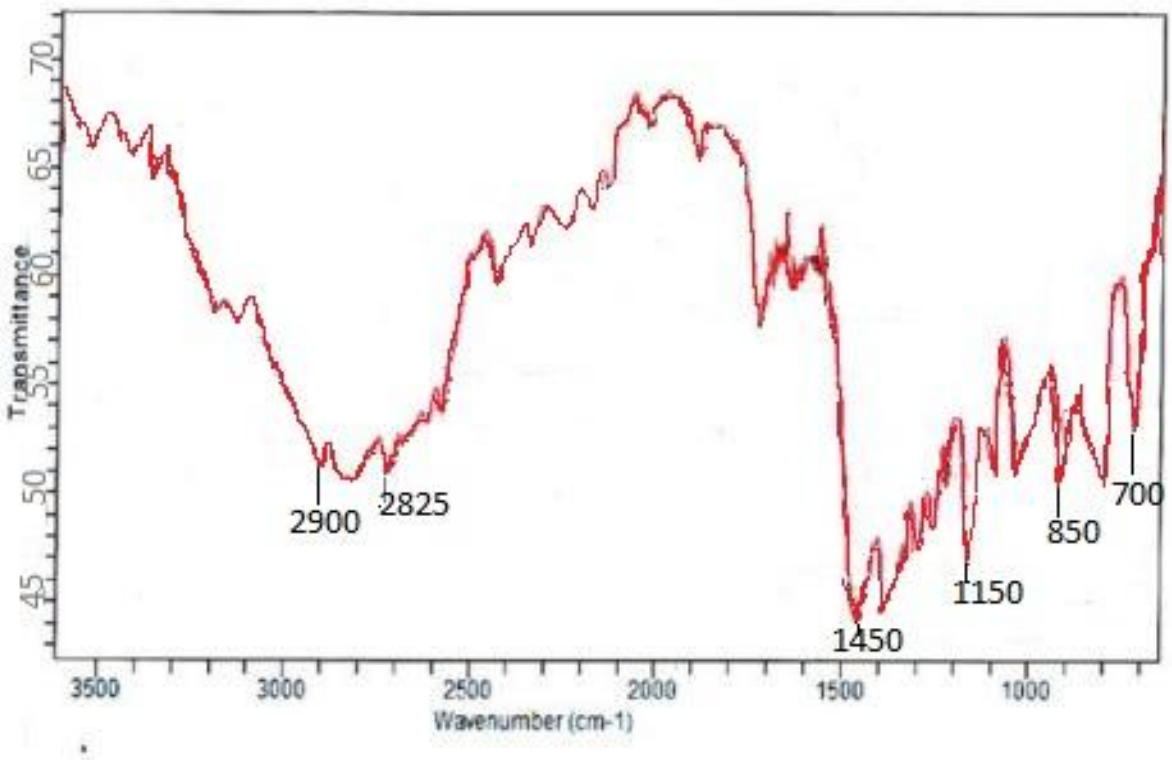


Fig. 30: FTIR spectrum for Microplastic particle obtained in station 3 in July, showing absorbance band at different wave numbers

The microplastics physically identified as filament in station 2 in August sample was not microplastic, therefore, no FTIR spectrum result.

