MICROPLASTIC POLLUTANTS IN *Clarias gariepinus* FROM IKPOBA RIVER, BENIN CITY, EDO STATE, NIGERIA.

BY

Collins OMOZOGIE,

AGR1700262

DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT

FACULTY OF AGRICULTURE

UNIVERSITY OF BENIN

BENIN CITY

SEPTEMBER, 2023

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

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CERTIFICATION

This is to certify that this project was carried out by Omozogie Collins, (AGR1700262) in the department of Aquaculture and Fisheries Management in the Faculty of Agriculture, University of Benin, Benin City, Nigeria.

Dr. O. M. Wangboje **Date**
Project Supervisor

Dr. O. M Nwangboje **Date**

Head of Department

DEDICATION

I dedicate this project to my Heavenly Father for His gracious goodness towards me and also to my parents; Mr. Felix Ogbewele and Mrs. Martha Omozogie for their encouragements and support throughout the duration of my program in the University of Benin.

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My sincere gratitude goes to God Almighty for His grace, faithfulness and strength throughout this academic year. I recognize that I do not deserve it, but He freely gave me anyway.

I wholeheartedly appreciate my Dad, Mum and siblings, whose support has made all of this possible. I will be forever grateful for all the time you all you have done for me, along the way.

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ABSTRACT

One of the most recent and emerging contaminants today, is plastic. These plastics through improper waste disposal and runoff, find their way to water bodies. This plastic when they fragment or occur in very small sizes (<5mm) are termed microplastic. They can be classified on physical characteristics or chemical characteristics. They pose risk to both fish and consumers of the fish.

The fish samples were analysed within 24hrs of collection. The fish were digested using 10% KOH, and purified using H_2O_2. The filtrates were examined under microscope to identify the microplastic particles, which were physically confirmed using the hot needle method and confirmed chemically using Fourier transfer infrared spectroscopy.

Microplastics occur at all stations through the three months under study. The microplastics found based on morphological characteristics are pellet, foams, fibre, filaments, and fragments, while on polymer characteristics, there were two namely; polypropylene and polyethene. The type of microplastic prevalent in each station had a relationship to the prevalent economic activities at the watershed. For example, at station 3 (Ikpoba bridge), the prevalent economic activities at the water fronts are car and rug washes, the prevalent plastic is polypropylene. The level of microplastic found in each station had a relationship with the population and rainfall, as it increases with increasing population and increasing rainfall. The plastic load for all samples was 1.37 microplastics particles per fish which is in agreement with the findings of several authors. The frequency of occurrence of microplastic ingestion of 59.2% which is far lower than those obtained by various authors which give 96% and 100% respectively.

CHAPTER ONE

1.0 INTRODUCTION

All freshwater, brackish, and marine water sources in the world have experienced some level of pollution due to human activity ever since the beginning of time. The majority of water bodies are mostly self-cleaning, but because of the rapid growth of the human population and the development of manufacturing technologies, the limit of most water bodies is almost, or has been exceeded. Microplastic is one of the more recent contaminants that has raised concerns. Plastic is now widely used in daily life. However, due to its rapid production, resistance to degradation, unsustainable usage, and inefficient waste management, plastic debris has accumulated extensively in aquatic habitats (Barnes *et al*., 2009).

Plastic is ubiquitously distributed, due to a variety of characteristics like its durability, lightweight, remarkable plasticity and flexibility, thermal and electrical insulation, corrosion resistance, and affordability. It is used in practically every industry. Such as textile, packaging, fashion, agricultural, transportation, and electronics industries. However, these qualities that make plastic so beneficial also make it a problem. This is because plastic, once introduced into the environment, can gradually disintegrate and produce various smaller plastic debris as a result of physical, chemical, and biological processes. (Zhang *et al.,* 2021). These smaller particles are called microplastics (Kershaw and Rochman, 2016).

Microplastics (MPs) are tiny plastic particles up to 5mm in diameter (Kershaw and Rochman, 2016; United Nations Environment Programme, 2022). Several studies have shown that microplastics are present in all areas of any given body of water, including sediments, the water surface, water columns, deep-sea floor, and aquatic animals (Reisser *et al*., 2013; Eriksen *et al*., 2014).

Microplastics come from both primary and secondary sources. Primary sources of microplastic are small-sized manufactured plastic products as is often the case with cosmetics, while the secondary sources are those formed as a result of chemical, biological, or physical weathering and fragmentation of larger-sized plastics (Carr *et al*., 2016; Lebreton *et al*., 2017). They can be found in various forms, including scrubbers, fragments, pellets, fibres, beads, foams, and films (Lusher *et al*., 2017).

Microplastics, especially when coloured, can be similar to larvae of several organisms, including plankton and this can result in their ingestion by other aquatic organisms (Besseling *et al*., 2014; Kaposi *et al*., 2014; Tanaka and Takada, 2016). Through this means, they enter the food chain from producers to top predators (Heinrich *et al.,* 2022). They have been identified in the alimentary canal of marine organisms, including whales, fish, and larvae (Burkhardt-Holm and N'Guyen, 2019). It is possible for toxic chemicals found in microplastics, which come from their manufacturing processes, to be absorbed into the aquatic environment (Koelmans *et al*., 2013; Nakashima *et al*., 2016), as well as for aquatic organisms to be directly exposed to these chemicals by ingesting the microplastics. This has been confirmed by numerous studies (Tanaka *et al*., 2015; Koelmans *et al*., 2016; Besseling *et al*., 2017). Chemical hazards eventually result from harmful compounds moving from microplastics into natural organisms and continuing up the food chain until they reach humans (Rochman *et al*., 2015; Wright and Kelly, 2017).

Microplastic-induced impairments in fish species can range from minimal biological systems disturbance to substantial unfavourable consequences that resulted in mortality (Mallik *et al*., 2021). growth retardation, hormone disruption, metabolic perturbation, oxidative stress, immunological and neurotoxicity malfunction, and genotoxicity behavioural alterations can all result from the buildup of MPs (Güven *et al*., 2017). Furthermore, a build-up of microplastics in the gut may lead to intestinal blockages, leading to starvation, reduced breeding, increased immobility, and the death of the organism (Gatidou *et al*., 2018). Studies on the toxicity of microplastics have shown that even their simple presence in aquatic ecosystems can have negative effects (Barboza *et al*., 2018; Syakti *et al*., 2019).

The impact of bioaccumulation of microplastics on humans has not yet been fully understood, However, animal studies have shown that microscopic particles are capable of passing through cell membranes, the blood-brain barrier, and the placenta. These effects include oxidative stress, DNA damage, cell damage, inflammation, and impaired energy allocation, which are similar to those reported for marine organisms (Vethaak and Leslie, 2016).

1.2 JUSTIFICATION OF THE STUDY

In the early 2000s, the issue of microplastics in aquatic environments began gaining attention. Researchers and scientists from all over the world have been interested in the contamination of aquatic habitats by microplastics (MPs), with a particular focus on the effects on food security and human health.

Numerous studies have revealed that microplastic is more prevalent in river segments close to inhabited and urban regions. This can be attributed to the transfer of household and industrial waste directly and indirectly into rivers. These rivers serve as a source of water, animal protein, and economic uses. Communities around rivers tend to depend on fish caught from these rivers for their animal protein. However, the introduction of microplastics into these waters through indiscriminate waste disposal and other economic activities that takes place at the fronts of these rivers such as car wash businesses, rug washing business, etc., calls for concern. Over the past few decades, concerns over microplastic pollution in the aquatic ecosystem have increasingly gained more attention. However, the amount of research done in freshwater environments is nothing compared to that in marine environments. Ikpoba River in Benin City, Edo state, Nigeria, is no different in this regard. Hence, this study aims to investigate the occurrence of microplastics in freshwater fish *Clarias gariepinus* in Ikpoba River.

Fish are an important biological element of freshwater ecosystems with significant economic and nutritional value worldwide (Oliveira *et al*., 2017). A good portion of people living in communities around Ikpoba River depends on the fish caught from the river for their animal protein. One of the most dominant fish species in Ikpoba River is *C. gariepinus*. It is one of the most sought-after fish in Nigeria and the most cultured fish species in Nigeria as well. This is because it is hardy, tolerates a wide range of water quality parameters, accepts different feedstuffs, and has a good food conversion ratio.

C. gariepinus is a bottom omnivorous feeder. Hence, there may be a high risk of bioaccumulation in *C. gariepinus* due to its position in the food web of Ikpoba River ecosystem. The continuous consumption of this fish by consumers will lead to biomagnification and possible toxicity in man. While studies exist on other pollutants such as heavy metals and polycyclic aromatic hydrocarbons, from the available literature, there has been no study on microplastic in *C. gariepinus* harvested from Ikpoba River. This study will thus attempt to fill this existing gap in knowledge.

1.2. Aim and Objectives of The Study

The aim of the study is to determine microplastic pollutants in *C. gariepinus* from Ikpoba River, Benin City, Edo State, Nigeria.

The specific objectives of the study are to:

1. Determine the level of microplastics in *C. gariepinus* in Ikpoba River.
2. Identify the types of microplastics based on physical composition in *C. gariepinus* in Ikpoba River.
3. Identify the types of microplastics based on chemical composition in *C. gariepinus* in Ikpoba River

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Plastic Production

Plastics are used daily throughout the world. The word plastic is a common term that is used for many materials of a synthetic or semi-synthetic nature. The term was derived from the Greek *plastikos*, which means "fit for moulding". Plastics are a wide variety of combinations of properties when viewed as a whole. They are used for shellac, cellulose, rubber, and asphalt. We also synthetically manufacture items such as clothing, packaging, automobiles, electronics, aircraft, medical supplies, and recreational items. The list could go on and on and much of what we have today would not be possible without plastics (Andrady *et al*., 2009).

In recent years, the amount of plastic in the environment has become a global concern. With the world population approaching eight billion, more and more plastic and plastic-derived products are being used and discarded. According to the statistical analysis, the global production of plastics reached 370 million tons in 2019 (PlasticsEurope, 2020). An estimated 367 million tonnes (367 billion kg) of plastic were produced in 2020 alone – about 12 tonnes (12,000kg) of plastic waste produced every second that year. With about 2.5 million tonnes of plastic waste annually (Temitope, 2022). Half of all plastics ever manufactured have been made in the last 15 years. Every year, about 8 million tons of plastic waste escapes into the oceans from coastal nations. That's the equivalent of setting five garbage bags full of trash on every foot of coastline around the world (Parker, 2019).

The expectation is that the production will have quadrupled (compared to 2019) to about 1480 million tons by 2050. About 40% of all plastic products are thrown away within one month. Between the 1950s and 2017, an estimated 9.2 billion tons of plastic have been manufactured.

Nigeria ranks ninth globally among countries with the highest contributions to plastic pollution. Unfortunately, over 88% of the plastic waste generated in Nigeria is not recycled. Instead, much of it ends up in water bodies – rivers, lakes, drains, lagoons and the ocean (Temitope, 2022) The low level of recycling – less than 12% – and inadequate waste collection pose a huge threat to plastic pollution management in Nigeria.

2.2 Sources of Plastic in Nigeria

Plastic in our oceans can arise from both land-based or marine sources. Plastic pollution from marine sources refers to the pollution caused by fishing fleets that leave behind fishing nets, lines, ropes, and sometimes abandoned vessels. There is often intense debate about the relative importance of marine and land sources for ocean pollution.

At the global level, best estimates suggest that approximately 80% of ocean plastics come from land-based sources and the remaining 20% from marine sources. Of the 20% from marine sources, it's estimated that around half (10 percentage points) arises from fishing fleets (such as nets, lines, and abandoned vessels). This is supported by figures from the United Nations Environment Programme (UNEP) which suggests abandoned, lost or discarded fishing gear contributes approximately 10% to total ocean plastics (United Nations Environment Programme, 2022).

Several studies indicate that water sachets and shopping bags are the major constituents of plastic waste in Nigeria. Educational institutions, markets, and households are among the major routes. They are indirect routes of entry of plastic waste, particularly into water bodies in Nigeria. The sources of plastic waste included tyre wear, cigarette butts, and electronic waste (mobile phone components, electronics, and electrical appliances). Others were fishing ropes, biosolids, cosmetics, clothing, food packs, and cellphone bags (Temitope, 2022).

2.3 Formation of Microplastics

There is an ongoing debate about the appropriate definition of microplastics. So far, the most widely used definition is that microplastics are particles less than 5 mm in their longest dimensions. This definition has been adopted in practical terms as it is considered the size under biological availability to aquatic biota occurs. (Joint Group of Experts on the Scientific Aspect of Marine Environmental Protection, 2015; The European Food Safety Authority, 2016; Lusher *et al.*, 2017).

Large plastics in the marine environment are broken down into smaller ones by mechanical action (plastic ageing process and forced crushing by weather), photo-oxidation, and biodegradation, resulting in the formation of microplastics (Novotna *et al.*, 2019). Not all microplastics are caused by fragmentation but may arise from the wearing out of car tyres, wearing and washing synthetic clothing, rinsing off toiletries and cosmetics, and the 'spilling' of nurdles, small plastic granules, by the plastics industry. Another widely accepted theory holds that large pieces of plastic are broken into smaller ones by mechanical wave stresses and ultraviolet (UV) radiation (Woodall *et al.*, 2014). The abundance of MP counts increases with decreasing size (Novotna *et al.*, 2019).

2.4 Types of Microplastic

Microplastic occurs and enters the aquatic environment in several forms. They can be classified based on origin, composition, and size/morphology. Based on their origin, they are classified as primary and secondary sources (Carr *et al.*, 2016). Based on morphology, they are classed into four types: pellets, fibres, films, and fragments (Harding, 2016).

2.4.1 Classification Based on Origin

2.4.1.1 Primary Microplastics (PMPs)

PMPs are artificial industrial products manufactured in a size range below 5mm. These microplastics (MP) result in the environment from their direct release into the water through the discharge of domestic sewage (Cole *et al*., 2011). They are mostly manufactured for their abrasive qualities (Joint Group of Experts on the Scientific Aspect of Marine Environmental Protection, 2015; Novotna *et al*., 2019). They include remnants of toothpaste, hair gel, cleansing milk, particle air fresheners pre-production resin pellets (Lusher *et al*., 2017), plastic nurdles, microbeads from personal care products (Carr *et al.,* 2016), microbeads, industrial scrubbers and industry abrasives (Joint Group of Experts on the Scientific Aspect of Marine Environmental Protection, 2015).

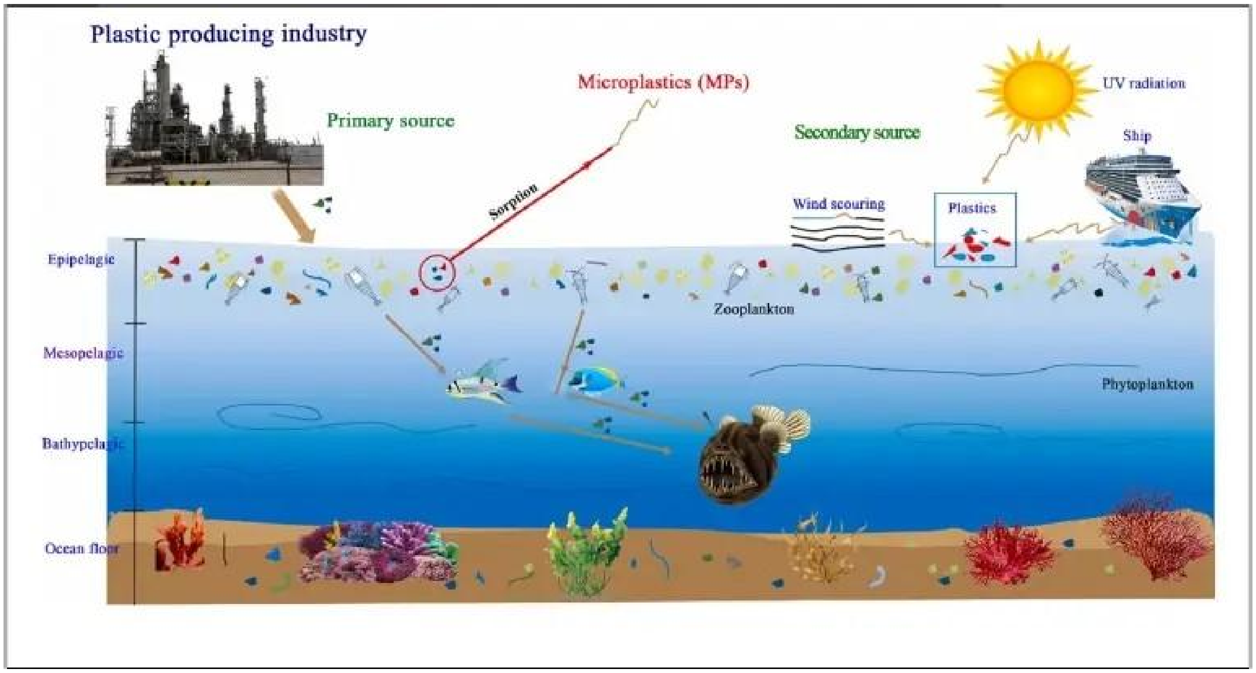

Fig 1: Sources of microplastics (Bhuyan, 2022)

2.4.1.2 Secondary Microplastics (SMPs)

These microplastics result from the gradual degradation, fragmentation, and breakdown of large plastic materials, due to weathering processes such as wave action, wind blasting, etc. (Joint Group of Experts on the Scientific Aspect of Marine Environmental Protection, 2015). Secondary microplastics (SMPs) are derived from meso (5 mm–25 mm)/macro (>25 mm) plastic waste through physical, chemical, and biological processes (Novotna *et al*., 2019). Potential sources of microplastic include tyre dust, synthetic textile, paints, fragmentation and degradation of meso (5 mm–25 mm)/macro (>25 mm) plastic (Browne *et al*., 2011; Napper and Thompson, 2016; Eunomia, 2017; Joint Group of Experts on the Scientific Aspect of Marine Environmental Protection, 2019). The generation of secondary microplastics may occur during the use of plastic or after disposal into the environment (Joint Group of Experts on the Scientific Aspect of Marine Environmental Protection, 2019).

