SCREENING FOR POTENTIALS OF MICROBIAL ASSEMBLAGES ON PLASTICS FROM SHORELINES OF RIVERS IN NIGER STATE TO UTILIZE PLASTICS

BY

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ABSTRACT

Plastics are widely used in virtually all sectors but are not easily degraded instead they fragment into smaller plastics (microplastics) and hence are persistent in the environment, becoming a major source of pollution. The study herein was conducted to isolate microorganisms that have potentials to degrade plastics from sediment samples of shorelines of rivers. Macro, meso and micro plastics in soil samples from the shorelines of four (4) different rivers Tagwai, Bosso, Shiroro and Chanchaga rivers in Niger State, North Central Nigeria were identified and quantified. The microbial communities attached to the plastics were isolated and screened for possible potentials to utilize plastic and the sampling was done at different points for each river. The optical density of the growth of bacteria and fungi were observed and measured at 600 nm for 32 days at 4 days interval. Five (5) bacterial isolates and three (3) fungal isolates from 20 samples were identified as *Eschericha coli, Alcaligene faecalis, Bacillus megaterium, Staphylococcus epidermis, Staphylococcus aureus, Aspergillus niger, Aspergillus flavus and Curvularia nodosa* respectively using cultural, biochemical and molecular identification techniques. Among the bacterial isolates, *Alcaligenes faecalis* (16 %) and *Bacillus megaterium* (12 %) utilized microplastics more than *Staphylococcus epidermis* (3 %), *Staphylococcus aureus* (2 %) and *Escherichia coli* (1 %). *Curvularia nodosa* (22 %) had the most percentage efficiency compared to *Aspergillus flavus* (7 %) and *Aspergillus niger* (5 %). The results obtained in the study showed that *Curvularia nodosa* could have huge potential in the utilization of plastics.

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LIST OF ABBREVIATIONS AND ACRONYMS

BPA	Bisphenol A
HDPE	High density polyethylene
ITS	Inter-genetic spacers
LDPE	Low density polyethylene
MP	Microplastics
PAHs	Polycyclic aromatic hydrocarbons
PE	Polyethylene
PET	Polyethylene terephthalate
PCR	Polymerase chain reaction
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
pH	Potential of Hydrogen
UV	Ultraviolet
VOCs	Volatile organic compounds

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Plastics are high molecular weight organic polymers made up of various elements. Plastics can either be synthetic or biologically based. However, the majority of plastic in use today is synthetic plastic because of the ease in manufacturing approaches involved in the processing of crude oil. Plastics are of great significance globally due to their wide use, which has enabled improvement in the quality of human life through ease of packaging of foods and other items, thus lengthening their shelf life (Demirbas, 2007). Based on their constituents and end use, plastics can be categorized into different types which include polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET), all of which are high molecular weight polymers whose ability to be broken down by microorganisms is relatively low or almost impossible, hence, plastics are persistent in the environment and are one of the sources of environmental pollution (Tokiwa *et al.,* 2009). Their disposal on the aquatic environment have resulted in their accumulation due to little, if any, biodegradation, making the environment unaesthetic, with possible health implications to humans and animals (Siddiqui *et al.,* 2008). Plastic pollution was first reported in the 1970s (Mohanan *et al.,* 2020).

After use, most of the plastics are collected and incinerated, however, this method has greater effects on human health and the environment in general (Andrady and Neal, 2009). Combustion of plastics result to the release of by-products which are harmful in the environment, especially to the health of living organisms. The frequently evolved byproducts of plastics during combustion are airborne particulate emission (soot) and solid residue ash (black carbonaceous colour) (Mohanan *et al.,* 2020). Several studies

have demonstrated that soot and solid residue ash possess a high potential of causing significant health and environmental effects. Aside from trying to eliminate plastic wastes, their production is also costly to the environment. It takes large amounts of chemical to produce plastics, as well as significant amounts of fossil fuels (Font *et al.,* 2004). Plastics in Nigeria are widely used for packaging purposes. The materials packed in plastics include food materials like fruits, cooked food and water. In addition, plastics especially polyethylene, are used in carrying purchased materials such as raw vegetables and fruits. Also, other materials carried or preserved using plastics are clothes.

After use, most of the plastics in Niger State, Nigeria is arbitrarily disposed into the environment due to lack of proper facilities for their orderly collection, infrastructure for recycling and lack of public awareness on the imminent danger associated with plastic litter in the environment. Some plastic wastes are collected for recycling but most remain scattered in the environment, especially in the aquatic environment. The aquatic environment is constantly under increasing and recurrent pressure from human activities majorly associated with production of plastics, pesticides, heavy metals and persistent organic pollutants (POPs) that undesirably affect the marine ecosystem (Auta *et al.,* 2017). Plastics enter the marine environment in different sizes and shapes and are mostly non-biodegradable, they fragment under ultraviolet light and are commonly referred to as microplastics (Andrady, 2017). Microplastics are tiny plastic particles that are smaller than five millimeter in size. They are either intentionally produced solely for specific industrial or cosmetic purpose such as exfoliating scrubs, tooth pastes and resin pellets used in the plastic industry (Primary microplastics) or formed from the fragmentation of large plastic products exposed to ultraviolet radiations or mechanical abrasion (secondary microplastics). Microplastics get into the marine ecosystem through a number of ways ranging from activities on land to domestic and industrial drainage systems and waste

water treatment plants (Auta *et al.,* 2017). Large plastics products from refuse dumps can be transported into the marine ecosystem either by wind or man-made activities. These fragmented plastics are easily seen as food and thus ingested by aquatic organisms like planktons and other larger animals due to the size. It has been reported that these tiny plastic particles also have the tendencies of absorbing toxic chemicals and transferring it into the food chain and ingestion of these tiny plastics could cause oxidative stress, reduced growth rate, reproductive complications, pathological stress and in some cases death (Auta *et al.,* 2017). Aquatic animals can accumulate these plastics in their tissues, serve as a vector for the distribution of pathogens, accumulate and absorb toxic pollutants (Siddiqui *et al.,* 2008). These fragmented plastics can cause adverse health effects such as cancer, impaired reproductive activity, oxidative stress, decreased immune response and other abnormalities in animals and humans when consumed via the food chain (Auta *et al.,* 2017). Many accumulate in the environment for long periods of time and are washed into the aquatic environment where they create concerns to human health and aquatic organisms. It would be both desirable and economical to have microorganisms capable of biodegradation of plastics as one solution to the problem of plastics accumulation and nuisance in the environment.

1.2 Statement of the Research Problem

The long-term build-up of plastics and microplastics in the aquatic environment have led to a decline in the survival rates of aquatic animals and in soil fertility (Cole *et al*., 2011). Some plastics are made with additives such as bisphenol A (BPA), which is harmful to aquatic animals and the food chain in general. Studies have demonstrated that freshwater invertebrates, fish and other marine biota can swallow microplastic particles, resulting to wounds, stress, contamination, bioaccumulation, and tumor development; immune

response disrupting feeding, and altering metabolic function. Plastic polymers do not biodegrade at timescales relevant for human society (Barnes *et al.,* 2009).

1.3 Aim and Objectives of the Study

The aim of this study was to screen for potentials of microbial assemblages associated with plastics from shorelines of rivers in Niger State to utilize plastics.

Objectives of this study were to:

i. Quantify and identify macro, meso and microplastics from sediment samples from shorelines of rivers at multiple locations in Niger State
ii. Isolate and identify the microbial communities attached to the plastics
iii. Screen the microorganisms for potentials to degrade plastics/microplastics

1.4 Justification for the Study

The interest in environmental issues is growing and there are increasing demands to develop material, which do not burden the already stressed environment significantly. Plastic pollution especially microplastic is a global menace that is fast eating into environmental health of the ecosystem. This global concern has carved the urgent need for either manufacturing alternative materials to plastics especially single-use plastics or an eco-friendly way of eliminating plastic waste. This research work will add to the existing body of knowledge on quantification of macro and microplastics in aquatic environment and their microbial communities with potentials to degrade these plastics. Biodegradation is necessary for water-soluble or water immiscible polymers because they eventually enter streams which can neither be recycled or incinerated. With the excessive use of plastics and increasing pressure being placed on capacities available for plastics waste disposal, the need for biodegradation of plastic waste has assumed increasing importance in the last few years (Shah *et al.,* 2008). Thus, a search for, and isolation from soil samples, of microorganisms capable of degrading these plastics can

mark the beginning of finding a solution to the problem of plastics accumulation and pollution in the environment.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Global Plastic Production

In the early 1900s, Bakelite, being the first plastic to be manufactured for commercial purposes was invented (Lear *et al.,* 2021). Production of plastics started on an industrial

scale between the 1940s and 1950s. Decades down the line, the global annual production of plastics exceeded 365 million metric tonnes (Plastics Europe, 2010). In the 1930s, the word plastics became a part of consumers' everyday language to describe a wide variety of this material which continues today. The use of plastics especially polythene (PE) is growing daily and every year millions of synthetic plastics are being accumulated in the sea coasts and terrestrial environment (Lee *et al.,* 1991). Plastic products have managed to infuse themselves into our industry, science and way of life very quickly, leaving many people unaware of its origins, benefits and negative effects. In 2015 global plastic production grew by 3.4 % compared to 2014. Compound annual growth rate (CAGR) from 1950 to 2015 is about 8.6 % (Plastic Europe, 2010). Asia with the leading country China accounts for more than 49 % of worldwide production. Polyolefins account for more than 55 % of global plastic materials demand followed by polyvinylchloride. World plastic production has increased exponentially since large-scale production first began in the 1950s. Global plastic production, excluding fibres, increased from 322 million tonnes (Mt) in 2015 to 348 Mt in 2017, fibres included, global production was estimated to be 381 Mt in 2015 with additives included, 407 Mt (Geyer *et al.,* 2017). Considering the estimated worldwide population growth rate and current consumption and waste habits, plastic production is predicted to double by 2025 and more than triple by 2050 of total non-fibre plastic production, 36 % is PE, 21 % is PP, 12 % is PVC, and less than 10 % each are PET, PUR and PS (Geyer *et al.,* 2017). The production of polyester PAs and acrylics fibre is the next largest group, much of which is PET, these seven groups account for 92 % of all plastics ever made (Geyer *et al.,* 2017). Intentional microplastic production represent less than one percent of the total plastic production based on European figures. Economically, the plastic industry provides employment to millions of people. The European plastic industry, for instance, is estimated to involve 60 000

companies employing 1.5 million people, with a turn-over of 355 billion Euros. The European industry represents 18.5 % of the annual global plastics market of 348 Mt in 2017 (Geyer *et al.,* 2017).

2.2 General Properties of Plastics

Plastic is a synthetic material formed from a broad range of organic polymers that have become an indispensable part of our everyday world. Plastics are classified based on their thermal properties.

2.2.1 Thermal properties

Plastics are classified into two groups based on their thermal properties: Thermoset and Thermoplastics polymers. Thermoset plastics are solid plastics that cannot be melted and modified (Raziya *et al.,* 2016). It undergoes a permanent chemical change when heated and hence these plastics cannot be recycled because they have a highly cross-linked structure, whereas thermoplastic are linear structured plastics. Examples include phenol-formaldehyde, polyurethanes (Raziya *et al.,* 2016). Thermoplastics are plastics polymers which can be hardened and softened by recurrent heating and cooling process. (Raziya *et al.,* 2016). These polymers do not change their chemical composition when heated. Example of these types of polymers are Polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC) and polytetrafluoroethylene (PTFE). They are also regarded to as common plastics, having molecular weight ranging from 20,000 to 500,000 AMU and have several numbers of repeating units derived from a simple monomer unit (Alshehrei, 2017). The thermoplastics are the main type of plastic used in packaging, and due to their non-biodegradable nature but tremendous profusion in plastic waste, Microplastic from thermoplastic materials pose a huge problem in plastic pollution in the oceans (Raziya *et al.,* 2016).

2.3 Classification of Plastics

Plastics can be classified based on its ability to be degraded by microorganism and based on size.

2.3.1 Based on biodegradability

There are two types of plastics based on biodegradability, they include non-biodegradable plastics and biodegradable plastics (Temoor *et al.,* 2018).

2.3.1.1 *Biodegradable plastics*

Biodegradable plastics are derived from renewable resources that are totally biodegradable in their natural forms, examples are components of living plants, animals and algae as source of cellulose, starches, protein and algal materials. They can also be produced by different microorganisms (Alshehrei, 2017). Factors such as UV, water, enzymes and gradual changes in pH brings about break down of biodegradable plastics (Alshehrei, 2017). Depending on the degree of biodegradability and microbial assimilation both bio-based and fossil-based polymers are considered as biodegradable plastics. Biodegradation of plastics involves enzymatic and non-enzymatic breakdown of water that is hydrolysis (Sumaira *et al.,* 2021). Type of organism, nature of pretreatment, and polymer characteristics are some of the factors affecting the efficiency of biodegradation processes. In addition, mobility, crystallinity, type of functional groups, chemical components, molecular weight, and additives present in polymers (Temoor *et al.,* 2018). Microorganisms secrete exoenzymes during degradation that disintegrate polymer complexes into smaller molecules like dimers and monomers. Therefore, small molecules pass through semi-permeable membranes of a bacterial cell to be used as energy as well as carbon source (Temoor *et al.,* 2018). Biodegradation reactions involve both aerobic and anaerobic mechanisms biodegradation (Temoor *et al.,* 2018).

2.3.1.2 *Non-biodegradable plastics*

They are commonly known as synthetic plastics, derived from petrochemicals. They are made up of several repetitions of small monomer units that account for their very high molecular weight (Alshehrei, 2017). Non-biodegradable plastics encompasses both fossil-based and bio-based polymers. Fossil-based synthetic polymers are the most commonly used non-biodegradable plastics, they are obtained from the derivatives of hydrocarbon and petroleum (petrochemicals) and have high molecular weight due to the extensive repetition of small monomer units (Temoor *et al.,* 2018). This type of plastics is highly stable and do not easily enter the degradation cycles of the biosphere (Temoor *et al.,* 2018). Nowadays, most of the commodity polymers used are either non-biodegradable or their degradation rate is too slow to be disintegrated completely (Temoor *et al.,* 2018). They have high molecular weight because of the reoccurrence of monomer units (Sumaira *et al.,* 2021). Examples are many of the regularly used plastics like PVC, PP, PS, PET, PUR, and PE (Sumaira *et al.,* 2021).