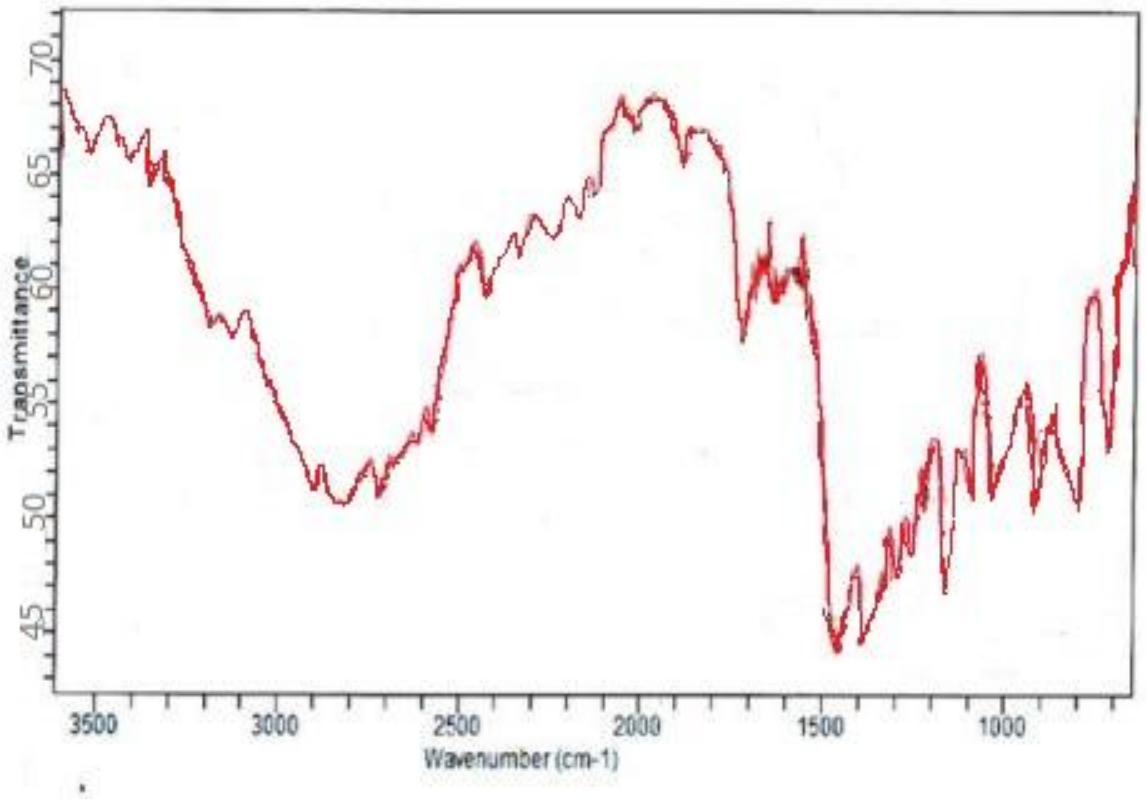


Fig. 31: FTIR spectrum for Microplastic particle obtained in station 3 in July, showing absorbance band at different wave numbers

August

There is no microplastic particles in august sample, therefore no FTIR spectrum result.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Level of Microplastic on *Clarias gariepinus*

Microplastics have been found in edible fish, according to various research, and as a result of biomagnifications, MPs penetrate human systems (Alfaro-Núñez *et al*., 2021; Goswami *et al*., 2020; James *et al*., 2020). Microplastics are synthetic, long-chain, and organic polymers that can be found in various ecosystems such as soils, subsurface systems, rivers, lakes, wetlands, oceans, and atmosphere (Kumar *et al*., 2021). The small size, microplastics can enter the human food chain through the consumption of seafood as well as other terrestrial food items, and subsequently can have impact on human health.

The level of microplastic varied from station to station and with months. Microplastic levels in station two has the highest abundance than that in station one and three. This can easily be explained by the level of urbanization, economic activities in the area which result in more direct and indirect introduction of plastic particles into the waters. There is a relationship between microplastic in the waters, sediment and fish from Ogba River. It is seen that the station two which had a higher amount of visible plastic particles.

The highest abundance in the month of June was at station three (1.37), while the highest abundance in the month of August occurred at station two (1.40) and the lowest abundance in the month of July is station one (0.60). For the month of August, the highest abundance was at station three (1.49) while the lowest occurred at station one (0.60). The greatest value occurred in station two in the month August, with the lowest occurring in station one in the month of July and August. The highest value for station three occurred in June, with the

lowest value occurring in July. The highest value for station one (0.77) occurred in the month of June and the lowest (0.60) in July and August.

