ISOLATION AND IDENTIFICATION OF PLASTICS-DEGRADING MICROORGANISMS FROM SOILS OF MOROGORO, TANZANIA

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ABSTRACT

Plastics, though widely used, have low biodegradability and hence are persistent in the environment, becoming a major source of pollution. The study herein was conducted to isolate, from soils, isolates of microorganisms capable of biodegradation of plastics. The soils were sampled from Morogoro Municipality in Tanzania. The pH of the soils ranged between 6.46 and 8.91. Organic carbon ranged from 0.20 and 1.23%, which was very low to low. Total nitrogen ranged from 0.01 and 0.09%, which was very low. Textures of the soils used were sandy, loamy sand and sandy clay loam. Isolation and the enumeration of bacteria, fungi and actinomycetes (*Streptomyces*) was done using nutrient agar, potato dextrose agar and starch casein agar, respectively. The total populations of microorganisms ranged from 1.60×10^4 to 1.57×10^5 CFU/g soil. The capability of microbial isolates to biodegrade ground polyethylene bags and bottles was tested using Bushnell and Haas agar. Many isolates were capable of degrading plastics as depicted by the diameters of clear zones around colonies, ranging between 1.0 and 66 mm for ground polyethylene bags and 1.0 to 73.7 mm for ground plastic bottles. Using molecular methods, identified bacterial isolates were *Bacillus cereus* and *Cellulosimicrobium sp*. Fungal isolates identified were *Eupenicillium rubidurum*, *Phoma sp*., *Neosartorya fischeri*, *Aspergillus terreus*, *Talaromyces islandicus* and *Aspergillus sp.* Actinomycetes were *Streptomyces werraensis* and *Streptomyces rochei*. Selected identified isolates showed significant ($P \leq 0.05$) differences in ability to degrade the plastics, with *Bacillus cereus*, *Streptomyces rochei*, and *Phoma sp*., being most efficient, with diameters of clear zones ranging, overall, from 30.0 to 66.0 mm for fungi and 19.3 to 47.5 mm for bacteria and actinomycetes. Further studies are needed to identify more isolates capable in degrading the different types of plastics which are routinely disposed of in the Tanzanian environment.

DECLARATION

I, Monica Daniel Nakei, do hereby declare to neither the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABI	Applied Biosystems
BPA	Biosphenol A
CRD	Completely Randomized Design
DEHP	di-2-ethylhexyl phthalate
DNA	Deoxyribonucleic acid
HDPE	high density polyethylene
ITS	intergenetic spacers
LDPE	low density polyethylene
MEGA	Molecular Evolutionary Genetics Analysis
ºC	degree Celsius
OC	organic carbon
PAHs	polycyclic aromatic hydrocarbons
PCDFs	polychlorinated dibenzofurans
PCR	polymerase chain reaction
pH	potential of Hydrogen
RAPD	Random Amplified Polymorphic DNA
rDNA	ribosomal DNA
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
SUA	Sokoine University of Agriculture
USA	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet
VOCs	volatile organic compounds

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Plastics are of great significance in today's world 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; Andrady and Neal, 2009). The plastics used include polyethylene, polypropylene, polystyrene, polyvinyl chloride and polyethylene terephthalate, all of which are high molecular weight polymers whose biodegradability is low. Hence, plastics are persistent in the environment and are one of the sources of environmental pollution (Tokiwa and Ugwu, 2007). Their disposal both on the land and the aquatic environment has resulted in their accumulation due to little, if any, biodegradation, making the environment unaesthetic, with possible health implications to humans, animals, and other organisms (Siddiqui *et al*., 2008).

After use, most of the plastics are collected and burned as one of the methods of removing them from the environment, but this has greater effects to human health and the environment in general. Combustion of plastics results in byproducts which are harmful in the environment, especially to the health of living organisms. The mostly evolved byproducts of plastics during combustion are airborne particulate emission (soot) and solid residue ash (black carbonaceous colour). Several studies have demonstrated that soot and solid residue ash possess a high potential of causing significant health and environmental effects. The soot, when generated, is accompanied with volatile organic compounds (VOCs), semi-VOCs, smoke (particulate matter), particulate bound heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzofurans (PCDFs) and dioxins (Valavanidids *et al*., 2008). These byproducts have the ability to travel thousands

of kilometers, depending on prevailing atmospheric conditions, before dropping back on earth and entering the food chain. Furthermore, the composition of byproducts of plastic combustion as to their type and concentration depends on the combustion temperature and the flame residence time (Lee *et al*., 1995). The common heating rates of 10 to 100 Kinetic rates/second are common under fire conditions but rare in thermal analysis. However, low heating rates can occur under real fires (Hirschler, 1986). For example, in burning the plastics through incineration or in open places some toxic acidic gases including hydrogen chloride, hydrogen fluoride, hydrogen bromide, sulphur oxides and nitrogen oxides are formed and emitted by incinerators. Exposure to oxides of nitrogen and sulphur has also been linked to adverse impacts on respiratory health (Simoneit, 2005).

Various researches have evaluated the toxicity of combustion products which are generated from the burning of plastics. These have significant environmental and health concerns, which include being carcinogens such as PAHs, nitro-PAHs and dioxins, all of which are present in the airborne particulate emissions. Moreover, these particulates are highly mutagenic (Lee *et al*., 1995), and PAHs which are in the range of 8-340 ppm in the soot are significantly high enough to cause cancer (Valavanidids *et al*., 2008). Various researches also show high concentration of persistent free radicals (unstable and highly reactive molecules) both in the soot and the solid residual ash, which are implicated in the creation of adverse health effects especially to human lungs (Simoneit, 2005).

A study of the combustion of PE [both low density (LDPE) and high density (HDPE) polyethylene] at different operating conditions detected more than 230 VOCs and semi-VOCs, especially olefins, paraffin, aldehydes and light hydrocarbons (Valavanidids *et al*., 2008). Among the VOCs, benzene is a known carcinogen and has been observed to be released in significant quantities during combustion of plastics. Some of the toxic semi-

VOCs, including benzo (alpha)pyrene and 1,3,5 trimethylbenzene, are also present in significant quantities in the emissions from plastic combustion (Simoneit *et al*., 2005).

Heavy metals including lead, cadmium, chromium and copper are also present in the smoke and the solid residue ash (Valavanidids *et al*., 2008). The DEHP (di-2-ethylhexyl phthalate) plasticizer compound used in plastic manufacturing is also a probable human carcinogen, a potential endocrine disruptor and is harmful by inhalation, generating possible health risks and irreversible effects (Simoneit *et al*., 2005). In addition, burning of plastics can sometimes result in toxic fumes (Valavanidids *et al*., 2008).

Aside from trying to eliminate plastic wastes, their production is also costly to the environment. It takes large amounts of chemical pollutants to create plastics, as well as significant amounts of fossil fuels (Font *et al*., 2004). Plastics in Tanzania are widely used for packaging purposes. The materials packed in plastics include food materials like fruits, take away cooked food and water. In addition, plastics especially polyethylene, are used in carrying the 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 Tanzania are haphazardly disposed into the environment due to the lack of facilities for their orderly collection and infrastructure for recycling. Some plastic wastes are collected for export and recycling but most remain scattered in the environment. Many accumulate in the environment for long periods of time. It would be desirable to have microorganisms capable of biodegradation of plastics as one solution to the problem of plastics accumulation in the environment. Some microorganisms, though of low abundance in the environment, mainly soils, have been isolated with ability of

attacking plastics because they produce enzymes that enable them to use the plastics as substrate (Tokiwa *et al*., 2009).

1.2 Justification

Discarded plastics affect the environment in different ways. Plastics affect habitats in the form of pollution, space-usage, contamination, especially because of their quality of persistence in the environment. Fortunately, society has recognized this problem, and efforts are underway of finding ways to reduce accumulation of plastics in the environment (Tokiwa and Ugwu, 2007). The discarded plastic in the ocean presents problems to aquatic animal life. Populations of marine animals, such as sea birds, turtles, fish, whales, and seals can be affected significantly due the disposal of plastics in the ocean, mainly in the form of discarded nets and equipment. Also large quantities of plastics are disposed of in soils of Tanzania, and these materials can have similar effects on terrestrial animals. Some plastics, especially those produced with the assistance of a substance called bisphenol A (BPA), which is a synthetic chemical compound, when ingested, can interfere with development and reproduction of animals, through interaction with estrogen (Siddiqui *et al*., 2008). Thus, a search for, and isolation from soils of, microorganisms capable of degrading these plastics can be the beginning of finding a solution to the problem of plastics accumulation in the environment.

1.3 Objectives

1.3.1 Overall objective

The overall objective this study was to obtain microorganisms capable of biodegradation of plastics from the soils of Morogoro Municipality.

1.3.2 Specific objectives

The specific objectives were

i. To isolate bacteria, fungi and actinomycetes (*Streptomyces*) from soils, including those into which plastics have been disposed of,

ii. To test the isolates for ability to biodegrade different types of plastics, and

iii. To identify the microbial isolates with the ability of biodegradation of plastics.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Global plastics production levels

Production of plastics started on an industrial scale between the 1940s and 1950s. In the last 15 years the global annual production of plastics has doubled, reaching 245 million tons in 2008 (Plastics Europe, 2009). The use of plastics, especially polythene, is growing day by day and every year 25 million tons of synthetic plastics are being accumulated in the sea coasts and terrestrial environment (Lee *et al*., 1991). Polythene constitutes 64% of the total synthetic plastic as it is being used in huge quantity for the manufacture of bottles, carry bags, disposable articles, garbage containers, margarine tubs, milk jugs, and water pipes (Lee *et al*., 1991). The annual plastics consumption per capita in Western Europe, Canada, USA and Mexico was 100 kg in 2005, which is ten times as much as in African countries and five times as much as in Asian countries, excluding Japan. This means that there is a large potential not only for further increased consumption but also for generation of plastics wastes that would call for removal from the environment (Plastics Europe, 2009).

There are several companies that produce plastics in Tanzania. Table 1 shows some of the companies where most of them manufacture both plastics and plastic products.

Table 1: Companies that manufacture plastics in Tanzania, their location and types of plastics produced

S/N	Company name	Type of plastics produced	Location
1	Plasco Ltd.	Plastics and rubber	Dar es salaam
2	Morogoro Plastics Ltd	Plastics and rubber	Morogoro
3	Tanga Pharmaceutical and Plastics Ltd	Plastics	Tanga
4	Somochem (T) Ltd	Plastics and plastic products	Dar es salaam
5	Unoplast (T) Ltd	Plastics and plastic products	Dar es salaam
6	Harsh Packaging CO. Ltd	Plastics and plastic products	Moshi-Kilimanjaro
7	Centaza Plastics Ltd	Plastics and plastic products	Dar es salaam
8	Cello Industries (T) Ltd.	Plastics and plastic products	Dar es salaam
9	Falcon Industries Ltd	Plastics and plastic products	Dar es salaam
10	JK Investment Company	Plastics and plastic products	Dar es salaam
11	Gunny Bags Manufacturers Company	Plastics and plastic products	Dar es salaam
12	Bajaber Packaging Ltd.	Plastics and plastic products	Tanga
13	B. H. Ladwa	Plastics and plastic products	Morogoro
14	Jambo Plastics Ltd.	Plastics and plastic products	Dar es salaam

Source: Wangwe *et al*., 2014; Donath and Sutton, 2012

2.2 Polythene and Polypropylene Plastics Production

The volume of plastics produced globally has increased from about five million tons in the 1950s to 260 million tons in 2007, before dropping to 245 million tons in 2008 following the recent financial crisis (Zheng and Yanful, 2005). The report by American Plastics Association shows that the percentage distribution of PP, High Density Polyethylene (HDPE), Linear Low Density Polyethylene (LLDPE) and Low Density Polyethylene

(LDPE) are 18.4%, 17.4%, 12.1% and 8.2%, respectively, in terms of sales and use in United States, Canada and Mexico (Zheng and Yanful, 2005). Non-degradable plastics accumulate in the environment at the rate of 25 million tonnes per year (Pometto and Lee, 1992).

Plastics production is spread around the world and can be expected to rise to meet the ongoing demand (Plastics Europe, 2009). According to GESAMP Report (2010), 25 percent of polyethylene and polypropylene was produced in Europe (EU 27 members states plus Norway and Switzerland), 23 percent in the NAFTA region including the USA, 16.5% in Asia (excluding China), 15 percent in China, 8 percent in the Middle East, 5.5 percent in Japan, 4 percent in South America and the rest of the world including Africa and Tanzania 3 percent. The GESAMP Report (2010) further reports that production and consumption of plastics vary from region to region. For example, Europe produced 55 million metric tonnes in 2009 but only consumed 45 in the same year (2009). Building materials account for 20%, with PVC as the main component followed by HDPE, epoxidised polysulphides (EPS) and polyurethane (PUR), while the automotive and electronics industries account for 7 and 6%, respectively, using a much wider range of materials. It is known that the cost of raw material may induce the substitution of different polymers for the same purpose in other regions, so the pattern of production and use is not consistent worldwide.

2.3 General Properties of Plastics

2.3.1 Structural chemistry of plastics

The structure of plastics is basically a polymer that is macromolecule chains formulated from monomeric units by chemical polymerization reactions. The chain assembling is due to typical reactions, which are polyaddition and polycondensation (Rolf, 2011).

Polyaddition can be as chain reaction or as a step reaction (Waterman, 1979). In polyaddition chain reaction, the polymerization occurs by chemical combination of a large number of monomer molecules, in which the monomers combine to form a chain either by orientation of the double bond or by ring splitting. The process involves energy consumption by light, heat, radiation or by use of catalysts. Examples of plastics formed by this type of reaction are polyethylene, polypropylene and polystyrene (Schwartz and Goodman, 1982). In polyaddition step reaction, the polymerization is by combination of monomer units without involvement of double bonds or separation of low molecular compounds (Peacock and Calhoun, 2012). Hydrogen atoms can change position during polyaddition process. The examples of plastics formed through this process are chlorinated polyethers, linear polyurethane, polyurethane and polyvinyl chloride. In polycondensation, plastics are formed by buildup of polyfunctional compounds (Belofsky, 1995).

The degradability of a plastic material depends on the constituents of a particular compound, which makes the plastic material to be hard or soft. For instance, polyvinyl chloride is different from polyethylene or polypropylene, whereby polyvinyl chloride contains chlorine in its structure while polyethylene and polypropylene do not. Polyvinyl chloride (PVC) which includes bottles, thin sheeting, transparent packaging materials, water and irrigation pipes and gutters is a hard, rigid material, unless plasticizers are added to make it more flexible (UNEP, 2009a).

The biodegradability of PVC is difficult unless it is plasticized. *Aspergillus fumigatusi* was observed to degrade plasticized PVC (Hamidi *et al*., 2011). Polyethylene (PE) plastic is divided into two main types which are low-density polyethylene (LDPE) and high density polyethylene (HDPE). LDPE is soft, flexible and easy to cut, with the feel of candle wax and it is used in the manufacture of film bags, sacks and sheeting and blow-moulded

bottles. HDPE is tougher and stiffer and is used for bags and industrial wrappings and soft drinks bottles (Khoo and Tan, 2010). Polypropylene is more rigid than PE, and can be bent sharply without breaking. It is used for stools and chairs, crates, pipes, fittings and food containers. In comparison of the susceptibility to biodegradation of polyethylene, LDPE is more easily degradable than HDPE, followed by polypropylene.

2.3.2 The physical properties of polymers

The physical properties of a polymer such as its strength and flexibility depend on chain length, branching, cross-linking and side groups (Smith and Lemstra, 1980). In general, the longer the chains the stronger the polymer; and polar side groups, including those that lead to hydrogen bonding give stronger attraction between polymer chains, making the polymer stronger (Han and Govaert, 2005). Straight and unbranched chains can pack together more closely than highly branched chains, giving polymers that have higher density, more crystalline and stronger (Jansen *et al*., 1999). If polymer chains are linked together extensively by covalent bonds, the polymer is harder and more difficult to melt (Giancaspro *et al*., 2009) and may be more difficult to biodegrade. The typical example of the plastics which explores these properties are polyethylene bags which are transparent plastic bags. It is also the simplest polymer, consisting of random-length but generally very long chains made up of two-carbon units (Smith and Lemstra, 1980).

2.4 Uses of Plastics

2.4.1 Plastics formulated into different mechanical equipment/parts

Car airbags, motorcyclists' helmets and protective clothing are made of plastics. Firefighters rely on flexible plastic clothing to protect against high temperatures and plastic equipment provides life-saving ventilation. Plastic packaging protects our food and drink from contamination while plastic flooring and furniture are easy to clean, preventing the spread of germs and bacteria. In healthcare, plastics are used in a variety of ways, for

blood pouches and tubing, artificial limbs and joints, contact lenses and artificial corneas, absorbable sutures, splints and screws that heal fractures (Andrady and Neal, 2009).

2.4.2 Other common uses of plastics

Different plastics have different uses in the society depending of the purpose for use and the materials used to manufacture which specifies them in their uses. Table 2 shows some of the types of plastics and their common uses.

Table 2: Common uses of plastics

Plastic type	Common uses
Polyethylene/Terepthalate	Mineral water, fizzy drink and beer bottles, pre-prepared food trays and roasting bags, boil in the bag food pouches, fibre for clothing and carpets, strapping, some shampoo and mouthwash bottles.
High Density Polyethylene	Credit cards, carpet backing and other floor covering, window and door frames, guttering, pipes and fittings, wire and cable sheathing, synthetic leather products.
Low Density Polyethylene	Films, fertilizer bags, refuse sacks, packaging films, bubble wrap, flexible bottles, irrigation pipes, thick shopping bags (clothes and produce), wire and cable applications, some bottle tops
Polypropylene	Most bottle tops, ketchup and syrup bottles, yoghurt and some margarine containers, potato crisp bags, biscuit wrappers, crates, plant pots, drinking straws, hinged lunch boxes, refrigerated containers, fabric/carpet fibres, heavy duty bags/tarpaulins
Polystyrene	Yoghurt containers, egg boxes, fast food trays, video cases, vending cups and disposable cutlery, seed trays, coat hangers, low cost brittle toys
Other	Nylon, acrylonitrile butadiene styrene (ABS), polycarbonate (PC), layered or multi-material mixed polymers

Source: Andrady and Neal, 2009.