2.3.2 Based on size

Plastic materials reach the aquatic environment in various sizes, among which are the large visible debris “macroplastics” that is larger than 1-10 millimeter, plastics that are defined as 5-10 millimeter in range are classified as meso plastics while plastics in form of tiny particles or fragments called “microplastics” (less than 5 millimeter) (Grazia and Gian, 2017). Microplastics accounts for 92.4 % of plastic waste and mainly contains PP, PS, PUR, PCV, PET, PETE (Sumaira *et al.,* 2021). In addition, the globally recognized definitions for these categories are yet to be established. Andrady (2017) in his review work compared the methodologies used in 68 studies for the quantification of microplastics in the marine environment and that most of them reported two main size ranges of microplastics, 500 μm-5 mm and 1-500 μm, or fractions that were retained on

filters, confirming that there is still not a universally adopted size range to define microplastics. Thus, the term “microplastics” is used in the literature to include a surprisingly broad range of particles sizes from ~5 mm to few microns in diameter (Andrady, 2017). A more practical scheme to classify plastic debris in the aquatic environment has been proposed by the European MSFD Technical Subgroup on Marine Litter (Grazia and Gian, 2017), a scheme which also includes the category “mesoplastics” in the size range 5 mm-2.5 cm. Plastic debris enters the marine environment in a wide range of sizes, from micrometer to meter range (Hidalgo-Ruz *et al.,* 2012), as well as in a broad range of color, shape, chemical composition and specific gravity. As far as the small size plastic debris is concerned, microplastics are typically categorized into primary and secondary (Grazia and Gian, 2017) Primary microplastics are microplastics that are manufactured to be of microscopic size for specific industrial or cosmetic purpose. (Auta *et al.,* 2017) Examples include plastic particles used in facial cleansers, tooth paste, resin pellets and cosmetics like shower/bath gels, scrubs, peelings (Cole *et al*., 2011), eye shadow, deodorant, blush powders, make up foundation, mascara, shaving cream, baby products, bubble bath lotions, hair coloring, nail polish, insect repellents and sunscreen, others include synthetic clothing, abrasives found in cleaning products, drilling fluids, and air-blasting media (Auta *et al.,* 2017). Secondary microplastics originate from the degradation of larger (macro plastics) plastic debris on sea and land. These macro plastics over time, when exposed to certain physical, chemical and biological factors fragment into smaller particles and end up as microplastics (Cole *et al*., 2011). A combination of several environmental factors (such as sunlight and temperature), and the properties of the polymer (size, density) influences the disintegration of microplastic debris. Exposure of larger plastic debris to ultraviolet (UV) radiation from the sun causes photo-

degradation of plastics. The ultra violet radiation in the sun causes oxidation of the polymer matrix which leads to the cleavage of bond (Auta *et al.,* 2017).

2.4 Structural Chemistry of Plastic

Polymer which is the basic structure of plastics is a macromolecular chain, framed and built from monomeric units by polymerization reactions. This chain assemblage is as a result of certain reactions known as polyaddition and polycondensation reactions which follow a step growth mechanism (Raziya *et al.,* 2016).

Addition polymer is a polymer that constitutes all the atoms in a monomer while condensation polymer is a polymer in which some monomer atoms are discharged into small molecules such as water. Some addition polymers are made from monomers comprising a double bond between carbon atoms. Such monomers are referred to as olefins and some marketable addition polymers are referred to as polyolefins. Furthermore, condensation polymers are made from monomers that have two dissimilar groups of atoms that can link together example: ester or amide links. They comprise polymers such as polyethylene, polypropylene, polystyrene, polyvinylchloride, polyurethane and polyethylene terephthalate (Raziya *et al.,* 2016).

2.4.1 Physical properties of polymers

The durability, flexibility and strength of a polymer is highly dependent on the side groups, branching, cross-linking and chain length of the polymer amongst other physical properties (Smith and Lemstra, 1980). In essence, the length of the chain determines the strength of the polymer and the polar side chains enhances the link between the chains making the polymer stronger. Unbranched chains cluster together more closely than split or branched chains resulting to polymers that are more crystalline, dense and strong (Jansen *et al.,* 1999). It is hard to melt or biodegrade polymers whose chains are cross-

linked by covalent bonds. Polyethylene bags are some of the plastics that exhibits these properties yet it is one of the simplest polymers that has lengthy chains comprising of two carbon chains (Smith and Lemstra, 1980).

2.5 Uses of Plastics

2.5.1 Common uses

The use of plastics in the fabrication of automobile materials and parts lead to greater fuel efficiency thereby making plastics very interesting in automobile industries. Cars are generally made of steel and over the past few years lightweight substitutes have progressively instituted their way into automobile designs. Polyurethane, polyvinylchloride and polypropylene are the three major types of plastics that are majorly used in the automobile industries. Plastics have also been used in the production of helmets, in the production of refrigerator insulators, food and drink packaging films, in the production of drainage pipes and in the health sector for the production and construction of some prosthesis, artificial implants and absorbable sutures (Cole *et al*., 2011). The importance of plastics in the society cannot be overemphasized. Plastics have diverse uses which are dependent on the purpose of use and materials used in the manufacturing. Some types of plastic can be used in the production of different materials while other types are more efficient in some products. In general, the choice of plastic is highly dependent on the purpose of use.

Table 2.1 Common Uses of Plastics

S/N	PLASTIC	USES
1	Polyethylene (PE)	Plastic bags, milk and water bottles, food packaging film, toys, irrigation and drainage pipes, motor oil bottles
2	Polyurethane (PU)	Tyres, gaskets, bumpers, in refrigerator insulation, sponges, furniture cushioning, and life jackets

3	Polyvinyl chloride (PVC)	Automobile seat covers, shower curtains, raincoats, bottles, visors, shoe soles, garden hoses, and electricity pipes
4	Polystyrene (PS)	Disposable cups, packaging materials, laboratory ware, certain electronic uses
5	Polypropylene (PP)	Bottle caps, drinking straws, medicine bottles, car seats, car batteries, bumpers, disposable syringes, carpet backings
7	Polycarbonate	Use for making nozzles on paper making machinery, street lighting, safety visors, rear lights of cars, baby bottles and for houseware. It is also used in sky-lights and the roofs of greenhouses, sunrooms and verandahs. One important use is to make the lens in glasses
8	Polytetrafluoroethylene (PTFE)	Use in various industrial applications such specialized chemical plant, electronics and bearings. It is met with in the home as a coating on non-stick kitchen utensils, such as saucepans and frying pans

Source: (Alshehrei, 2017)

2.6 Environmental Pollution by Plastic Waste

2.6.1 Marine pollution by plastic disposal

Synthetic plastics do not break down easily in the environment because they are resistant to microbial attack due to their excessive molecular mass, higher number of atomic rings, unusual bonds and halogen substitutions (Omar, 2017). As a result, they remain in the environment particularly in the biosphere without much deterioration and eventually get blown away by the wind or washed off by the rain through flood into the marine environment. Large scale accumulation of plastic waste has led to severe environmental

pollution. Plastic debris enter the marine environment in wide range of sizes, colours, shapes, specific gravity and chemical composition (Hidalgo-Ruz *et al.,* 2012). Plastics also get into the marine environment through anthropogenic activities such as indiscriminate disposal of plastics near or directly into water bodies (Andrady, 2011). The disposal of these plastics into aquatic environment causes marine pollution and endangers marine lives. This pollution may be as a result of leaching of the fragments of additives contained in the plastics and these additives can be degraded by UV light, chemicals or even microorganisms (Ryan *et al.,* 2009).

2.6.2 Pollution of soils by plastic disposal

Plastics can be classified as indispensable material in the world today however, it is harmful to the ecosystem due to its persistence in the soil and the environment at large (Barnes and Milner*,* 2015). Some biological effects in humans and animals especially aquatic animals have been observed due to the leaching of the chemical compounds used in the production of plastic materials. Examples of such chemicals and additives include Phthalates and BPA (Bisphenol-A) (Devi *et al.,* 2014). The indiscriminate disposal of plastic waste makes the environment unaesthetic and reduces the green areas in the environment which in turn affects human health. It also affects large space on the soil thereby affecting grazing animals and agricultural processes (Andrady and Neal, 2009). Municipal councils are now sensitive to this unethical plastic disposal on their soils. While some are still clueless on the best method to tackle this menace, others have resorted to combustion of some of these plastics which produces POP (persistent organic pollutants) known as dioxins and furans which affects the environmental health of the ecosystem (Jayasekara *et al.,* 2005).

2.7 Control of Plastic Pollution in Environment

Plastic materials are essential globally as they are involved in virtually all sectors of human activities, serving different purposes from packaging to medical and other areas. It is estimated that only 10 % of plastics are recycled 76 % goes to landfills or ends up in natural environment and just 14 % is incinerated (Mohanan *et al.,* 2020). In Nigeria, the ever-increasing use of single-use plastics and the inappropriate release of plastics in the aquatic environment are enabling prevalent microplastic pollution. About 60 million plastic pouch water bags popularly known as sachet or pure water are consumed and discarded daily in Nigeria. These pouches, bags and plastic products in general end up in the aquatic habitats because of the uncontrolled dumping and common communal practice of discarding waste at every available site ranging from road sides to drainage channels. It has been reported that Nigeria released up to 0.3 million tonnes of plastic fragments or remains into water bodies in 2010 and was listed as the ninth country in the world for pollution of aquatic environment. Microplastic leakage is estimated to increase by 2040 by 1.3-2.5 times and that equates to 3 million trillion pieces (Lear *et al.,* 2021).

2.7.1 Elimination by incineration

One of the popularly used methods of controlling plastics pollution in Nigeria is elimination of plastics by incineration. This entails the burning of plastic waste in an incinerator however, bio-hazardous plastics are known to emit toxic and harmful chemical pollutants when plastics are burned. Incineration method of plastic waste is a better alternative to landfill method as this uses limited land space compared to landfill method of plastic elimination (Bakht *et al.,* 2020). Incineration is limited by its ability to cause greenhouse gases and free radical exposure which are all detrimental to the overall environmental health of an ecosystem (Bakht *et al.,* 2020). It has been established that over 15,439 tonnes of plastic debris were generated globally as a result of the tragic

pandemic (COVID-19) which emerged fully in 2020 (Bakht *et al.,* 2020). Most hospital laboratories use incineration as a method of inactivating pathogens in residues. Among these wastes are majorly single use plastics which when burnt emit dangerous chemical pollutants and disposal of single use medical plastic supplies remain a potential danger to the environment whether it is disposed into landfills or incinerated (Bakht *et al.,* 2020).

2.7.2 Disposal of plastics through landfills

Landfill method of plastic disposal is another conventional method of plastic disposal. Plastic elimination through this method has persisted for more than 20 years due to insufficient oxygen in landfills (Hayden *et al.,* 2013). Plastics in landfills commonly undergo thermo-oxidation degradation and the anerobic conditions created in landfills slows down the rate of degradation. Plastic waste in landfills act as a source for other secondary pollutants (Hayden *et al.,* 2013). Sulphate reducing bacteria produces high concentration of hydrogen sulphide as a result the release of BPA from plastic and this high concentration of hydrogen sulphide is lethal to human and environmental health (Hayden *et al.,* 2013).

2.7.3 Elimination by recycling

The attraction behind the development of plastic recycling processes emerged as a result of the environmental setbacks of disposal through incineration and landfills methods (Ayodeji *et al.,* 2021). Plastic additives and other impurities can complicate the recycling procedures thereby decreasing both the yield and quality of the recovered product; however, recycling plastic is quite expensive and almost impossible in third war countries (Ayodeji *et al.,* 2021). These conventional methods of plastic disposal not only burden

the health of the environment and ecosystem at large it is also not sustainable. Thus, the need for a biologically based alternative for plastic disposal (Ayodeji *et al.,* 2021).

2.7.4 Elimination by biodegradation

Synthetic plastics are inevitable in our present day lives, its disposal and accumulation are a major challenge for human health and the environment at large (Mohanan *et al.,* 2020). Elimination of plastic through biodegradation process basically involves the use of microorganisms to degrade or eliminate plastics in an environment usually in landfills or refuse dumps (Poznyak *et al*., 2019). The microorganisms that have the ability to degrade plastics usually utilize these plastics as carbon and energy source (Poznyak *et al*., 2019). Conventional approaches to plastic elimination have enormous limitations making the need to study microbial interactions with pollutants a more beneficial alternative (Bishwambhar *et al*., 2020). Biodegradation is the ability of microorganisms to utilize biological materials in an environment and this ability help to recycle some elements in the environment. Some insects (waxworms and mealworms) and microorganisms have been reported to utilize these polymers and convert them into safe and economic products (Ayodeji *et al.,* 2021). Different types of plastics are degraded by various groups of microorganisms. Bacteria and fungi are majorly responsible for biodegradation. Petro-polymers such as polyvinyl chloride, polystyrene, polyethylene, polyurethane and polyethylene terephthalate are difficult to be utilized by microorganisms (Mohanan *et al.,* 2020). Some microorganisms that have the ability to breakdown these Petro-polymers under invitro conditions have been isolated and characterized and some of the enzymes involved in this degradation process have also been cloned. The rate of degradation by microorganism is dependent on molecular weight of the polymer, degrees of crystallinity, additives in the plastics, chemical structure and other exposure characteristics such as temperature, pH and moisture content of the

polymer (Mohanan *et al.,* 2020). In the environment, biodegradation can be used in the treatment of water, soil (bioremediation of contaminated sites, oil spillages) and also to preserve food, wood and agricultural products (Raziya *et al*., 2016).

2.7.4.1 ***Processes involved in plastic degradation***

Plastics can be broken down through different processes especially macro plastics. These methods include Photodegradation, thermal degradation, chemical degradation and biological degradation. In photodegradation, the chemical bonds or molecules absorb light energy or photon which results in a change in the physical and chemical properties of the plastic (Nakei, 2021). This process yields a low molecular weight polymer. When chain cleavage of plastics occurs, small molecules of plastics that are easily broken down by different microorganisms are produced in a mechanism mediated by the radicals (Iram *et al.,* 2019). During chemical degradation, chemicals are used to breakdown or degrade polymers in a process known as hydrolysis to yield products whose molecular weights are relatively low (Iram *et al.,* 2019). Chemical degradation of polymers can occur in different ways which include degradation by ozone attack (degradation by galvanic actions) corrosion of plastics and the reaction of chlorine with metal component (chlorine induced cracking) (Nakei, 2021). The breakdown of plastics at very high temperature or heat to give low molecular weight compounds is called thermal degradation. It can also be said to be the deterioration of plastics at extreme temperatures. Thermal degradation changes the structure and properties of the polymer resulting to alteration of the malleability of the polymer, molecular weight, cracking, discoloration and other physical features (Iram *et al.,* 2019). Thermal degradation of plastics involves some chemical reaction for the putrefaction process to be complete and these reactions include random chain cleavage in which a monomer can be removed at any location or site in the polymer chain, end chain cleavage in which the monomer is removed at the end of the polymer

chain or cross-linked whereby chemical bonds are created between the polymer chain (Nakei, 2021). In microbial degradation, the breakdown of polymers to oligomers and monomers is done by the action of microorganisms in a process known as biodegradation. Biodegradation of plastics begins with the attachment of the microorganism to the polymer surface by the secretion of some extracellular enzymes on the polymer which adheres to the polymer surface and this adherence results to the cleavage of the polymer chains to monomers that are absorbed by the semi permeable membrane of the cells of the microorganisms where it is further metabolized to useful non-toxic products (Fazakat and Hashmi, 2020). Biodegradation of plastic can occur under two conditions which are aerobic condition and anerobic condition, under aerobic conditions, biodegradation of plastics occur in the presence of oxygen. Oxygen is used as an electron acceptor and the metabolic end products are carbon dioxide and water (Fazakat and Hashmi, 2020). In Anerobic conditions, microorganisms degrade plastics in the absence of oxygen. Microorganisms use manganese, iron, nitrate, sulfate and carbon dioxide as electron acceptors thus end products released as a result of this process are carbon dioxide, water and methane (Fazakat and Hashmi, 2020). Compounds with side chains are not easily broken down as compared to those with straight chains. Polymers are difficult to degrade due to their strong chemical chains. The rate of biodegradation of plastics is affected by two major factors: The polymer characteristics such as size and shape, additives, molecular weight, biosurfactant and origin of the plastic, and the exposure characteristics such as moisture content, temperature, pH amongst other factors. pH affects the rate of hydrolytic reaction. The rate at which the microorganisms grow and the speed of degradation are affected by change in pH (Iram *et al.,* 2019). Products formed as a result of plastic degradation by microorganisms can change the acidic and basic condition of the environment thereby affecting the growth of the microorganisms (Fazakat and

Hashmi, 2020). Elevated temperatures decrease the breakdown capacity of enzymes. High melting point polyesters are less likely to be degraded by microorganisms. The lower the melting points of polyesters, the higher the efficiency of enzymes that degrade plastics (Iram *et al.,* 2019). For microorganisms to be activated, there is need for sufficient water content as this increases the hydrolytic activity of the microorganisms. Thus, rate of plastic degradation by microorganisms can be increased by availability of water (Fazakat and Hashmi, 2020).