2.4.2 Classification Based on Morphology

Table 1: Classification of plastic based on Morphology

Shape	**Other Terms Used**
Fragments	Irregularly shaped particles, crystals, fluff, powder, granules, shavings, flakes, films
Fibre	Filaments, microfibres, strands, threads
Foams	Polystyrene, Expanded Polystyrene
Beads	Grains, spherical microbeads, microspheres
Pellets	Resin, Pellets, nurdles, pre-production pellets, nibs

Source: Adapted from Lusher *et al*. (2017)

2.4.3 Classification Based on Polymer

Table 2: Classification of plastic based on polymer composition (Bajt, 2021)

Polymer	Abbreviation	Use
Polyethylene terephthalate	PET	Containers/bottles for beverages (juice, water, beer), detergents, butter jars, plastic film, microwavable packaging
High-density polyethylene	HDPE	Opaque milk, water, and juice containers, detergent and shampoo bottles, garbage bags, yogurt and margarine tubs, molded plastic cases
Low-density polyethylene	LDPE	Bread and frozen food bags, most plastic wraps, and squeezable bottles (honey, mustard), outdoor furniture, floor tiles, shower curtains
Polypropylene	PP	Bottle caps, ketchup bottles, yogurt and margarine containers, medicine and syrup bottles, drinking straws, opaque plastic containers, including baby bottles, plastic pressure pipe system
Polyvinyl chloride	PVC	Toys, clear food and nonfood packaging (e.g., cling wrap), some squeeze bottles, shampoo bottles, cooking oil and butter jars, detergent and window cleaner bottles, shower curtains, medical tubing, and numerous construction products, electrical cable/wire insulation
Polystyrene	PS	Food containers, egg cartons, disposable cups, plates, cutlery, plastic tableware, take-out food containers, plastic cutlery, compact disk cases
Polyamide	PA	Fibers, fishing line, toothbrush bristles, tubing
Polycarbonate	PC	Compact disks, eyeglasses, security windows, traffic lights and lenses

2.5 Deposition and Transportation of Plastic/Microplastic in River/Ocean System

More than half the 9.2 billion tons of plastic that have been produced up to now – about 5 billion tons – has ended up as waste in landfill or has simply ended up in the environment. Of this, between 5 and 13 million tons of plastic enter the oceans. Rivers, stormwater and sewage effluents are a major pathway of plastic debris to the ocean (Browne *et al*., 2011; Napper and Thompson, 2016; Lebreton *et al*., 2017).

Rivers are highly dynamic environments and along its course, a river will be subject to an accumulation of land-derived inputs, for example, road runoff, agricultural runoff, wastewater inputs and litter, all of which can contribute to the burden of microplastics within the watercourse (Lechner *et al*., 2014; Morritt *et al*., 2014; Nizzetto *et al*., 2016; Horton *et al*., 2017). The majority of microplastic particles entering the freshwater environment are likely to be derived from the breakdown of larger items, for example, single-use packaging items, tyre and road paint particles, or fibres from synthetic fabrics (Browne *et al*., 2011; Boucher and Friot, 2017; Horton *et al*., 2017).

Several studies have reported the presence of microplastics in Nigerian aquatic ecosystems, including rivers, lagoons, and coastal waters (Adeogun *et al*., 2020; Nnamdi *et al*., 2020). These microplastics originate from various sources, such as plastic waste, industrial effluents, and sewage discharge (Adeogun *et al*., 2020). The abundance of microplastics in Nigerian waters has raised concerns about their potential impact on aquatic organisms, including fish.

It is assumed that a proportion of microplastics (although not all) entering a river will be buoyant and easily transported downstream. Since the sources of (micro)plastic particles are anthropogenic, a site downstream of populated or industrial areas is likely to contain more microplastics than sites that have been subject to little anthropogenic input (McCormick *et al*., 2014; Dris *et al*., 2015; Horton *et al*., 2017). As such, sites further from the river source would be expected to be subject to a greater variety of inputs (Mani *et al*., 2015).

Propelled by wind, heavy rainfall, and tidal currents, MP contamination (Crawford and Quinn, 2017; Lusher *et al*., 2017; Wolf and Wheeler 2018; Bondelind *et al*., 2020 and Li, Zhang, *et al*., 2020) has spread to remote lakes (Zhang *et al*., 2016), rivers (Castañeda *et al.,* 2014; McCormick *et al.,* 2016; Nel *et al*., 2018), estuarine regions (Lima *et al*., 2014; Sadri and Thompson, 2014; Yonkos *et al*., 2014), seas (. Cózar, *et al*., 2015), oceans (Desforges *et al*., 2014) and even sea ice (Obbard *et al*., 2014). Their small size and relatively low density contribute to their long-range transport (Cózar *et al.,* 2017; Barboza *et al*., 2019) and global distribution (Cózar *et al*., 2014; Suaria *et al*., 2016). This allows them to drift over great distances within aquatic currents or over vast expanses of land by wind (Cole *et al*., 2011; Mathalon and Hill 2014). Plastic that is dumped inappropriately is carried to aquatic systems by pluvial flows (Faure *et al*., 2015)

The route of entry for primary microplastics into the environment will depend on their application. Primary microplastic can enter in the following ways: particles from cosmetic products will usually enter through wastewater; microplastics from abrasive blasting will enter through the atmosphere and wastewater, while primary microplastics used for raw materials may enter the environment through accidental loss during transportation and trans-shipment, or through runoff from processing plants. When too small for retention by wastewater treatment plants, primary microplastics may be passed directly into the oceans or pass through freshwater watercourses to subsequently enter the marine environment (Lusher *et al*., 2017).

There are multiple pathways for the entry of secondary microplastics into the environment, which include (1) particles from textiles may enter through wastewater following washing or through the air when drying (Browne *et al.*, 2011; Napper and Thompson, 2016); (2) weathering of plastics used in agricultural applications may enter the environment through surface runoff from soil; (3) abrasion of tyres during use generates microplastics that enter the environment through air and surface runoff; (4) fragmentation and weathering of items in landfills by UV light which may introduce microplastics into the atmosphere, rivers and the ocean by wind and surface runoff and (5), weathering of plastic litter in coastal areas and beaches which may remain in coastal sediments or be transported further offshore. The main environmental factors related to secondary microplastic generation are UV light exposure, temperature and abrasion (Lusher *et al.*, 2017).

2.6 Microplastic Ingestion

2.6.1 Microplastic Uptake

There is a growing body of evidence for microplastic ingestion by freshwater fish (Sanchez *et al.*, 2014; Biginagwa *et al.*, 2016; Peters and Bratton, 2016; Silva-Cavalcanti *et al.*, 2017). MPs can be taken by fish passively i.e. directly from the seawater (e.g. benthic fish consuming them inadvertently when feeding in sediments, gill water filtration (Graham and Thompson, 2009; Besseling *et al.*, 2013; Wright *et al.*, 2013; Watts *et al.*, 2014, Browne *et al.*, 2015b; Welden and Cowie, 2016a, b)) or actively through ingestion as a result of confusion with prey (Sussarellu *et al.*, 2016; Avio *et al.*, 2015a, Barboza *et al.*, 2020), and through the ingestion of contaminated prey, as suggested in previous studies with fish (Lusher *et al.*, 2013; Lusher, 2015; de Sá *et al.*, 2015; Campbell *et al.*, 2017; Ory *et al.*, 2018).

MPs can adhere to external appendages of aquatic organisms, including setae, swimming legs, and antennules of copepods (Cole *et al*., 2013; 2015). Recent results also indicate that microplastics in marine environments acquire a dimethyl sulphide signature, which acts as a keystone odorant in pelagic food webs (Savoca *et al*., 2016). This would imply that some aquatic organisms may also actively search out and ingest microplastic particles. They can also be taken up by fish through the gills. The uptake of microplastics through gills depends on the microplastic size and the morphology and efficiency of the filtering apparatus (Collard *et al*., 2017b).

Size contributes to prey perception by visual predators and microplastics with size comparable to prey are more prone to be actively ingested by fish (Galloway *et al*., 2017; Lehtiniemi *et al*., 2018). Several studies (De Sá *et al*., 2015; Ory *et al*., 2018a, b) suggest that at least part of microplastics ingested by fish are taken up actively because they were taken as food. In addition to colour, shape, size, and odour may also contribute to microplastic active ingestion by fish (Markic *et al*., 2018).

2.6.2 Factors Affecting Microplastic Ingestion

2.6.2.1 Physical properties

Microplastic size may be the most important factor in determining the range of organisms that ingest them (Andrady, 2011). Biological uptake is primarily dependent on the size of the particle relative to the natural prey items, while particle density determines the position in the water column and therefore the likelihood of encounter by an organism (Desforges *et al*., 2015). Organisms that inhabit surface waters are likely to encounter plastics with a specific density less than that of seawater such as polystyrene (PS), polypropylene (PP), and polyethene (PE), while benthic organisms are susceptible to more dense or fouled plastics, including polyethene terephthalate (PET) and polyvinyl chloride (PVC) (Cole *et al*., 2013).

Over time, particles may be resuspended through bioturbation, storms, or upwelling events and change their physical characteristics such as size, shape, and density. The processes of ageing and weathering contribute to the degradation of MPs and are driven by biotic (e.g.; microbial colonisation) and abiotic (e.g.; photo-oxidation) factors acting on the particle surface, resulting in modified surface topography and changes to the surface chemistry. As the surface area increases, so does the number of sites available for microbial colonisation altering the particle density, buoyancy and sinking rate (Rummel *et al*., 2017).

2.6.2.2 Presence of Biofilms

Biofouling may play a major role in the mistaken identity of plastic as a nutritious food source (Kooi *et al*., 2017). Research suggests that the formation of biofilms may not only increase the likelihood of MP ingestion by altering the vertical distribution of the particle but attract organisms relying on chemoreceptors to select prey through olfactory and gustatory cues. A recent study of foraging shorebirds suggests that the breakdown of biofilms on the plastic surface produces a distinct dimethyl sulphide odour commonly associated with organic matter (Savoca *et al*., 2016). Nelms *et al*., (2016) suggest sea turtles ingest plastics for the same reason, with visual cues also playing an important role. The active selection of fouled plastic particles is not restricted to higher trophic organisms, however, copepods exposed to both clean and fouled plastic particles ingested a higher frequency of aged particles exhibiting a biofilm (Vroom *et al*., 2016).

2.6.2.3 Physiological Traits

Assuming there is exposure, Physiological traits of fish, such as size, may determine whether an individual will ingest microplastics, and the number of particles the fish may ingest. For example, larger fishes will consume more in general due to increased energy demands

(Holker and Breckling, 2001), which increases their potential for ingestion of microplastic particles. Therefore, susceptibility to ingestion and volume of uptake, given exposure, will be determined by physiological characteristics. Combined, these two factors (exposure and likelihood of ingestion) are expected to determine the number of particles that an individual fish can ingest.

2.7 Bioavailability of Microplastics

The bioavailability of MPs increases with their decreasing size, making them easily available for lower trophic-level organisms (Browne *et al.*, 2008; Wright *et al.*, 2013)**.** Their tiny size and low density help them with long-range transport with prolonged time in water and other media, with at least a part of them being available to a variety of organisms or species level, including fishes, which form the human diet.

Microplastics can remain for many years in marine and other environments (Strungaru *et al.* 2018; Barboza *et al.*, 2019), at least part of them being available to a wide range of organisms, including species widely used in the human diet (Gallo *et al.*, 2018; Barboza *et al.*, 2018a). Ingestion of plastic can occur unintentionally, intentionally, or indirectly through the ingestion of prey species containing plastic. It has been documented for at least 233 marine species, including all marine turtle species, more than one-third of seal species, 59% of whale species, and 59% of seabirds (Kuhn *et al*, 2015) Ingestion by 92 species of fish and 6 species of invertebrates has also been recorded

The size of the ingested material is ultimately limited by the size of the organism. Very small particles such as plastic fibres can be taken up by small organisms such as filter-feeding oysters or mussels; larger materials such as plastic films, cigarette packets, and food packaging have been found in large fish species; and in extreme cases, documented cases of sperm whales have shown ingestion of very large materials including 9m of rope, 4.5m of hose, two flowerpots, and large amounts of plastic sheeting (Stephanis *et al*., 2014)

2.8 Effect of Microplastics in Fish

Many fish species, including endangered ones, are known to have been affected by plastics. Microplastics have been found in more than 100 aquatic species, including fish, shrimp, and mussels destined for our dinner plates. In many cases, these tiny bits pass through the digestive system and are expelled without consequence (Parker., 2019; Emmanuel, 2020).

There are several ways in which plastics can interact or influence wildlife. In the case of microplastics (particles smaller than 4.75 millimetres in diameter), the key concern is ingestion. Ingestion of microplastics has been shown to occur for many organisms. This can occur through several mechanisms, ranging from uptake by filter-feeders, swallowing from surrounding water, or consumption of organisms that have previously ingested microplastics (Galloway, 2017).

Three possible toxic effects of plastic particles have been suggested: the plastic particles themselves, the release of persistent organic pollutants adsorbed to the plastics, and the leaching of plastic additives (Teuten *et al*., 2009; Frias *et al*., 2010; Iniguez *et al*., 2017; Hahladakis *et al*., 2018; Vedolin *et al*., 2018). The potential impacts of ingested microplastics on aquatic organisms are driven by their physical and chemical effects, the latter being influenced by the presence of additives and adsorbed organic chemicals (Barboza *et al*., 2019).

There are several potential effects of microplastics at different biological levels, which range from sub-cellular to ecosystems, but most research has focused on impacts on individual adult organisms. Microplastic ingestion rarely causes mortality in any organisms. As such, ‘lethal concentration’ (LC_{50}) values which are often measured and reported for contaminants do not exist. There are a few exceptions: common goby exposure to polyethene and pyrene; Asian green mussels exposed to polyvinylchloride (PVC), and Daphnia magna neonates exposed to polyethene (Oliviera, 2013; Rist *et al*., 2016, Ogonowski *et al*., 2016).

Studies looking at the effects of MP ingestion on organisms have demonstrated a suite of reaction mechanisms including inflammation (Von Moos *et al*., 2012; Wright *et al*., 2013), increased immune activity (Browne *et al*., 2008), mortality of exposed individuals (Browne *et al*., 2013a;), and a reduction in feeding activity (Besseling *et al*., 2013; Cole *et al*., 2013; Wright *et al*., 2013; Watts *et al*., 2014; Cole *et al*., 2015), depletion of energy reserves (Wright *et al*., 2013; Watts *et al*., 2014), significant impacts on offspring (Sussarellu *et al*., 2016). Further evidence of reduced feed consumption includes, slower metabolic rate and survival in Asian green mussels (Rist *et al*., 2016), reduced reproducibility and survival in copepods (Cole *et al*., 2015), reduced growth and development of Daphnia (Ogonowski *et al*., 2016), reduced growth and development of langoustine (Welden *et al*., 2016), reduced energy stores in shore crabs and lugworms (Watts *et al.,* 2015; Wright *et al*., 2013).

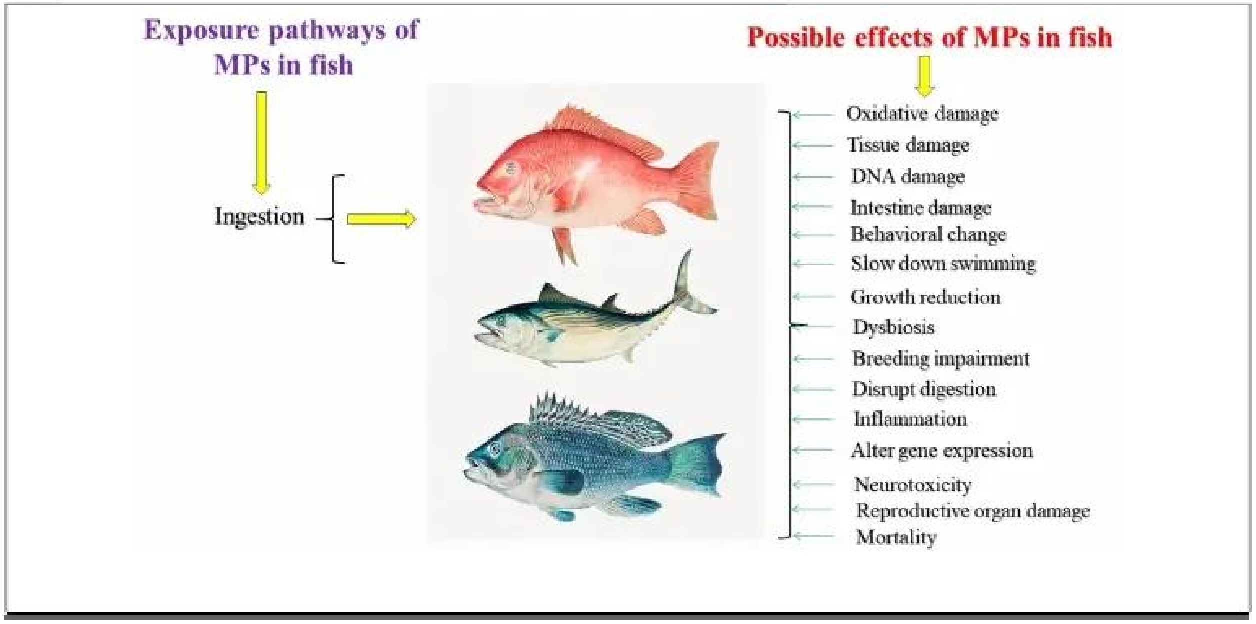

Figure 2: Possible effect of MPs on fish

Soruce: Bhuyan (2022)

In laboratory settings, biochemical responses to plastic ingestion have also been observed. These responses include oxidative stress, metabolic disruption, reduced enzyme activity, and cellular necrosis (Cedervall *et al*, 2012; Brown *et al*, 2013; Oliveira *et al*, 2013; Rochman *et al*, 2013). A new laboratory study shows that the effects extend beyond direct physical or chemical impacts, revealing that the presence of microplastics increases the severity of an important viral fish disease (Seeley *et al.*, 2023). Microplastics can also act as a vector of contaminants to amphipods, polychaetes (Besseling *et al.*, 2013; Browne *et al.*, 2013b), mussels (Avio *et al.*, 2015a) and fish (Oliveira *et al.*, 2013)

While the obvious physical impacts of ingestion include laceration, inflammation and in some cases starvation, the chemical effects of MP ingestion on an organism's daily functioning are less established. Plastics have been shown to accumulate various organic and inorganic contaminants from the surrounding water column (Rochman *et al.*, 2013a; Rochman *et al.*, 2014) The high surface area to volume ratio of small particles combined with non-polar surface facilitates the sorption of chemicals to the plastic surface, forming a complex mixture of contaminants available to marine organisms (Rochman *et al.*, 2013b). The toxicity of MPs is largely size dependent – generally the smaller the particle the further into the organism it can penetrate (Browne *et al.*, 2008), releasing toxic chemicals under acidic gut conditions. If there is a significant accumulation of environmental contaminants, there is the possibility that these concentrations could ‘biomagnify’ up the food chain to higher levels (Avio *et al.*, 2015).