2.5 Environmental Contamination by Waste Plastics

2.5.1 Water (marine) environmental contamination by plastics disposal

The disposal of plastics in water is due to transport by various sources such as floods or direct disposal by human beings (Andrady, 2011). The plastics in the marine environment

cause contamination and endanger the marine organisms. The contamination may be due to disposal or through leaching of the fragments of additives which are used to make plastics during polymerization, and some of the additives are broken down due to degradation by UV light, chemical or microorganisms (Ryan *et al*., 2009). Under the degradation conditions, no compound is persistent, but their instability within plastic products facilitates leaching and their high prevalence in aquatic environments, particularly in landfill leachates (Vom Saal and Myers, 2008). The fragments formed due to degradation are called microplastics (Zitko and Hanlon, 1991).

Due to the large surface-area-to-volume ratio of microplastics, marine biota may be directly exposed to leached additives after microplastics are ingested. Such additives and monomers may interfere with biologically important processes, potentially resulting in endocrine disruption, which in turn can impact upon mobility, reproduction and development, and lead to carcinogenesis (Barnes and Milner, 2005).

2.5.2 Contamination of soil by plastics disposal

Plastics are proved to be harmful to human, animal, and plant health. Plastics also harm habitats in the form of pollution, space-usage, contamination, and especially through its quality of persistence (Barnes and Milner 2005). The chemical compounds found in plastics are harming and causing biological effects in both humans as well as animals. Two broad classes of plastic-related chemicals are of critical concern for human health-bisphenol-A or BPA, and phthalates (Devi *et al*., 2014). Plastics wastes also take a large space in the soil environment and hence being unaesthetic for use especially in agriculture and grazing animals (Andrady and Neal, 2009). Several communities are now more sensitive to the impact of discarded plastics on the environment because of their persistence in our environment, including deleterious effects on wildlife and on the

aesthetic qualities of cities and forests. Improper disposal of plastics play significant role in potentially harming life through causing environmental pollution. In addition to this, the burning of polyvinylchloride (PVC) plastics produces persistent organic pollutants (POPs) known as furans and dioxins (Albertsson *et al*., 1987; Jayasekara *et al*., 2005).

2.6 Management of Plastics Contamination in Environment

2.6.1 Recycling to make new products

Plastics can be recycled into new products. Currently, two approaches used for the recycling of plastic include chemical and mechanical processing (Awaja and Pavel, 2005). Chemical processing of plastics is performed by carrying out chemolysis with one of a number of compounds, resulting in depolymerisation (hydrolysis), methanolysis (methanol production), glycolysis or aminolysis, for example of methylamine of the plastic (Sinha *et al*., 2010); Awaja and Pavel, 2005), resulting in different monomers that may be used as polymerisation materials to produce new plastic. Similarly, the mechanical processing, involves several individual steps (Awaja and Pavel, 2005) the first of which is the removal of as much contaminating material as possible. This involves sorting of plastic waste in order to separate the poly(ethylene terephthalate) (PET) from other plastics which is done manually, grinding PET into flakes and washing, either using 2% NaOH and detergent at 80 °C, followed by rinsing in cold water, or using tetrachloroethylene, drying under desiccation at ~170 °C for six hours, melting down and extruding into new forms.

2.6.2 Elimination by incineration

Another technique routinely used for disposal of plastic waste is incineration (Zhang *et al*., 2004). Plastic incineration overcomes some of the limitations placed on landfills in that it does not require any significant space, and there is even the possibility for energy recovery in the form of heat (Sinha *et al*., 2010). However, there is a significant trade-off in that

incineration of plastics leads to the formation of numerous harmful compounds, most of which are released to the atmosphere (Zhang *et al*., 2004). PAHs, PCBs, heavy metals, toxic carbon- and oxygen-based free radicals, not to mention significant quantities of greenhouse gases, especially carbon dioxide, are all produced and released when plastics are incinerated (Astrup *et al*., 2009). The significant environmental drawbacks of plastic disposal via both landfill and incineration were the driving force behind the development of plastic recycling processes.

2.6.3 Elimination by biodegradation

Different microorganisms have been identified to be a more efficient and environmentally safe method of eliminating the plastics in the environment. For example, bacteria have been a resource for remediation of pollution in the environment (Iranzo *et al*., 2001). Bacteria have been utilized in the clean-up of oil spills (Piedad *et al*., 2002; Rosa and Triguis, 2007) and heavy metals, such as arsenic, mercury, cadmium and lead (Takeuchi *et al*., 2007). There are sufficient examples to suggest that there are few if any substances that cannot be utilised at least in part by microbes for metabolic activities (Iranzo *et al*., 2001). Biodegradation is an attractive alternative to current practices for waste disposal, as it is generally a cheaper potentially much more efficient process and does not produce secondary pollutants, such as those associated with incineration and landfill (Pieper and Reineke, 2000).

The end products of biodegradation may be of economic benefit from microbial metabolism of pollutants, for example, ethanol for use as biofuel (Iranzo *et al*., 2001). Bioremediation of hydrocarbons, crude oil, for example, poses a number of practical difficulties. Firstly, Bacteria prefer aqueous nutrients (Iranzo *et al*., 2001) and hydrocarbons are often immiscible with water. Secondly, hydrocarbons are largely

deficient in certain essential elements, namely nitrogen, potassium and phosphorus (Rosa and Triguis, 2007). It has been shown in the literature that manually adding these elements in the form of fertilizer, for example ammonium sulphate, can significantly aid the degradation of hydrocarbons by bacteria (Rosa and Triguis, 2007). Thirdly, hydrocarbons also actively interfere with cell membranes, accumulating within and disrupting the phospholipid bilayer. However, some bacteria have even been isolated that resist organic solvents (Pieper and Reineke, 2000). Many studies have investigated the degradability of a wide range of polymers (Artham and Doble, 2009; Kondratowicz and Ukielski, 2009). Polymers with pure carbon backbones are particularly resistant to most methods of degradation, but polymers that include heteroatoms in the backbone, for example polyesters and polyamines, show higher susceptibility to degradation (Zheng *et al*., 2005).

2.6.4 Disposal of plastics through landfills

The first drawback associated with landfill disposal of plastic waste is the fact that landfill facilities occupy space that could be utilized for more productive means, such as agriculture (Zhang *et al*., 2004). This is compounded by the slow degradability of most plastics, as this means the occupied land is unavailable for long periods of time. Plastic components of landfill waste have been shown to persist for more than 20 years (Tansel and Yildiz, 2011). This is due to the limited availability of oxygen in landfills; the surrounding environment is essentially anaerobic (Tollner *et al*., 2011). The limited degradation that is experienced by many plastics is largely due to thermo oxidative degradation and the anaerobic conditions in landfills only serve to further limit degradation rates (Andrady, 2011).

Plastic debris in landfill also acts as a source for a number of secondary environmental pollutants (Zhang *et al*., 2004). Pollutants of not include volatile organics, such as

benzene, toluene, xylenes, ethyl benzenes and trimethyl benzenes, released both as gases and contained in leachate (Urase *et al*., 2008) and endocrine disrupting compounds, in particular, BPA leachate (Svenson *et al*., 2009). In addition to its endocrine disruption properties, BPA released from plastics in landfill, has also been shown to lead to an increase in production of hydrogen sulphide by sulphate-reducing bacteria in soil. High concentrations of hydrogen sulphide are potentially lethal to microbial populations (Tsuchida *et al*., 2011).

2.7 Processes Involved in Plastics Degradation

The degradation of plastics occurs through various processes, which include photo-induced degradation, thermal degradation, chemical degradation and biological degradation. Photo- induced degradation involves the photolysis of plastics to give low molecular weight molecules. It involves the use of electromagnetic waves with energy of visible light or higher, such as ultraviolet light, X-rays and gamma rays (Volke *et al*., 2002). Light - induced polymer degradation, or photodegradation, includes the physical and chemical changes caused by irradiation of polymers with ultraviolet or visible light (Rabek, 1996). In order to be effective, light must be absorbed by the substrate (polymeric system). Thus, the existence of chromophoric groups in the macromolecules is a prerequisite for the initiation of any photochemical reaction (Rabek, 1995).

Chemical degradation involves the use of chemicals through different process such as solvolysis, which is mainly hydrolysis, to give low molecular weight molecules and or ozonolysis whereby plastics form cracks by ozone attack (Weidner *et al*., 1996). Other chemical means of degradation of plastics include the galvanic action, which is the corrosion of plastics and the chlorine-induced cracking, which is the reaction of the metal component with chlorine (Konduri *et al*., 2010).

Thermal degradation involves the thermolysis of plastics at high temperatures of about 450^{o}C-500^{o}C to give small molecules, gases (largely in form of carbon monoxide, carbon dioxide and water vapour (Volke *et al*., 2002). It involves the number of chemical reactions in the decomposition of polymers (Hirschler, 1986). This includes random chain scission, which occurs at apparently random locations in the polymer chain, or end-chain scission, in which individual monomer units are successfully removed at the chain end and chain stripping, in which atoms or groups are not the part of the polymer whereby they are cleaved, or cross-linked whereby bonds are created between polymer chains (Kashiwagi *et al*., 1987).

Microbial biodegradation is widely accepted and is still underway for its enhanced efficiency (Starnecker and Menner, 1996). Biological degradation of plastics can be undertaken by microorganisms to give lower molecular weight molecules which are oligomers and monomers (Volke *et al*., 2002). Microbial degradation of plastics is caused by certain enzymatic activities that lead to a chain cleavage of the polymer into oligomers and monomers that can be further absorbed by the microbial cells where they are metabolized (Starnecker and Menner, 1996). Metabolism involves aerobic or anaerobic conditions. Aerobic metabolism results in carbon dioxide and water, whereas anaerobic metabolism results in carbon dioxide, water, and methane as the end products (Gu *et al*., 2000). The physical factors such as temperature, moisture and pressure deal with causing mechanical damage to the polymer so that the biological factors like the enzymes can then catalyze the process.

2.8 Microorganisms with Ability of Degrading Plastics

2.8.1 Types of microorganisms with ability of degrading the plastics

Different bacteria capable of degrading plastics have been observed in different environments with different capabilities in degrading the plastics. Chee *et al*. (2010)

observed some species of bacteria capable of degrading plastics, the isolates including *Bacillus megaterium*, *Pseudomonas sp*., *Azotobacter*, *Ralstonia eutropha,* and *Halomonas sp*. Majid *et al*. (2015) observed the ability of *Pseudomonas putida* S3A in biodegrading polyethylene, and *Pseudomonas putida* S3A was able to utilize nylons as a sole source of nitrogen and carbon, whereby the strain was isolated from soil contaminated with plastic waste. Similarly, Sharma and Sharma (2004) studied the degradation of low-density polythene (LDP) and polythene (PP) using *Pseudomonas stutzeri* under laboratory test condition. Throughout the investigation, both the plastic types were found to undergo qualitative and quantitative changes by the bacteria. Usha *et al*. (2011) demonstrated presence of polyethylene degrading microorganisms in a garbage soil in India, the organisms involved including *Comamonas acidovorans* bacterium. Sonil et al. (2010) investigated the biodegradation ability of *Brevibacillus*, *Pseudomonas*, and *Rhodococcus spp*. in degrading polyethylene.

Various studies were conducted to investigate the ability of different species of fungi in biodegrading plastics. The fungi including *Aureobasidium pullulans* and a group of yeasts and yeast-like fungi, including *Rhodotorula aurantiaca* and *Kluyveromyces spp*. isolated from the garbage soil in India, were observed to be capable of biodegrading polyethylene plastics (Usha *et al*., 2011). Similarly, Swift (1997) observed two fungal species (*Aspergillus glaucus* and *Aspergillus niger*) capable of degrading plastics. Immanuel *et al.* (2014) isolated strains of *Aspergillus japonicus* and *Aspergillus terreus* that could degrade low-density polyethylene (LDPE) and high-density polyethylene (HDPE) films in the delta mangrove of Niger. *Aspergillus flavus* and *Mucor sp.* were also observed to degrade polyethylene in India (Raaman *et al*., 2012).

Several actinomycetes (*Streptomyces*) species are also observed to be capable of degrading plastics. [Technically, actinomycetes are classified as being bacteria. However, because of their filamentous morphology as contrasted with simple bacterial cells, the author has chosen herein to retain the (historical) term "actinomycete" to distinguish them from simple bacterial forms]. Deepika and Jaya (2015) observed the ability of *Streptomyces sp.* in degrading low density polyethylene (LDPE). Other actinomycete sp. were also observed to be capable of degrading polyethylene (Swift, 1997; Chee *et al*., 2010). Other species of *Streptomyces* including *Streptomyces badius*, *S. setnii and S. viridosporous* in India are observed to cause the biodegradation of thermally oxidized low density polyethylene (LDPE), by causing the reduction of molecular weight, increasing carbonyl double bond groups and erosion of the surface of polyethylene (Arutchelvi *et al.*, 2007).

2.8.2 Enzymes catalyzing plastics degradation

Microorganisms (bacteria, fungi, algae) use plastics polymers as a source of carbon and energy for their continued existence. In acting upon the plastics, under the influence of extracellular inducible enzymes (endo- and exo-enzymes), the plastics polymers are degraded by the process of scission of the polymer chain, followed by oxidation of the shorter fragments (Chandra and Renu, 1998). These reactions can be effected by a great number of different enzymes, and the resulting increasingly smaller molecules produced enter into cellular metabolic pathways (such as the Kreb's cycle), generating energy and producing water, carbon dioxide, and other basic products of biotic decomposition (Kumar *et al*., 2013).

Some enzymes catalyzing plastics degradation have been identified. Examples of the identified enzymes are glucosidases from *Aspergillus flavus,* catalase and protease from *Aspergillus niger,* cutinase from *Fusarium*, lipase for *Rhizopus delemar, Penicillium,*

Rhizopus arrizus, and serine hydrolase from *Pseudomonas stutzeri.* The end products of enzymatic degradation are non-toxic and would not pose any threat in the environment (Andrej, 2012). Thus, in this way, it can be concluded that waste plastics could be collected from the environment, ground or macerated and subjected to biodegradation in industrial scale fermenters, thus effectively removing them from the environment (Andrej, 2012). The optimization of pH, temperature and the concentration of plastics is important although microorganisms differ in their performance in different levels of pH and temperatures and their efficiency can be tested to obtain the optimum pH and temperature. For example, the pH ranges of 6.5, 7, and 7.5 under the temperature of 30, 37 and 45 °C for fungi (Majid *et al*., 2015).

2.9 Identification of Microorganisms Capable of Degrading the Plastics

16S RNA gene sequencing is one of some techniques that have, of recent, been used in identifying microorganisms, including those that are capable of degrading plastics. 16S rRNA sequences for bacteria like *Rhizobium tropici, Rhizobium fredii, Rhizobium loti, Rhihizobium huakuii* have been applied (Cook and Meyers, 2003). Also 16S rRNA sequences have been used in identifying the actinomycetes, for example *Actinomadura, Gordonia, Rhodococcus*, *Saccharomonospora, Saccharopolyspora, Streptomyces* and *Tsukamurella* (Cook and Meyers, 2003). Molecular identification of fungi has been done using methods like Random Amplified Polymorphic DNA analysis (RAPD), which is one of the Polymerase Chain Reactions of Internally Transcribed Spacers (PCR-ITS/rDNA). Fungi like *Fusarium* (in wheat) and *Alternaria* (in carrot) have been identified using this technique (Dilip, 2004).

Organism that have been previously identified by these approaches have exhibited great efficiency in plastic degradation. Table 3 shows the efficiencies of some microorganisms

identified using these approaches in degrading some plastics. Those with higher efficiencies, for example, *Pseudomonas*, *Aspergillus*, and *Staphylococcus* (Table 3) could be candidates for further testing, leading to industrial utilization of that capacity.

Table 3: Plastic-degrading microbial species

Name of microbe	**Microbial degradation (% weight loss / month)**	
	Polythene bag	**Polypropylene**
Bacteria		
Pseudomonas sp.	20.54	3.97
Staphyloccoccus sp.	16.39	0.56
Moraxella sp.	7.75	8.16
Micrococcus sp.	6.61	1.02
Streptococcus sp.	2.19	1.07
Fungi		
Aspergillus glaucus	28.80	7.26
Aspergillus niger	17.35	5.54

*Source***:** Kathiresan, 2003

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Site

Soil samples for isolating microorganisms in order to evaluate their ability to degrade plastics were collected in the vicinity of the Morogoro-Iringa road at Kasanga village and within SUA maize fields close to the Department of Agricultural Economics and Agribusiness building which is within SUA campus. Sokoine University Agriculture is located at latitude 06°50' S and longitude 37°38' E, and at an altitude of 526 meters above sea level (526 m.a.s.l.). The study area is bordered by Uluguru Mountains from the South-East, Mindu and Lugala Hills to the north-west and Morogoro town the East. The study area experiences bimodal rainfall pattern ranging between 500 and 800 mm per year characterized by two rainfall peaks in a year with a definite dry season separating the short and long rains (New *et al*., 2002). The short rain season is from October to December while the long rain season starts from March and ends in May (Msanya *et al.*, 2003). In general, the climate of Morogoro District can be described to be of a sub-humid tropical type.

3.2 Soil Sampling

Different locations for soil sampling were identified within the study site, including those that have harbored waste plastic for different lengths of time, as well as those that visibly had apparently not encountered any plastic wastes. The soils with physical contacts with plastics were sampled at 5 to10 cm depth. Surface soils exposed to sunlight were avoided because any observation of degradation of waste plastics might be mainly due to UV radiation, rather than by microorganisms (Volke *et al*., 2002). The soil samples were collected in November 2014 and taken to the laboratory for isolation of actinomycetes,

bacteria and fungi, which were then subsequently tested for their ability to degrade plastics.