2.7.4.2 *Microorganisms with potential to degrade plastics*

A large number of microorganisms that have the ability to degrade or utilize plastics have been identified and reported. Biodegradation of petroleum-based plastic is an auspicious strategy for the breakdown of polymers to smaller molecular units for effective recycling processes or biomineralization to obtain useful end products (Mohanan *et al.,* 2020). While scouting for different ways to lower or eliminate microplastic pollution, scientist have discovered various microorganisms that have the ability to degrade plastics predominantly bacteria and fungi (Sumaira *et al.,* 2021). Auta *et al.* (2018) reported a weight loss of 4-6.4 % after 40 days in *Bacillus sp.* strain 27 and *Rhodococcus sp*. strain 36 isolated from mangrove environment and grown in aqueous synthetic media that contained polyproplyne microplastics. These microorganisms carry out degradation processes without creating more problem to and in the ecosystem. The by-products of the biodegradation process also offer useful economic values (Sumaira *et al.,* 2021).

Enormous number of microbial communities with the capacity to breakdown and utilize plastics have been reported in recent times and over 90 microorganisms have been identified (Mohanan *et al.,* 2020). Microorganisms with the ability to hydrolyse polyethylene have been isolated from activated sludge, compost, sea water and soil (Nakei, 2021). Bacteria and fungi species such as *Rhodococcus spp., Bacillus spp.,*

Pseudomonas spp., Aspergillus and Fusarium spp. have been shown to break down pretreated polyethylene. A pretreated polyethylene is a polyethylene that has been exposed to thermal treatment or UV or both thereby making the carbon chains of the polymer sensitive to microbial degradation (Mohanan *et al.,* 2020). The conversion of polyethylene through pyrolysis to polyhydroxyalkanoate in cells have been recorded in different species of *Pseudomonas* (Nakei, 2021). Biodegradation of untreated PE have also been shown in *Pseudomonas putida* 1RN19, *Micrococcus luteus* 1RN20 and bacterial strains from the genera *Comamonas, Delftia* and *Stenotrophomonas* (Mohanan *et al.,* 2020). Some insects like *Galleria Mellonella, Plodia interpunctella, Zophobas atratus*, lesser worms and snails have been able to eat and digest PE plastics (Ayodeji *et al.,* 2021). However, there are certain limitations associated with the use of insects to degrade plastics such as the need to sustain insect cultures to produce the larvae that feed on polyethylene, the high cost of maintaining these cultures and generation of nuisance microplastic due to incomplete degradation and lack of mineralization (Mohanan *et al.,* 2020). The utilization of plastics by microorganisms produces end products that may be of economic benefit example: ethanol to produce biofuel (Ayodeji *et al.,* 2021).

Table 2.2 Plastic Degrading Microbial Species

Polymer	**Species**	**Source**	**Degradation efficiency**
Polypropylene Microplastic	*Bacillus sp* strain and *Rodococcus sp* strain	Mangrove environment	4-6 % weight loss (Auta *et al,* 2018)
LDPE film	*Rodococcus ruber* C208	Dumping sites	4 % weight loss (Orr *et al.,* 2004)

LDPE film	*Bacillus subtilis* H1584	Marine water	1.75 % weight loss (Harshvardhan and Jha, 2013)
LDPE film	*Bacillus cereus* BF20	Marine water	2.5-10 % weight loss (Sudhakar *et al.,* 2008)
LDPE film	*Arthrobacter sp*. GMB5	Plastic waste dump sites	12-15 % weight loss (Balasubramanian *et al.,* 2010)
HDPE film	*Phomidium lucidum*	Domestic sewage water	3.5 % weight loss (Delacuvellerie *et al.,* 2019)

Source: (Ru *et al.,* 2020)

2.7.4.3 *Enzyme catalyzing plastic degradation*

Plastic degrading enzymes can be found in the digestive intestine of some invertebrates and in microorganisms from diverse environments. Algae, fungi and bacteria use plastics as a source of energy and carbon for their continual existence (Nakei, 2021). In biodegradation of plastics, the microorganisms excrete extracellular enzymes such as PETase, lipase, hydrolase, esterases, cutinase and other enzymes (depending on the microorganisms) on the plastic and these exoenzymes attach to the surface of the plastic and begins to cleave the polymer chains resulting to short intermediates that are absorbed by the semi permeable membrane of the microbial cells and metabolized, releasing end

products such as carbon dioxide and water under aerobic condition and carbon dioxide, water and methane under anaerobic conditions (Mohanan *et al.,* 2020).

2.8 Plastisphere and Biofilm Contribution to Plastic Degradation

Plastics serve as habitats and are colonized by certain microorganisms which form biofilms on the plastic surfaces. The term Plastisphere refers to microbial community that live in plastic environments or on plastics. The degradation rate, buoyancy and toxicity level of the epiplastic community appears to influence the fate and ecological effects of the marine plastic pollution. (Reisser *et al.,* 2014). Zettler *et al.* (2013) conducted the first comprehensive characterization of epiplastic microbial communities, which they named the ‘‘Plastisphere’’. These authors used scanning electron microscopy (SEM) and next generation sequencing to analyze three polyethylene and three polypropylene plastic pieces (approx. 2–20 mm long) from offshore waters of the North Atlantic. This pioneer study revealed a unique, diverse, and complex microbial community that included diatoms, ciliates, and bacteria (McCormick *et al.,* 2016). Microplastics interact with inorganic molecules, microorganisms and organic substances in the water resulting to various groups and sizes of organisms adhering to the surface of plastics (Sumaira *et al.,* 2021). The adherence of these microorganisms on plastics leads to the production of biofilms that consist of intricate ecosystem made up of organic and inorganic substance and microbes (Sumaira *et al.,* 2021). Several microbial biofilms have been found on plastic materials that has made its way into aquatic ecosystem (Reisser *et al.,* 2014). The composition of marine plastic biofilms results from a unique interaction of various factors such as substrate type (glass, wood), the geographical location, surrounding environment and the seasonal variation of environmental parameters (Kirstein *et al.,* 2019). Plastics serve as habitats and are taken over by certain microorganisms which form dense biofilms on the surface of the plastics. Biofilm affects the physical properties, surface roughness

and the potential risk of microplastics. Biofilms are composed of bacteria, archaea, and microbial eukaryotes attached to surfaces and embedded in an extracellular matrix of polymeric substances (McCormick *et al.,* 2016). The formation of biofilm is stimulated by hydrophobic nature of plastic surfaces, which support a wide range of metabolic activities, and drive succession of other macro- and micro-organisms (Reisser *et al.,* 2014). The formation of biofilm involves three stages (1) Attachment of microorganisms on the surface of the substrate (2) The secretion of extracellular polymeric substances (EPS)which includes protein, lipids, nucleic acid, polysaccharides and carbohydrate and (3) Proliferation of microorganism. The attachment of microorganism on the substrate occurs within different nanometer from the substrate surface and is propelled by Van der Waals force and electrostatic force. After the attachment of microorganisms to the surface, Extracellular polymeric substances are secreted which further shields the microorganism from physical and chemical stressors such as photo degradation, sand abrasion and water shear stress. Extracellular polymeric substances promote the formation of hetero-aggregates of microplastics, microorganism and chemicals by making microplastics sticky. Biofilm attains maturation stage with the proliferation of microorganisms (Sooriyakumar *et al*., 2022). Plastic biofilm community is majorly of the prokaryotic families such as *Flavobacteriaceae, Erythrobacteraceae, Hyphomonadaceae* and *Rhodobacteraceae* (Kirstein *et al.,* 2019). Studies have shown that there is little or no significant difference in mature biofilm formed on plastics when compared with biofilm formed on other substrate such as wood or glass and this implies that the shared core of the various biofilms is substrate unspecific. The microbial biofilm is essential for heterotrophic organic matter processing in aquatic habitats and offers an energy input to food webs, as they may be ingested directly or through their association with larger particles (McCormick *et al.,* 2016).

2.8.1 Biofilm formation

The major processes involved in biofilm formation are initial attachment followed by maturation and the eventual detachment of cells (Harrison *et al.,* 2018). Colonization of microplastics by microorganisms in the environment occurs rapidly within hours and several factors motivating the development of plastisphere communities are likely to be similar between freshwater and marine habitats. During the process of biofilm formation on other substrates, the surface properties (including roughness and hydrophobicity) of microplastics is vital (Fish *et al.,* 2016). The surface chemistry and structure of plastics can be modified by exposure to ultraviolet (UV) radiation and waves (via the formation of cracks and pits, a reduction in molecular weight, and an increase in surface oxidation), which may facilitate biofilm formation (Brandon *et al.,* 2016). Surface properties and buoyancy of polymers are affected by plastic-colonizing microorganisms, since microplastics are expected to be transported into marine environments via WWTP (Waste water transport pipes), rivers, and streams, elements contributing to initial colonization (such as surface roughness and attachment by pioneering colonizers) can be hypothesized to be particularly important within freshwaters (Harrison *et al.,* 2018). The effects of particle age and/or weathering on plastisphere consortia may be comparatively distinct within marine ecosystems where the residence times of plastic often exceed those within rivers and streams (Harrison *et al.,* 2018). Conversely, microplastics mount up within environments such as lakes, where they may persist for decades (similar to timescales predicted for marine habitats) and can be exposed to high levels of UV radiation (Harrison *et al.,* 2018). Local-scale differences in the composition of plastisphere assemblages between polymer types have been found, but it is unknown whether there are any general differences in the dominant types of plastic within freshwater and marine ecosystems. Although it is possible that ingestion of plastics by higher organisms could

have an impact on plastisphere colonization processes, this topic has not been investigated (Harrison *et al.,* 2018). Environmental conditions such as temperature, salinity, pressure, and the availabilities of light and oxygen are likely to influence the growth of plastic-associated biofilms (Harrison *et al.,* 2018). Several of these factors differ between freshwater and marine ecosystems, waste water treatment plants (WWTP) and unmanaged freshwaters. For instance, in the deep sea where the temperatures are low (<5C), absence of light, and elevated pressure are likely to impose selective forces on plastisphere assemblages that differ from those within shallow habitats (Harrison *et al.,* 2018). Numerous plastisphere members have been associated with pollutant degradation and it is apparent that several contaminants play a role in shaping biofilm formation and activities on polymers. Indeed, multiple types of pollutants, as well as heavy metals, are known to become adsorbed onto microplastics (Harrison *et al.,* 2018). In rivers, sediment movement is characterized using the concept of spiraling and the components of one spiral include downstream transport, deposition, bed load transport, and resuspension (Harrison *et al.,* 2018). This notion is a well-developed approach for modeling particle movement and is quantified using measurements of deposition length and velocity, turnover time, and the retention-export ratio. Each step in a spiral is likely to have implications for plastic-associated biofilm composition and activity, due to accompanying shifts in the surrounding environmental conditions (Harrison *et al.,* 2018). Studies of microplastic spiraling metrics will help estimate the spatial scales over which plastic particles move within lotic environments, enlightening how the related microbial community changes across multiple downstream spirals. Rivers are also characterized by flooding, which redistributes materials between riparian and aquatic components of the fluvial landscape (Harrison *et al.,* 2018). Flooding moves plastic from the riparian zone into aquatic habitats and increases stranding of plastic in debris dams (Harrison *et al.,*

2018). Despite their effects on plastisphere communities, the impacts of movement between aquatic and terrestrial habitats on plastic-associated biofilms have not been studied (Harrison *et al.,* 2018). Hydrology in most lakes embraces the single upstream inlet and downstream outlet, with water and particle residence times reliant on water volume and currents. These metrics will define microplastic residence times which are expected to influence microbial-plastic associations within several habitats, including the epilimnion, littoral, and benthic zones (Harrison *et al.,* 2018). The action of wind and wave can further influence the dispersal of microplastics within lakes (Harrison *et al.,* 2018), however it is unclear how transport of microplastics from freshwater to marine environments affects plastisphere assemblages, but they may undergo taxonomic and physiological shifts during this transition. For instance, exposing *Pseudomonas aeruginosa* to salt stress (0.5 M NaCl) was found to inhibit biofilm formation and reduce rates of benzoate degradation by this strain (Harrison *et al.,* 2018). Geographic and seasonal alteration in the structure and composition of freshwater plastisphere communities are so far not been investigated, but the spatiotemporal distribution of marine plastic-colonizing microbial consortia has recently been studied (Oberbeckmann *et al.,* 2016). Oberbeckmann *et al.* (2016) found location-dependent and seasonal variation in the structure and composition of plastisphere communities, based on 6-week in situ exposures of polyethylene terephthalate (PET) bottles in the North Sea. Similar variation was also reported by Amaral-Zettler *et al.* (2015). the authors stated latitudinal gradients in the species richness of plastic-colonizing assemblages, further to differentiate communities being discovered in the North Atlantic and North Pacific subtropical gyres. The taxonomic changes were also observed between polymer types, the data proposed that geography is a stronger predictor of plastisphere community composition at the scale of ocean basins (Harrison *et al.,* 2018).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sampling Sites

Soil samples from the shorelines of some rivers within Minna in Niger State, Nigeria were collected for the screening of the potentials of microorganisms associated with micro, meso and macro-plastics in soils from the shorelines of these rivers to utilize plastics. Four water bodies Shiroro lake, Tagwai lake, Bosso Lake and Chanchaga rivers were selected based on their location to represent Minna metropolis and Shiroro Local Government Area. Three (3) sampling sites were selected within each water body and the basis of selection were on the river status and accessibility. Anthropogenic activities that may contribute to the generation of macro-plastics, meso-plastics and microplastics were identified through observation of 2 km radius along each river. The table below delineates

the coordinates of the sampling sites, while the location of the sampling sites is presented in the table below.