2.9 Trophic Transfer of Microplastics

There is a growing concern for the possible trophic transfer of microplastics in aquatic, benthic and pelagic food webs (GESAMP, 2015; Lusher *et al.*, 2017). Laboratory experiments have established the trophic transfer of MPs from one trophic level to another in aquatic food webs (Cedervall *et al*., 2012; Farrel and Nelson, 2013; Watts *et al*., 2014; Besseling *et al*., 2014)

They have also been reported in an increasing number of marine organisms from different trophic levels, including zooplankton (Frias *et al*., 2014; Desforges *et al*., 2015), barnacles (Goldstein *et al*., 2013), bivalves (Van Cauwenberghe and Janssen, 2014), decapod crustaceans (Devriese *et al*., 2015), fish (Boerger *et al*., 2010; Lusher *et al*., 2013; Neves *et al*., 2015; Bellas *et al*., 2016), marine mammals (Besseling *et al*., 2015; Lusher *et al*., 2015) and seabirds (Avery-Gomm *et al*., 2012).

Microplastics occupy the same size range as plankton and grains of sand, making them accessible to a variety of organisms using different feeding strategies. Organisms may therefore ingest unknown quantities in conjunction with natural prey items, particularly non-selective feeders, which filter large quantities of water and sediment for organic nutrients (Browne *et al*., 2008; Cole *et al*., 2013; Farrell and Nelson, 2013). They are easily ingested by organisms and translocated to higher trophic levels through the food web (Xiang *et al*., 2022). Predatory organisms may indirectly accumulate microplastics during the ingestion of microplastic-contaminated prey, which may lead to bioaccumulation at upper trophic levels (Lusher *et al*., 2017).

Organisms within coastal food webs are more likely to ingest microplastics than those from offshore habitats, due to greater inputs from the land (Browne *et al*., 2010). Similarly, those inhabiting oceanic gyres and deep-sea sediments are more likely to encounter microplastic particles than organisms in pelagic environments, due to greater concentrations within these compartments (Van Cauwenberghe *et al*., 2013; Cózar *et al*., 2014; Eriksen *et al*., 2014; Woodall *et al*., 2014).

2.10 Biomagnification of Microplastics in Humans

2.10.1 Microplastic in Human Diet/Nutrition

There is a growing concern for the possible trophic transfer of microplastics in aquatic, benthic and pelagic food webs. Predatory organisms may indirectly accumulate microplastics during the ingestion of microplastic-contaminated prey, which may lead to bioaccumulation at upper trophic levels (Lusher *et al*., 2017). Human health could be at risk on account of microplastic ingestion. Microplastics can be retained for a longer time at the higher trophic levels where humans belong, thereby predisposing humans to serious health hazards (Emmanuel, 2020).

Fish is being contaminated with microplastics worldwide (Seqqueira *et al*., 2020), with the inevitable fact that fish consumption is a significant source of microplastic exposure in humans (Smith *et al.,* 2018). Research from Nigeria (Adeogun *et al*., 2020) on the occurrence and identification of microplastics in the abdomen of marketable fish species from a public water supply lake in Southwestern Nigeria showed that microplastics accumulated in most of the fish species sampled. These fishes will eventually be consumed as food by humans.

2.10.2 Biomagnification of Microplastics in Humans

While the biomagnification of organic pollutants from lower trophic levels to fish has been demonstrated (Kelly *et al*., 2007), there is, currently, very little evidence of the impact that microplastics can have on humans. For human health, microplastics are small enough to be ingested – that is of greatest concern. There are several ways by which plastic particles can be ingested: orally through water, consumption of marine products which contain microplastics, through the skin via cosmetics (identified as highly unlikely but possible), or inhalation of particles in the air (Revel *et al*., 2018).

Microplastics can be passed up to higher levels in the food chain. This can occur when a species consumes organisms of a lower level in the food chain which has microplastics in the gut or tissue (Galloway, 2015). The presence of microplastics at higher levels of the food chain (in fish) has been documented (Guven *et al*., 2017; Jabeen *et al*., 2017)

One factor which possibly limits the dietary uptake for humans is that microplastics in fish tend to be present in the gut and digestive tract — parts of the fish not typically eaten (Galloway., 2015). The presence of microplastics in fish beyond the gastrointestinal tract (e.g., in tissue) remains to be studied in detail (Bouwmeester., 2015). However, several studies have found microplastic in fish muscle/meat, which is mainly consumed by humans (Akhbarizadeh *et al*., 2018; Abbasi *et al*., 2018; Barboza *et al*., 2020; Thiele *et al*., 2021).

Despite seafood being a recognised source of contaminants in the human diet, the occurrence of microplastics in seafood is neither quantified nor regulated (Ziccardi *et al*., 2016). Seafood may be contaminated with microplastics through ingestion of natural prey, adherence to the organism's surface or during the processing and packaging phase (Cole *et al*., 2013; European Food Safety Authority, 2016). Organisms that are eaten whole present a greater risk of exposure. Microplastics in bivalves (mussels and oysters) cultured for human consumption have also been identified. However, neither human exposure nor potential risk has been identified or quantified extensively (Cauwenberghe and Janssen., 2014).

Levels of microplastic ingestion are currently unknown. Even less is known about how such particles interact in the body. It may be the case that microplastics simply pass straight through the gastrointestinal tract without impact or interaction (Wang *et al*., 2016). A study of North Sea fish, for example, revealed that 80% of fish with detected microplastics contained only one particle this suggests that following ingestion, plastic does not persist for long periods (Foekema *et al*., 2013). Concentrations in mussels, in contrast, can be significantly higher.

To date, there has been no clear evidence of the accumulation of persistent organic pollutants or leached plastic additives in humans. Continued research in this area is important to better understand the role of plastic within broader ecosystems and the risk to human health. Chemicals found in plastics such as BPA and phthalates have been found in humans and these affect hormones and cause issues with fertility and reproduction. Numerous chemicals used to produce plastic are known to be carcinogenic and to interfere with the body's hormone system causing reproductive, neurological, and immune disorders.

2.11 Effects of Microplastics in Humans

Medical studies on both rats and humans have demonstrated the translocation of PS and PVC particles<150 μm from the gut cavity to the lymph and circulatory system (Volkheimer, 1975; Hussain *et al*., 2001). Very fine particles are capable of crossing cell membranes, the blood-brain barrier and the placenta, with documented effects including oxidative stress, cell damage, inflammation and impairment of energy allocation similar to that reported for marine organisms. (Vethaak and Leslie, 2016; Muniasamy *et al*., 2020). Exposure to hydrophobic contaminants can occur by ingesting fish, birds or other organisms that have accumulated contaminants within their tissue from previously egested microplastics (Ziccardi *et al.,* 2016).

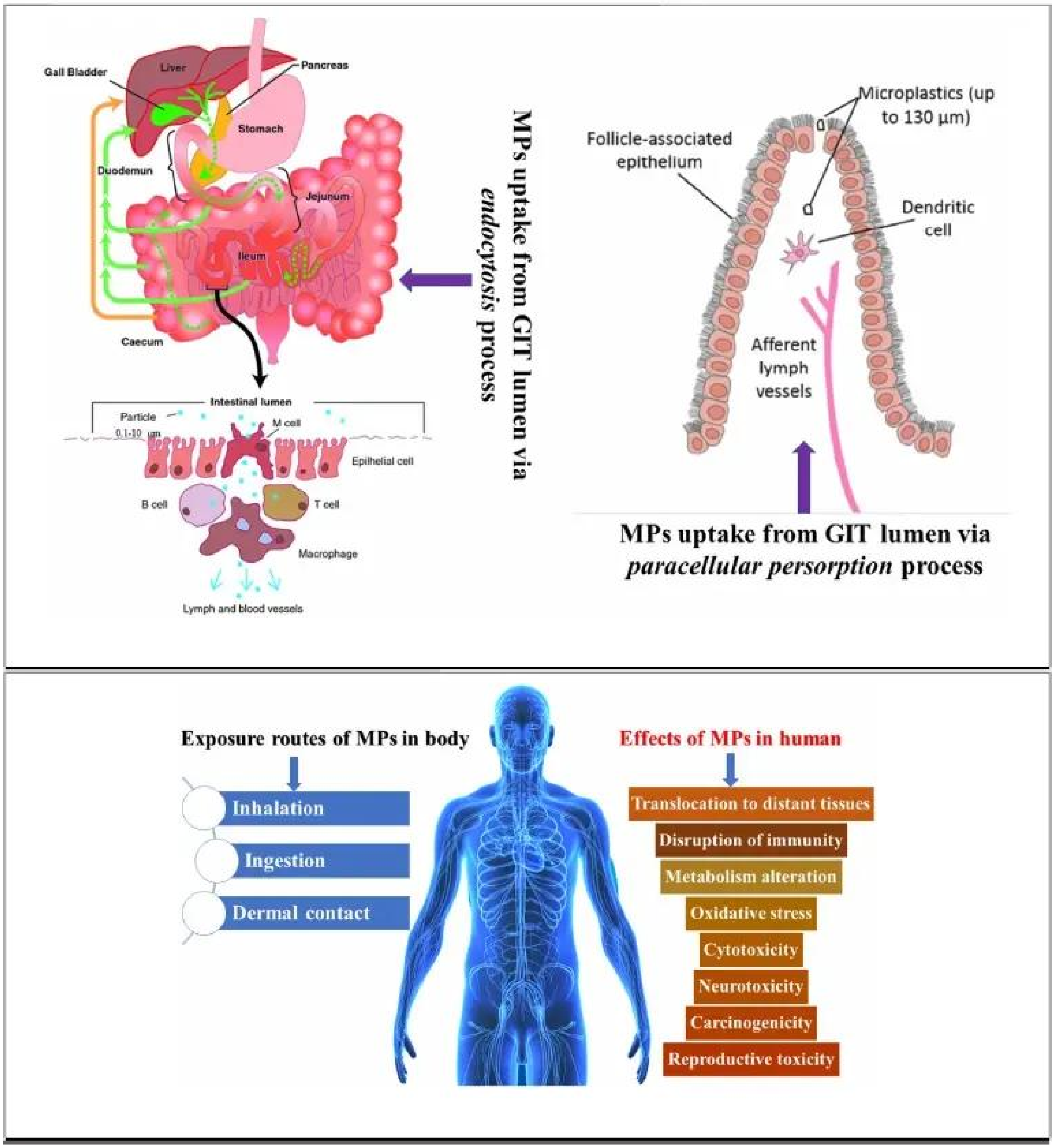

Figure 3: Possible effect of plastic in Humans

Source: Bhuyan (2022)

CHAPTER THREE

3.0 Materials and Methods

3.1 Study Area

Ikpoba River is located between Latitude 6° 190 12“N, longitude 5° 24’0” E, and Latitude 6° 220 48“N, longitude 5° 51’ 7.2” E in Benin City, Edo State, Nigeria. It flows in a south-westerly direction in a steeply incised valley and through sandy areas before passing through Benin City and joining the Ossiomo River (Atuanya *et al.,* 2012; Odigie, 2015). The river's upper stages are dendritic, and its sources come from the Ishan Plateau, which is 230 meters above sea level and is located in the eastern coastal plain, northeast of Benin City (Tabinda *et al.,* 2013; Odigie, 2015).

Local communities have access to a variety of resources in the Ikpoba 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 the dominant indigenous plant species in the study sections. However, a significant portion of natural vegetation has been lost as a result of anthropogenic activity such as widespread deforestation for agricultural purposes. Its rich alluvial train may be responsible for the landscape area's suitability for agricultural use.

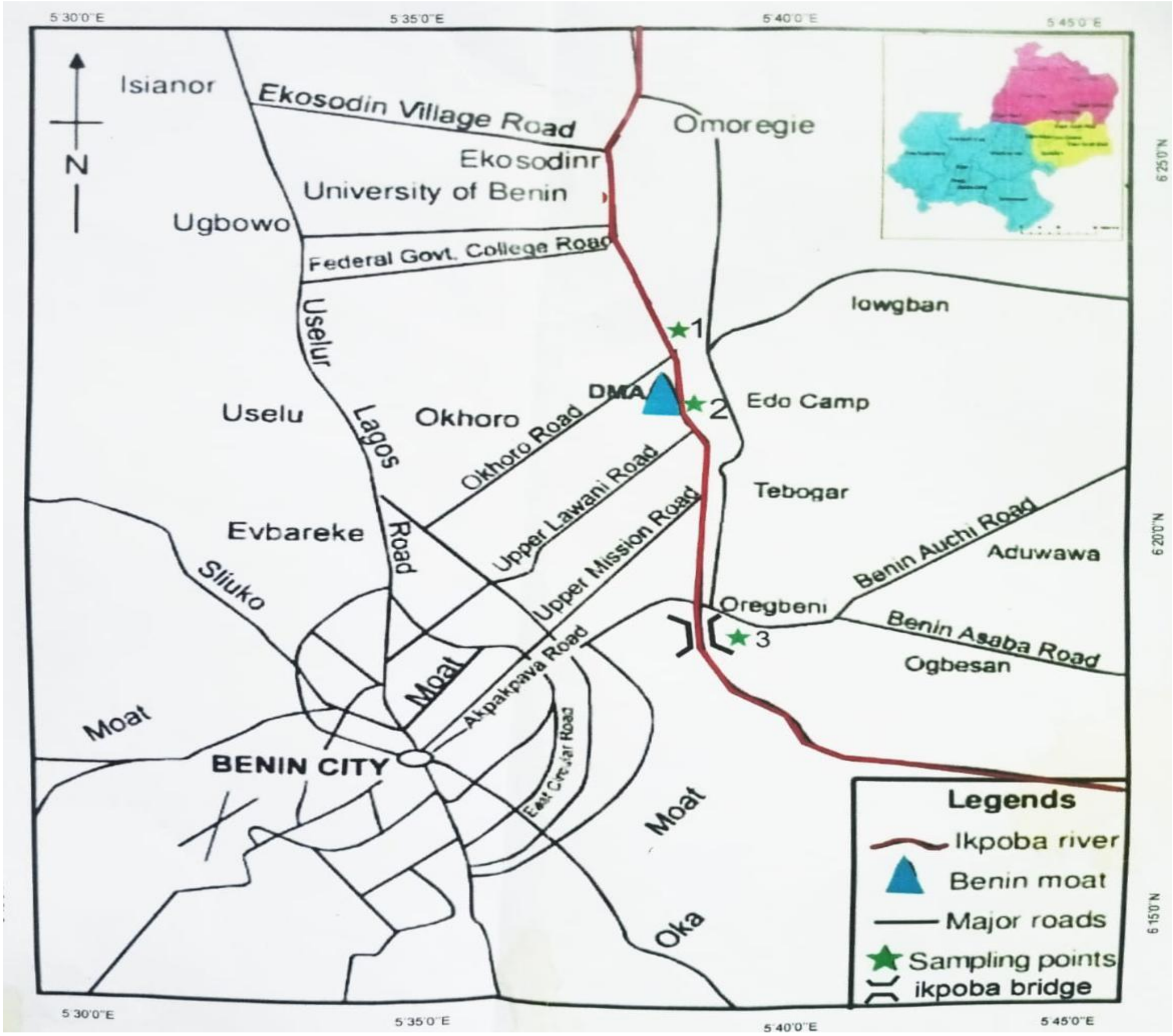

Fig 4: Map of Benin city, showing sampling station at Ikpoba River

3.2 Experimental Design

This experimental design will be a CRBD design with 3 stations x 3 months x four microplastic types x 1 fish species, replicated 3 times.

3.3 Stations

3.3.1 Okhoro Reservoir (Station 1)

The Okhoro reservoir is located at Latitude 6 22’ 37” N and Longitude 5 38’ 23” E. The reservoir is relatively closed. Economic activities in this area include retail stores, Fashion designing businesses, Sawmills and woodwork industry, Boutiques, Hairdressing, Tyre vulcanizing etc., all of which have the potential of generating plastic waste.