3.3 Soil Analysis

In the laboratory, portions of the soil samples were dried, ground and sieved through a 2 mm sieve for physico-chemical characterization. The soils were subjected to analysis of some essential parameters including total nitrogen, organic carbon, pH, particle size distribution and extractable phosphorus.

3.3.1 Total nitrogen

Total nitrogen was determined by the micro-Kjedahl digestion-distillation method previously described by Bremner (1996). One gram of soil was digested with concentrated Sulphuric acid (H_2SO_4) in the presence of a catalyst (K_2SO_4 + $CuSO_4$ + selenium powder; mixed in the ration of 10:10:1 by weight). The digest was distilled in the presence of 40% NaOH. The liberated NH_3 was collected in 4% boric acid (with mixed indicator) and titrated against a standard 0.05 M H_2SO_4. Afterwards, the titre was used to calculate the total nitrogen content of the soil sample.

3.3.2 Organic carbon

Organic carbon was determined by the wet digestion (oxidation) method of Walkley-Black previously described by Nelson and Sommers (1996). Briefly, 0.5 g soil sample, 10 ml of 1 M $K_2Cr_2O_7$ and 25 ml of concentrated H_2SO_4 were added and allowed to stand for 30 minutes to oxidize organic carbon. Two hundred ml of water was added to cool the mixture followed by of 10 ml of phosphoric acid. The amount of $K_2Cr_2O_7$ reduced was used to estimate the organic carbon content the soil by the titration of excess dichromate against a 0.5 N ferrous sulphate solution using diphenyl amine indicator.

3.3.3 Soil pH

The soil pH was measured electrochemically in water at the ratio of 1:2.5 (weight/volume) soil. To 10 g of the soil sample, 25ml of distilled water were added, and shaken on a reciprocating mechanical shaker for 30 minutes and pH measured using a pH meter (Thomas, 1996).

3.3.4 Particle size distribution analysis

Texture was determined by the hydrometer method (Bouyoucos, 1962). Briefly; 50 g oven-dry soil was placed into a soil dispersion cup and filled with distilled water. Then, 125 ml of 5% sodium hexametaphosphate (calgon) was added. The mixture was allowed to soak for 15 minutes. The cup was then attached to a mixer and mixed for 5 and 10 minutes for sandy and fine-textured soils, respectively. Then, the suspension was transferred into a sedimentation cylinder and filled up to 1000 ml mark with distilled water. The suspension was mixed with a plunger and hydrometer and temperature readings taken after 5 minutes and again after 5 hours. The percentage of sand, silt and clay were determined. The textural class was determined using the United States Department of Agriculture (USDA) textural class triangle.

3.4 Media for Microbial Isolation

3.4.1 Medium for isolation of bacteria

Medium used for bacteria isolation was nutrient agar prepared by mixing the following; 5 g of peptone, 3 g of beef extract, 1 g of yeast extract and 15 g of agar in 1 L of distilled water. The mixture was boiled prior to sterilization by autoclaving at 1.05 kg/cm^2 (15 lb./sq.in) and 121 ^{o}C for 20 minutes. The sterile medium was left to cool up to about 50 ^{o}C and then poured into sterile petri dishes and left to solidify. Cooled petri-dishes containing the medium were aseptically stored at 4 ^{o}C until used.

3.4.2 Medium for isolation of fungi

The potato dextrose agar (potato glucose agar) medium for isolation of fungi was prepared by adding 200 g of peeled and sliced potatoes into 500 ml of distilled water followed by cooking at 100 ^{o}C for 1 hour. The mixture was filtered through a cheese cloth. Then, 200 g of glucose and 15 g of agar were added to the filtered potato liquid and the volume adjusted to 1000 ml using distilled water. The medium was then sterilized by autoclaving at 1.05 kg/cm^2 and 121 ^{o}C for 20 minutes. The medium was left to cool to about 50 ^{o}C and then poured into petri dishes to solidify.

3.4.3 Medium for isolation of actinomycetes (Streptomyces)

Medium for isolation of actinomycetes was prepared as previously described by Kuster and Willams (1966). Briefly, starch-casein agar was prepared by mixing 10 g of starch, 0.3 g of casein (vitamin-free), 2.0 g of KNO_3, 2.0 g of NaCl, 2.0 g of K_2HPO_4, 0.05 g of $MgSO_4.7H_2O$, 0.02 g of $CaCO_3$, 0.01 g of $FeSO_4.7H_2O$ and 15 g of agar in 1000 ml of distilled water. The pH of the medium was adjusted to 7.2. The medium was sterilized in an autoclave at the pressure of 1.05 kg/cm^2 (15 1b/sq.in.) at 121^{o}C for 20 minutes. The medium was left to cool down to about 50 ^{o}C and then poured into petri dishes to solidify. Petri dishes were aseptically stored at 4 ^{o}C until use.

3.4.4 Medium for testing biodegradation of plastics

The Bushnell and Haas agar (Bushnell *et al*., 1941) was used for testing the ability of microorganisms in degrading plastics. The media was prepared by adding 0.2 g of $MgSO_4$, 1.0 g of KH_2PO_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 $FeCl_3$, and 20 g of agar into 1000 ml of distilled water. One gram plastic powder (polythene/polypropylene) was added into the Bushnell and Haas agar medium to make a final concentration of 0.1% (w/v) and the mixture was shaken for 1 hour in a mechanical

shaker. The pH of the medium was adjusted to 7.0 $\pm$ 0.2 and the medium autoclaved at 1.05 Kg/cm^2 and 121^{o}C for 15 minutes. The medium was left to cool to about 50 ^{o}C and then poured into petri dishes to solidify.

3.5 Isolation of Bacteria, Fungi and Actinomycetes from Soils

Ten-fold dilutions of soil samples were prepared prior to microbial isolation as previously described by Usha *et al*. (2011). 10 g of soil sample was transferred into a bottle containing 90 ml of sterile water and shaken vigorously to suspend the soil particles, thus making the 10^{-1} suspension. One ml of the above suspension was aseptically transferred to a bottle carrying 9 ml of sterile water and shaken to mix well, making a 10^{-2} suspension. Using a fresh sterile pipette, the 10^{-2} was transferred to make a 10^{-3} suspension. Thus, serial ten-fold dilutions were made up to the 10^{-6} dilution. Then, 1 ml aliquots from 10^{-3} to 10^{-6} were poured into different petri dishes in triplicates. To the petri dishes, about 15 ml of the molten starch casein agar for actinomycetes, nutrient agar for bacteria or potato dextrose agar for fungi were added. The petri dishes were then gently swirled clockwise, then anticlockwise and once forwards and once backwards to mix the soil suspensions and the molten media. Then, the petri dishes were left to stand for the media to solidify. The plates were then incubated, up-side down, at 30 °C for three to seven days for bacteria and fungi, and up to 14 days for actinomycetes, until visible colonies were seen. The developed colonies were sub-cultured respectively on respective agar plates previously prepared to get pure cultures and then preserved in slants of respective media at 4 °C.

3.6 Enumeration of Total Microbial Populations

The counting of the microbial populations was done depending on the nature of their growth. The counting for bacteria and fungi was initially done on the third day and for actinomycetes on 14th day when the colonies were clearly grown. Then after two days re-

counting of all the plates were done for all microorganisms. Colonies were counted on plates that showed a good distribution of colonies estimated at between 30 and 300. The colonies were counted and converted to microbial populations (CFU) per gram of soil sample.

3.7 Testing the Microbial Isolates for Ability to Biodegrade Different Types of Plastics

Fifteen ml of molten sterile medium containing the plastics powder (section 3.4.4) were poured into sterile petri dishes and left to cool and solidify. The isolated organisms were inoculated onto agar medium containing the ground plastics. The plastic powder was obtained from ground plastics sieved through a 0.6 mm sieve. The plates were incubated at 27 °C for up to 21 days, periodically observing clear zones surrounding the colonies. The colonies which showed the clear zones surrounding them were taken to be the ones with the ability of biodegrading plastics. The diameters of such colonies including the clear zones were measured using a ruler. These isolates were then purified by repeated sub-culturing in their normal media which were nutrient agar, potato dextrose agar and starch casein agar for actinomycetes, bacteria and fungi, respectively. Pure isolates of actinomycetes, bacteria and fungi were characterized to species level based on their macro-morphology and micromorphology, and subsequently identified using molecular methods.

After observing the microbial colonies which showed the clear zones surrounding them, colonies which had larger clear zones were selected and tested them for their comparative efficiency in degrading plastics using the completely randomized (CRD) statistical design, with four replications. The medium used was prepared as described in section 3.4.4 and the cultures were inoculated into the media containing plastics, to test the efficiency

between isolates. Diameters of clear zones surrounding the colonies were measured on day 5, 7, 9, 11 and 13 for fungi and on day 5, 8, 11, 14 and 17 for bacteria and actinomycetes. The diameters for each day of recording were subjected to analysis of variance and means compared using the Duncan's New Multiple Range Test at 5% level of probability.

3.8 Analysis of the Selected Colonies of Actinomycetes, Bacteria and Fungi

Representative colonies that displayed great potential to degrade plastics, as expressed by larger diameters of clear zones of degraded plastics surrounding the colonies, were selected and their morphologies described.

The macro-morphology of actinomycetes, bacteria and fungi was determined with the aid of the naked eye. Images of colonies were captured using a digital camera (Nikon, Hong Kong, China). Micromorphology was determined by preparing the fungal microscopic slides using lactophenol blue solution, a mounting medium and staining agent for fungi identification under the light microscope. Actinomycetes and bacteria smears were prepared using standard Gram stain procedure.

3.8.1 Staining of fungi using lactophenol cotton blue solution

Lactophenol cotton blue solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. The reagent was prepared by mixing 20 g of phenol crystals, 20 ml of lactic acid, 40 ml of glycerol, 20 ml distilled water and 0.075 g of methyl blue. The solution was well shaken to mix the contents. A drop of lactophenol cotton blue solution was placed on a slide, using an inoculating needle/loop, followed by careful spreading of fungal culture to obtain a thin preparation on the slide. A coverslip was placed on the drop and lowered to avoid air bubbles under the

coverslip and left for about 5 minutes. The slides were observed under a light microscope with low power for screening in low intensity as previously described by Alfred (2009).

3.8.2 Gram-staining of bacteria and actiomycetes

The Gram-staining of bacteria and actinomycetes were performed as previously described by Alfred (2009).

The actinomycetes and bacteria smears were prepared on microscopic slides. The slides were cleaned with alcohol, followed by drying using paper towels. The prepared slides were labelled by drawing a circle on the underside of the slide using a marker pen to clearly designate the area in which the smear will be prepared. The loop to be used was sterilized by heating it on a burner and cooled. With a sterile cooled loop, a drop of sterile normal saline solution was placed on the slide. The loop was sterilized and left to cool, and a very small sample of a bacterial and or actinomycete colony was picked up and gently stirred into the drop of normal saline on the slide to create an emulsion that was subsequently air dried. After the smear was air-dried, the slides were passed through the flame of a bunsen burner two to three times with the smear-side up ready to be stained. The fixed smear was flooded with crystal violet solution and allowed to stand for one minute. The crystal violet was rinsed off with distilled water, and slides were then flooded with iodine solution and allowed to remain for one minute. The iodine solution was rinsed off with distilled water, and the slides were flooded with acetic acid (decolourizer) for five seconds. The decolourizer was rinsed off with distilled water and the slides were flooded with safranin and allowed to stand for 30 seconds, after which safranin was rinsed off with distilled water. The slides were air dried by placing them in an upright position and smears were viewed using a light-microscope under oil-immersion at the 1000x magnification (Alfred, 2009).

3.9 Molecular Identification of Actinomycetes, Bacteria and Fungi

Actinomycetes and bacteria were identified based on 16S rDNA nucleotide sequencing followed by nucleotide identity search at GenBank using the Basic Local Alignment Search Tool (BLASTn). Nucleotide sequencing and identity search using BLASTn of 5.8S rDNA and the flanking intergenic spacer regions (ITS1 and 2) was used to identify fungi. Prior to nucleotide sequencing, DNA extraction and amplification of rDNA and integenic spacer regions was performed as described below.

3.9.1 DNA extraction

DNA was extracted from pure colonies of actinomycetes, bacteria and fungi using mini-spin columns (Qiagen, Hilden, Germany), as per manufacturer's instructions. Briefly, bacteria, actinomycetes and fungi were digested using proteinase K (20 mg/l) for three hours at 56 ^{o}C. Digestion was followed by lysis and precipitation of proteins by heating at 56 ^{o}C for 15 minutes and addition of ethanol. DNA was trapped in silica columns, washed using buffers and eluted using nuclease-free water. DNA was stored at -20 ^{o}C until used for polymerase chain reaction (PCR).

3.9.2 Polymerase chain reaction

Amplification of 16S rDNA of actinomycetes and bacteria was performed using universal 27F and 1492R primers, as previously described by Isik *et al*. (2014). The PCR amplification conditions included an initial denaturation at 95 ^{o}C for 10 minutes, followed by 40 cycles of denaturation at 95 ^{o}C for 1 minute, annealing at 62 ^{o}C for 30 seconds and extension at 72 ^{o}C for 30 seconds. PCR amplification was followed by a single final extension at 72 ^{o}C for 10 minutes. The amplification of 5.8S rDNA and flaking ITS regions of fungi was performed using universal ITS1 and ITS4 primers (Iwen *et al*., 2002; Balajee *et al*., 2007). The PCR amplification conditions included an initial denaturation at

95 ºC for 10 minutes followed by 40 cycles of denaturation at 95 ^{o}C for seconds, annealing at 57 ^{o}C for 45 seconds and extension at 72 ^{o}C for 1 minute, and a final extension at 72 ºC for 10 minutes.

3.9.3 Electrophoresis and sequencing of PCR products

The amplified DNA fragments were separated by electrophoresis through 1.2% agarose, and visualized and imaged using a gel documentation system after staining with GelRed. The PCR amplicons were sequenced by dideoxynucleotide cycle sequencing method using an ABI 3500 genetic analyzer (Applied Biosystems, Foster City, CA).

3.9.4 Identification of microorganisms through BLASTn

The quality of nucleotide sequences was analyzed visually using the sequence scanner v.1.0 (Applied Bosystems, Foster City, CA). The nucleotide sequences obtained using forward primers were overlapped with reverse complement sequences of reverse primers using notepad (Microsoft Windows 8.1, 2013). The obtained nucleotide sequences were used to search for similarity to other publicly available nucleotide sequences at GenBank using BLASTn. The identity of actinomycetes, bacteria and fungi was inferred based on highest nucleotide identity (≥99%) following BLASTn search.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Physico-chemical Properties of the Soils from the Study Area

The properties of the soils from the study area are given in Table 4. According to the USDA textural class triangle (Brady and Weil, 2002), the textural classes for selected sites were sandy (S), loamy sand 1, loamy sand 2 and sandy clay loam for Kasanga 1, Kasanga 2, Kasanga 3 and SUA farm soils, respectively.

Texture of the soil may have an influence on the population of actinomycetes, bacteria and fungi in the soil mainly because of the influence of texture on soil moisture - air relations. Fang *et al*. (2005) showed that soil types influence the structure of microbial communities, especially populations among soils of different textures, due to the variations in carbon sources and the moisture holding capacity. Coarse-textured soils do not hold moisture for long times, hence microorganisms fail to survive well under such conditions.

The pH values of soils from the study sites were 6.84, 6.46, 6.64 and 8.91 for Kasanga 1, Kasanga 2, Kasanga 3 and SUA farm, respectively (Table 4). The pH for Kasanga 1 and Kasanga 3 were rated as neutral while those of Kasanga 2 and SUA farm were rated as slightly acidic and strongly alkaline, respectively (Landon, 1991; Motsara and Roy, 2008). Different microorganisms survive and work efficiently in differing pH ranges. Bacteria, for example, are mostly harboured by the pH values which are suitable for them. Most of the bacteria are neutralphilic whereby some are acidophilic and alkaliphilic, but fungi have wide range of pH for their activities. The pH is one of the soil factors which affect microbial community structure directly by providing a suitable habitat for specific microorganisms, by rendering them of a maximum or minimum efficiency in their

functions (Girvan *et al.,* 2003). According to the pH vales obtained (Table 4), these soils should support substantial population sizes.

Percentage organic carbon (% OC) of Kasanga 1, Kasanga 2, Kasanga 3 and SUA farm soils were 0.20, 0.53, 1.23 and 1.09, respectively (Table 4). Accordingly, % OC of Kasanga 1 and Kasanga 2 soils were rated as being very low, while those of Kasanga 3 and SUA farm were rated as low (Landon, 1991; Motsara and Roy 2008). Low and very low organic carbon content may, in turn, affect the abundance of microorganisms due to low contents of substrates, and this may affect their functions and activity, including degradation of organic substrates like plastics.

Percentage total nitrogen (%N) for the soils of Kasanga 1, Kasanga 2, Kasanga 3 and SUA farm were, respectively, 0.01, 0.04, 0.08 and 0.09 (Table 4). All these values of % N for all soils under study were rated as very low (Landon, 1991; Motsara and Roy 2008). Very low total nitrogen may negatively affect microbial population and their activities in the soil. Microbial communities in the soil use the available carbon as the source of carbon for their activities, and the availability of sufficient carbon helps in increasing their population growth. Similarly, Hessen (1992) and Hiroki (1995) explained that microbial community in the soil uses carbon as the substrate to increase its number and biomass. Low to very low % OC and very low total nitrogen, together with the sandy soil textures (sandy soil, loamy sand, and sandy clay loam) may not support maximally the growth and activities of microorganisms due to insufficiency in basic nutrients and moisture hence the relatively low microbial populations in these soils (Table 5).