Table 3.1 Coordinates of the Study Area

Water Bodies	Local Government Area	Location	Sample Coordinates (Latitude)	Sample Coordinates (Longitude)
Chanchaga River	Chanchaga	**1**	9°36'50.4"N	6°33'25.2"E
		2	9°36'38.1"N	6°33'21.9"E
		3	9°36'34.8"N	6°33'13.6"E
Bosso Dam	Bosso	**1**	9°31'50.8"N	6°40'35.4"E
		2	9°31'39.6"N	6°40'40.6"E
		3	9°31'31.5"N	6°40'49.8"E
Shiroro River	Shiroro	**1**	9°57'25.6"N	6°49'55.8"E
		2	9°57'22.2"N	6°49'55.9"E
		3	9°57'19.5"N	6°49'59.2"E

Tagwai Dam	Chanchaga	**1**	9°57'34.9"N	6°06'14.9"E
		2	9°57'36.3"N	6°06'22.1"E
		3	9°57'39.0"N	6°06'28.7"E

Source: Department of Geography Federal University of Technology Minna, Niger State.

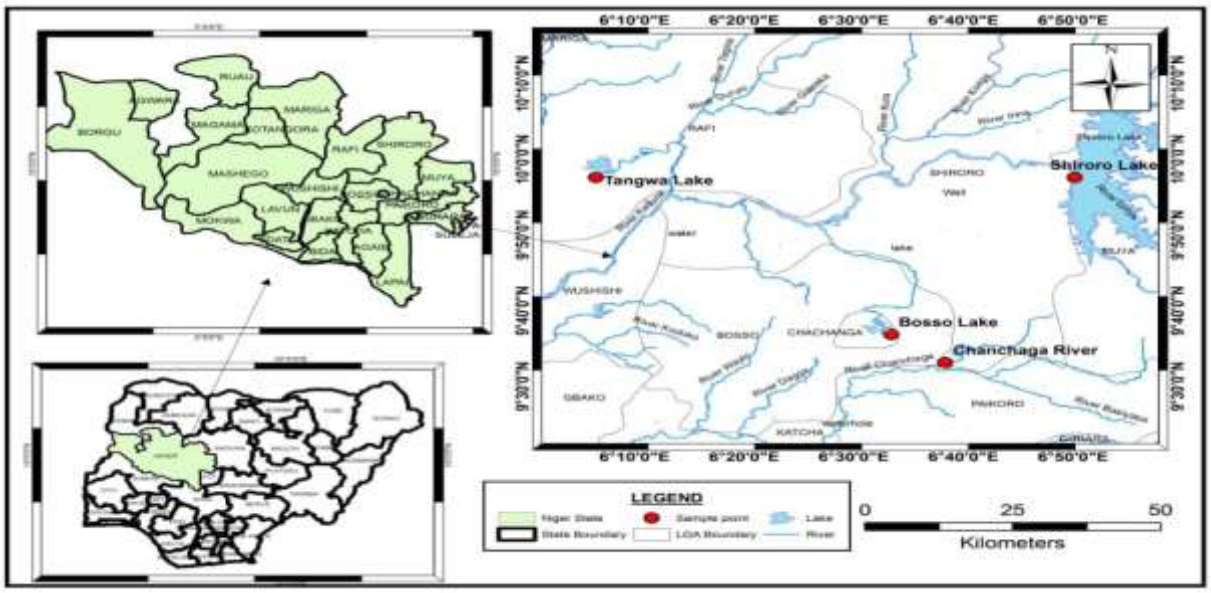

Figure 3.1 Map of Study Area
Source: Department of Geography Federal University of Technology Minna, Niger State

3.2 Study Area

(i) Shiroro lake

The Shiroro lake is a man-made lake that was created in May, 1984 by damming the Kaduna River at Shiroro village in Niger State. The reservoir has an estimated surface area of 312 km^2 and a mean depth of 22.4 meters and continues to grow. The Shiroro lake has an average annual rainfall of 1250 mm and annual temperature varies between 20.5 °C in December to 40.38 °C in March/April (Ovie and Adenji, 2014).

(ii) Tagwai lake

The Tagwai lake lies in the south-eastern part of Minna, Niger State. The dam is an earth filled dam, created in 1976 in tributary to Chanchaga river in Niger State to augment the source of raw water to the Chanchaga water treatment plant. The annual rainfall distribution pattern shows a maximum of 1300 mm and minimum of 1000 mm rainfall (Vulegbo *et al*., 2014).

(iii) Bosso dam

The Bosso Lake is a small body of water located in north of Minna Niger State with surface area of 294 m² and a mean depth of 6.1 m. The dam is shaded by shrub trees and bushes. The main use of the dam is to supply water for domestic use. The annual rainfall distribution pattern ranges between 1300 mm to 1000 mm and average temperature of 27.2 °C (Amadi and Olasehinde, 2010).

(iv) Chanchaga river

The Chanchaga river is located in the south of Minna, Niger State. The main use of this river is to supply water for domestic use. The river has a surface area of 342 km² and the area is estimated to have annual rainfall distribution of 1229 mm and average temperature of 27.7 °C.

3.3 Sampling Method

Soil samples from the shorelines of waterbodies (Shiroro lake, Tagwai lake, Bosso Lake and Chanchaga rivers) were collected. The samples were collected from different depth (4 cm) of the shorelines in a 0.04 m^2 range area between the shoreline of the water bodies using a sterile stainless-steel shovel, and kept in sealed plastic bags with labels (Jiang *et al.,* 2018). Samples below 4 cm depth were not collected this is to avoid sampling plastics

that may be undergoing degradation due to radiation or other factor rather than due to the activities of microorganisms (Volke *et al.,* 2002). A subset of these fragments was randomly sorted at each sampling point with sterilized forceps, rinsed with water and immediately transported to the laboratory for further analysis to isolate and identify the attached microbial communities and test their ability to utilize plastics.

3.4 Sample Collection and Laboratory Analyses

3.4.1 Physicochemical analysis

Soil sediment sample from each study location was poured into separate plastic container and transported to the Department of Soil Science Federal University of Technology Minna, Niger State for the determination of physicochemical parameter of the samples such as Soil texture, water holding capacity, temperature, total nitrogen, percentage chloride, moisture content, pH, Salinity and Organic matter.

(i) Organic carbon

This was carried out according to the wet digestion method of Walkley Black earlier described by Nelson and Sommers (1996). Organic carbon was oxidized by mixing 25 ml of concentrated Sulphuric acid, 10 ml of 1M potassium dichromate and 0.5 g of soil sample. It was allowed to stand for 30 minutes after which 200 ml of water was added to the mixture and an extra addition of 10 ml of phosphoric acid. The quantity of the potassium dichromate reduced was used to determine the organic carbon content in the soil by the titration of excess potassium dichromate against 0.5 N ferrous sulphate solution using diphenyl amine indicator.

(ii) Soil pH

The pH of the soil sample was measured in water at the ratio of 2:5 (weight/volume) soil. Fifty (50) ml of distilled water was added to 20 grams of the soil sample and shaken in a mechanical shaker for 30 minutes and the pH was measured using a pH meter (Kuti *et al.,* 2018).

(iii) Soil texture

Twenty-five (25) grams of sieved soil sample was added to 100 ml of water in a transparent jar, one teaspoon of Calgon was added to the mixture to help the clay settle out of the soil sample faster. The jar was shaken thoroughly and allowed to sit undisturbed for 2 days. Measurements were taken when the mixture was clear using Soil Texture Triangle (Kuti *et al.,* 2018)

(iv) Total nitrogen

Total nitrogen of a soil sample is the sum of nitrate-nitrite, ammonia and organically bonded nitrogen. For this study, the determination of the total nitrogen was done according to Kjedahl digestion-distillation method. The soil sample was mixed with concentrated sulphuric acid in the presence of a catalyst. The digest was distilled in the presence of 40 percent sodium hydroxide, ammonia was given off and collected in 4 percent boric acid and titrated against a standard 0.05 M of sulphuric acid and the titer was used to calculate the total nitrogen content of the soil sample.

3.5 Plastic extraction

All soil sediment samples were dried at 45 °C for 24 hours and three hundred (300) g of dried sediments sample of each location was investigated. Macro, meso and microplastics were extracted from each sample based on a density separation combined with filtration

method (Hanvey *et al.,* 2017). Each 300 g sediments were mixed with 750 mL of concentrated NaCl solution in a glass beaker for 2 min by stirring with a glass rod. The mixture was left standing for 1 hour and the resulting supernatant were wet sieved through a set of Tyler Sieves with 5.0 mm, 1.0 mm and 0.1 mm mesh sizes. The macro, meso and microplastics that were retained on the sieves were separated using forceps, and were then treated with 20 % alcohol solution overnight.

3.5.1 Identification and classification of plastics

Identification of plastics was conducted based on the morphological characteristics (type, size and color), by visual observation.

Table 3.2 Morphological Features of Plastics

Category	Classification
Type	Line (fibrous), Fragment (hard, jagged), Film (thin, flimsy), Foam (lightweight, sponge-like), Pellet (hard, rounded).
Size	<0.1 mm-5 mm, 5 mm and above
Colour	Transparent, Black, Blue, Red, Yellow, White, Others

Source (Free *et al.,* 2014)

3.6 Isolation of Microbial Communities on Plastics

(i) Isolation of bacteria

Nutrient Agar was prepared and aseptically poured into Petri dishes and allowed to solidify. Aseptically, a sterile forceps was used to pick fragments of microplastics/plastics and placed on a Nutrient Agar. The Nutrient Agar plates were inverted and incubated at 37 °C for 24 hours. A pure culture was made by sub culturing on nutrient agar plate using the streaked plate method. Morphological characteristics,

Gram staining and biochemical tests were used to identify the bacteria isolated (Nakei, 2021).

(ii) Isolation of fungi

Sabouraud Dextrose Agar (SDA) was prepared and aseptically poured into Petri dishes and allowed to solidify. Aseptically, sterile forceps were used to pick fragments of microplastics and placed on a Sabouraud Dextrose Agar plate. The plates were inverted and incubated at room temperature 25 °C for 5 days with constant checking. A pure culture was made by sub culturing on sterile Sabouraud Dextrose Agar plate using the point inoculation method and the isolates were identified based on their morphological and microscopic features. A small piece of mycelium was picked using a sterile inoculating needle and 2 drops of lactophenol placed on a clean glass and observed under the microscope for vegetative and reproductive structures (Nakei, 2021).

(iii) Gram reaction

Pure bacterial isolates were gram stained; under aseptic condition, using cooled, sterile wire loop, bacterial isolates were picked and smeared into a drop of normal saline on a clean, grease free glass slide, afterwards the smears were air-dried and then they were heat fixed. After which, the smears were flooded with crystal violet and left to stand for 1 minute, it was then drained off and passed over a running water. The stained smears were then flooded with Lugol's iodine, and left to stand for 1 minute, after which it was rinsed off gently under running tap water, followed by rapid decolorization by addition of few drops of 95 % ethanol and then rinsed off. The decolorized smears were counter stained by flooding with safranin, and left to stand for 30 seconds, and gently rinsed off using distilled water, after which it was air-dried. The slides were then viewed under light

microscope using oil immersion objective lens, bacterial cells were observed and observations were recorded (Cheesbrough, 2006).

3.7 Biochemical Reaction for Bacterial Isolates

(i) Catalase test

Aseptically, 2 drops of 3 % hydrogen peroxide were placed on 3 appropriately labelled clean, grease free slides. Clean, steriled glass rod was used to collect bacteria isolates and smeared on the appropriate slide containing hydrogen peroxide, except the slide labelled control. The slides were observed and findings were recorded (Cheesbrough, 2006).

(ii) Indole test

Aseptically, bacteria isolates were inoculated into 5 millilitres of sterile peptone broth and incubated aerobically at 37 °C for 48 hours. After the incubation period Kovac's reagent (5 grams dimethyl amino-benzaldehyde in 75 millilitres of amyl alcohol and 25 millilitres of concentrated hydrochloric acid) was added in a drop wisely to each cultured broth and were shook gently. The cultures were observed and findings were recorded (Cheesbrough, 2006).

(iii) Oxidase test

Aseptically, 3 drops of oxidase reagent(tetramethyl-p-phenylenediamine) was added on a sterile filter paper, bacteria isolates were collected and smeared on the wet filter paper using sterile glass rod. Observations were made and findings were recorded (Cheesbrough, 2006).

(iv) Citrate test

Aseptically, single sterile wire loop, bacteria isolates were inoculated by streaking on sterile Simmon Citrate Agar slants except from the control slant, the inoculated agar

slants were incubated aerobically at 37 °C for 96 hours, after which slants were observed, and compared to the control slant, and findings were recorded (Cheesbrough, 2006).

(v) Motility test

Aseptically, using sterile straight wire, bacteria isolates were inoculated on sodium chloride- peptone agar (10 grams of peptone, 5 grams of Agar agar, 5 grams of sodium chloride (NaCl)/litre) slants by stabbing except the control slant. The inoculated agar slants were then incubated aerobically for 24 hours at 37 °C. After which observations were made and findings were recorded (Cheesbrough, 2006).

(vi) Carbohydrate utilization test (acid and gas production from carbohydrate)

In an aseptic condition, bacteria isolates were inoculated into Tess tubes containing sterile phenol red peptone-sugar broth sugar broth (phenol red peptone broth containing sucrose, fructose, lactose, D-glucose, D-mannitol, arabinose, sorbitol, D-mannose) and Durham tubes, except the tubes that serve as control for all the different peptone-sugar broth. After which the inoculated broth was incubated aerobically for a period 48 hours at 37 °C. After period incubation period observations were made and findings were recorded (Cheesbrough, 2006).

(vii) Methyl red and voges proskauer test

Aseptically, isolates were inoculated into Two tubes containing 2 millilitres of Sterile glucose phosphate peptone broth, and were incubated 37 °C. After which 4 drops of methyl red was added to the cultured broth using Pasteur's pipette, and shaken gently, for proper mixing. Observations were then made and the findings documented. Aseptically, in two other cultured VP broth, 1 millilitre of 40 % potassium hydroxide (KOH) and 3 millilitres of 5 % alcoholic alpha-naphthol was added and properly shaken,

and left to stand for 3 minutes, which Observations were added and findings were documented (Cheesbrough, 2006).

3.8 Biodegradation of Plastics

This medium was prepared by dissolving 1.8 g dipotassium phosphate, 4.0 g ammonium chloride, 0.1 g sodium chloride,0.2 g of magnesium sulfate heptahydrate, 0.01 g iron sulphate heptahydrate in 1 L of distilled water. Aseptically the pH was adjusted to 6.90 before dispensing the medium into different 50 ml conical flasks containing 1g of plastic (plastic disc of 0.3 cm diameter) each, then autoclaved at 121 °C for 15 mins. The media was allowed to cool before the organisms were inoculated into each conical flask using a sterilized wire loop. The Bushnell and Haas medium (BHM) (Bushnell and Haas, 1941) was used for testing the ability of fungi isolates in degrading plastics. The media was prepared by adding 0.2 g of $MgSO_4$, 1.0 g of K_2HPO_4, 1.0 g of NH_4NO_3, 0.02 g of $CaCl_2$, 0.05 g of $FeCl_3$ into 1000 ml of distilled water which was dispensed into different 50 ml conical bottles containing 1g of plastic each (plastic disc of 0.3 cm diameter) and the mixture was thoroughly shaken before autoclaving for 121 °C for 15 minutes and the pH of the medium was adjusted to 7.0. The medium was left to cool to about 50 °C before inoculating the fungi isolates using sterile needle under aseptic condition. The 50 ml bottles containing the microorganisms were left in a shaker.