3.3.2 Upper Lawani (Station 2)

The Upper Lawani waterfront is located at Latitude 6° 22’ 32” N and Longitude 5° 38’ 45” E. Several activities are carried out at the waterfront including fishing and religious rituals. Economic activities in this area include retail stores, hairdressing businesses, sawmills woodwork industry etc. Solid plastic waste can be seen at the waterfront.

3.3.3 Ikpoba Bridge (Station 3)

Ikpoba Hill waterfront is located at Lat. 6 21’ 5” N and Long 5 38’ 49” E. The river provides the riparian communities with water, fish and selected aquatic plants. Several commercial activities are carried out in the surrounding communities. However, the most prevalent at the waterfront are car wash, rug wash, and slaughterhouses.

3.4 Collection of Samples

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

3.5 Preparation of Samples for Analysis

3.5.1 Cleaning the Samples

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

3.5.2 Preparing the Digesting Solution

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

3.5.3 Digestion of samples

The cleaned fish sample was cut open on ventral side and the intestine carefully extracted. The extracted intestine was rinsed under running water. The intestine was placed in the 10% KOH solution in a beaker (Foekema *et al*., 2013; Rochman *et al*., 2015). The beaker was covered. The sample was left to digest in a closed vial 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).

3.5.4 Purification of Digested Samples

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)

3.5.5 Sieving the Digested Sample

The resultant liquified samples was sieved using 5mm cellulose nitrate filters to ensure the capturing of microplastics of the smallest sizes. The filter was rinsed into a glass petri-dish using pure water (Cauwenberghe and Janssen, 2014).

3.6 Identification of Microplastics

The Petri dishes was subjected to a temperature of 100^{o}C for 12hrs in an oven. The resultant dried filtrate was transferred to a slide for visual identification using a microscope, counting the number of each type of microplastic (fibre, foam, fragment and film) present (Roch *et al.*, 2020)

3.7 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 difficult 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 (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2015). The method to be used will be the tagging method. This is because it is fast and cheap while producing relatively accurate results.

3.7.2 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 (Vandermeersch *et al*., 2015; Canesi *et al*., 2015; Devriese *et al*., 2015; Bellas *et al*., 2016).

3.7.3 Analysis of Microplastics

The suspected microplastic particles were analysed for different types of microplastics based on polymer type using Fourier transform infrared imaging (FTIR) at Splendidstan Research Laboratory, 100 New Lagos Road, Iguosa, Benin City, Edo State. The polymer type of each microplastic was identifed using Fourier Transform Infrared (FTIR) Spectrometer. The suspected particles were placed into the FTIR, which was appropriately programmed to collect the sample spectrum. After receiving the sample spectrum, polymer identification was made. Before placing each sample, the background signal was updated, and the ATR-crystal was cleaned with acetone.

3.8 Measurement of Microplastic Indices

The indices to be evaluated include

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

3.8.1 Plastic load (PL)

The average amount of microplastic per fish is referred to as the plastic load (PL). This value will include 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).

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

3.8.2 Frequency of Occurrence of Microplastic Ingestion (FO)

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

$$\text{FO} = \frac{\text{Number of fish with at least one microplastic particle.}}{\text{Number of fish sampled}}$$

3.9 Contamination Controls

All work surfaces, vials and utensils were cleaned beforehand with 70% ethanol solution before use and in-between individual samples to prevent cross-contamination. Throughout all processing and analysis, strict protocols were observed to ensure that contamination risk was 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 was cleaned methodically before any work was carried out and between each fish (Karami *et al*., 2017).

3.10 Statistical Analysis

Genstat software (version 12.1) was used for statistical analysis of the data obtained from this study. ANOVA will be used to determine differences between mean values of MPs at 5%

probability, while significant means ($P<0.05$) will be separated using New Duncan Multiple Range Test.

CHAPTER FOUR

4.0 RESULT

The result of microplastic pollutants in *C. gariepinus* harvest from Ikpoba river, Benin city, Nigeria, is shown in this chapter.

4.1 Mean Level of Microplastics

The level of plastic informs the abundance of microplastic in fish from the stations. The values range from 0.357 to 4.65. There is no significant difference ($p<0.05$) between all stations in the month of June. There is no significant difference ($p<0.05$) between station two and station three, but there is a significant difference ($p<0.05$) between station one and the other two stations. There is no significant difference ($p<0.05$) station one and two in the month of august, while there is a ($p<0.05$) significant difference between station three and the other two stations in the month of august.

There is no significant difference ($p<0.05$) between the means in June and August, while a significant difference ($p<0.05$) occurs between July and the other months. There is no significant difference ($p<0.05$) in station two across all months. There is no significant difference ($p<0.5$) between June and July but exist as against August.

Table 3: Mean level of microplastics from harvested fish according to stations

Month	Station 1 (Okhoro)	Station 2 (Upper Lawani)	Station 3 (Ikpoba Bridge)
June	0.36 ± 0.57[a]	1.29 ± 1.52[a]	0.36 ± 0.57[a]
July	2.36 ± 1.52[b]	0.38 ± 0.57[a]	0.70 ± 1.15a
August	0.67 ± 0.57[a]	1.10 ± 1.00[a]	4.65 ± 1.73[b]

4.2 Plastic Load

This is the number of plastic particles per fish sampled. In the table below, there is no significant difference ($p<0.05$) in August across all stations. There is no significant difference ($p<0.05$) between station two and three in July, however significant difference ($p<0.05$) does exist between station one and the others. In august, there is no significant difference ($p<0.05$) between station one and two, while there is between station three and the other stations. There is no significant difference ($p<0.05$) between June and august in station, however it does occur between July and the other months. There is no significant difference ($p<0.05$) in station two across all months. There is significant difference ($p<0.05$) between the mean value for June and July, however it does exist between august and the other months.

Table 4: Mean plastic load from harvested fish according to stations

Month	Station 1 (Okhoro)	Station 2 (Upper Lawani)	Station 3 (Ikpoba Bridge)
June	0.33 ± 0.57^{a}	1.33 ± 1.52^{a}	0.33 ± 0.57^{a}
July	2.67 ± 1.52^{b}	0.33 ± 0.577^{a}	0.67 ± 1.16^{a}
August	0.67 ± 0.57^{a}	1.10 ± 0.577^{a}	5.00 ± 1.70^{b}

4.3 Frequency of Occurrence

There is significant difference ($p<0.05$) across all months in station two, as well as station one. There is significant difference ($p<0.05$) between August and the other months in station three. There is significant difference ($p<0.05$) between station one and three in the month of June, while it exists between station two and the other stations. Significant differences ($p<0.05$) occur between station one and the other stations in the month of July. There is no significant difference($p<0.05$) between all stations in the month of August

Table 5: Mean frequency of occurrence of microplastics from harvested fish according to stations

Month	**Station 1 (Okhoro)**	**Station 2 (Upper Lawani)**	**Station 3 (Ikpoba Bridge)**
June	0.29 ± 0.05^{a}	0.68 ± 0.02^{c}	0.29 ± 0.05^{a}
July	0.86 ± 0.15^{c}	0.31 ± 0.02^{a}	0.35 ± 0.02^{a}
August	0.65 ± 0.03^{b}	0.60 ± 0.04^{b}	0.93 ± 0.03^{b}

4.4 Classification of Microplastics

4.4.1 Morphological Classification

From the table 6 below, it can be observed that the

Table 6: Types of Microplastic Particles Found Based on Morphological Classification

	June			July			August		
	Station 1	**Station 2**	**Station 3**	**Station 1**	**Station 2**	**Station 3**	**Station 1**	**Station 2**	**Station 3**
R1	A	C	A	B, C	A	A	D	B, E	B, C, E
R2	C	C	A	B, E	E	A	A	B	B, E
R3	A	A	B	C	A	B, C	C	A	B,C, E

A = No plastic, B = Filament, C = Fragment, D = Foam, E = Pellet, F = Fibre

4.4.2 Polymer Classification

The polymer composition of microplastics were identified by FTIR. Polypropylene (Slootmaekers *et al*., 2019; Collard *et al*., 2018) and the polyethylene (PE) were reported more frequently (Horton *et al.,* 2018; Biginagwa *et al*., 2016; Andrade *et al.,* 2019).

4.4.2.1 Station 1 (Okhoro)

June

The FTIR spectrum of microplastic obtained from station 1 in June can be seen in plate 1 below. The FTIR spectrum show absorbance band at different wave numbers. There is a peak at 2910cm-1 which is a characteristics absorption of asymmetric CH2 stretching. There is also, a peak at 2820cm^{-1} which is a characteristic of symmetric CH2 stretching.

A peak at 1445cm^{-1} is a characteristics CH2 scissoring.

There is a peak at 710cm^{-1} which is a characteristic of CH_2 rocking. The peaks at 2910cm-1, 1445cm^{-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**.**

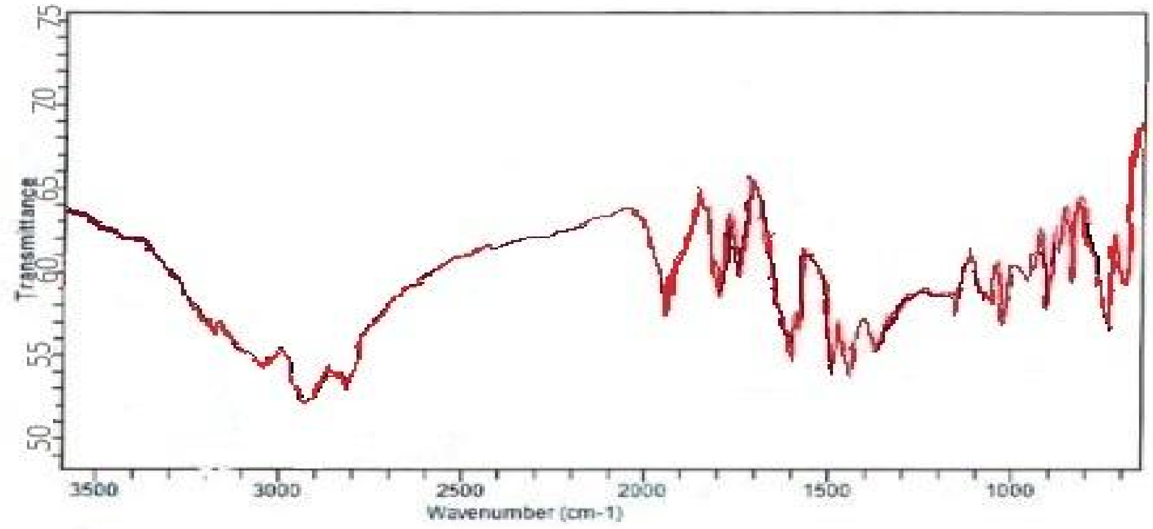

Plate 1: FTIR spectrum of microplastic particle obtained at station 1 in June, showing absorbance band at different wave numbers

July

The FTIR spectrum of one of the microplastic particles obtained from station 1 in July can be seen in plate 2 below. The FTIR spectrum show absorbance band at different wave numbers. There is a peak at 2910cm$_{-1}$ which is a characteristics absorption of asymmetric CH_2 stretching.

There is also, a peak at 2830cm^{-1} which is a characteristic of symmetric CH_2 stretching.

A peak at 1470cm^{-1} is a characteristics CH_2 scissoring.

There is a peak at 710cm^{-1} which is a characteristic of CH_2 rocking.

The peaks at 2910cm^{-1}, 1470cm^{-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**.**

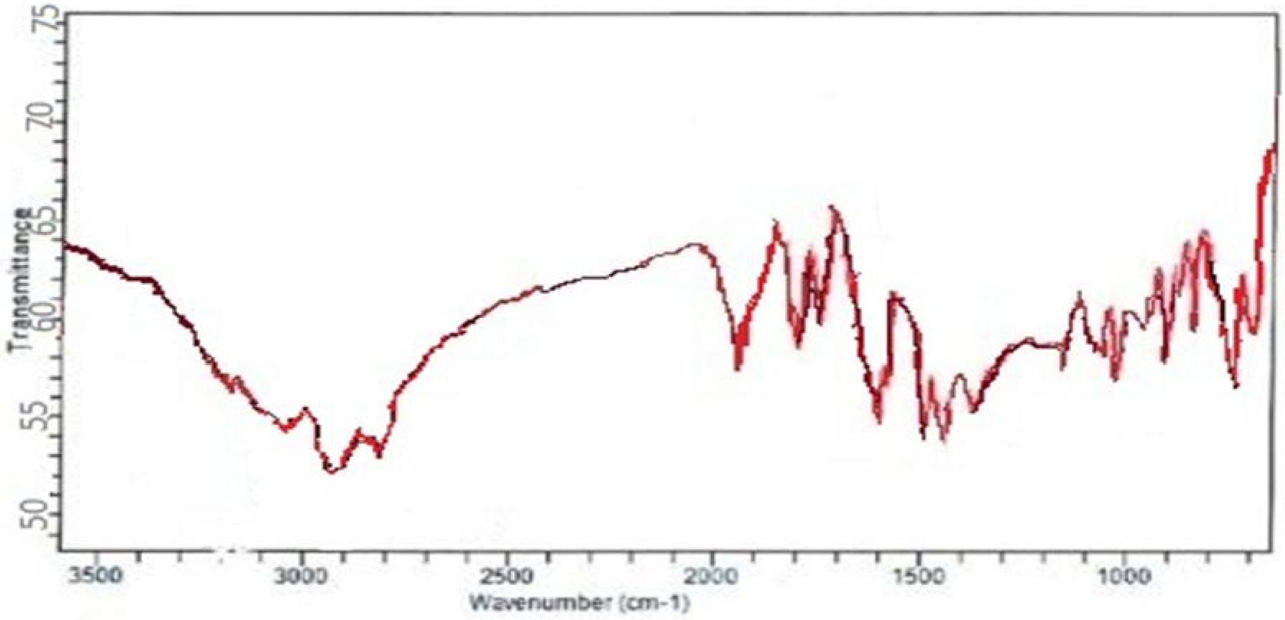

Plate 2: FTIR spectrum of one of the microplastic particle obtained at station 1 in July, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 1 in July can be seen in plate 3 below. The FTIR spectrum show absorbance band at different wave numbers.

There is a peak at 2920cm^{-1} which is a characteristics absorption of asymmetric CH_2 stretching.

There is also, a peak at 2830cm^{-1} which is a characteristic of symmetric CH_2 stretching.

A peak at 1455cm^{-1} is a characteristics CH_2 scissoring.

There is a peak at 710cm^{-1} which is a characteristic of CH_2 rocking. The peaks at 2920cm^{-1}, 1455cm-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**.**

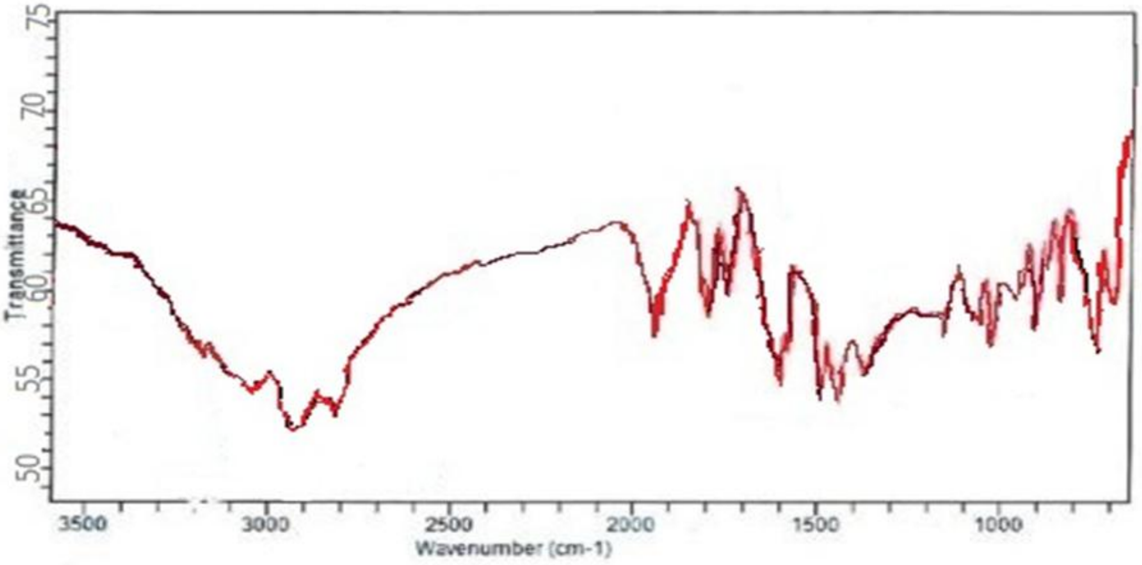

Plate 3: FTIR spectrum of one of the microplastic particles obtained at station 1 in July, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 1 in July can be seen in plate 4 below. The FTIR spectrum show absorbance band at different wave numbers. 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.