Table 4: Physico-chemical properties of the soils used in the study

Soil	pH in H_2O	OC, %	Total N, %	Texture
Kasanga 1	6.84	0.20 VL*	0.01 VL	Sandy
Kasanga 2	6.46	0.53 VL	0.04 VL	Loamy Sand
Kasanga 3	6.64	1.23 L	0.08 VL	Loamy Sand
SUA Farm	8.91	1.09 L	0.09 VL	Sandy Clay Loam

*L = Low, and VL = Very Low. These ratings were according to Landon, 1991 Motsara and Roy 2008.

4.2 Microbial Populations of the Soils Used

Table 5 shows total counts of microbial populations of soils used in the present study. These results seem to indicate a relatively low capacity of the soils to sustain higher microbial populations above 10^5 CFU/g of soil.

Table 5: Microbial populations of the study soils

Soil	CFU/g soil		
	Bacteria	Fungi	Actinomycetes
Kasanga 1	1.00×10^5	3.73×10^4	1.04×10^5
Kasanga 2	1.12×10^5	8.10×10^4	1.34×10^5
Kasanga 3	1.00×10^5	1.60×10^4	2.99×10^5
SUA Farm	1.21×10^5	4.70×10^4	1.57×10^5

A combination of factors including poor moisture conditions linked to the coarse textured soils and poor substrate availability induced by very low values of % OC and % N (Table 4) cause most microorganisms may fail to survive under such conditions. This is similar to the observations made by Carney and Matson (2005) that fine textured soils support more microbial populations than coarse-textured soils. The distribution of microorganisms in these various soil textural classes might also be related to soil moisture and nutrient

contents as explained by Heritage *et al*. (2003), that sandy soils cannot retain water very well and drain very quickly and fail to hold nutrients for longer periods of time.

The microbial populations in all soils varied from as low as 1.60 x 10^4 CFU/g soil (fungi, Kasanga 3 soil) (4.20 as log of CFU per g soil) to 1.57 x 10^5 CFU/g soil which is the population of actinomycetes from SUA farm soil (5.20 as log of CFU per g soil). Similar observations were made by Najmadeen *et al*. (2010) as they compared levels of microbial populations isolated from sandy loam and silty loam soils.

4.3 Degradation of Ground Plastics by Fungal, Actinomycetal and Bacterial Isolates

As an example, the growth patterns of fungi on a medium containing plastics powder is shown in Fig. 1, with the ability of the microorganisms to degrade the plastics shown by a clear zone surrounding the colonies. Similar observations were made for bacteria and actinomycetes, similar to those shown in Fig. 1. The diameters of clear zones as indication of plastics biodegradation are summarized in Tables 6 and 7.

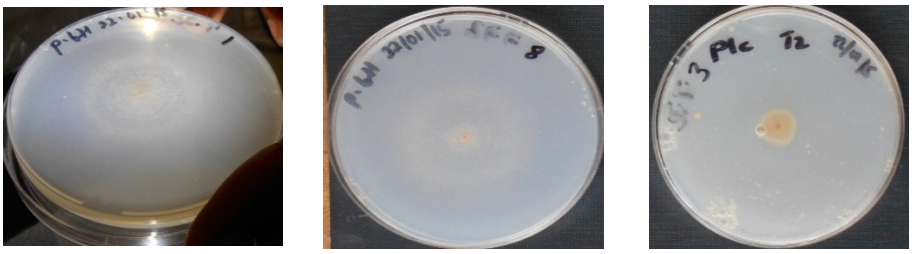


Figure 1: Clear zone (plastic degradation) surrounding a microbial colony: an example for fungi

4.3.1 Degradation of ground plastic bags by fungal, actinomycete and bacterial isolates

Several isolates from experimental soils tested for ability to degrade plastic bags (Table 6) showed differences in abilities to degrade ground plastic bags as depicted by differences in the diameters of clear zones surrounding the colonies.

Using ground plastic bags, the minimum diameter of clear zones for bacteria was 1.0 mm for an isolate from SUA farm soil and the maximum diameter was 54.0 mm for an isolate from SUA farm soil. The minimum diameter for fungi was 5.3 mm for an isolate from Kasanga 3 soil and the maximum diameter was 66.0 mm for an isolate from Kasanga 1 soil. The minimum clear zone diameter for actinomycetes was 11.0 mm for an isolate from Kasanga 2 soil while the maximum diameter was 58.3 mm for isolates from the same Kasanga 2 soil.

Among actinomycetes, bacteria and fungi, it was presently observed that there were differences in their comparative ability to degrade ground plastic bags. This observation is in line with that of Kathiresan (2003) who identified five bacterial species, including.

Table 6: Degradation ability of bacteria, fungi and actinomycetes for ground plastic bags

Soil + Organism	Number of isolates tested	Number showing biodegradation ability	Diameter of clear zone			
			Smallest (mm)	Largest (mm)	Mean (mm)	SD
a) Kasanga 1						
Bacteria	14	13	9.7	32.0	15.1	5.6
Fungi	13	13	28.7	66.0	39.1	10.2
Actinomycetes	5	5	22.0	52.0	35.3	12.0
b) Kasanga 2						
Bacteria	-	-	-	-	-	-
Fungi	5	5	15.7	57.7	32.6	24.1
Actinomycetes	20	19	11.0	58.3	27.2	18.2
c) Kasanga 3						
Bacteria	16	16	9.7	34.0	16.6	5.9
Fungi	16	14	5.3	52.7	27.7	15.8
Actinomycetes	5	5	22.0	51.0	34.8	12.6
d) SUA farm						
Bacteria	11	11	1.0	54.0	30.0	16.2
Fungi	8	8	22.0	57.0	33.2	19.1
Actinomycetes	7	7	36.0	52.9	25.9	4.7

(-) = not observed,

SD = Standard Deviation

Streptococcus and *Staphylococcus*, and eight fungal species of *Aspergillus* with different abilities of degrading plastic film and polythene. In a different study, Deepika and Jaya (2015) reported significant differences in weight loss of low density polyethlene (LDPE) as compared to initial weights, being the result of degradation by *Pseudomonas sp*, *Aspergillus niger* and *Aspergillus flavus*.

Among actinomycetes, bacteria and fungi, it was presently observed that there were differences in their comparative ability to degrade ground plastic bags. This observation is in line with that of Kathiresan (2003) who identified five bacterial species, including *Streptococcus* and *Staphylococcus*, and eight fungal species of *Aspergillus* with different abilities of degrading plastic film and polythene. In a different study, Deepika and Jaya (2015) reported significant differences in weight loss of low density polyethlene (LDPE) as compared to initial weights, being the result of degradation by *Pseudomonas sp*, *Aspergillus niger* and *Aspergillus flavus*.

The ability of a microorganism to degrade plastics, like polyethylene plastic bags, depends on its ability and efficiency in the production of enzymes for utilizing or degrading the substrate, mainly because the degradation is due to the action of extracellular enzymes secreted by the organism. Augusta and Widdecke (1993) reported that the zone of clearance around a colony was due to extracellular plastics-hydrolyzing enzymes secreted by the target organism into polyester granules suspended in the agar medium.

The differences in the abilities of same organism but from different environment to degrade plastics might be due to differences in the ecology of the environment from where the organisms were originally isolated. Sonil *et al.* (2010) investigated the same phenomenon by comparing three *Pseudomonas sp*. from three different isolation sources,

namely sewage sludge dump, household garbage dump and textile effluents drainage site. They observed that *Pseudomonas sp.* from sewage sludge dump degraded polyhydroxyalkanoate (PHA), a natural plastic, more efficiently by 46.2%, as compared to its ability to degrade by 29.1% synthetic polyethylene. In contrast, *Pseudomonas sp*. from the household garbage dump gave the lowest biodegradability of 31.4% and 16.3% for the natural plastic and synthetic polyethylene, respectively. However, *Pseudomonas sp*. isolated from the textile effluents drainage site gave an intermediate biodegradability of 39.7% and 19.6% for the natural plastic and synthetic polyethylene, respectively. So, differences within species of a microorganism, as presently observed using the diameters of the clear zones, can always be expected.

4.3.2 Degradation of ground plastic bottle by fungal, actinomycetal and bacterial isolates

Different numbers of microbial isolates tested for their abilities to degrade ground plastic bottles in the different soils, are presented in Table 7. Among microbial groups of bacteria, fungi and actinomycetes, there were clear differences in abilities to degrade ground plastic bottles. For all soils from which the microorganisms were isolated, the minimum diameter of clear zone for bacteria was 1.0 mm for an isolate from Kasanga 1 soil and the maximum diameter was 56.0 mm for an isolate from SUA farm soil (Table 7). In the case of fungi the minimum clear zone diameter was 5.0 mm for an isolate from Kasanga 3 soil and the maximum diameter was 73.7 mm for an isolate from Kasanga 2 soil (Table 7). The minimum clear zone diameter for actinomycetes was10.0 mm for an isolate from Kasanga 1 soil while the maximum diameter is 60.0 mm for an isolate from Kasanga 2 soil (Table 7).

Differences in degrading ground plastic bottles between bacteria, fungi and actinomycetes, may be due to similar reasons (e.g. differences in enzymes produced and/or differences in ecology of the microorganisms) as already discussed for plastic bags (section 4.3.1).

Table 7: Degradation ability of bacteria, fungi and actinoycetes fo ground plastic bottles

Soil + Organism	Number of isolates tested	Number of isolates showing biodegradation ability	Diameter of clear zone			
			Smallest (mm)	Largest (mm)	Mean	SD
a) Kasanga 1						
Bacteria	14	13	1.0	42.7	12.8	10.5
Fungi	13	13	16.7	55.0	39.7	12.4
Actinomycetes	5	5	10.0	48.0	32.2	13.8
b) Kasanga 2						
Bacteria	-	-	-	-	-	-
Fungi	5	5	47.3	73.7	59.3	11.1
Actinomycetes	20	19	30.0	60.0	36.9	14.8
c)Kasanga 3						
Bacteria	16	16	5.0	46.0	17.3	8.9
Fungi	16	14	5.0	58.7	23.3	18.3
Actinomycetes	5	5	22.0	43.0	32.3	8.8
d) SUA farm						
Bacteria	11	11	8.7	56.0	30.3	16.7
Fungi	8	8	12.0	66.7	48.2	19.5
Actinomycetes	7	7	16.0	43.0	30.0	10.1

- = not observed

SD = Standard Deviation

4.4 Morphology of Plastic-degrading Microorganisms

Macro and micromorphological features of all bacterial, fungal and actinomycete isolates were examined both on plates as seen by naked eye (macromorphology) and under the light microscope (micromorphology) as detailed hereafter. The preliminary identification of representative bacteria, actinomycetes and fungi capable of degrading plastics was done by describing their macro- and micromorphological observations as shown in Fig. 2. More details are provided in Appendix 2.

On the whole, the actinomycetes were mainly large dry colonies, with colours varying from white to greyish to blue-grey and the reverse colour was almost brownish for all actinomycetes. Bacterial colonies were slimy and shiny on the surface, with whitish to yellow colours. Fungal colonies were more profuse, with substantial sporulation. The microscopic features shows actinomycetes and fungi to be filamentous, but bacteria to be single celled entities.

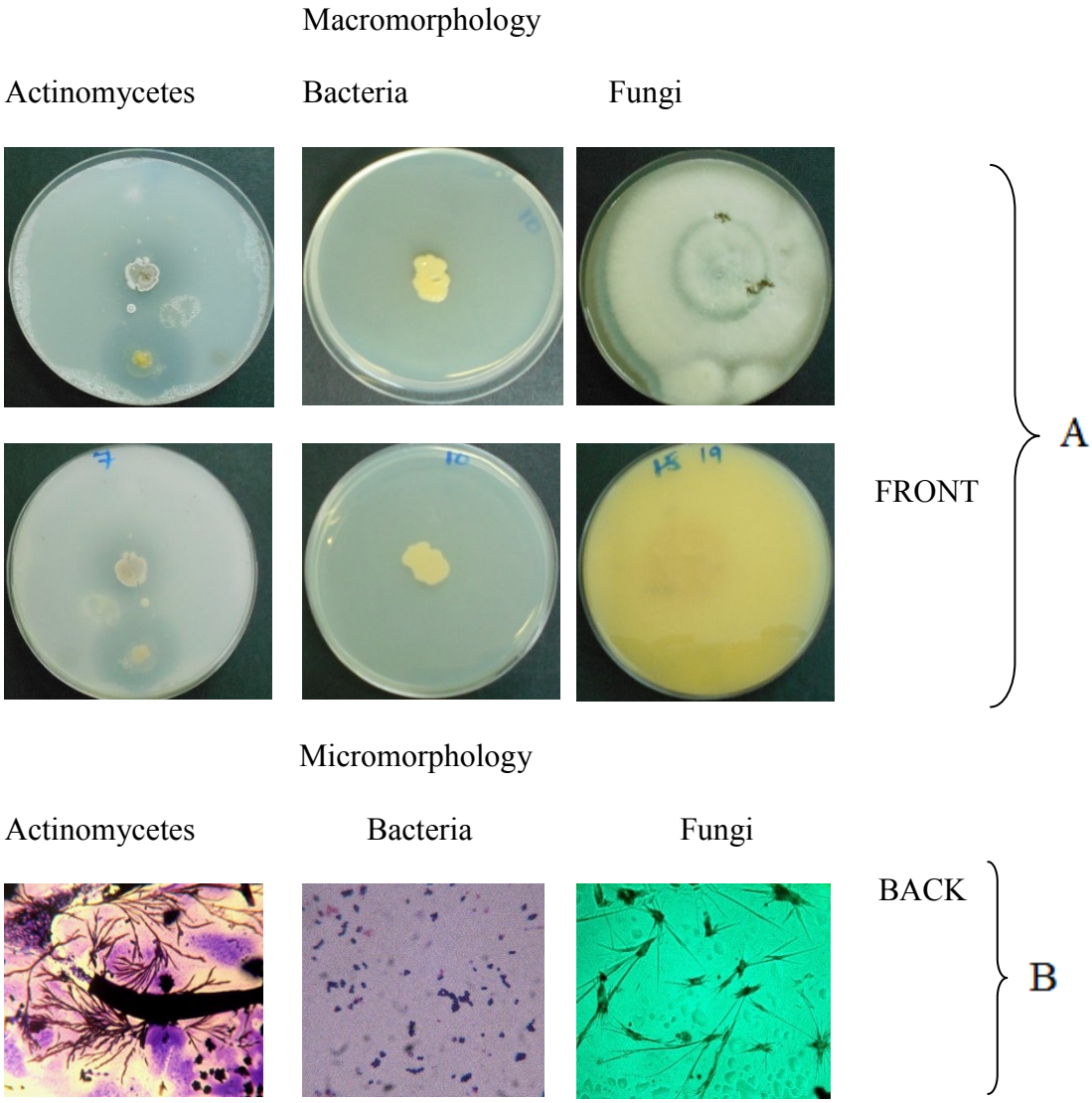


Figure 2: Macromorphology (A) and micromorphology (B) of representative actinomycetes, bacteria and fungi

4.4.1 Morphology of plastic-degrading bacteria

The details for macroscopic and microscopic features of bacteria are summarized in Table 8. The shapes of the colonies, cells, colour and the Gram reaction for most of the bacterial isolates varied from one to another. The colour of the colonies varied from yellow to white, with small to relatively large colonies. The cells varied from cocci to rod chains and/or groups. The rod-like bacteria could be of the genus *Bacilus*.

Table 8: Morphology of plastic-degrading bacteria isolated from study soils

Isolate	Colony morphology	Shape of bacteria	Colour	Gram stain
B2	Yellow, large with raged ends	Cocci in short chains	Purple	Gram +
B3	White, small, round	Cocci rods	Pink	Gram -
B4	Yellow, small	Cocci in groups	Pink	Gram -
B5	White, small	Cocci in groups	Purple	Gram +
B6	White, large, raged ends	Cocci in tetrads	Purple	Gram +
B7	White, large, irregular shape	Large rods with ovoid ends	Purple	Gram +

4.4.2 Morphology of plastic-degrading actinomycetes

The macroscopic and microscopic features of Actinomycetes (*Streptomyces*) are summarized in Table 9. The colony characteristics of the actinomycetes varied in their appearance (colour), but were larger in size. The colours of the colonies varied from white, greyish to bluish, characteristic of the genus *Streptomyces* when grown/cultured in Starch Casein Agar (SCA). They were dry on the surface and filamentous.

Table 9: Morphology of plastic-degrading actinomycetes from study soils

Isolate number	Macromorphology: Colony appearance	Micromorphology: (Shape of the cell)	Gram reaction
A2	White, large and dry colony	Filamentous	Gram +
A3	White to dark, large and dry colony	Filamentous	Gram +
A4	Greyish, large and dry colony	Filamentous	Gram +
A5	Whitish surrounded by brown shadow, large and dry colony	Filamentous	Gram +
A6	Bluish, large and dry colony	Filamentous	Gram +
A7	Whitish, large and dry colony	Filamentous	Gram +
A8	Whitish, large and dry colony	Filamentous	Gram +

Under microscopic observation, some of the isolates developed a substrate mycelium (black), with sporulated aerial mycelium. The observations of the present study are similar to the phenomenon explained by Sykes and Skinner (1973), that *Streptomyces* are capable

of forming a non-fragmenting substrate mycelium which may bear spores, and, in most genera, a well-developed aerial mycelium with spore chains which can be long or very short.

4.4.3 Morphology of plastic-degrading fungi

The morphological features of fungi are summarized in Table 10. The colours of the colonies of the fungi varied from white – brown, greyish – brown to deep green in the front side of the colonies with moderate sporulation, while the reverse of the colonies for most of the cultures showed a deep dirty brown colour. The microscopic features observed were conidiophores, conidia and phialides. Most of the fungi had the characteristics of *Aspergillus* and *Penicillium* which, in their common microscopic features, contain conidia, conidiophores, phaliades and the vesicles (mostly in *Aspergillus*), as was also observed by Alfred (2009).