3.8.1 Determination of microbial growth

Sample was drawn from each 50 ml bottle and placed into a transparent cuvette and the absorption was measured relative to medium alone. Optical density (OD) is directly proportional to the biomass in the cell suspension in a given range (Stevenson *et al.,* 2016). The optical density of the microbial growth was observed and measured at the wavelength of 600 nm for a period of 32 days at 4 days interval in 3 replicates and a

negative control containing non inoculated media supplemented with microplastics was maintained. At the end of 32 days, the plastics were recovered from the MSM and BHM media through filtration and sieving and the microbial film attached to the microplastics were removed by washing the microplastics with 70% ethanol before drying in hot air oven at 50^0 C prior to weighing. Weight loss was calculated by using the formular in equation (3.1) (Auta *et al.,* 2017).

3.8.2 Determination of final weight of plastics

$$\text{Weight loss} = W_o - W \quad (3.1)$$

Where W_o is the Initial weight of the microplastics (g) and W is the residual/weight of the plastic (g).

The weight loss in percentage (%) was calculated using the formular in equation (3.2).

$$\text{Percentage (\%) weight loss} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100 \quad (3.2)$$

The rate constant of microplastic was determined by using the first order kinetic model based on the initial and final weights along specific intervals (4days) (Auta *et al.,* 2018)

$$K = 1/t \ (\text{In } w/w_0) \quad (3.3)$$

Where k is the first order rate constant for microplastic uptake per day, t is time in days, w is the final weight of the microplastics (g)

Half-life ($t_{1/2}$) was calculated in accordance to equation (3.3)

$$(t_{1/2}) = \text{In } (2)/k \quad (3.4)$$

Where t refers to time, In (2) = 0.69 and K is rate constant after 32 days (Auta *et al.,* 2017).

3.8.3 Molecular identification of bacterial and fungal isolates

3.8.3.1 *DNA extraction*

(i) Fungal isolate

Genomic DNA extraction was carried out with solution-based JENA bioseience Animal and Fungi DNA Preparation Kit following manufacturer's instructions. Fungi cells were harvested from 1000 µl aliquot of broth culture using a microcentrifuge at 15,000 g for 1 min. The residual pellet was resuspended in 300 µl of Resuspension buffer and 1.5µl of proteinase K Solution. The mixture was homogenized by inverting several times thereafter incubated at 55 °C for 1 hour. Resuspended cells were recovered centrifugation and lysed by adding 300 µl of Lysis solution. The mixture was vortexed vigorously and centrifuged at 15,000 g for 3 mins after adding 100 µl of protein precipitation solution to precipitate the protein present in the cells. The supernatant was transferred to a clean 2 µl microcentrifuge tube containing 300 µl of Isopropanol and mixed gently by inverting for 1 minute to precipitate the DNA. DNA was pelleted by centrifugation at 15,000 g for 1min, washed with 500 µl washing buffer and allowed to completely air-dry after the washing buffer was discarded, 50 µl hydration solution and 1.5 µl RNase A was added to the air-dried DNA pellet. The sample was subjected to initial incubation at 37 °C for 1 hour followed by a final incubation at 65°C for 1 hour to completely hydrate the dried DNA pellet (Tomar *et al.,* 2019).

(ii) Bacterial isolate

Genomic DNA extraction was carried out with column-based JENA Bioscience Bacteria DNA Preparation Kit following manufacturer's instructions. Bacteria cells were harvested from 500 µl aliquot of bacterial broth culture using a microcentrifuge at 10,000 g for 1 min. The residual pellet was resuspended in 300 µl of Resuspension Buffer and 2

µl of Lysozyme Solution. The mixture was homogenized by inverting several times thereafter incubated at 37 °C for 1 hour. Resuspended cells were recovered by centrifugation and lysed by adding 300 µl of Lysis Buffer after which 2 µl RNase A and 8 µl proteinase K solution was added, followed by incubation at 60 °C for 10 mins. The tube was cooled on ice for 5 min, 300 µl binding buffer was added to the mixture and vortexed briefly; the mixture was cooled on ice for 5 mins and thereafter centrifuged at 10,000 g for 5 min. The supernatant was transferred directly into the spin column and centrifuged at 10,000 g for 1min to trap the DNA. The trapped DNA was washed twice with washing buffer after which it was eluted with 50 µl elution buffer into a clean Eppendorf tube (Tomar *et al.,* 2019).

(iii) ITS Region amplification (fungal extract)

Polymerase chain reaction mixture consisted of 12.5 µl mastermix, 1µL each of forward primer ITSI-TCCGTAGGTGAACCTGCGG and reverse primer ITS4 TCCTCCGCTTATTGATATGC, 1µl DNA template and 9.5µl sterile nuclease free water to make up a total reaction volume of 25µl. Polymerase chain reaction amplification was carried out in an Applied Biosystem 2720 Thermocycler. The mixture was subjected to an initial denaturation at 95°C for 5mins; followed by 35 cycles of denaturation at 94°C for 45s, annealing at 55°C for 45s and extension at 72°C for 45s; and a final extension at 72°C for 5mins (Tomar *et al.,* 2019).

(iv) 16s rRNA Amplification (bacterial extract)

Each PCR reaction mixture consisted of 12.5µl mastermix (2x JENA Ruby hot start mastermix), 1µl (10pmol) each of forward primer 27F 5'AGA GTT TGA TCM TGG CTC AG3' and reverse primer 1492R-5' TAC GGY TAC CTT GTT ACG ACT T 3', 1µl DNA template and 9.5µl sterile nuclease free water to make up a total reaction

volume of 25 µl. Polymerase chain reaction amplification was carried out in an Applied Biosystem 2720 Thermocycler. The mixture was subjected to an initial denaturation at 94°C for 3 mins followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 60s and extension at 72°C for 60 seconds; and a final extension at 72°C for 10 mins (Tomar *et al.,* 2019).

3.8.3.2 *Gel electrophoresis*

Polymerase chain reaction products were visualized on a 2 % agarose gel containing ethidium bromide in 0.5x Tris-borate buffer (pH 8.0) using blue led transilluminator (image attached). PCR products were purified and sequenced by Sanger sequencing method using AB1 3730XL sequencer and done by Inqaba biotec, Pretoria, South Africa.

3.9. Data Analysis

Data generated from this research was subjected to one-way analysis of variance (ANOVA) test followed by a post-hoc TukeyHSD test to determine which pairs of mean differ significantly from each other. The data were evaluated in duplicate and presented as mean standard error of mean, P-values ≤ 0.05 were considered statistically significant. The data were statistically evaluated using R statistical software

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Physicochemical parameters of soil sediment

The properties of the soil samples from the sampling sites were ascertained by determining the type of soil, the pH which influences mineral absorption and the constituents of the soil such as mineral particles, organic matter and moisture content. The soil samples used in this study had varying low levels of percentage organic carbon and total nitrogen ranging from 0.32-0.56 % and 0.24-0.38 % respectively and a pH range of 6.64-7.46. In highly acidic soil, Aluminum and Manganese becomes more available and more toxic thereby reducing microbial activities. The moisture content of the four sampling sites ranged from 5.25-7.80% which equally served as a pointer to the soil type of each sampling site.

Table 4.1 Physicochemical Parameter of Soil Samples from the Study Sites

Parameters	Bosso	Tagwai	Chanchaga	Shiroro
Moisture content (%)	6.94	6.71	5.25	7.80
Total nitrogen (%)	0.38	0.32	0.35	0.24
Organic carbon (%)	0.44	0.56	0.48	0.32
Organic matter (%)	0.76	0.96	0.82	0.55
Sand (%)	76	46	68	52
Silt (%)	15	38	24	32
Clay (%)	9	16	8	16
Chlorine (mg/kg)	226	184	302	155
Temperature (° C)	28.7	28.3	28.7	27.9
Electrical conductivity (μS/cm)	82	124	108	76
pH value	6.64	7.21	7.46	6.81

4.1.2 Mean occurrence of plastic debris across sampling sites

The occurrence of plastic in the four different sampling sites as shown in Figure 4.1 indicated that Shiroro had the highest number of plastics recovered from sediment samples (Mean: 13.3±9.45), followed by Chanchaga (mean: 10±5.57), while Bosso had

the least (Mean: 8.67±5.86). However, no statistically significant difference was observed between these locations P>0.05

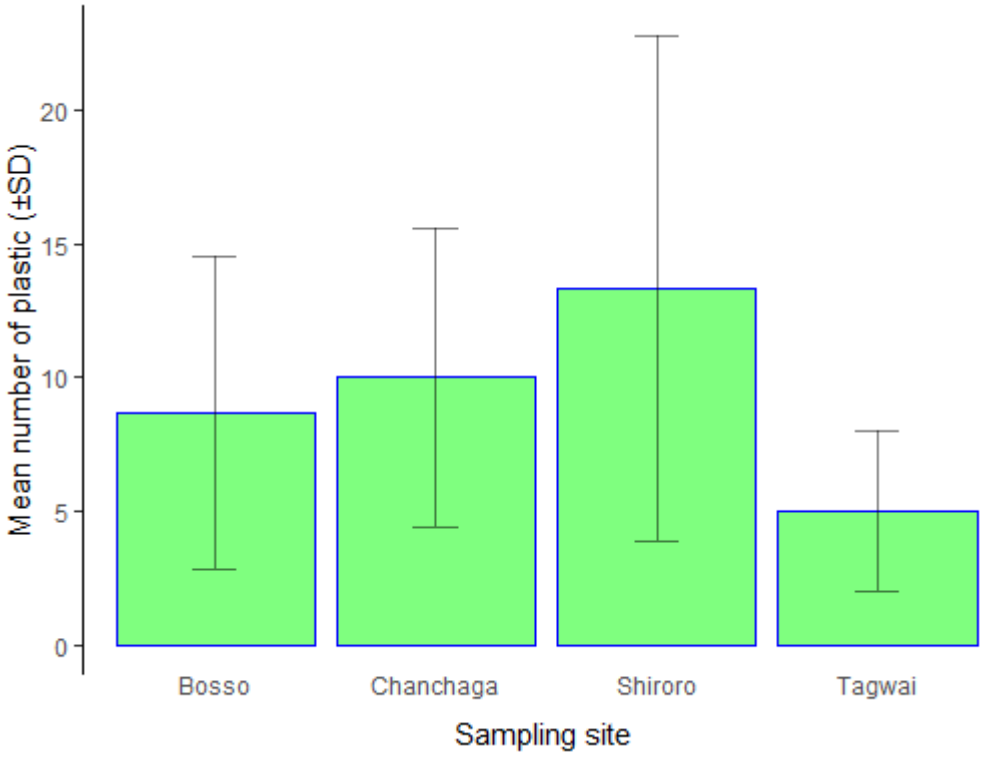

Figure 4.1 Mean occurrence/number of plastic debris across sampling sites

(i) Mean occurrence of detected plastic debris according to size

The mean occurrence of the three plastic types by size is illustrated in Figure 4.2. Mean number of plastic types ranged from 6 ±3.7 for macro plastic to 11.8±5.7 for micro plastic. Although micro plastic was found to occur more than other plastic sizes, there was no statistically significant difference in the occurrence of the three plastic types.

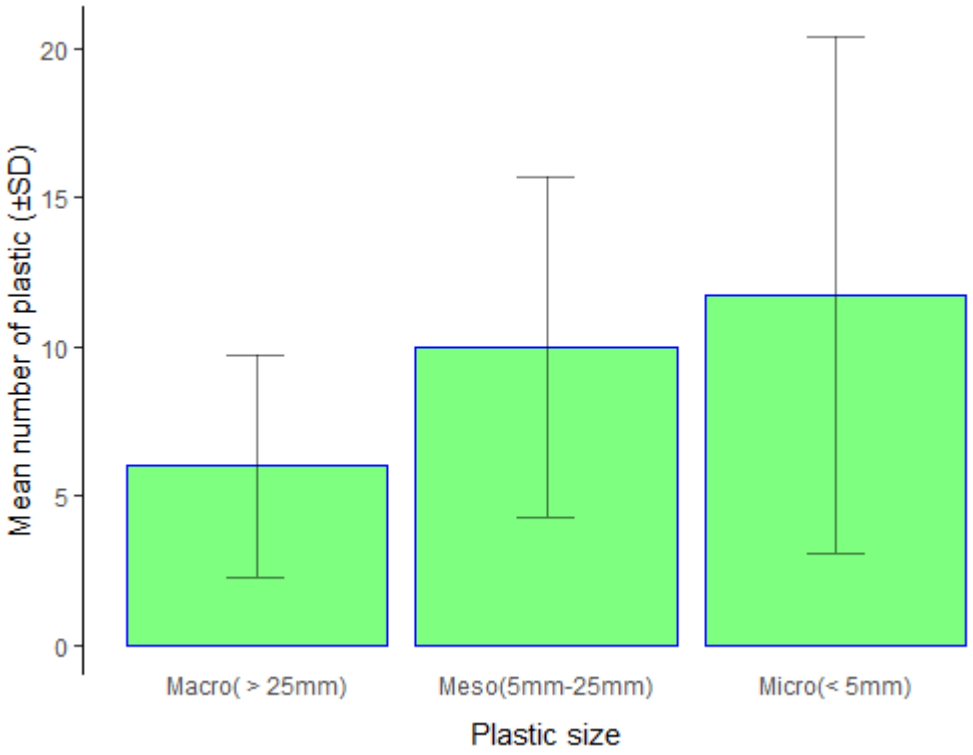

Figure 4.2 Mean occurrence of detected plastic debris according to size

(ii) Distribution of plastic type by shape across sampling sites

The distribution of plastic by shape, in percentage, across sampling sites is shown in Figure 4.3. Analysis of variance revealed that there was significant difference between the occurrence of plastic type with fragment occurring more than film and line in each site. However, pairwise comparison test/ post-hoc test (TukeyHSD) revealed that line and film did not significantly differ in their occurrence. As regards the distribution of plastic types across sampling sites, there was no evidence to conclude that there was significant difference across the sampling site.