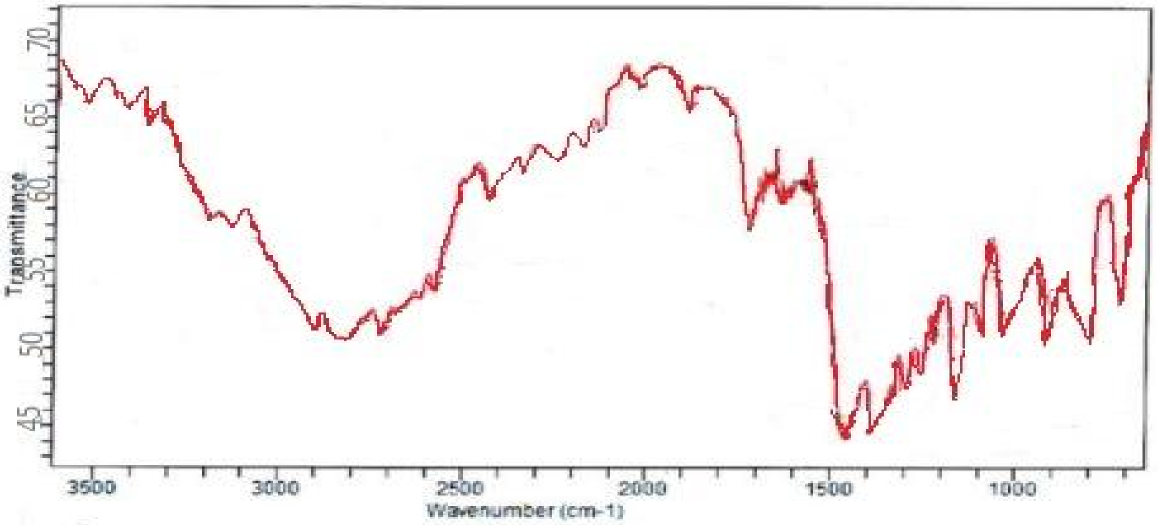

Plate 4: FTIR spectrum of one of the microplastic particle obtained at station 1 in July, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 1 in July can be seen in plate 5 below. The FTIR spectrum show absorbance band at different wave numbers There is a peak at 2885cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching. There is also, a peak at 1440cm^{-1} which is a characteristic of CH_2 scissoring.

A peak at 1135cm^{-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 2885cm^{-1}, 1440cm^{-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.

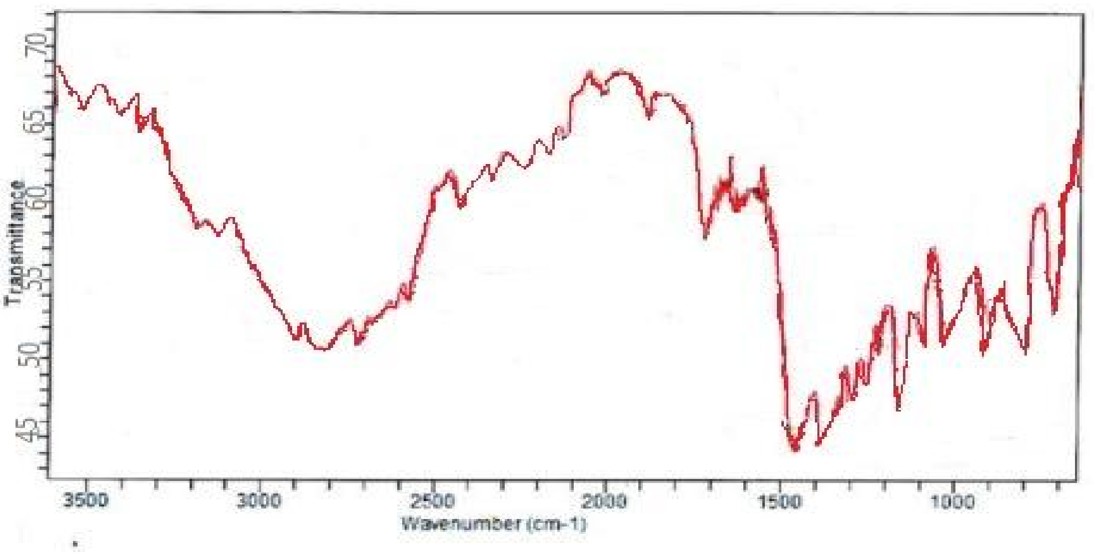

Plate 5: FTIR spectrum for one of the microplastic particle obtained at station 1 in July, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 1 in July can be seen in plate 6 below. The FTIR spectrum show absorbance band at different wave numbers

There is a peak at 2910cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching.

There is also, a peak at 1440cm^{-1} which is a characteristic of CH_2 scissoring.

A peak at 1140cm^{-1} is attributed to the asymmetric stretching of oxygen atom.

There is a peak at 920cm^{-1} is a characteristics of CH rocking.

The peaks at 2910cm^{-1}, 1440cm^{-1} and 920cm^{-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.

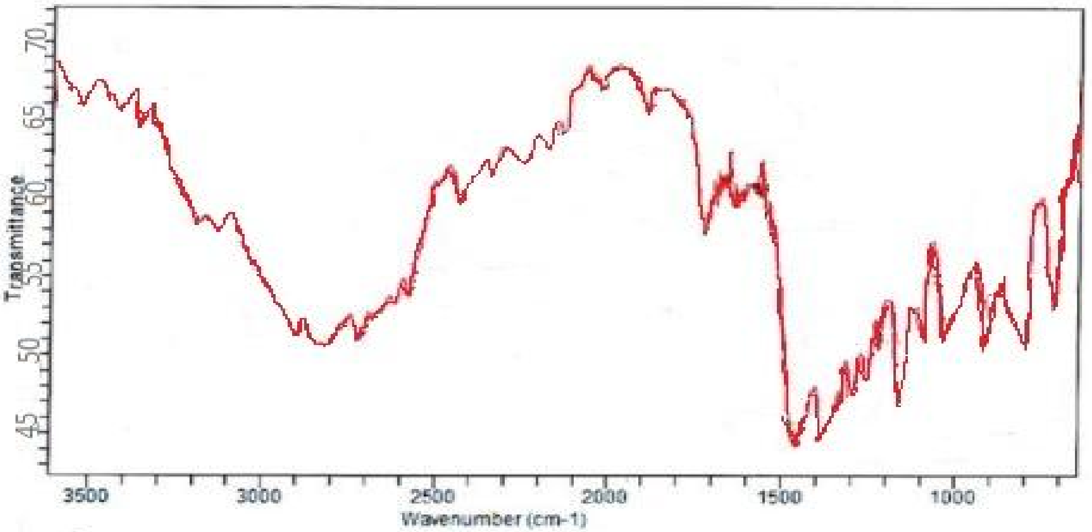

Plate 6: FTIR spectrum of one of the microplastic particle obtained at station 1 in July, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 1 in July can be seen in plate 7 below. The FTIR spectrum show absorbance band at different wave numbers. There is a peak at 2915cm^{-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 1145cm^{-1} is attributed to the asymmetric stretching of oxygen atom.

There is a peak at 700cm^{-1} is a characteristics of CH rocking.

The peaks at 2915cm^{-1}, 1450cm^{-1} and 700cm^{-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.

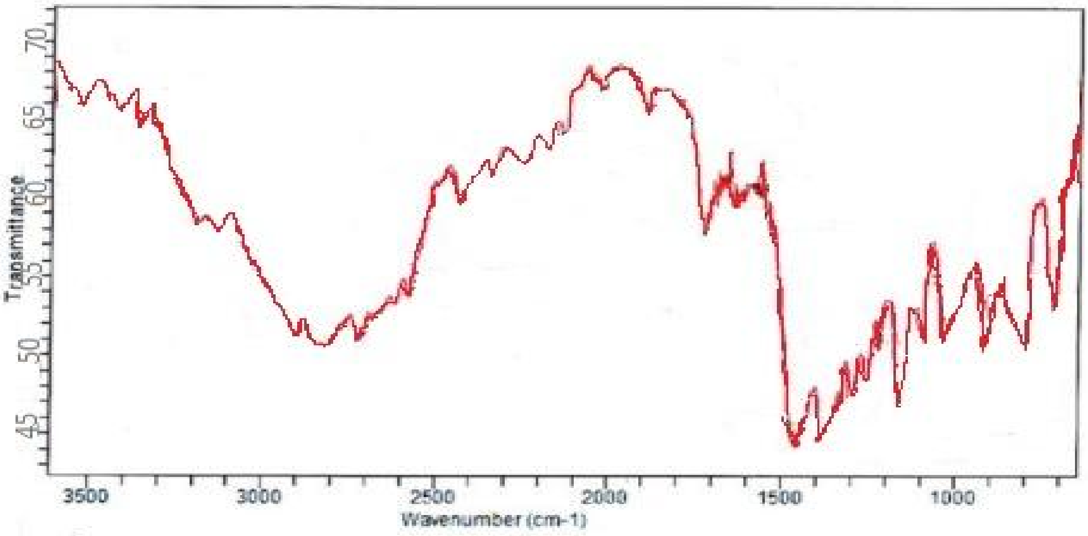

Plate 7: FTIR spectrum of one of the microplastic particle obtained at station 1 in July, showing absorbance band at different wave numbers

August

The FTIR spectrum of microplastic obtained from station 1 in August can be seen in plate 8 below. The FTIR spectrum show absorbance band at different wave numbers. There is a peak at 2880cm-1 which is a characteristics absorption of asymmetric CH2 stretching.

There is also, a peak at 2820cm^{-1} which is a characteristic of symmetric CH_2 stretching.

A peak at 1445cm^{-1} is a characteristics CH_2 scissoring.

There is a peak at 710cm-1 which is a characteristic of CH_2 rocking.

The peaks at 2880cm-1, 1445cm^{-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**.**

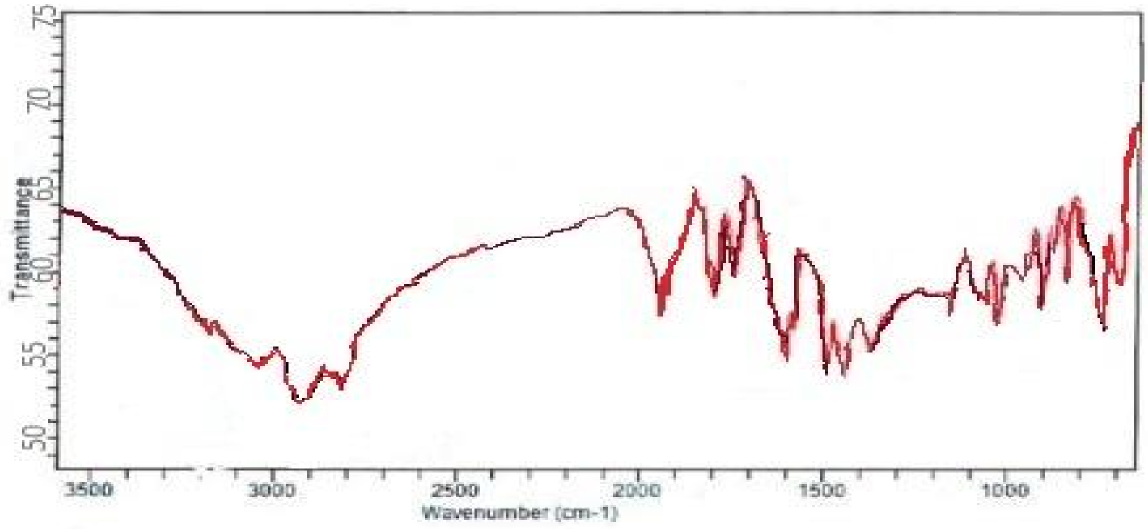

Plate 8: FTIR spectrum of microplastic particle obtained at station 1 in August, showing absorbance band at different wave numbers

The other particle physically identified as microplastic did not produce any FTIR result, hence it is not a microplastic.

Station 2

June

The FTIR spectrum of one of the microplastic particles obtained from station 2 in June can be seen in plate 9 below. The FTIR spectrum show absorbance band at different wave numbers. There is a peak at 2925cm^{-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 1155cm^{-1} is attributed to the asymmetric stretching of oxygen atom.

There is a peak at 720cm^{-1} is a characteristics of CH rocking.

The peaks at 2925cm^{-1}, 1450cm^{-1} and 720cm^{-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.

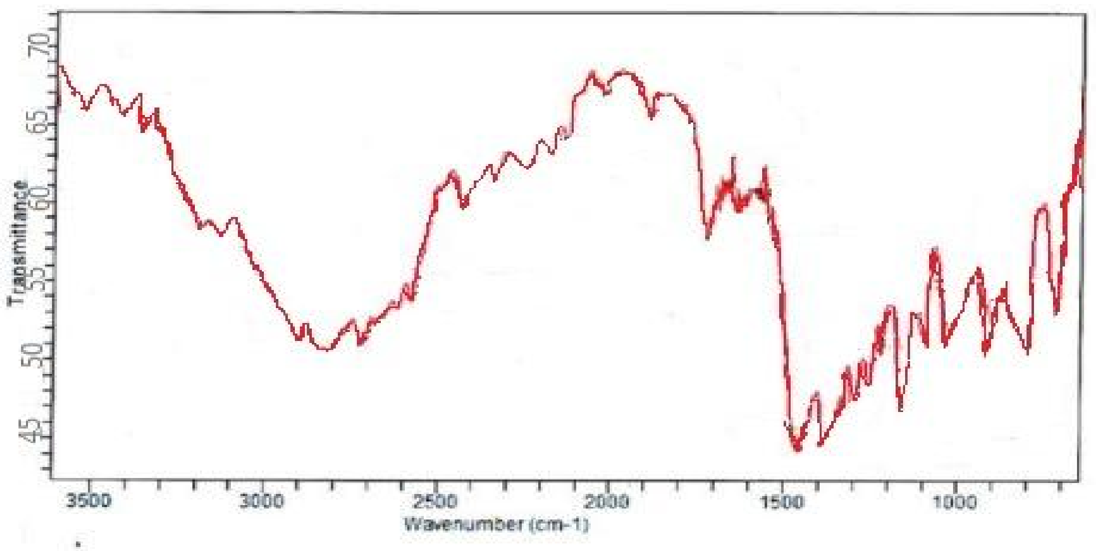

Plate 9: FTIR spectrum of one of the microplastic particle obtained at station 2 in June, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastics obtained from station 2 in June can be seen in plate 10 below. The FTIR spectrum show absorbance band at different wave numbers.

There is a peak at 2920cm^{-1} which is a characteristics absorption of asymmetric CH$_2$ stretching.

There is also, a peak at 2830cm^{-1} which is a characteristic of symmetric CH$_2$ stretching.

A peak at 1465cm^{-1} is a characteristics CH2 scissoring.

There is a peak at 710cm-^{1} which is a characteristic of CH$_2$ rocking.

The peaks at 2920cm-1, 1465cm^{-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.

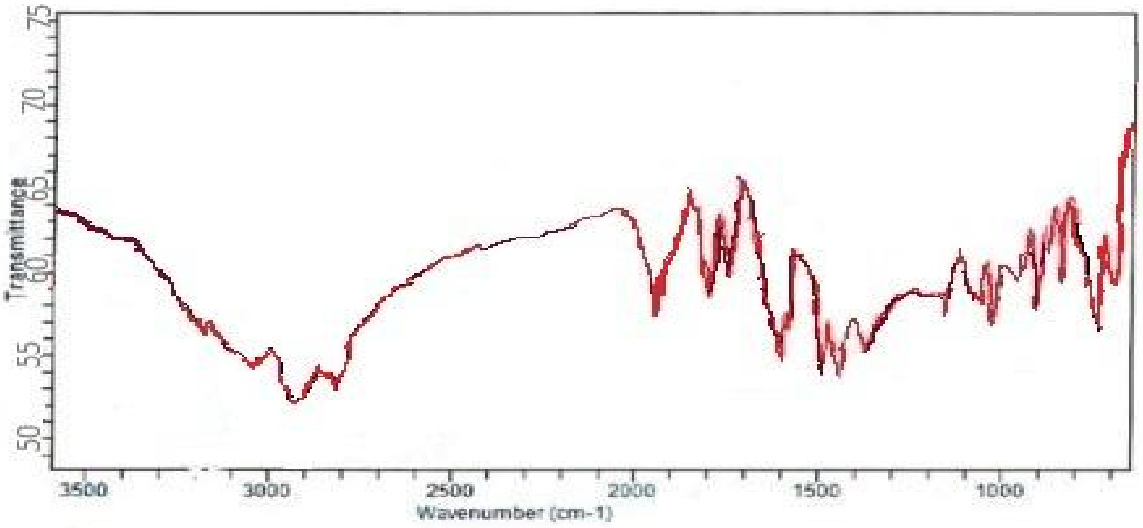

Plate 10: FTIR spectrum of one of the microplastic particles obtained at station 2 in June, showing absorbance band at different wave numbers

The FTIR spectrum of the microplastic obtained from station 2 in June can be seen in plate 11 below. The FTIR spectrum show absorbance band at different wave numbers.

There is a peak at 2910cm^{-1} which is a characteristics absorption of asymmetric CH2 stretching.

There is also, a peak at 2840cm^{-1} which is a characteristic of symmetric CH2 stretching.

A peak at 1470cm^{-1} is a characteristics CH_2 scissoring.

There is a peak at 710cm^{-1} which is a characteristic of CH_2 rocking.

The peaks at 2910cm^{-1}, 1470cm^{-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**.**

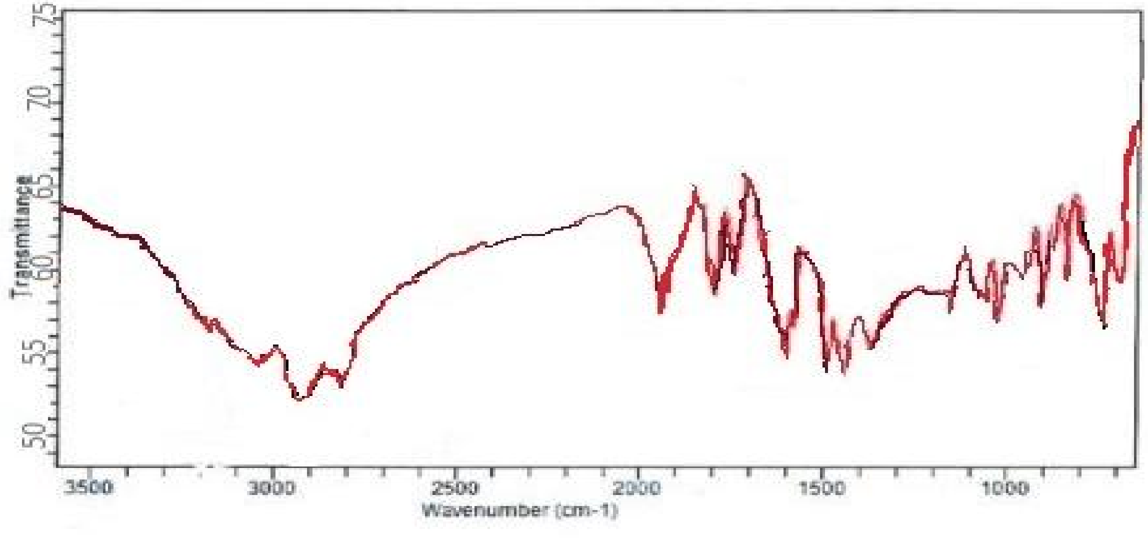

Plate 11: FTIR spectrum of one of the microplastic particles obtained at station 2 in June, showing absorbance band at different wave numbers

July

The FTIR spectrum of microplastic obtained from station 2 in July can be seen in plate 12 below. The FTIR spectrum show absorbance band at different wave numbers.