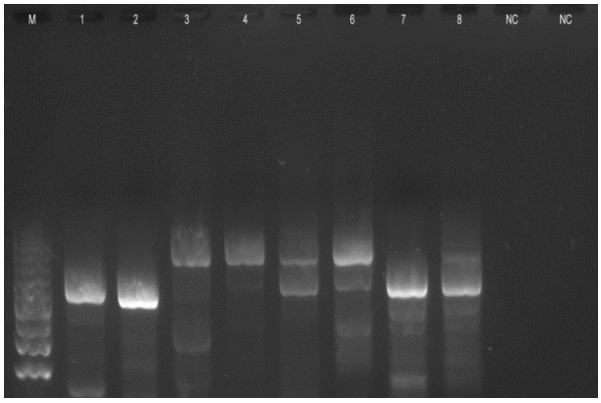
Table 10: Morphology of plastic-degrading fungi isolated from experimental soils

Isolate code	Colony morphology (colour)		Sporulation	Microscopic features
	Front	Back		
F2	greyish-brown, powdery/granular	Brownish colour	Moderate	Conidia a produced in abundance within the pycnidia on narrow thread-like phialides, which are pycnidial wall cell, Conidia globose to cylindrical
F3	Whitish-bluish, cotton like	Deep brownish	Moderate	Short conidiophores branching from one foot cell, globose to hemispherical vesicle, branched straight phialides
F4	White to brown	Deep dirty brown	Moderate	Long conidiophores branching from one foot cell, globose to hemispherical vesicle, branched straight phialides
F5	White to brown	Deep dirty brown	Moderate	Long conidiophores branching from one foot cell, globose to hemispherical vesicle, branched straight phialides
F6	Deep green, with white periphery	Brown	Moderate	Coninia globose to subglobose,conidophore are on the surface hyphae
F7	White to brown	Deep dirty brown	Moderate	Long conidiophores branching from one foot cell, globose to hemispherical vesicle, branched straight phialides
F8	White to brown	Deep dirty brown	Moderate	Long conidiophores branching from one foot cell, globose to hemispherical vesicle, branched straight phialides

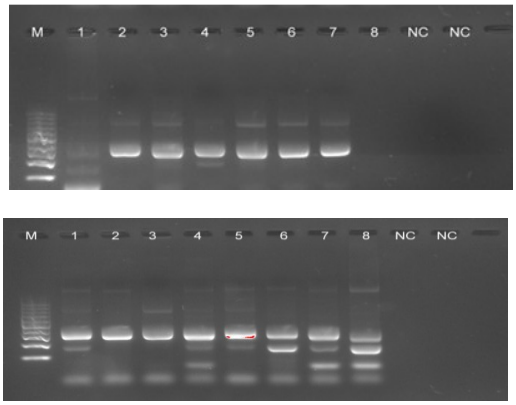
4.5 Molecular Identification of Plastic-degrading Microorganisms

Amplification of 16S rDNA for bacteria and actinomycetes, and of 5.8S rDNA and flaking ITS1 and ITS2 for fungi, produced PCR products with the size ranging between 300 and 800 bp (Fig. 3). The DNA were of sufficient quality and quantity for DNA sequencing.

A: Fungi



B: Actinomycetes (top), Bacteria (bottom)



M = Molecular weight marker, 1 -8 = Bands for DNA samples and NC = Negative control

Figure 3: Agarose gel electrophoresis bands of PCR products from the microbial isolates

Results of 16S rDNA gene nucleotide sequencing of bacteria and actinomycetes with abilities to degrade plastics and their subsequent identifications are presented in Table 11. The results further show that different genera and species of bacteria and actinomycetes had ability to degrade plastics. All microbial isolates of the present study were identified after standard comparison with known species in the (American) National Institutes of Health (NIH) genetic sequence database (GenBank) using BLASTn.

The identified bacterial species included *Bacillus cereus, Sinomonas sp*. and *Cellulosimicrobium sp*. while actinomycetes included *Streptomyces werraensis and Streptomyces rochei*, as shown in Table 11. Additional particulars of the organisms found

to resemble the current bacterial and actinomycete isolates, their countries and environmental media of isolation, are presented in Appendices 4 and 6.

Various genera/species of bacteria and *Streptomyces* were similarly observed to biodegrade plastics, including polyethylene, as shown by El-Shafei *et al*. (1998) in that *Streptomyces* were capable of degrading polyethylene containing 6% starch. Deepika and Jaya (2015), in their study on screening the ability of different microorganisms in degrading polyethylene, observed that *Streptomyces sp*. were more efficient than bacteria and fungi. Rowe (2002) observed the ability of *Bacillus subtilis* to degrade polyurethane.

Following sequencing of the 5.8S rDNA, the fungi isolates which were capable of degrading the plastics included *Eupenicillium rubidurum, Phoma sp*., *Neosartorya fischeri, Aspergillus terreus, Aspergillus sp*. and *Talaromyces islandicus* (Table 12). *Aspergillus terreus* appeared in two different soils of Kasanga 2 (LS 1) and SUA farm (SCL). Further particulars of fungi isolated elsewhere but resembling the isolates of this study are shown in Appendix 5 and 7.

Various researches have also reported on the abilities of different genera/species of fungi in degrading different types of plastics. Raaman *et al.* (2012) reported biodegradation of plastics by *Aspergillus spp*., including *Aspergillus terreus*, isolated from polythene polluted sites around Chennai in India. Other studies have observed *Eupenicillium sp*., and *Talaromyes sp*. and *Penicillium simplicissimum* to have the ability of degrading polyethylene (Sowmya *et al*., 2014).

In general, results presented on Tables 11 and 12, of involvement of different genera/species, further confirm the diversity of bacteria, fungi and actinomycetes genera

and species that can degrade plastics. Other studies have similarly shown that different genera/species of bacteria, including *Pseudomonas sp* (Sonil *et al*., 2010), *Streptococcus spp*., *Staphylococcus spp*., *Micrococcus spp*. *and Moraxella spp*., *Bacillus subtilis*, *Bacillus amylolyticus and Arthobacter defluvii* (Prabhat *et al.,* 2013), actinomycetes, including *Streptomyces sps* (Deepika and Jaya, 2015), and fungi, including *Aspergillus niger*, *A. japonicus*, *A. terreus A. flavus* and *Mucor* sp. (Ibrahim *et al*., 2011; Raaman *et al*., 2012), exhibited the ability to degrade low density polyethylene (LDPE).

The isolates identified herein were a selection of those which exhibited greater ability to degrade the plastics as depicted by the larger diameters of clear zones around the isolates cultured in the medium containing the ground plastics as sole source of carbon. The identified isolates hold the potential to be exploited industrially (in fermenters) or environmentally (in landfills) to degrade waste plastics. Continued search could reveal even more efficient microbial genera/species/strains; thus their potential may be unlimited. It should be cautioned that not all organisms or strains occurring in nature bearing the identified names may exhibit this ability. This is because they might be different or distinct strains. For example, two *Cellulosimicrobium* isolates were presently obtained from the Kasanga 3 soil, but one isolate resembled by 100 % a strain isolate from China while the other resembled by 99 % another strain isolated from India (Appendix 4). Thus, the current two isolates obtained herein may, indeed, be two distinct strains as the China and India isolates may also be different. Both of the current strains degraded ground plastic bottle (Table 11). Similarly, two isolates of the fungus *Aspergillus terreus* were each isolated from Kasanga 2 soil and SUA farm soil. The one from Kasanga soil resembled by 100 % a strain from India (Table 12) while the one from SUA farm soil resembled the Indian isolate by 99 % (Appendix 6). They degraded both ground plastic bottle and plastic bag. These may not be exactly the same strain.

Table 11: Identity of plastic-degrading actinomycetes and bacteria isolated from experimental soils

			Similarity of current isolate to GenBank		
Current isolate/strain	**Isolation source (soil type)***	**Identification (species level)**	**Species from GenBank with highest identity**	**% identity**	**Type of plastic degraded**
Bacterium (B1)	SUA farm (SCL)	*Bacillus cereus*	KC683896	100	Plastic bag
Bacterium (B2-1)	SUA farm (SCL)	*Sinomonas sp*	HE793513	100	Plastic bottle
Bacterium (B2-2)	SUA farm (SCL)	*Sinomonas sp*	KJ504159	100	Plastic bottle
Bacterium (B4-1)	Kasanga 3 (LS2)	*Cellulosimicrobium sp*	EU307933	100	Plastic bottle
Bacterium(B4-2)	Kasanga 3 (LS2)	*Cellulosimicrobium sp*	LN846832	99	Plastic bottle
Actinomycete (A2)	Kasanga 3 (LS2)	*Streptomyces werraensis*	KM215730	99	Plastic bag
Actinomycete (A8)	Kasanga 1 (S)	*Streptomyces rochei*	KF444515	100	Plastic bottle

SCL = sandy clay loam, LS = loamy sand, S= sandy

Table 12: Identity of plastic-degrading fungi isolated from experimental soils

Current isolate/strain	Isolation source (Soil type)*	Identification (species level)	Similarity of current isolate to GenBank		Type of plastic degraded
			Species from GenBank with highest identity	% identity	
Fungus (F1)	Kasanga 3 (LS2)	*Eupenicillium rubidurum*	HQ608058	100	Plastic bag
Fungus (F2)	Kasanga 1 (S)	*Phoma sp*	EF423518	100	Plastic bag
Fungus (F3)	Kasanga 3 (LS2)	*Neosartorya fischeri*	AF455538	99	Plastic bag
Fungus (F4)	Kasanga 2 (LS1)	*Aspergillus terreus*	KC119206	100	Plastic bottle
Fungus (F5)	Kasanga 2 (LS1)	*Aspergillus terreus*	KC119206	100	Plastic bag
Fungus (F6)	SUA farm (SCL)	*Talaromyces islandicus*	NR_103664	100	Plastic bottle
Fungus (F7)	Kasanga 1 (S)	*Aspergillus sp.*	KF367546	100	Plastic bottle
Fungus (F8)	SUA farm (SCL)	*Aspergillus terreus*	KM491895	99	Plastic bottle

S= sandy, LS =loamy sand and SCL = sandy clay loam

It is also possible that a given organism, for example *Aspergillus terreus*, may have other strains that have greater ability to degrade a given type of plastic while others do not. Ibrahim *et al*. (2011) observed strains of *Aspegillus terreus* that degraded polyethylene by 58.0 % as tested by using colony diameter on petri dishes. However, another *Aspergillus terreus* isolate degraded the polyethylene only by 0.08 % using the same test in a different study (Nowak *et al*., 2012).

This is an indication that while the organism used in these two different studies cited was *A. terreus*, they must be two distinct strains as depicted by their huge difference in extent of their degradation of polyethylene. Therefore, it cannot be assumed that any isolates of the same genus/species will automatically have equal capability of degrading a given plastic. Each isolate will have to be tested in its own right.

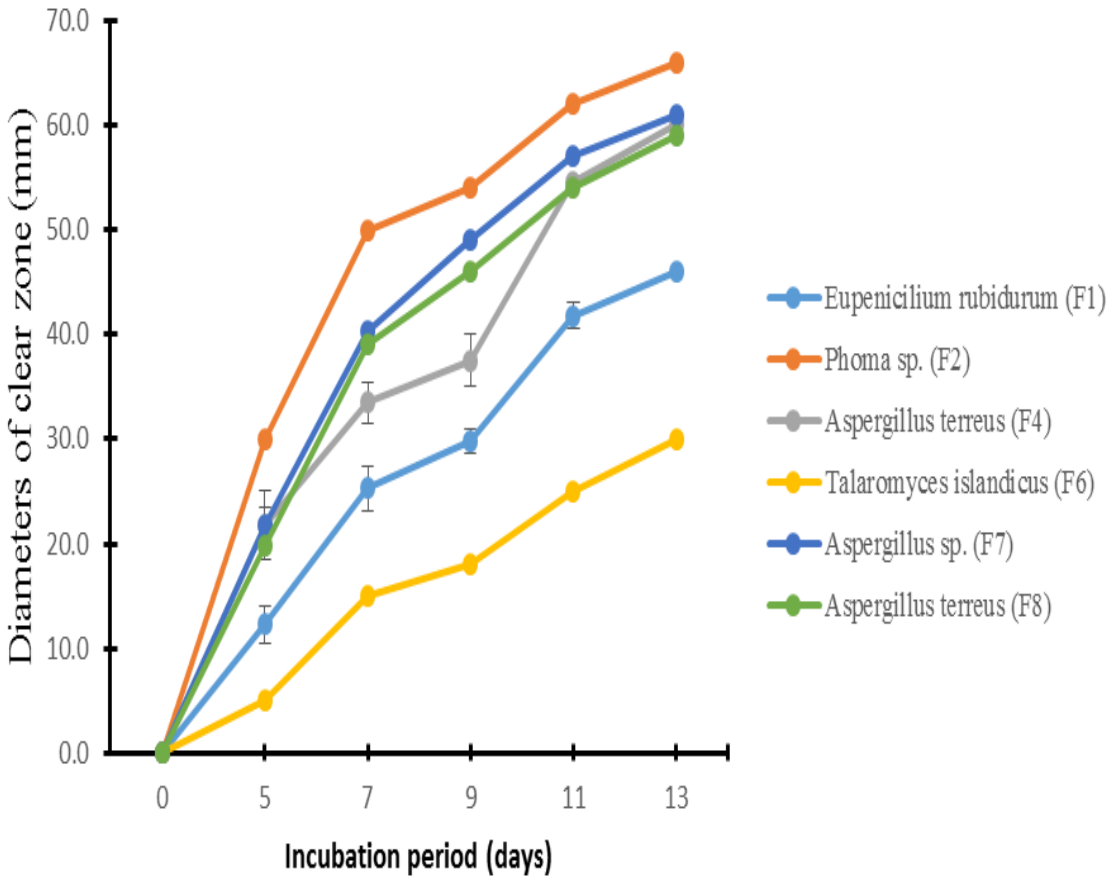
4.6 Comparative Ability of Different Microorganisms in Degrading Ground Polyethylene Bags

Different microorganisms were observed to have different abilities in degrading plastics. This was proved by their rate of degrading plastics as depicted by sizes of clear zones. The clear zones were observed to increase day after day and in some isolates after sometime the growth was abrupt, indicating that they had acquired greater capability of utilizing plastics as a sole source of carbon. The details of the differences in the abilities of different microorganisms in degrading plastics bags are indicated in Figures 4 and 5.

4.6.1 Comparative abilities of different fungi in degrading ground polyethylene bags

There was difference in growth among species/genera of fungi from 5^{th} to 13^{th} day. In the 5^{th} day the minimum diameter of 5.0 mm was observed in *Talaromyces islandicus* and the

maximum diameter was 30.0 mm which was observed in *Phoma sp*. The growth continued to vary from 7th, 9th and 11th days and, finally, in the 13th day the minimum clear zone diameter was 30.0 mm which was observed in the same isolate which is *Talaromyces islandicus* and the maximum diameter of 66.0 mm was observed in the same species which was *Phoma sp*. The diameters of clear zones for other fungal species, including *Eupenicillium rubidurum*, *Aspergillus terreus* which appeared twice and *Aspergillus sp*., varied between those of *Talaromyces islandicus* and *Phoma sp*.



Error bars represent mean ± SD of four independent biological replicates

Figure 4: Comparative ability of different fungi in degrading ground plastics bags

The differences in biodegradation of plastics by different fungi as captured on the 13th (last) day of diameter measurement are shown in Table 13. The growth extent was

indicated by different letters and others by the same letters, the same letters indicating no difference between the particular species and different letters, for example a and f, indicating significant (P≤0.05) difference (mm) (P≤0.05) in biodegradation between the species (for example 30.0a for *Talaromyces islandicus* and 66.0f for *Phoma sp*) (Table 13).

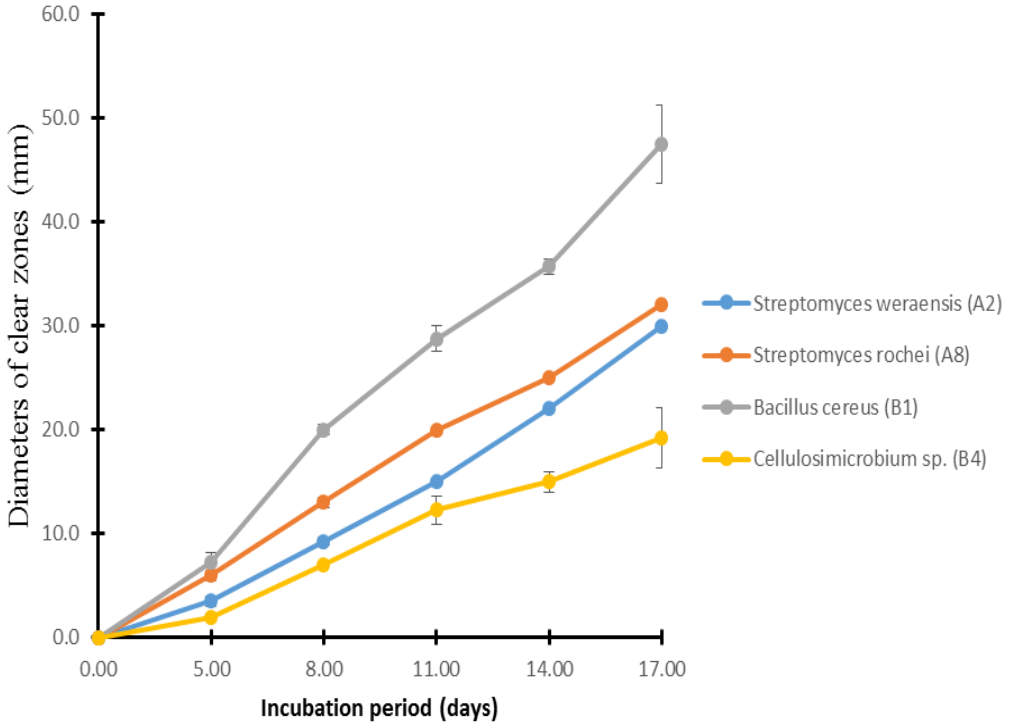
Table 13: Biodegradation (colony diameters) of plastics by different fungi species on the 13th day of growth

Isolate/strain	Colony diameter, mm after 13 days
Eupenicilium rubidurum (F1)	46.0b
Phoma sp. (F2)	66.0f
Aspergillus terreus (F4)	60.0d
Talaromyces islandicus (F6)	30.0a
Aspergillus sp. (F7)	61.0e
Aspergillus terreus (F8)	59.0c

Means within the column followed by different letters were different according to Duncan's New Multiple Range Test at P≤0.05

4.6.2 Comparative ability of different actinomycetes and bacteria in degrading ground polyethylene bags

The variation in the diameters of clear zones for actinomycetes and bacteria followed the same trend to those of fungi discussed in Section 4.6.1. The minimum clear zone diameter for actinomycetes and bacteria in the 5th day was 2.0 mm observed in *Cellulosimicrobium sp*. and the maximum diameter in the 17th day was 7.3 mm observed in *Bacillus cereus*. The differences in biodegradation of plastics by different bacteria and actinomycetes on the 17th day of diameter measurement are shown in Table 14 and *Bacillus cereus* was the most (P≤0.05) effective in degrading the plastic bags.