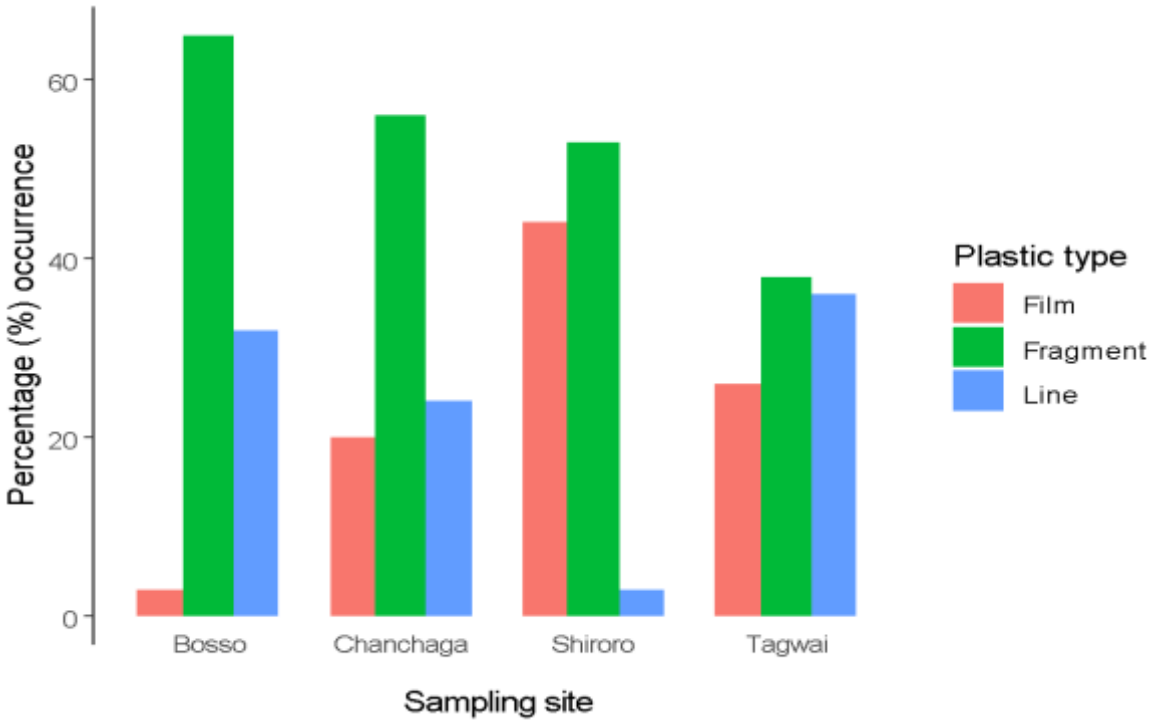

Figure 4.3 Distribution of Plastic Type by Shape across Sampling Sites

(iii) Percentage occurrence of plastic debris according to colour

The percentage occurrence of plastic debris represented by different colour is illustrated in the Figure 4.4. The highest percentage of colored plastics was observed in Shiroro sampling sites which may be attributed to anthropogenic activities located within and around the site. Different colours of plastic were observed but black, brown blue, black and white coloured plastics were predominantly visible in the sampling sites.

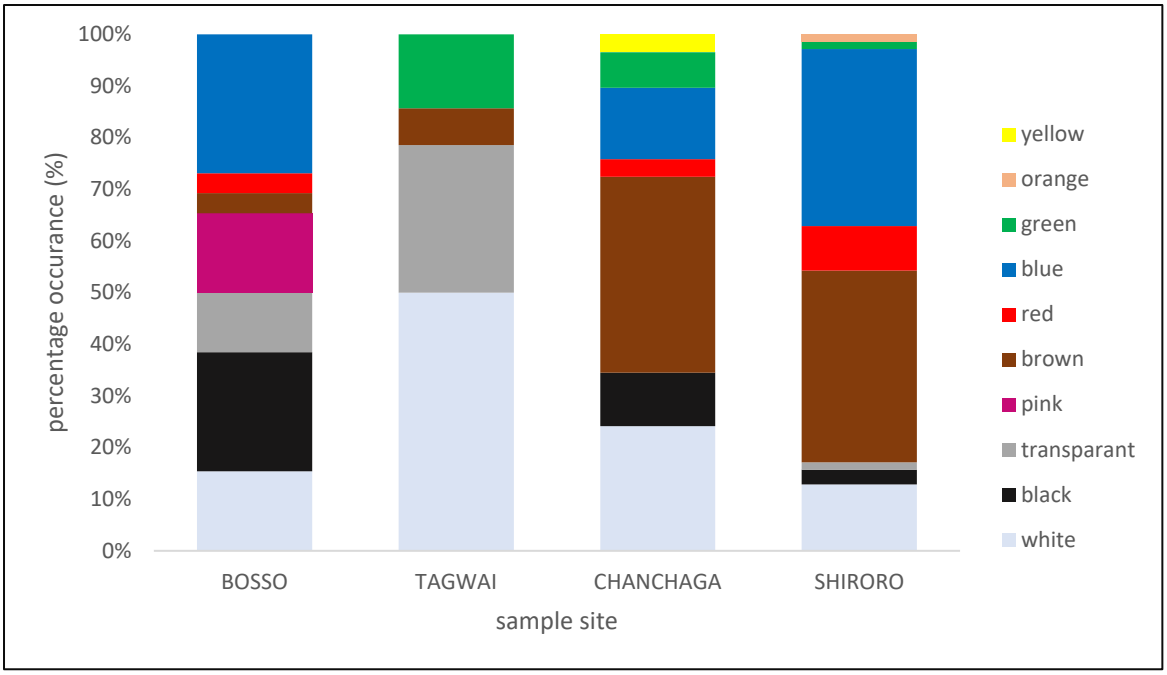

Figure 4.4 Percentage Occurrence of Plastic Debris according to Colour

4.1.3 Morphological cultural and biochemical reaction for bacterial isolates

The result from the microbial analysis revealed forty-nine (49) isolates which were separated into genera and species. They were five (5) bacteria isolated and they include *Eschericha coli Alcaligenes feacalis*, *Bacillus megaterium, Staphylococcus epidermis* and *Staphylococcus aureus* biochemical test result for bacterial isolates presented in Table 4.2 showed that all the bacterial isolates were subjected to gram staining and all the isolates retained the crystal violet dye except *Eshericha coli*.

Table 4.2 Morphological, Cultural and Biochemical Analysis of Bacterial Isolate

	Bacterial Isolates				
	Eschericha coli	*Alcaligenes faecalis*	*Bacillus Megaterium*	*Staphylococcus epidermis*	*Staphylococcus aureus*
Gram's reaction	-	-	+	+	+
Shape	Rod	Rod	Rod	Cocci	Cocci
Motility	+	+	-	-	-
Oxidase	-	+	-	-	-
Catalase	+	+	+	+	+
Methyl-Red	+	+	+	-	+
Voges-Proskauer	-	+	-	+	-
Citrate	-	+	-	-	+
Indole	+	-	-	-	-

Key: - = Negative, + = Negative

(i) Macrosopic and microscopic features of bacterial isolates

The bacterial isolates were of different forms, elevations, textures and colours. When viewed under the microscope the following shapes was observed rod, rod, rod, cocci and cocci for *Eschericha coli, Alcaligenes faecalis, Bacillus megaterium, Staphylococcus epidermis* and *Staphylococcus aureus respectively*.

4.1.4 Morphological feature of fungal isolates from the sampling sites

The Fungi isolated during this study were three (3) and they include *Aspergillus niger, Aspergillus flavus* and *Curvularia nodosa* with *Aspergillus flavus* having the highest occurrence across the sampling sites.

4.1.5 Percentage (%) degradability of micro plastics

The percentage (%) degradation of the microplastics was evaluated in terms of percentage weight loss by using Equation (2). *Eschericha coli* had the least percentage degradability (1 %) and the weight loss of the plastic was almost negligible when compare to the organisms. The weight loss of plastic exposed to *Curvularia nodosa* was 0.36 g. *Alcaligenes faecalis* (25 %) utilized plastic the most amongst the bacterial isolates.

Table 4.3 Percentage (%) Degradability of Micro Plastics

Isolates	Initial Weight (g)	Final Weight(g)	Weight loss(g)	Degradation (%)
Alcaligenes faecalis	1	0.75	0.25	25.00
Bacillus megaterium	1	0.78	0.22	22.00
Eschericha coli	1	0.99	0.01	1.00
Staphylococcus epidermis	1	0.93	0.07	7.00
Staphylococcus aureus	1	0.98	0.02	2.00
Curvularia nodosa	1	0.64	0.36	36.00
Aspergillus flavus	1	0.84	0.16	16.00
Aspergillus niger	1	0.88	0.12	12.00

4.1.6 Rate constant(K)

The rate constant (K) for microplastic uptake per day at 32 days was evaluated using the organisms that had the highest percentage degradability *Alcalegenes faecalis* and *Curvularia nodosa* was evaluated.

Table 4.4 K Value at Different Periods (Day)

Isolates	Day 0-4	Day 0-8	Day 0-12	Day 0-16	Day 0-20	Day 0-24	Day 0-28	Day 0-32	Half-life after 32days
Alcaligenes faecalis	0.72	0.36	0.02	0.02	0.01	0.01	0.01	0.01	76
Curvularia nodosa	0.11	0.06	0.04	0.03	0.02	0.02	0.02	0.01	49

4.1.7 Molecular identification of microbial isolates

The result of the gel electrograph with the sizes1500 bp are shown in plates 1 and 2. The isolates identified via molecular identification techniques are those that displayed greater abilities to degrade microplastics as depicted by their percentage degradability rates. The microbial isolates *Alcaligenes feacalis* and *Curvularia nodosa* (bacteria and fungi respectively) with the highest degradation percentage (%) in this study were identified by the 16S rRNA sequence analysis. The sequences obtained were blasted in GenBank of NCBI. BLAST (Basic local alignment search tool) result revealed that the test organisms bacterial and fungi were similar to *Alcaligenes faecalis* (NR13111) and *Curvularia nodosa* (NR154865.1) at 98.94 % and 98.52 % similarities respectively.

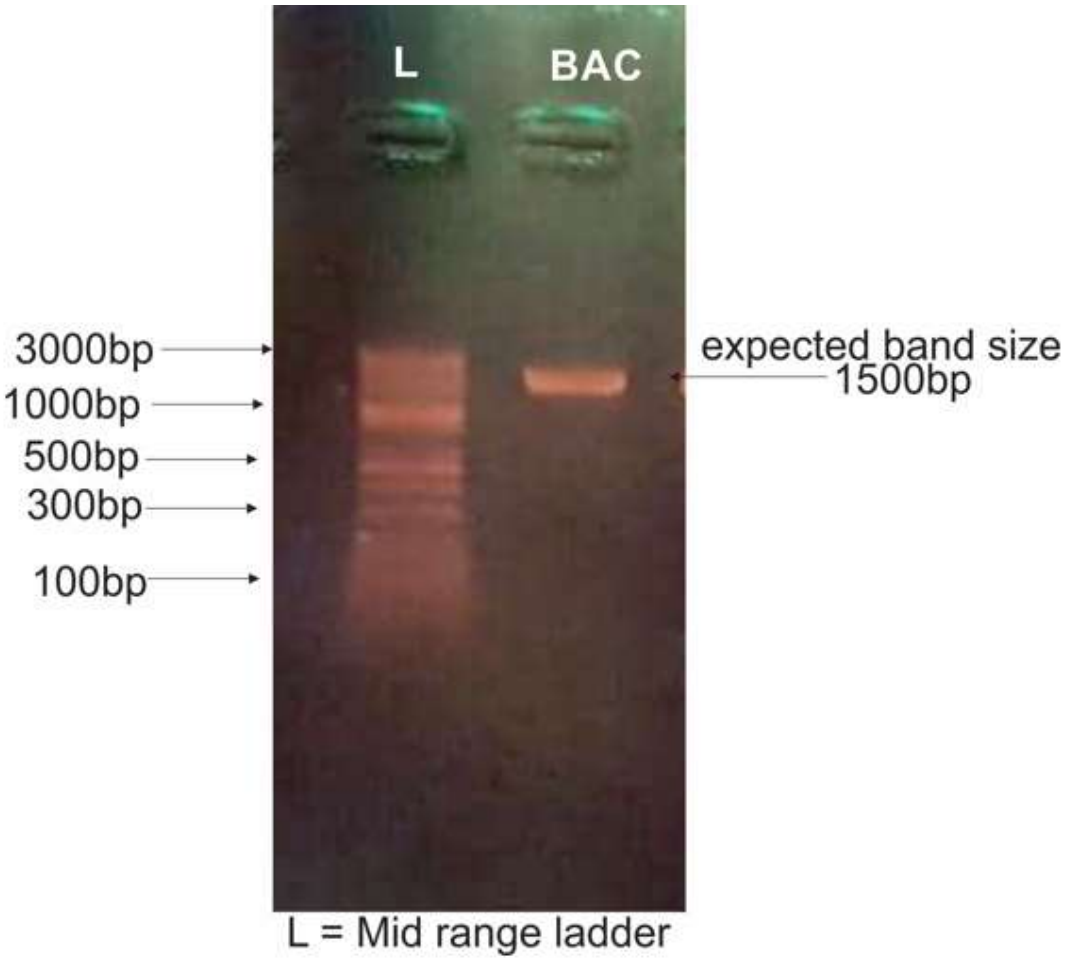

Plate 1: Agarose gel electrograph of the PCR products of bacterial isolate

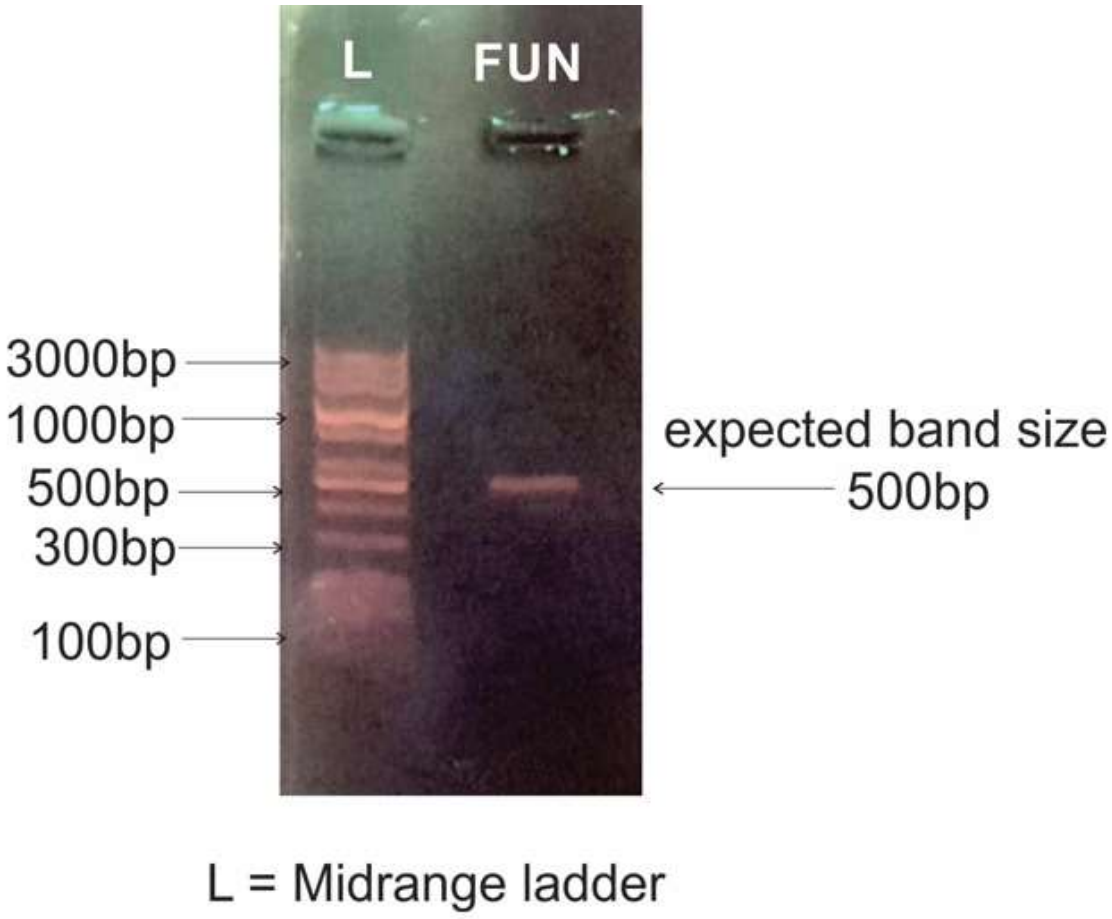

Plate 2: Agarose gel electrograph of the PCR products of fungal isolate

Table 4.5 Molecular Identification of Bacterial and Fungal Isolates

Sample ID	Scientific name	Max Score	E value	%Identity	Accession number
Bacteria	*Alcaligenes faecalis*	1462	0.0	98.94 %	NR13111.1
Fungi	*Curvularia nodosa*	239	7e-64	98.52 %	NR154865.1

4.1.8 Growth rate of bacterial isolates by day

Growth rate of all bacteria isolates except *Eshericha coli* generally increased from Day 4 and peaked at Day 20. There was a significant increase in the absorbance rates of isolates across the days. However, the rate of increase from Day 0 and Day 4 were not

significantly different. After Day 20, all isolates began to show decrease in the rate of absorbance which maybe as a result of nutrient depletion or death. There was a significant different in the rate of absorbance, with *Alcaligenes faecalis* showing the highest absorbance rate across the days. The growth pattern of *Alcaligenes faecalis* from day 0 to day 4 acclimatized to the medium first before it maintained a steady log phase.