There is a peak at $2880cm^{-1}$ which is a characteristics absorption of asymmetric CH_2 stretching.

There is also, a peak at $2820cm^{-1}$ which is a characteristic of symmetric CH_2 stretching.

A peak at $1450cm^{-1}$ is a characteristics CH_2 scissoring.

There is a peak at $710cm^{-1}$ which is a characteristic of CH_2 rocking.

The peaks at $2880cm^{-1}$, $1450cm^{-1}$ 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.

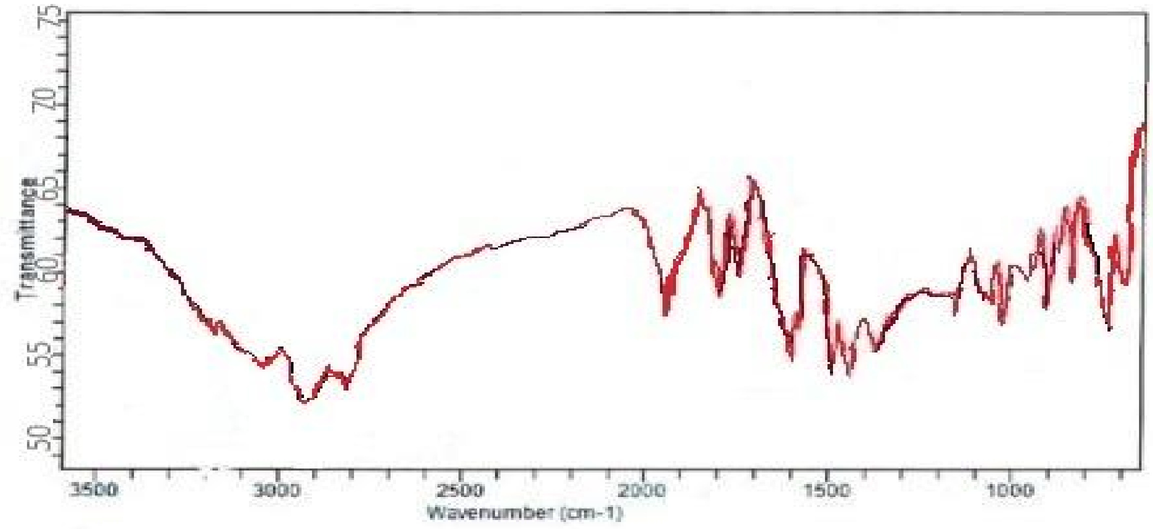

Plate 12: FTIR spectrum of one of the microplastic particles obtained at station 2 in July, showing absorbance band at different wave numbers

August

The FTIR spectrum of one of the microplastics obtained from station 2 in August can be seen in plate 13 below. The FTIR spectrum show absorbance band at different wave numbers. There is a peak at 2910cm^{-1} which is a characteristics absorption of asymmetric CH_2 stretching.

There is also, a peak at 2820cm^{-1} which is a characteristic of symmetric CH_2 stretching.

A peak at 1445cm^{-1} is a characteristics CH_2 scissoring.

There is a peak at 710cm^{-1} which is a characteristic of CH2 rocking.

The peaks at 2910cm^{-1}, 1445cm^{-1} and 710cm 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**.**

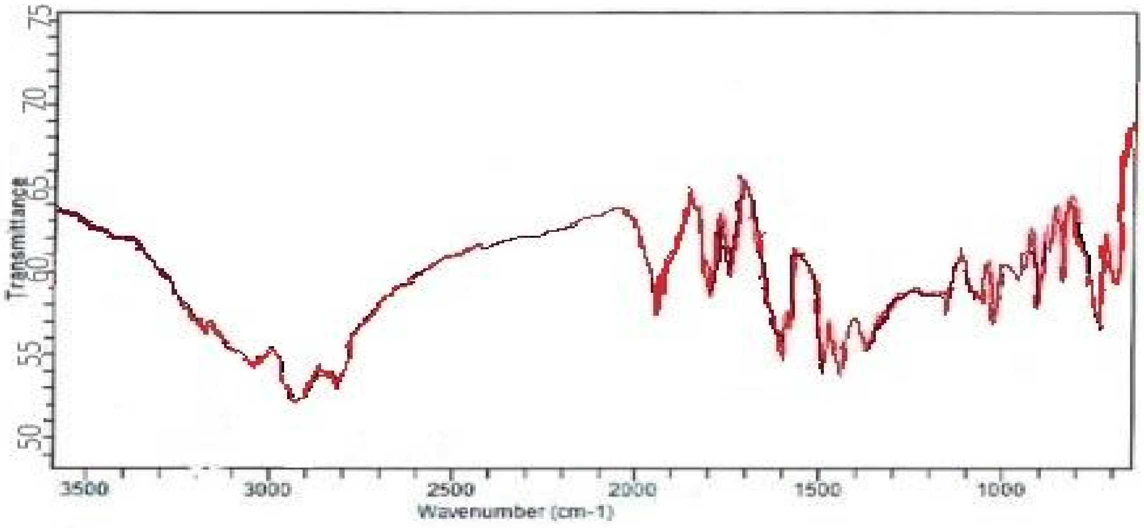

Plate 13: FTIR spectrum of one of the microplastic particles obtained at station 2 in August, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastics obtained from station 2 in August can be seen in plate 14 below. The FTIR spectrum show absorbance band at different wave numbers.

There is a peak at 2910cm^{-1} which is a characteristics absorption of asymmetric CH_2 stretching.

There is also, a peak at 2820cm^{-1} which is a characteristic of symmetric CH_2 stretching.

A peak at 1470cm^{-1} is a characteristics CH_2 scissoring.

There is a peak at 710cm^{-1} which is a characteristic of CH_2 rocking.

The peaks at 2910cm^{-1}, 1470cm^{-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**.**

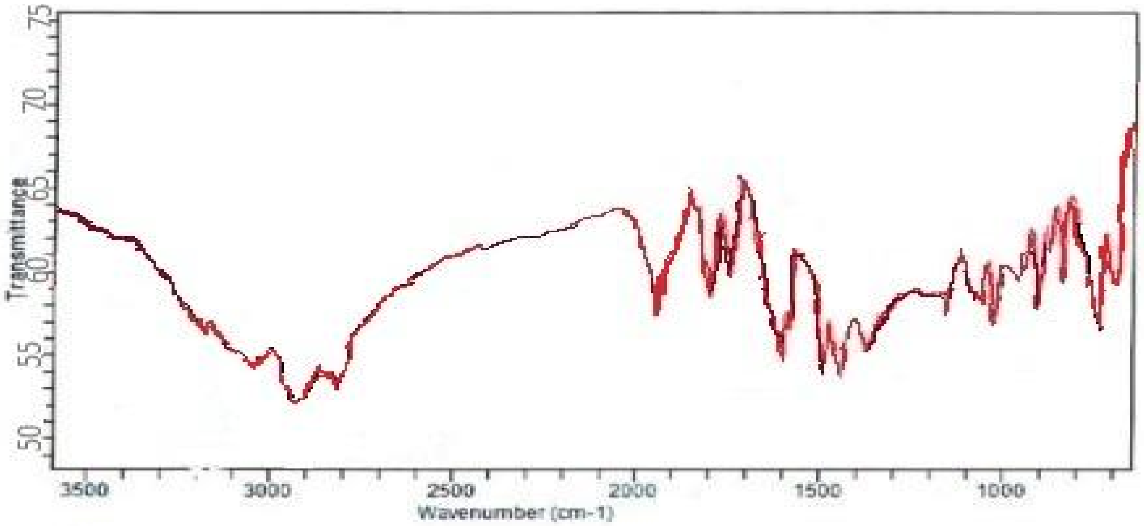

Plate 14: FTIR spectrum of one of the microplastic particles obtained at station 2 in August, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 2 in August can be seen in plate 15 below. The FTIR spectrum show absorbance band at different wave numbers

There is a peak at 2855cm^{-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 1155cm^{-1} is attributed to the asymmetric stretching of oxygen atom.

There is a peak at 830cm^{-1} is a characteristics of CH rocking.

The peaks at 2855cm^{-1}, 1450cm^{-1} and 830cm^{-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.

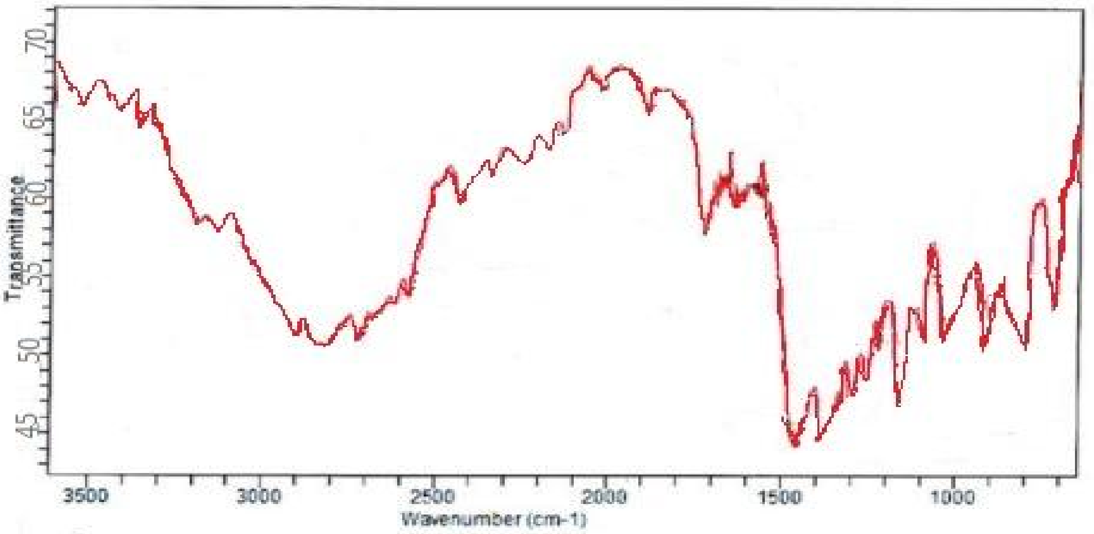

Plate 15: FTIR spectrum of one of the microplastic particle obtained at station 2 in August, showing absorbance band at different wave numbers

Station 3

June

The FTIR spectrum of microplastic particle obtained from station 3 in June can be seen in plate 16 below. The FTIR spectrum show absorbance band at different wave numbers. There is a peak at 2905cm^{-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 1145cm^{-1} is attributed to the asymmetric stretching of oxygen atom.

There is a peak at 750cm^{-1} is a characteristics of CH rocking.

The peaks at 2905cm^{-1}, 1450cm^{-1} and 750cm^{-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.

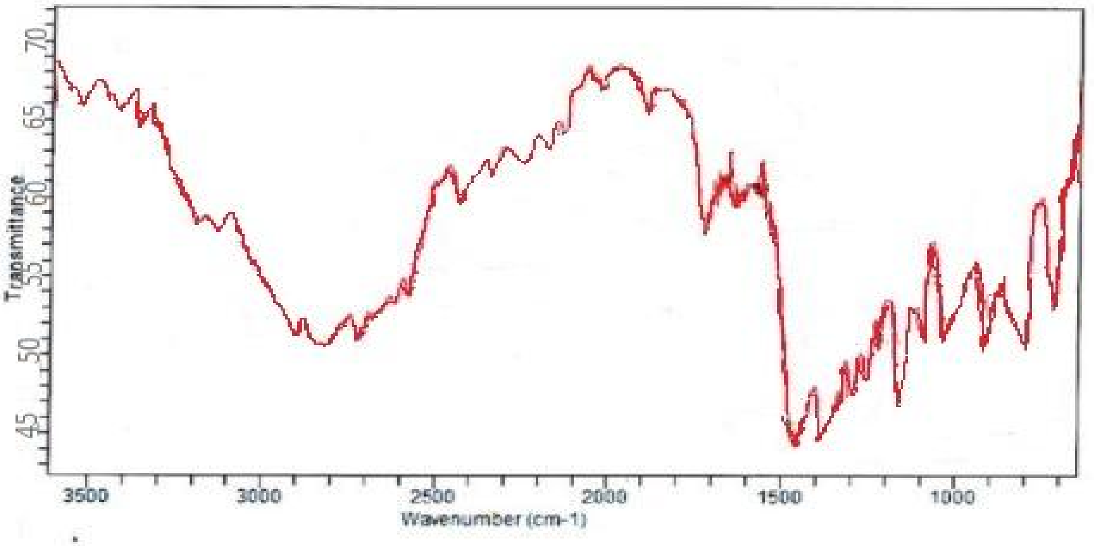

Plate 16: FTIR spectrum of one of the microplastic particle obtained at station 3 in June, showing absorbance band at different wave numbers

July

The FTIR spectrum of one of the microplastic particles obtained from station 3 in July can be seen in plate 17 below. The FTIR spectrum show absorbance band at different wave numbers

There is a peak at 2925cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching.

There is also, a peak at 1470cm^{-1} which is a characteristic of CH_2 scissoring.

A peak at 1170cm^{-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 2925cm^{-1}, 1470cm^{-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.

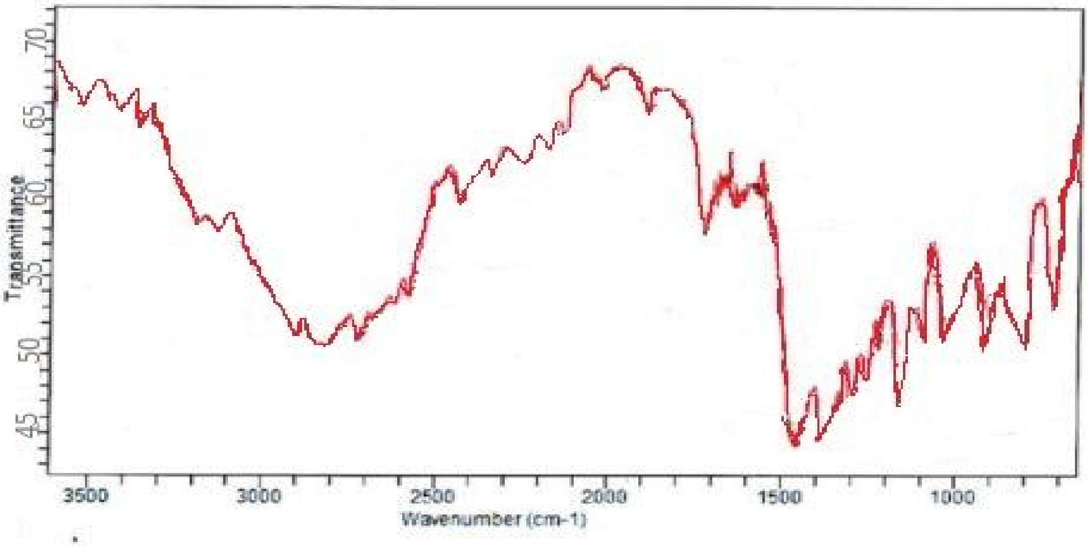

Plate 17: FTIR spectrum of one of the microplastic particle obtained at station 3 in July, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 3 in July can be seen in plate 18 below. The FTIR spectrum show absorbance band at different wave numbers

There is a peak at 2930cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching.

There is also, a peak at 1445cm^{-1} which is a characteristic of CH_2 scissoring.

A peak at 1130cm^{-1} is attributed to the asymmetric stretching of oxygen atom.

There is a peak at 880cm^{-1} is a characteristics of CH rocking.

The peaks at 2930cm^{-1}, 1445cm^{-1} and 880cm^{-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.

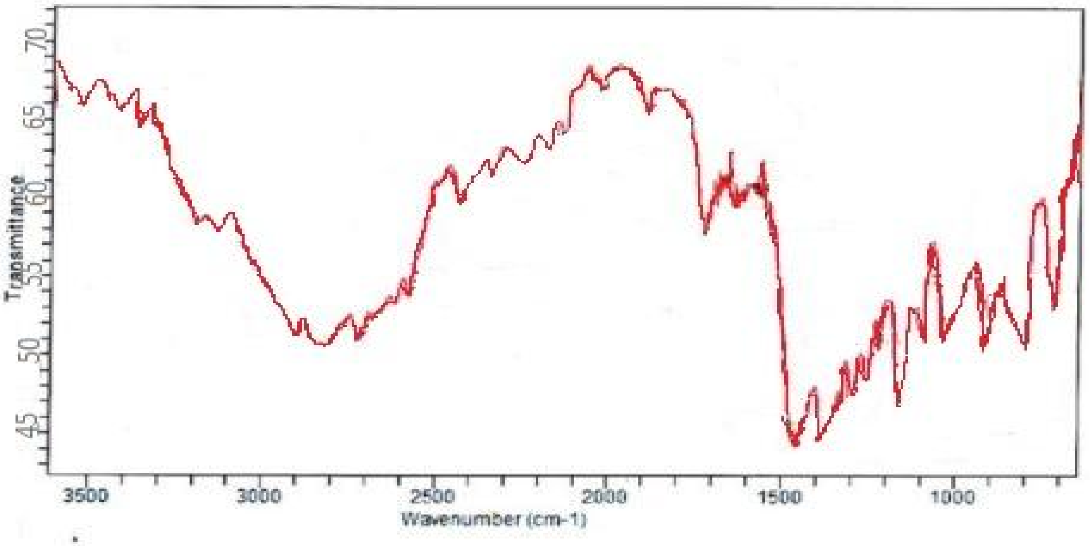

Plate 18: FTIR spectrum of one of the microplastic particle obtained at station 3 in July, showing absorbance band at different wave numbers

August

The FTIR spectrum of one of the microplastic particles obtained from station 3 in August can be seen in plate 19 below. The FTIR spectrum show absorbance band at different wave numbers

There is a peak at 2925cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching.