Error bars represent mean ± SD of four independent biological replicates

Figure 5: Comparative ability of different actinomycetes and bacteria (isolated from experimental soils) in degrading ground plastics bags

Table 14: Comparative ability of different actinomycetes and bacteria in degrading ground plastics bags, measured as diameter (mm) of clear zone

S/N	Isolate/strain	Colony diameter, mm after 17 days
1	*Streptomyces weraensis* (A2)	30.00b
2	*Streptomyces rochei* (A8)	32.00b
3	*Bacillus cereus* (B1)	47.50c
4	*Cellulosimicrobium sp.* (B4)	19.25a

Means within the column followed by different letters were significantly different according to Duncan's New Multiple Range Test at P≤0.05.

The difference in the ability of microorganisms to biodegrade plastics depends on the active enzyme produced by a particular microorganism. Sonil *et al*. (2010) observed that

microbial degradation of plastics is caused by enzymatic activities leading to cleavage of the polymer into micromolecules after which they are further metabolized by the microbial cells. Carbon dioxide and water are produced under aerobic metabolism (Starnecker and Menner, 1996) and anaerobic metabolism results in carbon dioxide, water, and methane as the end products (Gu *et al.*, 2000).

The growth rate of the microorganisms was very slow in the first two to three days since plating. This indicates that the organisms were not well adapted to the available carbon source. From 5^{th} since plating to 13^{th} days for fungi and up to 17^{th} day for bacteria and actinomycetes the growth started to speed up, implying that microorganisms were now well adapted to the available foreign carbon source. Similarly, Sonil *et al*. (2010) observed the growth rate of *Pseudomonas sp*., whereby the degradation rate was almost steady until day 21 after which it took a sudden leap. He assumed that *Pseudomonas sp*. metabolized the available basal media nutrients for about 3 weeks and after their limitation, it geared up to utilize the carbon sources from the polyethylene. Organisms differ in their ability of degradation; others have high efficacy while others have low, and this might be due to the capacity of the enzymes produced to catalyze the plastics. Shah *et al*. (2008) also concluded that the active enzymes produced by microorganisms, for example *Pseudomonas*, catalyze the degradation process as they adhere to the polyethylene surface rather than being solitary in the liquid media. Whatever the rationale may be, biodegradation depends upon polymer characteristics, organism type and nature of plastics pre-treatment.

4.7 Microorganisms that Resulted in Larger Diameters of Clear Zones (Plastics Degradation) but Remained Unidentified

Some of the microorganisms (bacteria and actinomycetes) that degraded plastics but could not be sequenced (Table 15) were among those whose additional provisional information

is presented in Tables 8 and 9 by macromorphology and micromorpholgy, and in Appendices 1 and 2.

Table 15: List of microbial isolates with ability to degrade plastics that could not be sequenced

Bacterial isolate	Actinomycete isolate
B3	A3
B5	A4
B6	A5
B7	A6
	A7

There are several reasons for the failure of DNA sequencing. It is difficult to pinpoint to any stage of the process because for the DNA sample to be obtained and sequenced, a chains of steps are involved. The problem may be in the culture, DNA extraction level, PCR level, sample handling during transport or during sequencing.

According to Secugen (2013), some possible reasons for the failure of DNA sequencing include the following: too low DNA template concentration, no primer was added to the reaction; binding between the DNA and the primer cannot occur in either of these instances. In our case the cultures were proved to be pure, DNA extraction was done carefully and at PCR level all the steps were done carefully, the DNA concentration was high enough as their quality and quantity are indicated by gel electrophoresis.

The most probable reasons for the failure of the DNA sequencing in the case of this study could be transportation and handling of the DNA samples before sequencing, whereby the DNA could undergo degradation; for the DNA to be sequenced, must remain intact. The second possible reason could be that the machine for sequencing, was not well maintained.

However, these reasons may not apply here because the processes of DNA preparation and sequencing were repeated twice, and the same answers were obtained. So, for these reasons, further study on the DNA sequencing of these samples, perhaps in a different laboratory, is required to obtain more information about these isolates.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 Microbial populations in the soils

The soils used in this study had low to very low levels of % OC and total N ranging between 0.20 to 1.23 % and 0.01 to 0.09 %for OC and total N, respectively, and a wide pH range of 5.5 to 9.0. The lowest population of bacteria and actinomycetes were obtained in the soils with lower %OC and total nitrogen while the highest population was encountered in soils with the higher values. For fungi, the lowest and highest population was observed in the soil with pH around 6.6. Overall, the soils supported modest populations of microorganisms of generally around 10^5 CFU/g soil.

5.1.2 Ability of microorganisms on degrading plastics

Most of the microbial isolates were capable of degrading polyethylene plastic bags and bottles as indicated by clear zone formation around colonies during their growth in media containing plastics. The minimum diameter of the clear zone exhibited by the microbial isolates tested in degrading ground plastic bags was 1.00 mm for bacteria and the maximum diameter was 66 mm exhibited by a fungal isolate. For plastic bottle the minimum clear zone diameter was observed in the same group of bacteria which was 1.00 mm and the maximum one in fungi, which was 73.67 mm. Some isolates failed to grow on the medium containing plastics, indicating their inability of degrading the plastics.

5.1.3 Morphological and genetic identification of microorganisms on degrading plastics

Different morphological groups were encountered for all microbial groups. For bacteria, white colour macromorphology was dominant (about 60%) followed by cocci bacteria in

groups. The dominant colony characteristics for actinomycetes were whitish, large and dry, with the Gram positive reaction. The dominant colours for fungal colonies were white with deep brown reverse, and they contained long conidiophores with the presence of conidia and phialides.

The isolates of bacteria, actinomycetes and fungi were subjected to molecular identification and matched with those at GenBank by 99 - 100%. For example, bacteria of the genera *Bacillus, Sinomonas* and *Cellulosimicrobium* were identified. The dominant fungal genera identified were *Aspergillus*, *Phoma*, *Penicillium*, *Talaromyces and Neosartorya.* The actinomycetes were *Streptomyces warraensis* and *Streptomyces rochei*. However, there were other isolates of bacteria and actinomycetes that could not be sequenced and identified.

5.1.4 Comparative efficiency of selected microorganisms in degrading plastics

In an experiment to test the relative efficacy of some identified microorganisms in degrading the plastics, it was observed that some genera/species were more efficient than others. It is concluded that some of the microorganisms displayed potential for degrading plastics which could be exploited to degrade waste plastics.

5.2 Recommendations

Based on the findings in the current study, it is recommended that:

i. Further studies need to be continually undertaken to identify more isolates of microorganisms which are capable in degradation of different types of plastics, especially those which are the major sources of pollution in the environment.

ii. These further studies should be carried out to test the ability of the identified isolates in degrading a greater variety of different kinds of plastics used for various purposes.

iii. There is a need for studies to identify the enzymes produced by these microbes that are responsible for catalyzing the degradation of plastics.

iv. Further efforts should be directed at identifying those microorganisms that presently displayed substantial potential to degrade plastics but could not be identified/sequenced in the present study.

v. It is recommended that further studies be conducted on in situ biodegradation of plastics.

REFERENCES

Albertsson, A. C., Andersson, S. O and Karlsson, S. (1987). The mechanism of biodegradation of polyethylene. *Polymer Degradation and Stability* 18:73-87.

Alfred, B. A. (2009*). Benson's Microbiological Applications: Laboratory Manual in General Microbiology*. Short Version, Eleventh Edition. McGraw-Hill. New York. 47pp.

Andrady, A. L. (2011). Microplastics in the marine environment. *Marine Pollution Bulletin* 62: 1596-1605.

Andrady, A. L. and Neal, M. A. (2009). Applications and societal benefits of plastics. *Philosophical Transactions of the Royal Society B* 364: 1977-1984.

Andrej, K. (2012). *Biodegradable polymers and plastics.* European Regional Development Fund. Europe. 8pp.

Artham, T. and Doble, M. (2009). Biodegradation of physicochemically treated polycarbonate by fungi. *Biomacromolecules* 11: 20-28.

Astrup, T., Møller, J. and Fruergaard, T. (2009). Incineration and co-combustion of waste: Accounting of greenhouse gases and global warming contributions. *Waste Management and Research* 27: 789-799.

Augusta, J. M. R. and Widdecke, H. (1993). A Rapid evaluation plate-test for the biodegradability of plastics. *Applied Microbiology and Biotechnology* 39: 673-678.

Arutchelvi, J., Sudhakar, M., Ambika, A., Mukesh, D., Sumit, B. and Parasu, V. U (2008). Biodegradation of polyethylene and polypropylene. *Indian Journal of Biotechnology* 7: 9-22.

Awaja, F. and Pavel, D. (2005). Recycling of PET. *European Polymer Journal* 41: 1453-1477.

Baath, E. (1996). Adaptation of soil bacterial communities to prevailing pH in different soils. *FEMS Microbiology Ecology* 19: 227-237.

Balajee, S. A., Sigler, L. and Brandt, M. E. (2007). DNA and the classical way: identification of medically important molds in the 21st century. *Medical Mycology* 45: 475-490.

Barnes, D. K. A. and Milner, P. (2005). Drifting plastic and its consequences for sessile organism dispersal in the Atlantic Ocean. *Marine Biology* 146: 815-825.

Belofsky, H. (1995). Plastics: In: *Product Design and Process Engineering*, Hanser, Munich. German.

Bouyoucos, G. J. (1962). Hydrometer method improved for making particle size analysis of soils. *Agronomy Journal* 54: 464-465.

Brady, N. C and Weil, R. R (2002). *The Nature and Properties of Soils*. 13th Ed. Prentice Hall. Upper Saddle River. New Jersey. 960pp.

Bremner, J. M. (1996). Total Nitrogen. In: *Methods of Soil Analysis, Part 3. Chemical Methods*. *Soil Science Society of America and American Society of Agronomy*. Inc., Madison, Wisconsin. 1085-1123pp.

Bushnell, L. D. and Haas, H. F. (1941). The utilization of certain hydrocarbons by microorganisms. *Journal of Bacteriology* 41: 653-673.

Carney, K. M. and Matson, P. A. (2005). Plant communities, soil microorganisms, and soil carbon cycling. *Ecosystems* 8: 928-940.

Chandra, R. and Renu, R. (1998). Biodegradable Polymers. *Progress in Polymer Science* 23: 1273-1335.

Chee, J. Y., Yoga, S. S., Lau, N. S., Ling, S. C., Abed, R. M. M. and Sudesh, K. L. (2010). Bacterially produced polyhydroxyalkanoates (PHA): converting renewable resources into bioplastics. In: Méndez-Vilas, A. (ed). *Current research, technology and education topics in microbiology and microbial biotechnology*. Formatex Research, Spain. 1395-1404pp.

Cook, A. E. and Meyers, P. R. (2003). Rapid identification of filamentous actinomycetes to the genus level using genus specific 16S rRNA gene restriction fragment patterns. *International Journal of Bacteriology* 53: 1907-1915.

Deepika, S. and Jaya, M. R. (2015). Biodegradation of low density polyethylene by micro-organisms from garbage soil. *Journal of Experimental Biology and Agricultural Sciences* 3(1): 16-21.

Demirbas, A. (2007). Biodegradable Plastics from Renewable Resources. *Journal of Recovery, Utilization and Environmental Effects* 29(5): 419-424.

Devi, G. P., Kumar, M. S. C., Madhavi, B., Guntur, G. C. W. and Guntur, A. R. S. (2014). Adverse effects of plastic on environment and human beings. *Journal of Chemical and Pharmaceutical Science Special Issue* 3: 56-58.

Dilip, K. A. (ed). (2004). *Fungal Biotechnology in Agricultural, Food, and Environmental Applications*. Marcel Dekker Inc. New York, U.S.A.

Donath, O. and Sutton, J. (2012). *An enterprise map of Tanzania*. International Growth Sector (IGC) and London Publishing Partnership (publishers). London.

El-Shafei, H. A., El-Nasser, N. H. A., Kansoh, A. L. and Ali, A. M. (1998). Biodegradation of disposable polyethylene by fungi and Streptomyces species. *Polymer Degradation and Stability* 62: 361-365.

Fang, M., Kremer, R. J., Motavalli, P. P. and Davis, G. (2005). Bacterial diversity in rhizospheres of nontransgenic and transgenic corn. *Applied and Environmental Microbiology* 71: 4132-4136.

Font, R., Aracil, I., Fullana, A. and Conesa, J. A. (2004). Semivolatile and volatile compounds in combustion of polyethylene. *Chemosphere* 57: 615-627.

GESAMP (2010). Proceedings of the GESAMP International Workshop on plastic particles as a vector in transporting persistent, bio-accumulating and toxic substances in the oceans. GESAMP Reports and Studies No. 82.

Giancaspro, J., Papakonstantinou, C. and Balaguru, P. (2009). Mechanical behaviour of fire-resistant biocomposite. *Composites: Part B* 40: 206-211.

Girvan, M. S., Bullimore, J., Pretty, J. N., Osborn, A. M. and Ball, A. S. (2003). Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. *Applied and Environmental Microbiology* 69: 1800-1809.

Gu, J. D., Ford, T. E., Mitton, D. B., and Mitchell, R. (2000). *Microbial corrosion of metals*. The Uhlig Corrosion Handbook. Wiley. New York. USA.

Hamidi, A. A., Jyy, L. T., AbuAhmed, M H., Muhammad, U. and Nordin, A. M. (2011). Leachate treatment by swim-bed bio fringe technology. *Desalination* 276(1-3): 278-286.

Han, E. H. M. and Govaert, L. E. (2005). Mechanical performance of polymer systems. The relation between structure and properties. *Progress in Polymer Science* 30: 915-938.

Heritage, J., Evans, E. and Killington, R. (2003). *Microbiology in action*. Cambridge University Press. New York.

Hessen, D. O. (1992). Dissolved organic carbon in a humic lake. Effects on bacterial production and respiration. *Hydrobiology* 229: 115-123.

Hiroki, M. (1995). Study on carbon flow by microbial community in the soils of temperate zone. *Soil Science and Plant Nutrition* 41: 262-266.

Hirschler, M. M. (1986). Thermal Decomposition of Poly (vinyl chloride) Compounds under a Variety of atmospheres and heating rates. *European Polymer Journal* 22: 153-160.

Ibrahim, N. I., Anwar, M., Khalid, M. H., Ismail, M. S. and Hamzah, M. M. (2011). Assessment of potential plastic-degrading fungi in Jordanian habitats. *Turkish Journal of Biology* 35: 551-557.

Immanuel, O. M., Ibiene, A. A. and Stanley, H. O. J. (2014). Enhanced biodegradation of polyethylene by fungus isolated from the koluama mangrove swamp in the Niger Delta. *Journal of Microbiology and Biotechnology Research* 4 (2): 1-9.

Iranzo, M., Sainz-Pardo, I., Boluda, R., Sánchez, J. and Mormeneo, S. (2001). The use of microorgansims in environmental remediation. *Annals of Microbiology* 51: 135-143.

Isik, K., Gencbay, T., Özdemir-Kocak, F., and Cil, E. (2014). Molecular identification of different actinomycetes isolated from East Black Sea region plateau soil by 16S rDNA gene sequencing. *African Journal of Microbiology Research 8*(9): 878-887.

Iwen, P. C., Hinrichs, S. H. and Rupp, M. E. (2002). Utilization of the internal transcribed spacer regions as molecular targets to detect and identify human fungal pathogens. *Medical Mycology* 40: 87-109.

Jansen, B. J. P., Rastogi, S., Meijer, H. E. H. and Lemstra, P. J. (1999). Rubber modified glassy amorphous polymers prepared via chemically induced phase separation. Comparison of properties of semi- and full-IPNs, and copolymers of acrylate-aliphatic epoxy systems. *Macromolecules* 32(19): 6290-6297.

Jayasekara R, Harding I, Bowater I, Lornergan, G. (2005). Biodegradability of selected range of polymers and polymer blends and standard methods for assessment of biodegradation. *Journal of Polymers and the Environment* 13:231-251.

Kashiwagi, T., Inabi, A., Brown, J. E., Hatada, H., Kitayama, T. and Masuda, E. (1987). Effects of weak linkages on the thermal and oxidative Degradation of poly (methyl methacrylates). *Macromolecules* 19: 2060-2168.

Kathiresan, K. (2003). Polythene and plastics degrading microbes from mangrove soil. *Tropical Biology and Conservation* 51(3-4): 629-634.

Khoo, H. H. and Tan, R. B. H. (2010). Environmental impacts of conventional plastic and bio-based carrier bags: Part 2: End-of-life options. *International Journal of Life Cycle Assessment* 15: 338-345.

Kondratowicz, F. L. and Ukielski, R. (2009). Synthesis and hydrolytic degradation of poly (ethylene succinate) and poly (ethylene terephthalate) copolymers. *Polymer Degradation and Stability* 94: 375-382.