5.2 Level of Microplastic on *Oreochromis niloticus*

The highest abundance in the month of August was at station one (0.86). The highest abundance in the month of July occurred at station one (0.85) and the lowest abundance in the month of June is station one (0.04). For the month of June, the highest abundance was at station three (0.20) while the lowest occurred at station one (0.04). The greatest value occurred in station one in the month July, with the lowest occurring in station one in the month of June. The highest value for station three occurred in August (0.78), with the lowest value occurring in June (0.20). The highest value for station two (0.74) occurred in the month of August and the lowest (0.08) in June.

5.3 Frequency of Occurrence on *Clarias gariepinus*

The frequency of occurrence was relatively higher in the month June. This might be attributable to increased levels of microplastics in the river as a result of increased runoff into the river, drawing from the abundant rain. It was also relatively higher in the month of July except for the month August. The highest value of frequency occurrence was in station three (1.03) in the month of June, while the lowest value of frequency occurrence was in station one (0.40) in the month of August. The highest value for station two (0.92) occurred in July, with the lowest value occurring in August (0.66). This might be attributed to the fact that station three is more susceptible to improper plastic and waste disposal.

5.4 Frequency of Occurrence on *Oreochromis niloticus*

The frequency of occurrence was relatively higher in the month June, as a result of increased runoff into the river, drawing from the abundant rain. The highest value of frequency occurrence was in station two (0.56) in the month of June, while the lowest value of frequency occurrence was in station three (0.36) in the month of August. The highest value for station three (0.55) occurred in June, with the lowest value occurring in August (0.36). the highest value in station one occurred in July (0.39), while the lowest values occurred in June (0.37) and August (0.37).

5.5 Plastic load on *Clarias gariepinus*

The plastic load was relatively higher in the month of June, this may be as a result of the increased levels of Microplastic in the river. In the month of July, it was relatively high except for the month of August. The highest value of plastic load is in station three (1.03) in the month of June. The lowest value of plastic load was in station one (0.40) in the month of August. It was low in the station one (0.40) in the month of August while it was relatively high in June (0.70) in station one.

5.6 Plastic Load on *Oreochromis niloticus*

The highest value of plastic load is in station one (0.74) in the month of August. The lowest value of plastic load was in station one (0.05) in the month of June. The highest value of plastic load is in station two (0.45) in the month of August. The lowest value of plastic load was in station two (0.35) in the month of July. The highest value of plastic load is in station three (0.63) in the month of August. The lowest value of plastic load was in station three (0.05) in the month of June.

5.7 Physical Classification

The dominant microplastic particles in *Clarias gariepinus* in this study were fragments, with a small percentage of fibre, pellets, foam and filaments. While Majority of the microplastic particles in *Oreochromis niloticus* in this study was filament. These potentially coming from plastic bags, soft food packaging, acrylate and paint chips etc. It is well known that microplastics are accumulated in some organs of the fish body. Fish may consume microplastics by mistake since the sizes of microplastics are similar to the food particles. Previous field studies have revealed microplastic ingestion by many commercial fish species from the Yellow Sea (Sun *et al.,* 2019), the Bohai Sea (Wang *et al.,* 2021), the North Sea (Kühn *et al.,* 2020), the East China Sea (Wu *et al.,* 2020), and the North America (Baechler *et al.,* 2020).

5.8 Chemical classification

The microplastic particles produced FTIR results that showed they were made of polypropylene and polyethene. Microplastic of polyethylene identity was more prevalent. From the FTIR result in *Clarias gariepinus*, while in microplastic of polypropylene identity was confirmed more in *Oreochromis niloticus.* it is observed that there is a clear pattern to the type of microplastic found at each station.

CHAPTER SIX

6.1 Summary

This study establishes the level of microplastic concentrations in *Clarias gariepinus* and *Oreochromis niloticus* from Ogba River, Benin City, Nigeria. It also determines the frequency of occurrence and plastic load. It also identifies and classifies the microplastic based on physical and chemical characteristics.