There is also, a peak at 1445cm^{-1} which is a characteristic of CH_2 scissoring.

A peak at 1140cm^{-1} is attributed to the asymmetric stretching of oxygen atom.

There is a peak at 910cm^{-1} is a characteristics of CH rocking.

The peaks at 2925cm^{-1}, 1445cm^{-1} and 910cm^{-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.

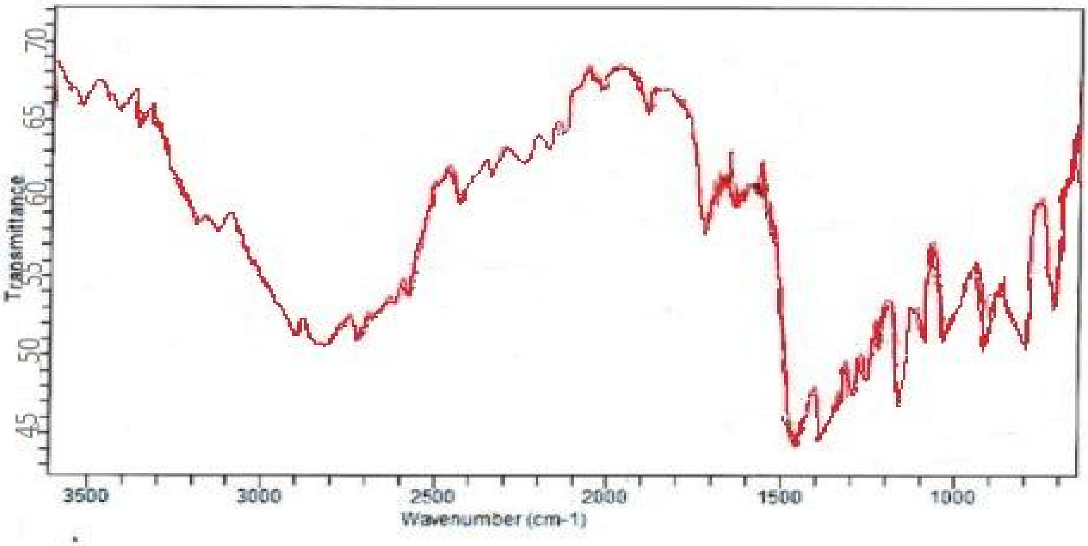

Plate 19: FTIR spectrum of one of the microplastic particle obtained at station 3 in August, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 3 in August can be seen in plate 20 below. The FTIR spectrum show absorbance band at different wave numbers

There is a peak at 2925cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching.

There is also, a peak at 1460cm^{-1} which is a characteristic of CH_2 scissoring.

A peak at 1160cm^{-1} is attributed to the asymmetric stretching of oxygen atom.

There is a peak at 700cm^{-1} is a characteristics of CH rocking.

The peaks at 2925cm^{-1}, 1460cm^{-1} and 700cm^{-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.

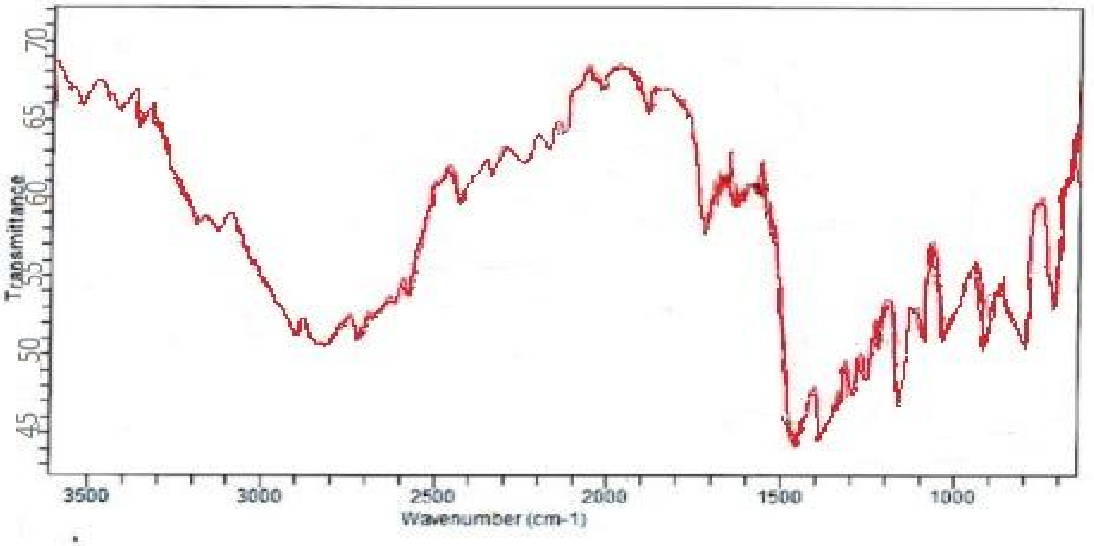

Plate 20: FTIR spectrum of one of the microplastic particle obtained at station 3 in August, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 3 in August can be seen in plate 21 below. The FTIR spectrum show absorbance band at different wave numbers

There is a peak at 2945cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching.

There is also, a peak at 1455cm^{-1} which is a characteristic of CH_2 scissoring.

A peak at 1145cm^{-1} is attributed to the asymmetric stretching of oxygen atom.

There is a peak at 820cm^{-1} is a characteristics of CH rocking.

The peaks at 2945cm^{-1}, 1455cm^{-1} and 820cm^{-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.

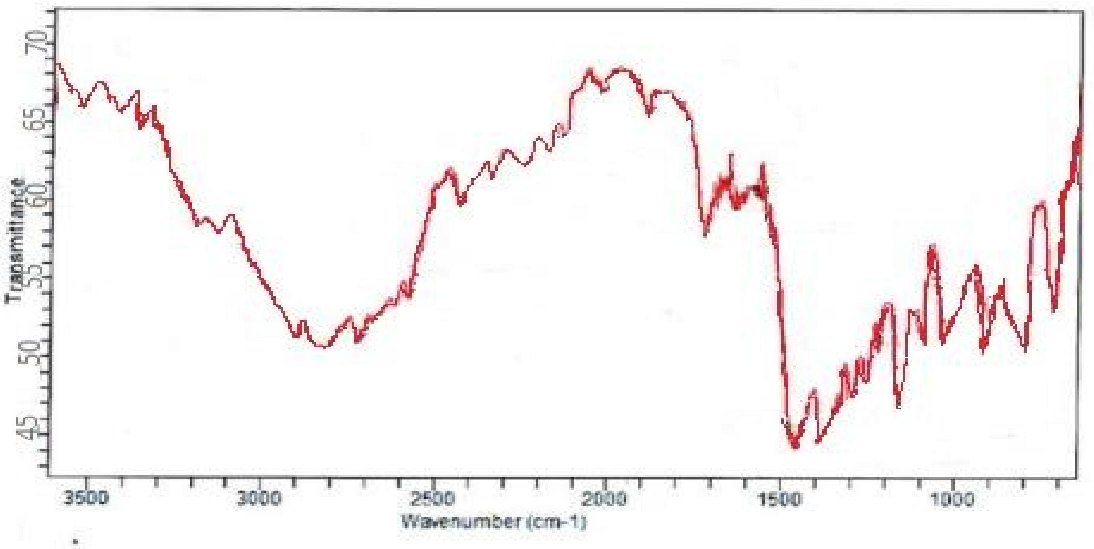

Plate 21: FTIR spectrum of one of the microplastic particle obtained at station 3 in August, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 3 in August can be seen in plate 22 below. The FTIR spectrum show absorbance band at different wave numbers

There is a peak at 2905cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching.

There is also, a peak at 1445cm^{-1} which is a characteristic of CH_2 scissoring.

A peak at 1165cm^{-1} is attributed to the asymmetric stretching of oxygen atom.

There is a peak at 840cm^{-1} is a characteristics of CH rocking.

The peaks at 2905cm^{-1}, 1445cm^{-1} and 840cm^{-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.

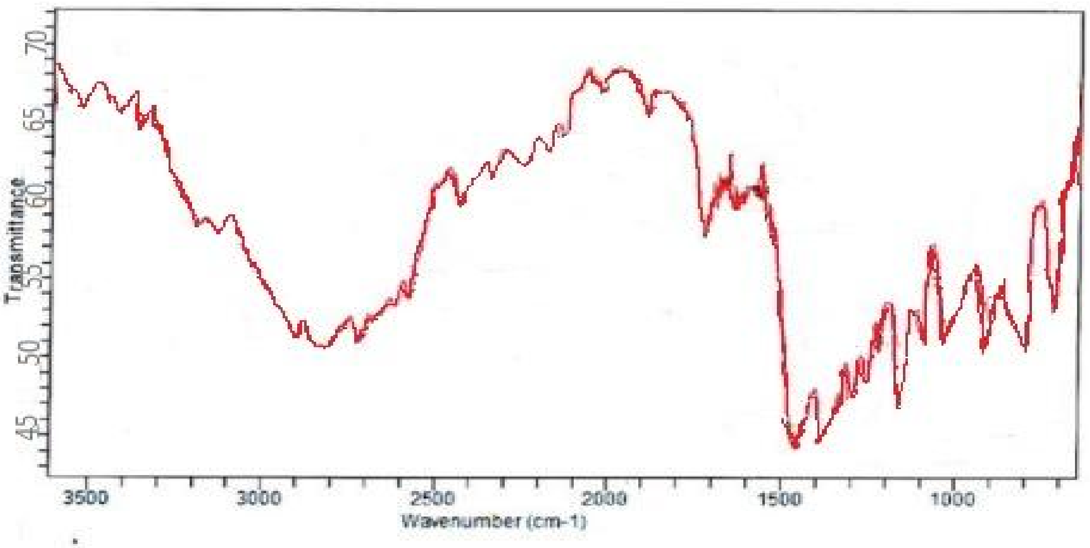

Plate 22: FTIR spectrum of one of the microplastic particle obtained at station 3 in August, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 3 in August can be seen in plate 23 below. The FTIR spectrum show absorbance band at different wave numbers

There is a peak at $2980cm^{-1}$ which is a characteristics absorption of symmetric CH_2 stretching.

There is also, a peak at $1440cm^{-1}$ 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 $2980cm^{-1}$, $1440cm^{-1}$ 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.

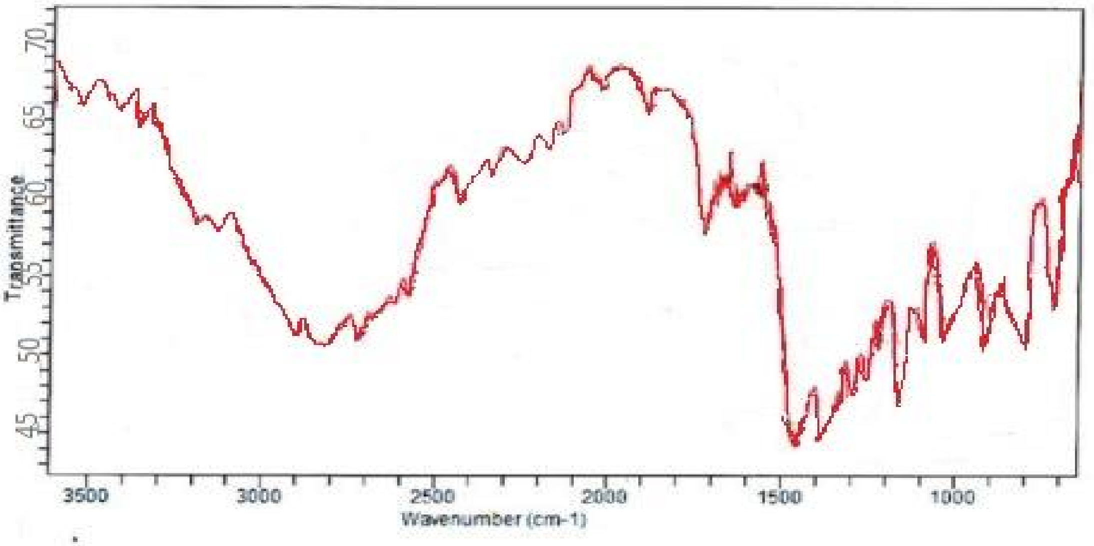

Plate 23: FTIR spectrum of one of the microplastic particle obtained at station 3 in August, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 3 in August can be seen in plate 24 below. The FTIR spectrum show absorbance band at different wave numbers. There is a peak at 2905cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching.

There is also, a peak at 1465cm^{-1} which is a characteristic of CH_2 scissoring.

A peak at 1170cm^{-1} is attributed to the asymmetric stretching of oxygen atom.

There is a peak at 790cm^{-1} is a characteristics of CH rocking.

The peaks at 2905cm^{-1}, 1465cm^{-1} and 790cm^{-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.

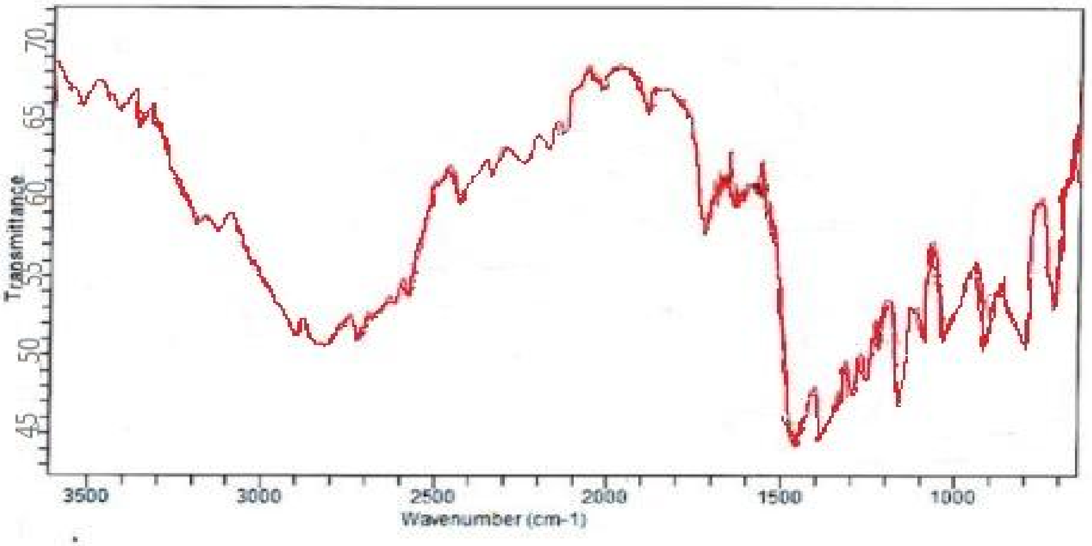

Plate 24: FTIR spectrum of one of the microplastic particle obtained at station 3 in August, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 3 in August can be seen in plate 25 below. The FTIR spectrum show absorbance band at different wave numbers

There is a peak at 2925cm^{-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 1135cm^{-1} is attributed to the asymmetric stretching of oxygen atom.

There is a peak at 820cm^{-1} is a characteristics of CH rocking.

The peaks at 2925cm^{-1}, 1450cm^{-1} and 820cm^{-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.

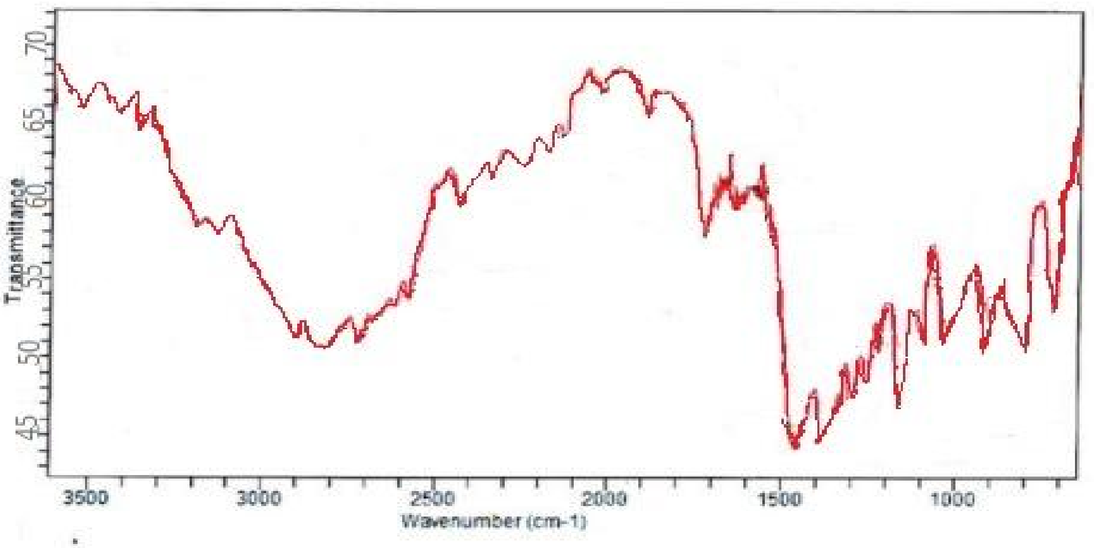

Plate 25: FTIR spectrum of one of the microplastic particle obtained at station 3 in August, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastic particles obtained from station 3 in August can be seen in plate 26 below. The FTIR spectrum show absorbance band at different wave numbers

There is a peak at 2940cm^{-1} which is a characteristics absorption of symmetric CH_2 stretching.