Konduri, M. K. R., Anupam, K. S., Vivek, J. S., Kumar, R. D. B. and Narasu, M. L. (2010). Synergistic Effect of Chemical and Photo Treatment on the Rate of Biodegradation of High Density Polyethylene by Indigenous Fungal Isolates. *International Journal of Biotechnology and Biochemistry* 6: 157-174.

Kumar, S., Das, P. M., Rebecca, L. J. and Sharmila, S. (2013). Isolation and identification of LDPE degrading fungi from municipal solid waste. *Journal of Chemical and Pharmaceutical Research* 5(3): 78-81.

Kuster, E. and Williams, S. T. (1966). Selection of media for isolation of streptomycetes. *Nature (London*) 202: 928-929.

Landon, J. L. (1991). *Booker Tropical Soil Manual*. A handbook for soil survey and agricultural land evaluation in the tropics and subtropics. Longman Group FE Limited. New York. 113-138pp.

Lee, B., Pometto, A. L., Fratzke, A. and Bailey, T. B. (1991). Biodegradation of degradable plastic polyethylene by phanerochaete and streptomyces species. *Applied and Environmental Microbiology* 57: 678-685.

Lee, H., Wang, L. and Shin, J. F. (1995). Mutagenicity of particulates from the laboratory combustion of plastics. *Mutation Research* 346: 135-144.

Majid, H., Al-Jailawi, R., Ameen, S. and Ali, A. A. (2015). Polyethylene degradation by *Pseudomonas putida* S3A. *International Journal of Advanced Research in Biological Sciences* 2(1): 90-97.

Motsara, M. R. and Roy, R. N. (2008). *Guide to laboratory establishment for plant analysis*. Food and Agriculture Organization of the United Nations, Rome. 204pp.

Msanya, B. M., Kaaya, A. K., Araki, S., Otsuka, H. and Nyadzi, G. I. (2003). Pedological Characteristics, General Fertility and Classification of Some Benchmark Soils of Morogoro District, Tanzania. *African Journal of Science and Technology* 4(2): 101-112.

Najmadeen, H. H., Mohammad, A. O. and Mohamed-Amin H. H. (2010). Effects of soil texture on chemical compositions, microbial Populations and carbon mineralization in soil. *Egyptian Journal of Experimental Biology* 6(1): 59-64.

Nelson, D. W. and Sommers, L. E. (1996). Organic Carbon in Soils. In: *Methods of soil analysis*. ASA Monograph No. 9. 570-571pp.

New, M., Lister, D., Hulme, M. and Makin, I. (2002). A high-resolution data set of surface climate over global land areas. *Climate Research* 21: 1-25.

Nowak, B., Pajak, J. and Karcz, J. (2012). Biodegradation of Pre-Aged Modified Polyethylene Films. *Scanning Electron Microscopy* 32: 643-670.

PlasticsEurope (2009). The Compelling Facts about Plastics. An analysis of European plastics productivity, demand and recovery for 2008.

Pometto, A. L and Lee, B. (1992). *Process of biodegradation of high molecular weight polyethylene by aerobic lignolytic microorganisms*. United States Patent 51457798.

Peacock, A. J. and Calhoun, A. (2012). *Polymer Chemistry*: *Properties and Application*. GmbH Co KG. Carl Hanser Verlag.

Piedad, D. M., Boyd, K. G., Grigson, S. J. W. and Burgess, J. G. (2002). Biodegradation of crude oil across a wide range of salinities by an extremely halo tolerant bacterial consortium MPD-M, immobilized onto polypropylene fibres. *Biotechnology and Bioengineering* 79: 145-153.

Pieper, D. H. and Reineke, W. (2000). Engineering bacteria for bioremediation. *Current Opinion in Biotechnology* 11: 262-270.

Prabhat, S., Bhattacharyya, S., Vishal1, V., Kalyan, R.K., Vijai, K., Pandey, K.N. and Singh, M. (2013). Studies on Isolation and Identification of Active Microorganisms during Degradation of Polyethylene/Starch Film. *International Research Journal* of *Environment Sciences* 2(9): 83-85.

Raaman, N., Rajitha, N., Jayshree, A. and Jegadeesh, R. (2012). Biodegradation of plastic by *Aspergillus spp*. isolated from polythene polluted sites around Chennai. *Journal of Academia and Industrial Research* 1(6): 313-316.

Rabek, J. F. (1995). *Polymer photodegradation: mechanisms and experimental methods*. Springer Science and Business Media.

Rabek, J. F. (1996). *Polymer photodegradation of polymers: physical characteristic and applications*. Beelin: Springer- verlag.

Rolf, K. (2011). *Laser Welding of Plastics.* 1st Ed. Wiley-VCH Verlag GmbH and Co. KGaA. New York.

Rosa, A. P. and Triguis, J. A. (2007). Bioremediation process on Brazil shoreline. Laboratory experiments. *Environmental Science and Pollution Research* 14: 470-476.

Rowe, L. and Howard, G.T. (2002). Growth of *Bacillus subtilis* on polyurethane and the purification and characterization of a polyurethanase-lipase enzyme. *International Biodeterioration and Biodegradation* 50: 33-40.

Ryan, P. G., Moore, C. J., van Franeker, J. A. and Moloney, C. L. (2009). Monitroing the abundance of plastic debris in the marine environment. *Philosophical Transactions of the Royal Society B* 364: 1999-2012.

Schwartz, S.S. and Goodman, S.H. (1982). *Plastics Materials and Processes*. Van Nostrand Reinhold. New York.

Secugen, S.L. (2013). DNA sequencing Sanger. In: *Technical Solutions Guide*. Madrid, Spain. 1-11pp.

Shah, A. A., Hasan, F., Hameed, A. and Ahmed, S. (2008). Biological degradation of plastics: A Comprehensive review. *Biotechnology Advances* 26: 246-265.

Sharma, A. and Sharma, A. (2004). Degradation assessment of low-density polyethylene (LDPE) and polyethylene by an indigenous isolate of *Pseudomonas stutzeri*. *Journal of Scientific and Industrial Research* 63: 293-296.

Siddiqui, M. N., Gondal, M. A. and Redhwi, H. H. (2008). "Identification of Different Type of Polymers in Plastic Wastes". *Journal of Environmental Science and Health Part A* 43(11): 1303-1310.

Simoneit, B. R. T., Medeiros, P. M. and Didyk, B. M. (2005). Combustion products of plastics as indicators for refuse burning in the atmosphere. *Environmental Science Technology* 39: 6961-6970.

Sinha, V., Patel, M. R., Patel, J. V. (2010). PET waste management by chemical recycling: A review. *Journal of Polymers and the Environment* 18: 8-25.

Smith, P. and Lemstra, P. J. (1980). Ultrahigh strength polyethylene filaments by solution spinning/drawing. Influence of drawing temperature. *Polymer* 21(11): 1341-1343.

Sonil, N., Smiti, S. S. and Jayanthi, A. (2010). Studies on the biodegradation of natural and synthetic polyethylene by *Pseudomonas spp*. *Journal Applied Science and Environmental Management* 14 (2): 57-60.

Sowmya, H.V., Ramalingappa, B., Nayanashree, G., Thippeswamy, B. and Krishnappa, M. (2014). Polyethylene Degradation by Fungal Consortium. *International Journal of Environmental Research* 9(3): 823-830.

Starnecker, A., and Menner, M. (1996). Assessment of biodegradability of plastics under simulated composting conditions in a laboratory test system. *International Biodeterioration and Biodegradation* 37(1), 85-92.

Svenson, A., Sjöholm, S., Allard, A.S. and Kaj, L. (2009). Antiestrogenicity and estrogenicity in leachates from solid waste deposits. *Environmental Toxicology and Chemistry* 26: 233-239.

Swift, G. (1997). *Non-medical biodegradable polymers: environmentally degradable polymers*. *Handbook of biodegradable polymers*. Hardwood Acedemic, Amsterdam. 473-511pp.

Sykes, G. and Skinner, F. A. (1973). *Actinomycetales: Characteristics and practical importance*. Academic Press. London. New York. 14-64pp.

Takeuchi, M., Kawahata, H., Gupta, L.P., Kita, N., Morishita, Y., Ono, Y. and Komai, T. (2007). Arsenic resistance and removal by marine and non-marine bacteria. *Journal of Biotechnology* 127: 434-442.

Tansel, B. and Yildiz, B. S. (2011). Goal-based waste management strategy to reduce persistence of contaminants in leachate at municipal solid waste landfills. *Environment, Development and Sustainability* 13: 821-831.

Thomas, G. W. (1996). Exchangeable cations: In *Methods of Soil Analysis*. ASA Monograph No. 9. 149-157pp.

Tokiwa, Y. and Ugwu, C. U. (2007). Biotechnological production of (*R*)-3-hydroxybutyric acid monomer. *Journal of Biology* 132: 264-272.

Tokiwa, Y., Calabia, B. P., Ugwu, C. U. and Aiba, S. (2009). Biodegradability of plastics. *International Journal of Molecular Science* 10: 3722-3742.

Tollner, E. W., Annis, P. A. and Das, K. C (2011). Evaluation of strength properties of polypropylene-based polymers in simulated landfill and oven conditions. *Journal of Environmental Engineering* 137: 291-296.

Tsuchida, D., Kajihara, Y., Shimidzu, N., Hamamura, K. and Nagase, M. (2011). Hydrogen sulfide production by sulfate-reducing bacteria utilizing additives eluted from plastic resins. *Waste Management and Research* 29: 594-601.

UNEP (2009a). Marine litter - a global challenge, United Nations Environment Programme. 232pp.

Urase, T., Okumura, H., Panyosaranya, S. and Inamura, A. (2008). Emission of volatile organic compounds from solid waste disposal sites and importance of heat management. *Waste Management and Research* 26: 534-538.

Usha, R., Sangeetha, T. and Palaniswamy, M. (2011). Screening of polyethylene degrading microorganisms from garbage soil. *Libyan Agriculture Research Center Journal International* 2 (4): 200-204.

Valavanidids, A., Iliopoulos, N., Gotsis, G. and Fiotakis, K. (2008). Persistent free radicals, heavy metals and PAHs generated in particulate soot emissions and residual ash from controlled combustion of common type of plastics. *Journal of Hazardous Materials* 156: 277-284.

Volke, S. T., Saucedo, C. G., Gutierrez, R. M., Manzur, A. and Favela, T. E. (2002). Thermally Treated Low Density Polyethylene Biodegradation by *Penicillium pinophilum* and *Aspergillus niger*. *Journal of Applied Polymer Science* 83: 305-314.

Vom Saal, F. S. and Myers, J. P. (2008). Bisphenol A and risk of metabolic disorders. *Journal of the American Medical Association* 300: 1353–1355.

Wangwe, S., Donald, M., Jehovanes, A., Neema, R., Thadeus, M. and Abel, K. (2014). The Performance of the Manufacturing Sector in Tanzania. Challenges and the Way Forward. Working Paper No. 22.

Waterman, N. A. (1979). *The Selection and Use of Engineering Materials*. Design Council. London.

Weidner, S., Kuhn, G., Freiedrich, J. and Schroder, H. (1996). Plasmaoxidative and chemical degradation of poly (ethylene terepthalate) studied by matrix-assisted laser desorption/ionisation mass spectrometry. *Rapid Communications in Mass Spectrometry* 10(1): 40-46.

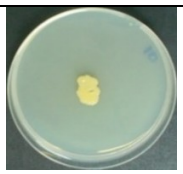
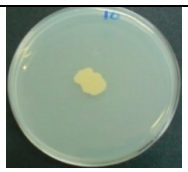
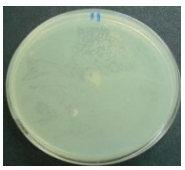
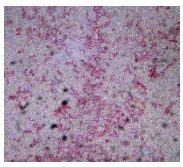
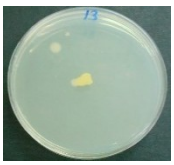
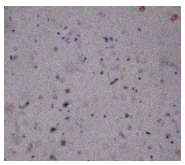
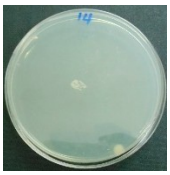
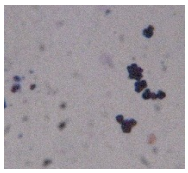
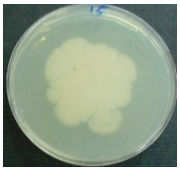
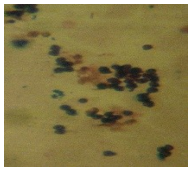
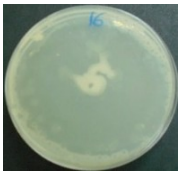
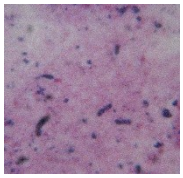
Zhang, J., Wang, X., Gong, J. and Gu, Z. (2004). A study on the biodegradability of polyethylene terephthalate fibre and diethylene glycol terephthalate. *Journal of Applied Polymer Science* 93: 1089-1096.

Zheng, Y. and Yanful, E. K. A. (2005). A Review of plastic waste degradation. *Critical Reviews in Biotechnology* 25: 243-250.

Zitko, V. and Hanlon, M. (1991). Another source of pollution by plastics: Skin cleaners with plastic scrubbers. *Marine Pollution Bulletin* 22: 41-42.

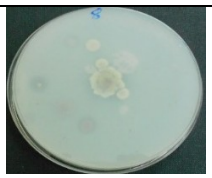
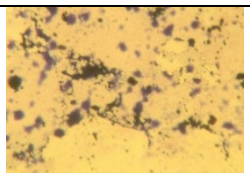
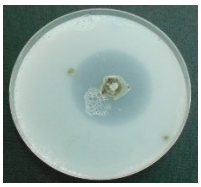
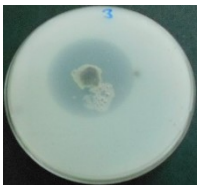
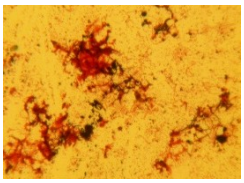
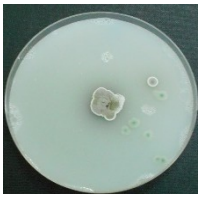
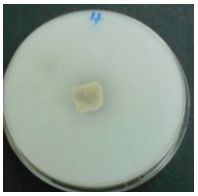
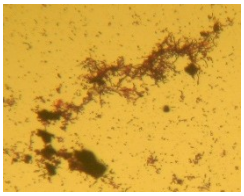
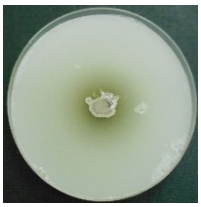
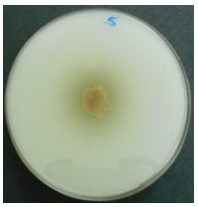
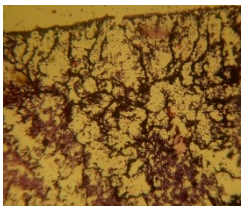
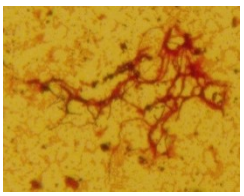
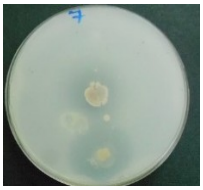
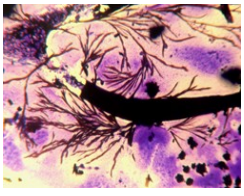
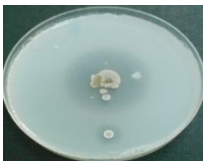
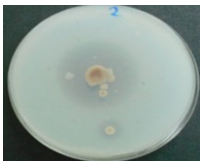
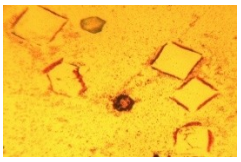
APPENDICES

Appendix 1: Morphology and identity of plastic-degrading bacteria

	Macromorpholgy		Micromorphology	Identity
SAMPLE	FRONT	BACK		
B2				*Sinomonas sp*
B3				*
B4				*Cellulosimicrobium sp*
B5				*
B6				*
B7				*

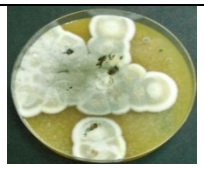
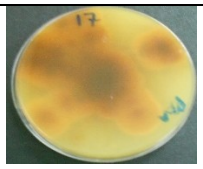
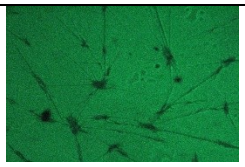
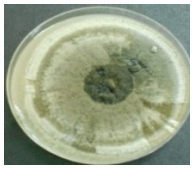
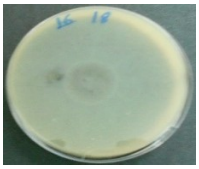
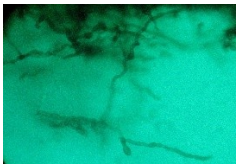
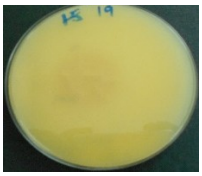
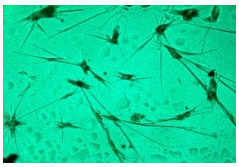
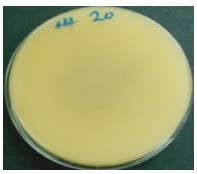
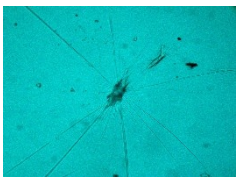
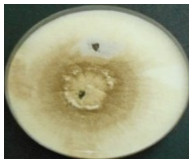
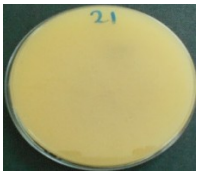
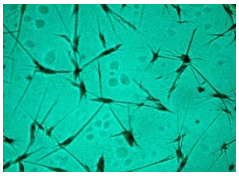
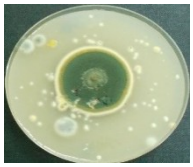
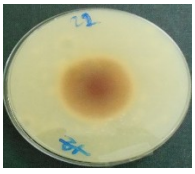
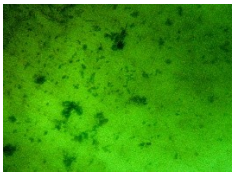
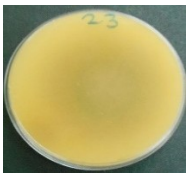
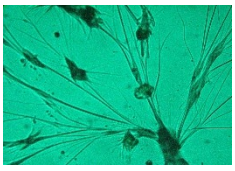
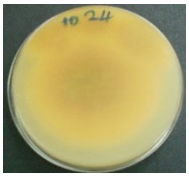
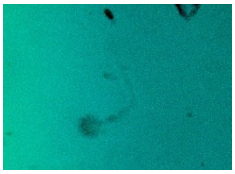
*= DNA not sequenced