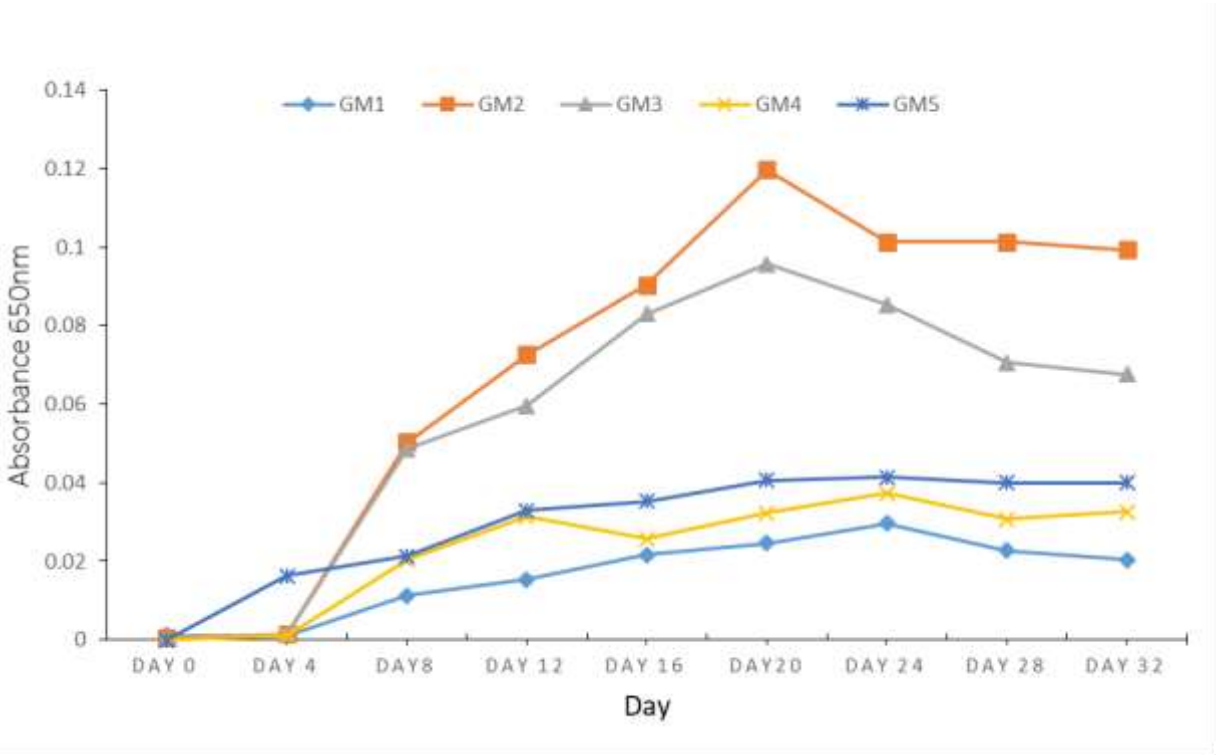

Figure 4.5 Growth rate of bacteria isolates by day. Key: GM1= *Eschericha coli,* GM2= *Alcaligenes feacalis,* GM3= *Bacillus megaterium,* GM4= *Staphylococcus epidermis,* GM5= *Staphylococcus aureus*

(i) Mean distribution of the absorbance rate of plastics among bacteria isolates

The mean plastic absorbance rate differed significantly among bacterial isolates. *Alcaligenes feacalis* showed the highest mean absorbance rate of 0.07 ± 0.04 (mean ±sd), followed by *Bacillus megaterium* with a mean of 0.06± 0.03. *Eschericha coli* had the least ability in degrade plastic debris (mean ± sd: 0.02 ± 0.01). It is worthy of note that

these observed statistically significant differences in the mean absrobance rate of isolates did not occur between some isolates. Post-hoc test (TukeyHSD) revealed that *Staphylococcus epidermis* and *Eschericha coli*, *Staphylococcus aureus* and *Eschericha coli*, *Bacillus megaterium* and *Alcaligenes feacalis* and *Staphylococcus aureus* and *Staphylococcus epidermis* were not significantly different from each other respectively as shown in Figure 4.6.

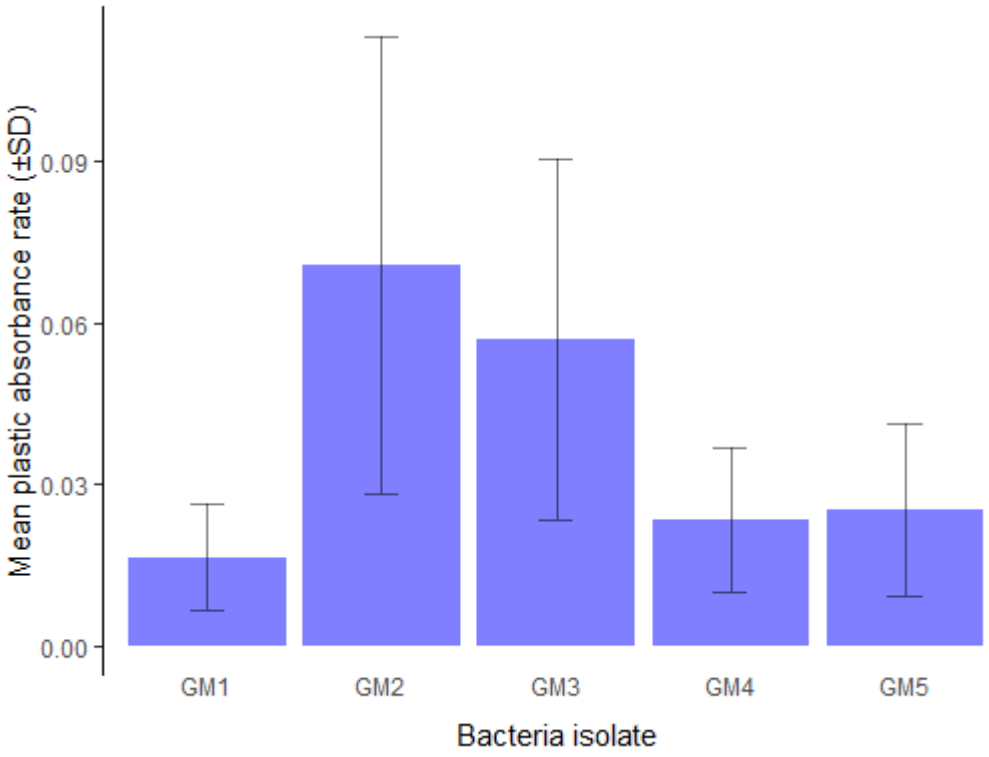

Figure 4.6 Mean distribution of the absorbance rate of plastics among bacteria isolates.

Key: GM1: *Eschericha coli*, GM2: *Alcaligenes feacalis*, GM3: *Bacillus megaterium*, GM4: *Staphylococcus aureus,* GM5*: Staphylococcus epidermis*

(ii) Mean distribution of the absorbance rate of plastics among fungi isolates

The mean plastic absorbance rate of fungi isolates ranged from 0.04 ± 0.03 to 0.07 ± 0.04 for *Aspergillus flavus* and *Curvularia nodosa* respectively. Differences observed between isolates are statistically significant. However, a closer look at the differences by running

a pairwise comparison test (TukeyHSD) revealed that *Aspergillus niger*, and *Aspergillus flavus* were not significantly different.

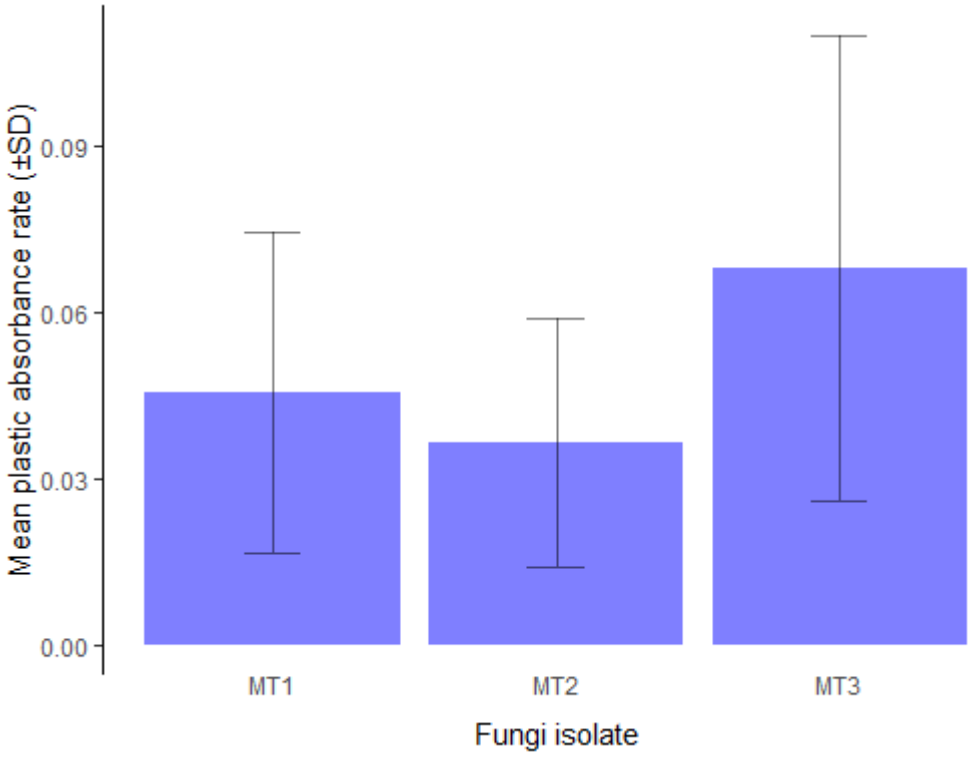

Figure 4.7 Mean distribution of the absorbance rate of plastics among fungal isolates. MT1= *Aspergillus niger,* MT2= *Aspergillus flavus,* MT3= *Curvularia nodosa*

(iii): Mean absorbance rate of bacteria and fungi

The mean plastic absorbance rate was observed to be significantly different between bacteria and fungi. Fungi was more efficient at degrading plastic debris than bacteria. The means absorbance rate of fungi is 0.039 ± 0.03 while that of bacteria is 0.05 ± 0.03.

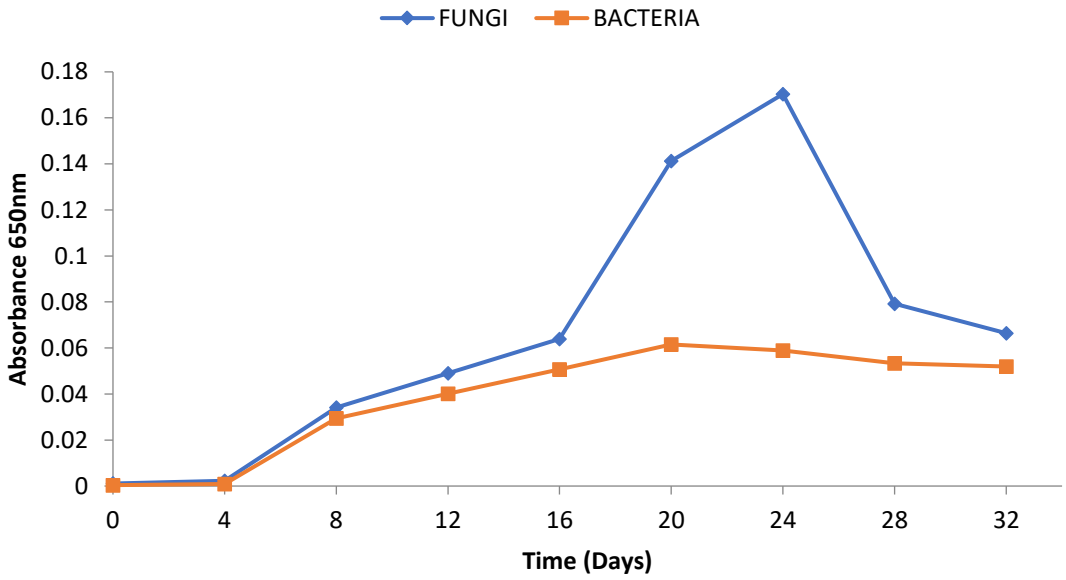

Figure 4.8 Mean absorbance rate of bacteria and fungi

4.2 Discussion of Results

4.2.1 Physicochemical parameters of soil sediment

Plastics have become an essential part of our everyday life since the beginning of their mass production in the 1940s. Fragmented plastics smaller than 5 mm are called microplastics and are relatively new type of pollutants that are widely distributed in the marine environment so, understanding the distribution, accumulation and the microbial communities associated with this form of pollution is crucial for environmental risk assessment (Boskovic *et al.,* 2021). Different soil sediment sampling locations that were identified within the shorelines of Shiroro lake, Tagwai lake, Bosso Lake and Chanchaga rivers visibly had plastic debris over various period of time. The physicochemical parameters of the study sites presented were compared with similar study by Edori and Iyama (2017). The soil pH of Bosso and Shiroro sampling sites were neutral, therefore the soil was neither alkaline nor acidic while Chanchaga and Tagwai sampling site had slightly alkaline pH. However, the acidity and alkalinity of a sampling site greatly influence the availability of nutrients to different organisms found in the soil. The value of the electrical conductivity ranged from 76-124 μs/cm in the various study site. The low conductivity values implied the presence of low concentration of soluble salts in the soil thereby resulting to increase in activities of soil microorganisms (Edori and Iyama, 2017). Sediment samples with lower percentage of total nitrogen and organic carbon had the lowest population of bacteria while soils with higher percentage of total nitrogen and organic carbon had the highest population of bacteria. The lowest and highest population of fungi was observed in the soils with the pH range of 6.6-6.9. The percentage of sand, clay and silt of the sediment samples from all four-study site, were within the range of 52-76 % (sand), 15-38 % (clay) and 8-16 % (silt) respectively. The textural class of the soil was sandy-clay-loam (SCL). Soil texture is a measure of the physical properties of

the soil (Kuti *et al.,* 2018). These properties include plasticity of the soil, water retention capacity, soil productivity, soil permeability and ease or toughness of tillage of the soil which directly affect the activity of microorganisms in the soil (Kuti *et al.,* 2018). The total moisture content of the four-study sites ranged from 5.25-7.80 %. The amount of water remaining in a soil drained to field capacity and the amount that is available are functions of the soil type. The chloride content of all four-study site ranged between 155-302 mg/kg which is very high and might affect the activities of microbes present in the soil (Edori and Iyama, 2017).