There is also, a peak at 1445cm^{-1} which is a characteristic of CH_2 scissoring.

A peak at 1155cm^{-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 2940cm^{-1}, 1445cm^{-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.

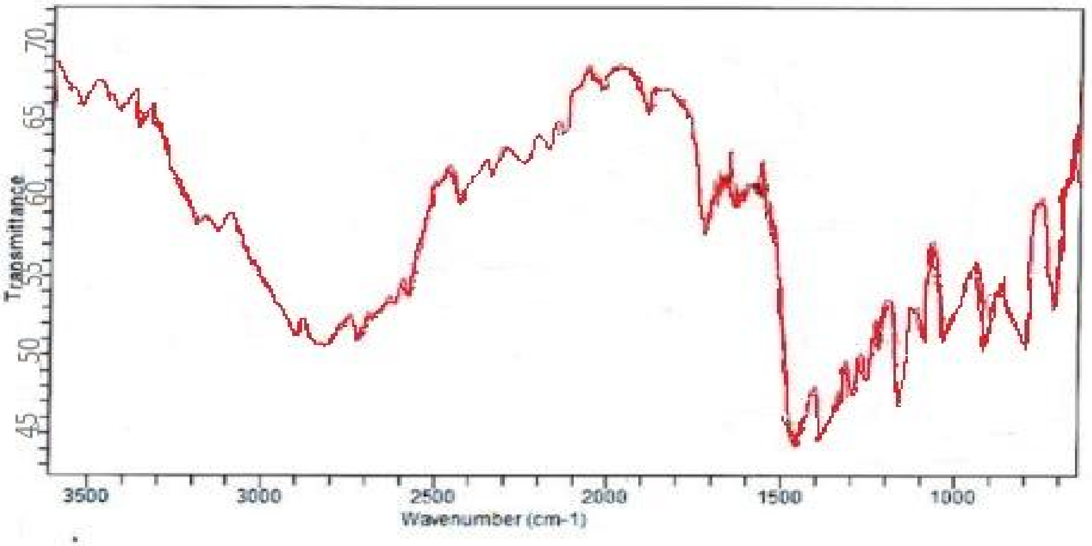

Plate 26: FTIR spectrum of one of the microplastic particle obtained at station 3 in August, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastics obtained from station 3 in August can be seen in plate 27 below. The FTIR spectrum show absorbance band at different wave numbers.

There is a peak at 2920cm^{-1} which is a characteristics absorption of asymmetric CH2 stretching.

There is also, a peak at 2820cm^{-1} which is a characteristic of symmetric CH2 stretching.

A peak at 1445cm^{-1} is a characteristics CH2 scissoring.

There is a peak at 710cm^{-1} which is a characteristic of CH2 rocking.

The peaks at 2920cm-1, 1445cm^{-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**.**

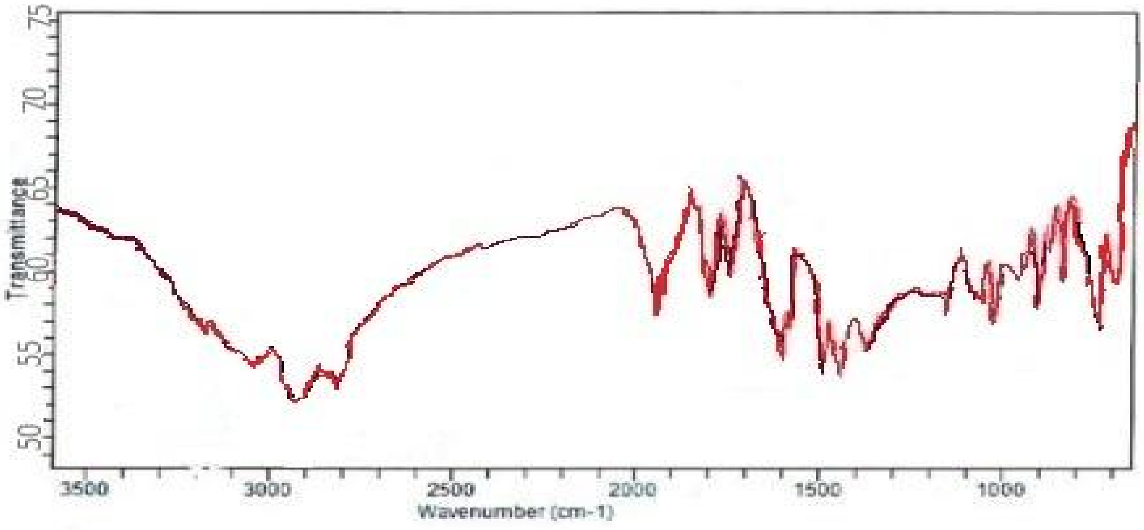

Plate 27: FTIR spectrum of one of the microplastic particles obtained at station 3 in August, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastics obtained from station 3 in August can be seen in plate 28 below. The FTIR spectrum show absorbance band at different wave numbers.

There is a peak at 2920cm^{-1} which is a characteristics absorption of asymmetric CH2 stretching.

There is also, a peak at 2840cm^{-1} which is a characteristic of symmetric CH_2 stretching.

A peak at 1460cm^{-1} is a characteristics CH_2 scissoring.

There is a peak at 710cm^{-1} which is a characteristic of CH_2 rocking.

The peaks at 2920cm^{-1}, 1460cm^{-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**.**

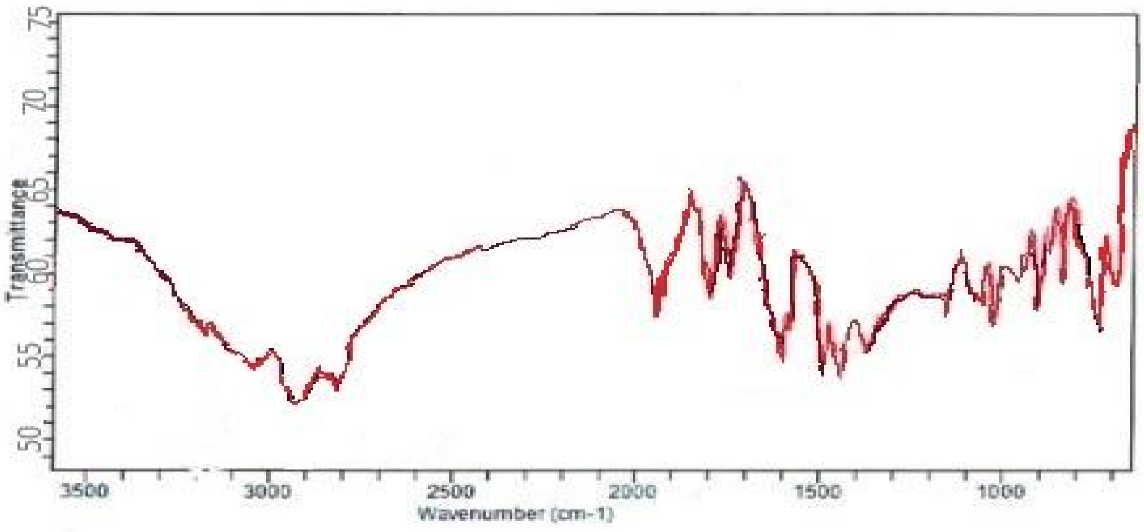

Plate 28: FTIR spectrum of one of the microplastic particles obtained at station 3 in August, showing absorbance band at different wave numbers

The FTIR spectrum of one of the microplastics obtained from station 3 in August can be seen in plate 29 below. The FTIR spectrum show absorbance band at different wave numbers. There is a peak at 2910cm^{-1} which is a characteristics absorption of asymmetric CH_2 stretching.

There is also, a peak at 2830cm^{-1} which is a characteristic of symmetric CH_2 stretching.

A peak at 1445cm^{-1} is a characteristics CH_2 scissoring.

There is a peak at 710cm^{-1} which is a characteristic of CH_2 rocking.

The peaks at 2910cm^{-1}, 1445cm^{-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**.**

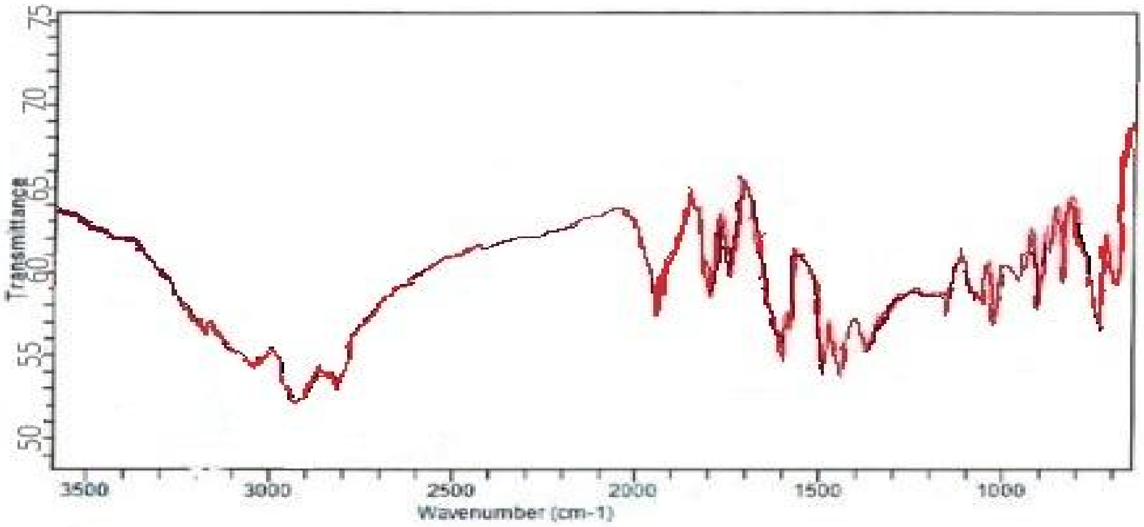

Plate 29: FTIR spectrum of one of the microplastic particles obtained at station 3 in August, showing absorbance band at different wave numbers

Four others suspected microplastic particles did not produce any FTIR results, hence they were not microplastics.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Level of Microplastic

Of the 37 suspected microplastic particles tested, 29 were confirmed to be microplastics, hence a success rate of visual identification of 78.3%. This is in agreement with the findings of Su Lei *et al*., (2018). The level of microplastic per individual ranged from 0-6 per individuals, largely agreeing with the result of Su Lei *et al*., (2018), Rodrigues *et al*., (2019) and Hamilton *et al*., (2021). Many factors, including the presence of wastewater effluent, economic activities, industrial activities hydrological conditions and sedimentation, have been shown to drive microplastic concentrations in freshwater (Eriksen *et al*., 2013).

Microplastic levels in station three was more than that in station two and one. This can easily be explained by the level of urbanization, economic activities in the area, and at the waterfront. Ikpoba river waterfront is located in a relatively more urbanized area. There are also several economic activities taking place at the waterfront. This result in more direct and indirect introduction of plastic particles into the waters.

It can be said that there is a strong relationship between microplastic in the waters and sediment and that in fish from Ikpoba River. From visual observation only, it is seen that the Ikpoba waterfront (station 3) which had a higher amount of visible plastic particles, also presented the highest abundance in terms of microplastics in fish harvested from the stations.

The level of microplastic varied from station to station and with months. The highest abundance in the month of June was at station two, while the highest abundance in the month of July occurred at station one and the lowest at station two. For the month of August, the highest abundance was at station three while the lowest occurred at station one. The greatest value occurred in station one occurred in July, with the lowest occurring in June. The highest mean value for station two occurred in June, with the lowest mean value occurring in July. The highest mean value for station three occurred in the month of August and the lowest in June.

5.2 Plastic Load

The average plastic load was 1.37 plastic particles per fish. This value is higher than that reported by Lusher *et al*., (2016) with an average microplastic count per individual fish of 0.13. it is however, closer to those recorded by Boerger *et al*., (2010) with average microplastic count per fish to be 2.1, and Brownlow *et al*., (2018) with average microplastic count per fish of 1.8 plastic particles per fish. This theoretically means for *every C. gariepinus* harvested from Ikpoba River, a consumer is consuming at least one microplastic particle. This should be a relative cause for probable concern for the government at all levels and consumers.

5.3 Frequency of Occurrence of Microplastics Ingestion

Of the twenty-seven fishes sampled, 16 were found to have ingested microplastic, giving an overall frequency of occurrence 59.2%. This is significantly higher than the result of Faure *et al*., (2015) and Zhang *et al*., (2017), in their study of freshwater fish sampled from China and Switzerland (7.5%-26%), and lower than the result of Hamilton *et al*., (2021), in their study of the prevalence of microplastics and anthropogenic debris within a deep-sea food web in The Monterey Bay which gave 96% and Feng *et al*., (2021), Prihadi *et al*., (2019), which gave a 100%. This may be as a result of varied levels of contamination in these waters, feeding habit of species, net feeding and capacity of functioning waste disposal service (Lusher *et al*., 2016); as well as variation in extraction method. It however is close to the 68% obtained by Merill *et al*., (2023). This result largely corresponds with the result of Wooton *et al*., (2021).

The frequency of occurrence was relatively higher in the month August. 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 at station three except for the month June, where the mean value was 0.29 ± 0.05 and less than mean value for station two. This might be attributed to the fact that station three is more susceptible to improper plastic and waste disposal, which will only have been compounded by the increasing amount of rainfall across the months to August.

5.5 Morphological Classification

Majority of the microplastic particles in this study were fragments, with a small percentage of fiber, pellets, foam and filaments. These potentially coming from plastic bags, soft food packaging, acrylate and paint chips etc. This in agreement with other findings (Lusher *et al*., 2013, 2016; Neves *et al*., 2015; Rochman *et al*., 2015; Bellas *et al*., 2016). This contrast with the finding of Hamilton *et al*., (2021), whose, of the total microparticles quantified, 84.7% were fibers, 14.4% were fragments, and 0.1% were beads, as well as Wooton *et al*., (2021), where the majority of them were fibres and films. This is in agreement with findings from Markic *et al*., (2018); Rochman *et al*., (2015), which opines that microplastic being investigated are often made up of fibres in developed nations and fragments in developing and underdeveloped nations. Brownlow *et al*., (2018) suggested that this is due to the large amount of waste water effluents carrying synthetic fibres from washing machines, which are more common in developed nations.

5.6 Polymer Classification

All the suspected microparticles were analysed and validated using ATR-FTIR analysis. The microplastic particles produced FTIR results that showed they were made of propylene and polyethene; with propylene being the more prevalent. Polypropylene (Collard *et al*., 2018) and the polyethylene (PE) were reported more frequently. This result is in partial agreement with Merill *et al*., (2023) in whose study, most common polymer was polyethylene and PE, and is in agreement with the findings of Biginagwa *et al*., (2016), Horton *et al.,* (2018), Collard *et al*., (2018), Andrade *et al*., (2019) Denutsui *et al*, (2020), Frank *et al*., (2022), Mardiansyah *et al*., (2022). From the FTIR result, it is observed that there is a clear pattern to the type of microplastic found at each station, with station three being predominantly polypropylene while in station one, polyethene is most prevalent. This can be explained by the type of economic activities carried out within the watershed of the river at each station.

While all stations receive an unreasonable amount of plastic wastes resulting from mostly domestic and commercial uses, these are often in the form of cellophanes, disposable plastic packs, PET bottles and polyethylene bags. At station three however, there is a lot of mechanic workshops car washing, and rug washing business which results in the unequal and sizable deposition of polypropylene particles.

The wave number ranged from 2920cm^{-1} to 2870cm^{-1} for asymmetric stretching, 2820cm^{-1} to 2840cm^{-1} for symmetric stretching, 1445cm^{-1} to 1470cm^{-1} for CH_2 scissoring. These ranges correspond to the peaks for polyethylene.

The wave number for the polypropylene spectrum ranged from 2905cm^{-1} to 2940cm^{-1} for symmetric stretching, 1445cm^{-1} to 1470cm^{-1} for CH_2 scissoring, 1135cm^{-1} to 1170cm^{-1} for O_2 stretching, 700cm^{-1} to 920cm^{-1}. These are characteristic peaks for polypropylene.

CHAPTER SIX

6.0 Summary, Conclusion and Recommendation

6.1 Summary

This study establishes the level of microplastic in *C. gariepinus* harvested from the Ikpoba River. 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 microplastics in Ikpoba River which is seen in the amount of microplastics found in *C. gariepinus* harvested from the river at different stations. The microplastic type found based on morphological characteristics includes fragments, filaments, fibre, pellets and foam, with fragments being the most occurring. Based on polymer characteristics, there were two types, namely polypropylene and polyethene.

The types of microplastic prevalent at the different stations are fairly indicative of their sources and has a relationship with the economic activities in the area. The level of microplastics 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. Government on federal, state and local level should implement proper waste management services and practices, as improper waste disposal is the major source of plastics that find their way to water bodies.

2. 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.
3. Frequent and regular water body clean-up operations should be carried out to remove plastics that find a way to water bodies.
4. A model for determining safe consumption level for fish contaminated with microplastic
5. Further studies need to be carried out to establish dietary exposure to microplastic consumption
6. Studies on the absorption process of MPs is highly recommended to know the exact damages caused by these microplastics.
7. It is also recommended to study the waste management and plastic degradation process to have complete idea about microplastic sources

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