6.2 Conclusion

From the study, it can be concluded that there is the occurrence of microplastic in Ogba River which is seen in the amount of Microplastic found in *Clarias gaariepinus* and *Oreochromis niloticus* harvested from the river. The microplastic type based on physical characteristics includes filaments, foam, fragments, pellets and fibre, with fragments being the most occurring. Based on chemical characteristics, there are two types, namely polypropylene and polyethene. The level of microplastic in the river has a relationship with the amount of rainfall, population and waste disposal practices in the area.

6.3 Recommendation

From the findings of this study, the following recommendation can be made;

1. In determining safe consumption level for fish contaminated with microplastics.
2. Further studies need to be carried out to estimate the daily intake of microplastic consumption.
3. Proper waste management services and practices should be implemented by the Government in all level, as improper waste disposal is the major source of plastics that find their way to water bodies.

4. Government on all levels, NGOs, civil society organizations, University and tertiary institutions should carry out sensitization and awareness campaigns to put into the consciousness of the populace, the need for proper waste disposal.

5. Frequent water body clean-up operations should be carried out to remove plastics that may have found a way into water bodies.

It is also recommended to study the waste management and plastic degradation process to have complete idea about microplastic sources.

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GenStat Release 12.1 (PC/Windows Vista) 26 September 2023 09:04:12

Registered to: The NULL Corporation

GenStat Twelfth Edition
GenStat Procedure Library Release PL20.1

```
 1  %CD 'C:/Users/Anthony/Documents'
 2  "Data taken from unsaved spreadsheet: New Data;1"
 3  %PostMessage 1129; 0; 83588512 "Sheet Update Completed"
 4  "Data taken from unsaved spreadsheet: New Data;1"
 5  %PostMessage 1129; 0; 83588512 "Sheet Update Completed"
 6  "Data taken from unsaved spreadsheet: New Data;1"
 7  %PostMessage 1129; 0; 83588512 "Sheet Update Completed"
 8  "Data taken from unsaved spreadsheet: New Data;1"
 9  %PostMessage 1129; 0; 83588512 "Sheet Update Completed"
10  "Data taken from unsaved spreadsheet: New Data;1"
11  DELETE [REDEFINE=yes] MONTH,Station_1,Station_2,Station_3
12  UNITS [NVALUES=*]
13  TEXT [NVALUES=9] MONTH
14  READ MONTH
```

Identifier	Minimum	Mean	Maximum	Values	Missing
MONTH				9	0

```
17  TEXT [NVALUES=9] Station_1
18  READ Station_1
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Station_1				9	0

```
20  TEXT [NVALUES=9] Station_2
21  READ Station_2
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Station_2				9	0

```
23  TEXT [NVALUES=9] Station_3
24  READ Station_3
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Station_3				9	0

```
26
27 %PostMessage 1129; 0; 83588512 "Sheet Update Completed"
28 "Data taken from unsaved spreadsheet: New Data;1"
29 DELETE [REDEFINE=yes] MONTH,Station_1,Station_2,Station_3
30 UNITS [NVALUES=*]
31 FACTOR [MODIFY=yes; NVALUES=9; LEVELS=3; LABELS=!t('August,2023',\
32 'July,2023','June,2023'); REFERENCE=1] MONTH
33 READ MONTH; FREPRESENTATION=ordinal
```

Identifier	Values	Missing	Levels
MONTH	9	0	3

```
35 VARIATE [NVALUES=9] Station_1
36 READ Station_1
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Station_1	0.0000	0.5844	1.000	9	0

```
38 VARIATE [NVALUES=9] Station_2
39 READ Station_2
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Station_2	0.01000	0.4378	0.8700	9	0

```
41 VARIATE [NVALUES=9] Station_3
42 READ Station_3
```

Identifier	Minimum	Mean	Maximum	Values	Missing
Station_3	0.1100	0.5389	0.8900	9	0