Appendix 2: Morphology and identity of plastic-degrading actinomycetes

SAMPLE	Macromorphology FRONT	Macromorphology BACK	Micromorphology	Identity
Actinomycete (A2)				*Streptomyces werraensis*
A3				*
A4				*
A5				*
A6				*
A7				*
Actinomycete (A8)				*Streptomyces rochei*

*= DNA not sequenced

Appendix 3: Morphology and identity of plastic-degrading fungi

SAMPLE	Macromorphology		Micromorphology	Identity
	FRONT	BACK		
Fungus (F1)				*Eupenicillium rubidurum*
Fungus (F2)				*Phoma sp*
Fungus (F3)				*Neosartorya fischeri*
Fungus (F4)				*Aspergillus terreus*
Fungus (F5)				*Aspergillus terreus*
Fungus (F6)				*Talaromyces islandicus*
Fungus (F7)				*Aspergillus sp.*
Fungus (F8)				*Aspergillus terreus*

Appendix 4: The current isolates of bacteria and actinomycetes capable of degrading the plastic bags and bottle matched to those identified elsewhere

Isolate	Isolation Soil	Similarty with GeneBank species	Starin name form GeneBank	Accesion Number	Country of isolation	Isolation medium/source	Authors	Year	Status
B1	SUA farm (SCL)	*Bacillus cereus*	AMDK	KC683896	India	fermented mustard	Manhar,A.K., Mandal,M. and Saikia,D.	2013	Unpublished
		Bacillus cereus	SPL01	KP455735	China	Not indicated	Pan,K.C. and Zhang,D.M.	2015	Unpublished
		Bacillus cereus	F3-25	KP462870	India	rhizosphere soil	Thomas,J., Josephine, R.C.M. and Chatterjee,S.	2015	Unpublished
		Bacillus cereus	VRT2	KP636422	India	soil	Thamke,V.R.	2015	Unpublished
		Bacillus thuringiensis	270-3	KP636422	Argentina	Not indicated	Sauka,D.H., Lopez,N.E. and Benintende,G.B.	2015	Unpublished
		Bacillus thuringiensis	270-2	KP675939	Argentina	Not indicated	Sauka,D.H., Lopez,N.E. and Benintende,G.B.	2015	Unpublished
B2-1	SUA farm (SCL)	*Sinomonas sp*	S1D	HE793513	India	50 feet beneath the surfacecoal bed	Roy,R., Mukherjee,A. and Ghosh,W.	2012	Unpublished
		Sinomonas sp	S3D	HE793512	India	51 feet beneath the surfacecoal bed	Roy,R., Mukherjee,A. and Ghosh,W.	2013	Unpublished
		Sinomonas sp	S1C	HE793511	India	52 feet beneath the surfacecoal bed	Roy,R., Mukherjee,A. and Ghosh,W.	2013	Unpublished
		Sinomonas sp	S2B	HE793510	India	53 feet beneath the surfacecoal bed	Roy,R., Mukherjee,A. and Ghosh,W.	2013	Unpublished
		Corynebacterium cyclohexanicum	2M1	KC119155	India	coal mine soil sample	Saini,R., Kapoor,R., Kumar,R. and Kumar,A.	2012	Unpublished
B2-2	SUA farm (SCL)	*Sinomonas sp*	gx11	KJ504159	China	oil shale	Jiang,Y.S.	2014	Unpublished
		Sinomonas sp	R-NB-7	KM083585	Taiwan (R.O.C.)	red soil	Huang,Y.S. and Shen,F.T.	2014	Unpublished
		Sinomonas sp	R-NB-15	KM083584	Taiwan (R.O.C.)	red soil	Huang,Y.S. and Shen,F.T.	2014	Unpublished
		Sinomonas sp	R-NB-11	KM083583	Taiwan: Miaoli	red soil	Huang,Y.S. and Shen,F.T.	2014	Unpublished

					County				
B4-1	Kasanga 3 (LS2)	*Cellulosimicrobium sp.*	3-H	EU307933	China	soil sample from chemical factory	College of Life science	2007	Unpublished
		Cellulosimicrobium funkei	CCTCC NO M 2013564	KM032184	China	soil	Sun,L., Sun,R., Zhang,N. and Qi,D.	2014	Unpublished
		Cellulosimicrobium sp	X-d4	JX997906	China	oil production water	Sun,J.-Q., Xu,L. and Wu,X.-L.	2012	Unpublished
		Cellulosimicrobium funkei	BAB1015_J 15-1	JX081369	India	Microbial epository of Biodiversity	Joshi,Y.,*et al*	2012	Unpublished
B4-2	Kasanga 3 (LS2)	*Uncultured bacterium*	IJ1ZXBL01 B4Q0T	KM334385	Taiwan	earthworm cast and soil	Chang,B.-V. and Yang,C.-W.	2014	Unpublished
		Cellulosimicrobium sp	JC363	LN846832	India	Water	Azmatunnisa,M.,*et al*	2015	Unpublished
		Cellulosimicrobium funkei	0312TES29 C8	LN774498	Spain	air sample	Dominguez Monino,I.	2014	Unpublished
		Cellulosimicrobium sp	BAB-694	KM388756	India	soil	Joshi,M.N., *et al*	2014	Unpublished
A2	Kasanga 3 (LS2)	*Uncultured bacterium*	TERI-AS9	KC820825	India	agricultural soil	Cheema,S., Lavania,M. and Lal,B.	2013	Unpublished
		Streptomyces cyaneus	ITD-19	KM215731	India	Rhizosphere Bacteria	Kumar,S.,*et al*	2014	Unpublished
		Streptomyces werraensis	ITD-18	KM215730	India	Rhizosphere Bacteria	Kumar,S., *et al*	2014	Unpublished
		Streptomyces werraensis	P119	KJ023428	USA	cloaca	Su,H. *et al*	2014	Unpublished
		Streptomyces werraensis	HB-11	KC710334	India	water	Bhosale,H.J.	2014	Unpublished
		Streptomyces caelestis	BAB1706	JQ964031	India	NOT INDICATED	Gosai,S., *et al*	2012	Unpublished
A8	Kasanga 1 (S)	*Streptomyces rochei*	ICTA126	KF444515	India: Medak, Andhra Pradesh	soil from sunflower fields	Kumar,C.	2013	Unpublished
		Streptomyces rochei	T178.2	JF793511	China	rhizosphere	Fei,L.	2011	Unpublished
		Streptomyces rochei	AL14	KP797910	Thailand	air	Lertcanawanichakul,M.	2015	Unpublished

Appendix 5: Identity possibilities for bacteria and actinomycetes isolates in the present study

Isolate	Species	PCR method	% identity	Identities	Gaps
B1	*Bacillus cereus*	16S rRNA	100	820/820	0/820
	Bacillus cereus	16S rRNA	99	819/822	2/822
	Bacillus cereus	16S rRNA	99	819/822	2/822
	Bacillus cereus	16S rRNA	99	819/822	2/822
	Bacillus thuringiensis	16S rRNA	99	819/822	2/822
	Bacillus thuringiensis	16S rRNA	99	819/822	2/822
B2-1	*Sinomonas sp*	16S rRNA	100	854/854	0/854
	Sinomonas sp	16S rRNA	100	854/854	0/854
	Sinomonas sp	16S rRNA	100	854/854	0/854
	Sinomonas sp	16S rRNA	100	854/854	0/854
	Corynebacterium cyclohexanicum	16S rRNA	99	849/855	2/855
B2-2	*Sinomonas sp*	16S rRNA	100	114/114	0/114
	Sinomonas sp	16S rRNA	100	114/114	0/114
	Sinomonas sp	16S rRNA	100	114/114	0/114
	Sinomonas sp	16S rRNA	100	114/114	0/114
B4-1	*Cellulosimicrobium sp.*	16S rRNA	100	270/270	0/270
	Cellulosimicrobium funkei	16S rRNA	99	270/271	1/271
	Cellulosimicrobium sp	16S rRNA	99	270/271	1/271
	Cellulosimicrobium funkei	16S rRNA	99	270/271	1/271
B4-2	*Uncultured bacterium*	16S rRNA	100	307/307	0/307
	Cellulosimicrobium sp	16S rRNA	99	306/307	0/307
	Cellulosimicrobium funkei	16S rRNA	99	306/308	0/307
	Cellulosimicrobium sp	16S rRNA	99	306/309	0/307
A2	*Uncultured bacterium*	16S rRNA	100	265/265	0/265
	Streptomyces cyaneus	16S rRNA	99	264/265	0/265
	Streptomyces werraensis	16S rRNA	99	264/265	0/265
	Streptomyces werraensis	16S rRNA	99	264/265	0/265
	Streptomyces werraensis	16S rRNA	99	264/265	0/265
	Streptomyces caelestis	16S rRNA	99	264/265	0/265
A8	*Streptomyces rochei*	16S rRNA	100	260/260	0/260
	Streptomyces rochei	16S rRNA	100	260/260	0/260
	Streptomyces rochei	16S rRNA	100	259/259	0/259
	Streptomyces rochei	16S rRNA	100	259/259	0/259

Appendix 6: The current isolates of fungi capable of degrading the plastic bags and bottle matched to those identified elsewhere

Isolate	Isolation Soil	Similarity with GeneBank species	Strain name form GeneBank	Accesion Number	Country of isolation	Isolation medium/source	Authors	Year	Status
F1	Kasanga 3 (LS2)	*Eupenicillium rubidurum*	CY249	HQ608058	USA: Bull Creek Park, Texas	Cyphomyrmex wheeleri nest	Rodrigues, A.,*et al*	2011	Published journal
		Eupenicillium rubidurum	CY131	HQ607978	USA: Bull Creek Park, Texas	Cyphomyrmex wheeleri nest	Rodrigues, A.,*et al*	2011	Published journal
		Penicillium sp	ATT170	HQ607875	USA: Bastrop, Texas	Atta texana nest	Rodrigues, A.,*et al*	2011	Published journal
		Eupenicillium rubidurum	L7	HE962577	Netherlands	Cannabis sativa	Kusari, P.,	2012	Unpublished
		Penicillium pimiteouiense	a4s2_20	KC344973	Malaysia	beach soil	Teh,L.Y. and Latiffah,Z.	2012	Published journal
F2	Kasanga 1 (S)	*Phoma sp*	P009	EF423518	Panama: Prorena Nursery	Brosimum alicastrum	Gilbert, G.S. and Webb,C.O.	2007	Published journal
		Phoma sp	P9E4	JN207265	Venezuela	Chloris barbata	Loro,M.,	2011	Unpublished
		Phoma multirostrata	A2S1-D32	KJ767077	Malaysia	beach soil	Teh,L.Y. and Latiffah,Z.	2014	Unpublished
		Alternaria porri	AP-14	JF422728	India	leaf and flower stalk	Sharma,P. and Prakasam,V.	2011	Unpublished
F3	Kasanga 3 (LS2)	*Neosartorya fischeri*	wb171	AF455538	Austria	Nasal mucus	Buzina,W.,	2003	Published journal
		Aspergillus fumigatiaffinis	UOA/HCPF 9455	FJ878682	Greece	emerging mold pathogens	Velegraki,A. and Arabatzis,M.	2009	Published journal
		Neosartorya fischeri	CY159	HQ607997	USA: Bull Creek Park, Texas	Cyphomyrmex wheeleri nest	Rodrigues,A.,	2011	Published journal
		Neosartorya fischeri	IFM 54311	AB369900	Japan	soil	Sano,A.,*et al*	2007	Unpublished
F4	Kasanga 2 (LS1)	*Aspergillus terreus*	KAML04	KC119206	India	Not indicated	Amutha,K. and Lavanya,M.	2012	Unpublished
		Aspergillus terreus	KARVS02	KC119206	India	Not indicated	Amutha,K. and Shalini,R.V.	2012	Unpublished
		Aspergillus terreus	TN01	KC119198	India	Not indicated	Arasappan,S., Kalyanaraman,R. and Karthik,G.	2012	Unpublished

		Aspergillus terreus	NHRC-F-05-2-1	AJ413985	Russia:Moscow	Bronchoalveolar lavage	Vasilenko,O.V. and Bezmelnitsyn,N.V.	2001	Unpublished
F5	Kasanga 2 (LS1)	*Aspergillus sp.*	7 BRO-2013	KF367546	Portugal	untreated drinking water sources	Oliveira,B.R.,*et al*	2013	Published journal
		Aspergillus terreus	KAML04	KC119206	India	Not indicated	Amutha,K. and Lavanya,M.	2012	Unpublished
		Aspergillus terreus	KARVS02	KC119198	India	Not indicated	Amutha,K. and Shalini,R.V.	2012	Unpublished
		Aspergillus terreus	TN01	JX290029	India	Not indicated	Arasappan,S., *et al*	2012	Unpublished
F6	SUA farm (SCL)	*Talaromyces islandicus*	CBS 338.48	NR_103664	The Netherlands	Not indicated	Samson,R.A.,*et al*	2011	Published journal
		Talaromyces islandicus	CICC 4034	KJ783270	China	Industrial Culture	Bai,F.R.	2014	Unpublished
		Talaromyces islandicus	CBS 117284	KF984882	The Netherlands	Industry	Yilmaz,N.,*et al*	2014	Published journal
		Penicillium islandicum	MUCL 14074	FR670311	FRANCE	soil	Diguta,C.F., *et al*	2011	Published journal
F7	Kasanga 1 (S)	*Aspergillus sp.*	7 BRO-2013	KF367546	Portugal	untreated drinking water sources	Oliveira,B.R.,*et al*	2013	Published journal
		Aspergillus terreus	KAML04	KC119206	India	Not indicated	Amutha,K. and Lavanya,M.	2012	Unpublished
		Aspergillus terreus	KARVS02	KC119198	India	Not indicated	Amutha,K. and Shalini,R.V.	2012	Unpublished
		Aspergillus terreus	TN01	JX290029	India	Not indicated	Arasappan,S.,*et al*	2012	Unpublished
F8	SUA farm (SCL)	*Aspergillus terreus*	NOT INDICATED	KM491895	Portugal	surface water	Penetra,A., *et al*	2014	Unpublished
		Aspergillus sp.	7 BRO-2013	KF367546	Portugal	untreated drinking water sources	Oliveira,B.R., *et al*	2013	Unpublished
		Aspergillus terreus	KAML04	KC119206	India	Not indicated	Amutha,K. and Lavanya,M.	2012	Unpublished
		Aspergillus terreus	KARVS02	KC119198	India	Not indicated	Amutha,K. and Lavanya,M.	2012	Unpublished

Appendix 7: Identity possibilities for fungi isolates in the present study

Isolate	Species	PCR method	% identity	Identities	Gaps
F1	*Eupenicillium rubidurum*	5.8S rRNA	100	594/594	0/594
	Eupenicillium rubidurum	5.8S rRNA	99	594/595	1/595
	Penicillium sp	5.8S rRNA	99	589/594	0/594
	Eupenicillium rubidurum	5.8S rRNA	99	585/592	0/592
	Penicillium pimiteouiense	5.8S rRNA	99	570/571	0/571
F2	*Phoma sp*	5.8S rRNA	100	569/569	0/569
	Phoma sp	5.8S rRNA	99	559/560	1/560
	Phoma multirostrata	5.8S rRNA	99	544/545	1/545
	Alternaria porri	5.8S rRNA	99	544/545	1/545
F3	*Neosartorya fischeri*	5.8S rRNA	99	607/610	2/610
	Aspergillus fumigatiaffinis	5.8S rRNA	99	608/609	0/609
	Neosartorya fischeri	5.8S rRNA	99	605/609	0/609
	Neosartorya fischeri	5.8S rRNA	99	606/610	2/610
F4	*Aspergillus terreus*	5.8S rRNA	100	641/641	0/641
	Aspergillus terreus	5.8S rRNA	100	641/641	0/641
	Aspergillus terreus	5.8S rRNA	100	641/641	0/641
	Aspergillus terreus	5.8S rRNA	100	640/641	0/641
F5	*Aspergillus sp.*	5.8S rRNA	100	644/644	0/644
	Aspergillus terreus	5.8S rRNA	100	644/644	0/644
	Aspergillus terreus	5.8S rRNA	100	644/644	0/644
	Aspergillus terreus	5.8S rRNA	100	644/644	0/644
F6	*Talaromyces islandicus*	5.8S rRNA	100	605/605	0/605
	Talaromyces islandicus	5.8S rRNA	99	600/602	0/602
	Talaromyces islandicus	5.8S rRNA	100	586/586	0/586
	Penicillium islandicum	5.8S rRNA	99	601/607	6/607
F7	*Aspergillus sp.*	5.8S rRNA	100	639/639	0/639
	Aspergillus terreus	5.8S rRNA	100	639/639	0/639
	Aspergillus terreus	5.8S rRNA	100	639/639	0/639
	Aspergillus terreus	5.8S rRNA	100	639/639	0/639
F8	*Aspergillus terreus*	5.8S rRNA	99	641/642	1/642
	Aspergillus sp.	5.8S rRNA	99	640/642	1/642
	Aspergillus terreus	5.8S rRNA	99	640/642	1/642
	Aspergillus terreus	5.8S rRNA	99	640/642	1/642