4.2.2 Mean occurrence/number of plastic debris across sampling sites

The analyses of the soil samples collected from the four (4) different sampling sites in Niger state confirmed the presence of plastic pollutant in the sediments. The largest number of plastic debris was recorded at Shiroro sampling site (71 plastics items/750 g sediment), followed by Chanchaga with 30 plastics items/750 g sediment, then Bosso with 26 plastics items/750 g sediment and Tagwai with 15 plastics items/750 g sediment). The largest number of plastic wastes observed at Shiroro sampling site may be as a result of the anthropogenic activities as it serves as a tourist centre as well as a fish market where different species of fish are caught and sold. All four-study site are in close proximity to urban settlement and this accounts for high generation of waste in and around the rivers and most likely a potential factor to the emergence of a large number of plastic debris found at the study sites. At Bosso Lake and Chanchaga river meso-plastics were dominantly found while at Shiroro lake, micro-plastics were dominantly found. Auta *et al.* (2017) showed that microplastics debris in aquatic environment are majorly derived from the fragmentation and disintegration of larger plastic, this therefore explained why the number of microplastic debris from Shiroro sampling site was higher than the microplastics in other sampling sites considering the anthropogenic activity close

to Shiroro lake. Tagwai lake had the least number of meso-plastic and Bosso Lake had the least number of macro-plastics. The predominance of plastics debris less than 5 mm (microplastics) at the study sites might be due to the presence of fragmented plastic which usually appear to be in this size. Martin *et al.* (2017) reported a similar result on the extraction and quantification of macro, meso, and microplastic debris in a floodplain lake.

4.2.3 Distribution of plastic type by shape across sampling sites

Different colours of plastics were seen in the sampling sites however, white was the dormant colour and this may be attributed to the prolonged leaching of the colour additives contained in the plastics. Colours such as blue and brown were also found mostly in all the sampling sites. Shiroro sampling site had remarkable variety of colours followed by Bosso while Tagwai had the least. The abundance of diverse plastic colours in Shiroro sampling sites can be attributed to the regular influx of tourist and the presence of fisher men who also sell fishes. The percentage (%) occurrence of plastic debris according to shape (Fragment, line and film) as shown above where Bosso lake had 65 % fragment shaped, 32 % line shaped plastics and 3 % film shaped plastics, sediment samples from Tagwai lake had 38 % fragment shaped plastics, 35 % line shaped and 25 % film shaped plastics, samples from Chanchaga river had 56 % fragment shaped plastics, 24 % line shaped plastics and 20 % film shaped plastics and samples from Shiroro lake had 53 % fragment shaped plastics, 3 % line shaped and 44 % film shaped plastics. Sediment samples from Bosso Lake had the highest number of fragment shaped plastic while Tagwai lake had the least. The highest percentage (%) of line shaped plastics was also observed in sediment samples from Tagwai lake with the least percentage (%) observed in Shiroro lake. Perfetti-Bolano *et al.* (2022) observed an average of 13 plastic particles per 50 ml sample to be fragment shaped and film abundance of 15 plastic particles per 50 ml sample. The low percentage of film shaped plastics observed across

the sampling sites maybe attributed to the principal component analysis which shows the relationship between films and intertidal sediments whereas fragments present a relationship with soil (Perfetti-Bolano *et al.,* 2022). Predominance of fragment is associated with high concentration of organic matter which was observed in the study areas.

4.2.4 Percentage (%) degradability of the micro plastics

The degradation of plastics under normal conditions is known to be a very slow process however, the capacity to degrade plastics has been found in many bacteria and microscopic fungi where plastics serve as sole energy source (Jeyakumar *et al.,* 2013). Biodegradation of plastic is assessed by formation of biofilm, decrease in the molecular weight of the polymer and by increase in the surface hydrophilicity (Ru *et al.,* 2020). In this study, plastic degradation was studied using spectrophotometric method to monitor the optical density of microbial culture medium containing plastic as the sole energy source, most of the isolates were capable of degrading microplastics at different levels and their degradation abilities were assessed. Subsequently, the weight loss of the plastics and the percentage degradability of each isolate were calculated. The growth pattern exhibited by the microorganisms were evaluated and the results presented in the figure above, a lag growth response was observed for all the microorganisms from day 0 to day 4. Increasing growth rates on day 4 to day 8 did not imply high response and performance of the Isolates upon exposure to microplastics, it only showed that the period was favourable for the interaction between the bacterial cell membrane and microplastics and therefore allowed for rapid metabolism. The growth of the bacterial isolates accelerated towards a positive growth pattern from 0.025 OD600 nm to 0.040 OD600 nm on the 20^{th} day which depicted the highest growth rate for the bacterial isolates whereas growth pattern from 0.030 OD600 nm to 0.170 OD600 nm on the 24^{th} day showed the highest

growth rate for fungal isolates. A sharp decline in the growth rate of the isolates on the 20th and 24th for bacteria and fungi respectively up until the 32nd day of exposure to microplastic was observed. The decline phase attained by the isolates was possibly due to lysis of cells, nutrient depletion or presence of inhibitory products in the culture media. Auta *et al.* (2018) showed plastic specimen incubated with microbial culture exhibited 6.4 % decrease of plastic weight in 40 days. Similarly, Balasubramanian *et al.* (2010) showed plastic from dump sites incubated with microbial culture exhibited 12-15 % weight loss. Fungi possess enzyme systems that can survive under challenging environmental conditions and at low levels of nutrient and water supply, they have strong hyphae that can penetrate the cracks and hollows on the plastic surface (Jeyakumar *et al.,* 2013). Motta *et al.* (2009) showed polystyrene mass loss of 2-5 % in 3 months by *Curvularia* and the incubation of Alcaligenes in a media containing polystyrene as the sole source of carbon and energy led to 12 % substrate depletion per month.

4.2.5 Rate constant(K) at different periods of the degradation

The value of the rate constant is temperature dependent and a large value of the rate constant means that the reaction is relatively fast while a small value of the rate constant means that the reaction is relatively slow (Auta *et al.,* 2018). The rate constant is the proportionality constant relating the rate of the reaction to the concentrations of reactants.

4.2.6 Molecular identification of microbial isolates

The isolates identified via molecular identification techniques are those that displayed greater abilities to degrade microplastics as depicted by their percentage (%) degradability rates in a medium containing microplastics as source of carbon. The microbial isolates *Alcaligenes feacalis* and *Curvularia nodosa* (bacteria and fungi respectively) with the highest degradation percentage (%) in this study were identified after standard comparism with known specie the GenBank using BLASTn. The Result

of 16SrRNA gene nucleotide of bacteria with ability to degrade plastics showed that GM2 is *Alcaligenes faecalis* strain NBRC13111 with 98.94 % identity and NR113606 specie from GenBank with highest identity. The result for fungal isolate MT3 is *Curvularia nodosa* CPC28800 with 98.52 % identity with the specie in GenBank. Generally, *Curvularia nodosa* exhibited more tolerance between the 4^{th} and 24^{th} day of exposure to the microplastic than *Alcaligenes faecalis*. Aatikah *et al.* (2022) showed that *Alcaligenes faecalis* degraded LLDPE by 3.5 %, HDPE by 5.8 % and Polyester by 17.3 %. *Alcaligenes faecalis* are commonly found in water and soil environment and some *Alcaligenes* species are known to utilize quite a number of aromantic compounds for energy and carbon source (Thomas, 1999). A study by Mohanan *et al.* (2020) showed many strains of *Pseudomonas, bacillus, Aspergillus, Staphylococcus, Alcaligenes* and microorganisms have been observed to degrade complex compounds and have also been associated with the partial degradation of a wide range of petro- plastics. Vimala and Mathew (2016) reported the ability of *Bacillus spp* to utilize polyethylene using UV treatment as the physical means of pretreatment to enhance the ability of microbes to assimilate PE and this bacteria species was found to utilize PE as carbon source. The ability of *Alcaligenes faecalis* and *Curvularia nodosa* to degrade microplastics isolated from the sampling sites may be attributed to the type of extracellular enzymes they produce. *Curvularia* is a dermatophyte that can be found in soil, nails and hair and it produces keratinase, lipase and protease and these enzymes have shown potentials of plastic degradation, *Alcaligenes faecalis* produced CMcase, protease, xylanase and lipase which degraded plastic surface (Nag *et al.,* 2021).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Biodegradation of petroleum derived plastics have been a ground-breaking area of research focused on solving plastic pollution in the environment. This work was based on quantification and identification of macro, meso and microplastics isolated from sediment samples from the shorelines of 4 (four) rivers in Niger State, Nigeria, identifying and isolating the microbial communities associated with these microplastics and screening their abilities to utilize these plastics were tested. Most of the

microorganisms isolated were capable of degrading plastics at different rates however the degradation rate of *Alcaligenes faecalis* was high compare to the other bacterial isolates; *Alcaligenes faecalis* (16 %) and *Bacillus megaterium* (12 %) utilized microplastics more than *Staphylococcus epidermis* (3 %), *Staphylococcus aureus* (2 %) and *Escherichia coli* (1 %) while *Curvularia nodosa* degraded plastics the most when compared to the fungal isolates *Aspergillus flavus* (7 %), *Aspergillus niger* (5 %) and the entire microbial community that was studied in this work. Based on the result obtained from the screening of the abilities of these microorganisms to utilize plastics, *Alcaligene faecalis* and *Curvularia nodosa* may have the potential to degrade plastics.

5.2 Recommendations

Based on the findings of this study, the following are recommended

1. Proper sensitization of the general public on the health and environmental risk associated with indiscriminate disposal of plastics
2. Minimal use of plastics should be encouraged while finding alternative to plastics
3. Further study on the ability of *Alcaligenes faecalis* and *Curvularia nodosa* to degrade plastics and the specific enzymes involved is recommended.

5.3 Contribution to Knowledge

This thesis contributes to the growing body of knowledge about plastic pollution and its impact in soil ecosystems. It also identifies specific microorganisms that could be harnessed for potential bioremediation strategies, offering a more sustainable approach to dealing with plastic waste. The thesis represents a significant contribution to the understanding of the presence of plastics in soil samples and their interaction with various microbial species. The study established the presence of plastics in soil samples collected from different study sites thereby highlighting the ubiquity of plastic pollution in various

environments. The recorded quantity of plastics in each location (Bosso, Tagwai, Chanchaga and Shiroro) gives clearer picture of the extent of plastic contamination in different areas. By quantifying the number of plastic pieces in a specific weight of soil (300 grams), the study provides concrete data on the density of plastic pollution in the examined sites. The variations in the number of plastic pieces at different locations suggest potential differences in pollution sources or accumulation rates. One of the most crucial aspects of the study is the evaluation of the degradation efficiency of different microorganisms on the attached plastics. The study's screening analyses reveal the extent to which each microbial species can break down plastics. The recorded percentages (ranging from 1% to 22%) provide valuable information about the potential of each microorganism to contribute to plastic degradation. Based on the degradation efficiency findings, the study identifies two microorganisms, *Alcaligenes faecalis* and *Curvularia nodosa* as having promising potential for plastic utilization. This thesis stands as an original contribution to the field of environmental science by offering valuable insights into the distribution of plastics, their interaction with microbial species and the potential of certain microorganisms to contribute to plastic degradation. Its findings not only deepen our understanding of plastic pollution but also suggest a path toward more sustainable solution to mitigate its effects.

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APPENDIX I

S/N	ISOLATES	ORGANISMS (NA and MK)
1	BMB	*B.subtilis*
2	BMA	*Staphylococcus sp*
3	BMB	*Staphylococcus epidermis*
4	SMS	*Eschericha coli*
5	TMT	*Bacillus sp*

6	CMC	*Aspergillus sp*
7	CMC	*Bacillus megaterium*
8	TMT	*Staphylococcus aureus*
9	SMS	*Staphylococcus sp*
10	BMB	*Eschericha coli*
11	BMB	*Staphylococcus epidermis*
12	SMS	*Alcaligenes faecalis*
14	CMC	*Bacillus megaterium*
15	GM1	*Eschericha coli*
16	TMT	*Bacillus sp*
17	TMT	*Staphylococcus aureus*
18		*Eschericha coli*
19	BMB	*Eschericha coli*
20	SMS	*Bacillus sp*
21	TMT	*Eschericha coli*
22	CMC	*Eschericha coli*
23	CMC	*Staphylococcus sp*
24	SMS	*Bacillus megaterium*
25	TMT	*Staphylococcus epidermis*

26	BMB	*Eschericha coli*
27	BMB	*Staphylococcus sp*
28	BMB	*Staphylococcus sp*
29	SMS	*Bacillus sp*
30	SMS	*Alcaligenes faecalis*
31	SMS	*Bacillus sp*
32	CMC	*Bacillus megaterium*
33	TMT	*Eschericha coli*

Key: NA= Nutrient Agar MK= Macconky Agar

APPENDIX II

Macroscopic and Microscopic Features of Bacterial Isolates

	Eschericha coli	*Alcaligenes faecalis*	*Bacillus megaterium*	*Staphylococcus epidermis*	*Staphylococcus aureus*
Form	Circular	Circular	Large	Circular	Circular
Size	Small	Medium	Irregular	Pinhead	Pinhead
Texture	Mucoid	Mucoid	Dull	Smooth	Mucoid

Colour	Greenish metallic sheen EMB agar	Milky-white	White	Golden yellow	Whitish grey
Elevation	Raised	Convex	Convex	Convex	Convex
Margin	Entire	Entire	Umbonate	Entire	Entire
Microscopy	Rod	Rod	Rod	Cocci	Cocci

APPENDIX III

Morphological Features of Fungi Identified from the Sampling Sites

Isolate	**Macroscopy**	**Microscopy**
Aspergillus niger	Black and powdery appearance (Surface view). White yellowish (Reverse view)	Conidiophores smooth walled. The conidial heads appear radial and split as the isolate continues to age
Aspergillus flavus	Bright green fluggy appearance.	Rough conidiophores

		Radiating conidial heads
Curvularia nodosa	White (surface view) to reddish brown (Reverse view) appearance.	Macroconidia and spherical microconidia with smooth walls, appearance of colony cottony growth

APPENDIX IV

Statistical analysis of *Alcaligenes faecalis and Curvularia nodosa*.

	MICROOGANISMS		
Time (Days)	BACTERIA	FUNGI	P- value
0	0.0004±0.0001	0.0011±0.0002	0.467
4	0.0009±0.0002	0.0023±0.0003	0.152
8	0.0294±0.0045	0.0341±0.0048	0.106
12	0.0400±0.0059	0.0490±0.0035	0.001
16	0.0507±0.0079	0.0639±0.0050	0.001
20	0.0615±0.0103	0.1412±0.0588	0.082

24	0.0589±0.0077	0.1703±0.1014	0.019
28	0.0533±0.0078	0.0792±0.0055	0.015
32	0.0520±0.0076	0.0664±0.0048	0.012

P<0.05