```
44
45 %PostMessage 1129; 0; 83588512 "Sheet Update Completed"
46 DESCRIBE [SELECTION=mean,median,min,max,q1,q3,var,sd,sem; GROUPS=MONTH] Station_1,\
47 Station_2,Station_3
```

Summary statistics for Station_1: MONTH August,2023

Mean =	0.85
Median =	0.93
Minimum =	0.62
Maximum =	1
Lower quartile =	0.698
Upper quartile =	0.983
Standard deviation =	0.202
Standard error of mean =	0.117
Variance =	0.0409

Summary statistics for Station_1: MONTH July,2023

Mean =	0.867
Median =	0.9
Minimum =	0.7
Maximum =	1
Lower quartile =	0.75
Upper quartile =	0.975
Standard deviation =	0.153
Standard error of mean =	0.0882
Variance =	0.0233

Summary statistics for Station_1: MONTH June,2023

Mean =	0.0367
Median =	0.01
Minimum =	0
Maximum =	0.1
Lower quartile =	0.0025
Upper quartile =	0.0775
Standard deviation =	0.0551
Standard error of mean =	0.0318
Variance =	0.00303

Summary statistics for Station_2: MONTH August,2023

Mean =	0.747
Median =	0.8
Minimum =	0.57
Maximum =	0.87
Lower quartile =	0.627
Upper quartile =	0.853
Standard deviation =	0.157
Standard error of mean =	0.0906
Variance =	0.0246

Summary statistics for Station_2: MONTH July,2023

Mean =	0.48
Median =	0.33
Minimum =	0.29
Maximum =	0.82
Lower quartile =	0.3
Upper quartile =	0.698
Standard deviation =	0.295
Standard error of mean =	0.170

Variance =	0.0871

Summary statistics for Station_2: MONTH June,2023

Mean =	0.0867
Median =	0.02
Minimum =	0.01
Maximum =	0.23
Lower quartile =	0.0125
Upper quartile =	0.178
Standard deviation =	0.124
Standard error of mean =	0.0717
Variance =	0.0154

Summary statistics for Station_3: MONTH August,2023

Mean =	0.787
Median =	0.87
Minimum =	0.6
Maximum =	0.89
Lower quartile =	0.667
Upper quartile =	0.885
Standard deviation =	0.162
Standard error of mean =	0.0935
Variance =	0.0262

Summary statistics for Station_3: MONTH July,2023

Mean =	0.627
Median =	0.76
Minimum =	0.33
Maximum =	0.79
Lower quartile =	0.438
Upper quartile =	0.782
Standard deviation =	0.257
Standard error of mean =	0.149
Variance =	0.0662

Summary statistics for Station_3: MONTH June,2023

Mean =	0.203
Median =	0.2

Minimum =	0.11
Maximum =	0.3
Lower quartile =	0.133
Upper quartile =	0.275
Standard deviation =	0.0950
Standard error of mean =	0.0549
Variance =	0.00903

```
48  "One-way design"
49  DELETE [REDEFINE=yes] _ibalance
50  A2WAY [PRINT=aovtable,information,means; TREATMENTS=MONTH; FPROB=yes; PSE=diff,lsd,\
51  means; LSDLEVEL=5; PLOT=*; EXIT=_ibalance] Station_1; SAVE=_a2save
```

Analysis of variance

Variate: Station_1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
MONTH	2	1.35069	0.67534	30.12	<.001
Residual	6	0.13453	0.02242		
Total	8	1.48522			

Information summary

All terms orthogonal, none aliased.

Tables of means

Variate: Station_1

Grand mean 0.584

MONTH	August,2023	July,2023	June,2023
	0.850	0.867	0.037

Standard errors of means

Table	MONTH
rep.	3
d.f.	6
e.s.e.	0.0865

Standard errors of differences of means

Table	MONTH
rep.	3

d.f.	6
s.e.d.	0.1223

Least significant differences of means (5% level)

Table	MONTH
rep.	3
d.f.	6
l.s.d.	0.2992

```
 52  IF _ibalance.eq.0 .OR. _ibalance.eq.1
 53    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 54    AKEEP [SAVE=_a2save[2]] MONTH; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 55    AKEEP [SAVE=_a2save[2]] #_resid; DF=_rdf
 56    AMCOMPARISON [METHOD=duncan; DIRECTION=ascending; PROB=0.05] MONTH
```

Duncan's multiple range test

MONTH

	Mean	
June,2023	0.0367	a
August,2023	0.8500	b
July,2023	0.8667	b

```
 57  ENDIF
 58  SET [IN=*]
 64  "One-way design"
 65  DELETE [REDEFINE=yes] _ibalance
 66  A2WAY [PRINT=aovtable,information,means; TREATMENTS=MONTH; FPROB=yes; PSE=diff,lsd,\
 67  means; LSDLEVEL=5; PLOT=*; EXIT=_ibalance] Station_2; SAVE=_a2save
```

Analysis of variance

Variate: Station_2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
MONTH	2	0.66142	0.33071	7.80	0.021
Residual	6	0.25433	0.04239		
Total	8	0.91576			

Information summary

All terms orthogonal, none aliased.

Message: the following units have large residuals.

units 5 0.340 s.e. 0.168

Tables of means

Variate: Station_2

Grand mean 0.438

MONTH	August,2023	July,2023	June,2023
	0.747	0.480	0.087

Standard errors of means

Table	MONTH
rep.	3
d.f.	6
e.s.e.	0.1189

Standard errors of differences of means

Table	MONTH
rep.	3
d.f.	6
s.e.d.	0.1681

Least significant differences of means (5% level)

Table	MONTH
rep.	3
d.f.	6
l.s.d.	0.4113

```
68  IF _ibalance.eq.0 .OR. _ibalance.eq.1
69    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
70    AKEEP [SAVE=_a2save[2]] MONTH; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
71    AKEEP [SAVE=_a2save[2]] #_resid; DF=_rdf
72    AMCOMPARISON [METHOD=duncan; DIRECTION=ascending; PROB=0.05] MONTH
```

Duncan's multiple range test

MONTH

	Mean	
June,2023	0.0867	a
July,2023	0.4800	ab
August,2023	0.7467	b

```
 73  ENDIF
 74  SET [IN=*]
 80  "One-way design"
 81  DELETE [REDEFINE=yes] _ibalance
 82  A2WAY [PRINT=aovtable,information,means; TREATMENTS=MONTH; FPROB=yes; PSE=diff,lsd,\
 83  means; LSDLEVEL=5; PLOT=*; EXIT=_ibalance] Station_3; SAVE=_a2save
```

Analysis of variance

Variate: Station_3

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
MONTH	2	0.54509	0.27254	8.06	0.020
Residual	6	0.20300	0.03383		
Total	8	0.74809			

Information summary

All terms orthogonal, none aliased.

Tables of means

Variate: Station_3

Grand mean 0.539

MONTH	August,2023	July,2023	June,2023
	0.787	0.627	0.203

Standard errors of means

Table	MONTH
rep.	3
d.f.	6
e.s.e.	0.1062

Standard errors of differences of means

Table	MONTH
rep.	3
d.f.	6
s.e.d.	0.1502

Least significant differences of means (5% level)

Table	MONTH
rep.	3
d.f.	6
l.s.d.	0.3675

```
 84  IF _ibalance.eq.0 .OR. _ibalance.eq.1
 85    DELETE [REDEFINE=yes] _mean, _rep, _var, _rdf
 86    AKEEP [SAVE=_a2save[2]] MONTH; MEAN=_mean; REP=_rep; VARIANCE=_var; RTERM=_resid
 87    AKEEP [SAVE=_a2save[2]] #_resid; DF=_rdf
 88    AMCOMPARISON [METHOD=duncan; DIRECTION=ascending; PROB=0.05] MONTH
```

Duncan's multiple range test

MONTH

	Mean	
June,2023	0.2033	a
July,2023	0.6267	b
August,2023	0.7867	b

```
 89  ENDIF
 90  SET [IN=*]
```