RAPID BIOCONVERSION OF RICE STRAW USING CELLULOLYTIC CULTURES FOR IMPROVED AND SUSTAINABLE CROP PRODUCTIVITY AND SOIL FERTILITY IN MWEA, KENYA

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DECLARATION

I, Anncarol W. Karanja hereby declare that this thesis is my original work and has not been presented in any other university for the award of a degree or for any other award.

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DEDICATION

I dedicate this work to my dear parents Mr and Mrs Nahashon Karanja.

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ABBREVIATIONS AND ACRONYMS

ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
bp	Base Pairs
C: N	Carbon to Nitrogen ratio
CBM	Cellulolysis Basal Medium
CEC	Cation Exchange Capacity
CFU	Colony Forming Unit
CH_4	Methane
CO	Carbon Monoxide
CO_2	Carbon Dioxide
CTAB	Cetyl Trimethyl Ammonium Bromide
CMC	Carboxymethyl cellulose
DNA	Deoxyribonucleic Acid
EC	Electrical Conductivity
EM	Effective Microorganisms
GI	Germination Index
HCL	Hydrochloric acid
HI	Humification Index
ITS	Internal Transcribed Spacer
NCBI	National Centre for Biotechnology Information
NO_x	Nitrogen oxides

PAHs	Polyaromatic Hydrocarbons
PCR	PCR Polymerase Chain Reaction
PDA	Potato Dextrose Agar
RSA	Rice Straw Agar
SO_2	Sulphur dioxide
SPSS	Statistical Package for Social Sciences
TBE	Tris-Borate-EDTA
Tg	Teragrams
UV	Ultra Violet Radiation
WERL	Woods End Research Laboratory

ABSTRACT

Proper management strategies for huge amounts of crop residue generated from agricultural farms need to be obtained. Composting is a good strategy in rice straw management. Traditional methods of composting rice straw are limited in timely production of good quality compost due to its recalcitrant nature. There is need to develop and formulate microbial starter cultures that are adapted to local climatic conditions to ensure efficiency in using microorganisms in composting. This study aimed at assessing and presenting appropriate starter cultures for bioconverting rice straw into bioorganic fertilizer for use in crop production. Bacteria and fungi with lignocellulolytic potential were isolated and used alongside other starter cultures to enhance bioconversion of recalcitrant and abundant rice straw in Mwea, the main rice producing area in Kenya. The microbial isolates were selected through screening procedures and then characterized morphologically, biochemically and genetically. Rice straw was composted by treating it with the selected microorganisms, chicken droppings, commercial effective microorganisms and donkey dung in separate setups. The resultant compost types were characterized in respect to their maturity, heavy metal content and nutrient concentrations. Changes in microbial population densities and diversity during a natural composting process were also studied. The lignocellulolytic microorganisms selected for use in composting the rice straw in this study included 20 bacterial and 11 fungal isolates. Results from various identification techniques used showed that most of the bacterial isolates belonged to Genus *Bacillus* while most of the fungi were mainly in Genus *Trichoderma*. Mean values for temperature, pH and electrical conductivity (EC) among the five treatments of the study revealed significant differences at 5 % level of confidence. Using the starter cultures of the study, composting the rice straw was successfully completed within 62 days. The five compost types produced by the composting experiments were physicochemically different as demonstrated by the significant differences revealed by analysis of variance (ANOVA) of their cation exchange capacity, phosphorus, nitrogen and carbon content. The composts were observed to have attained biological maturity as revealed by the germination index, plant growth index and C: N ratio values recorded. Microbial analysis of compost samples taken from the natural composting experiment indicated notable variations in the number of bacterial cells at different phases of composting ranging from 8.7×10^5 to 2.1×10^6 CFU/ g. It was observed that fungi were less prevalent in the compost during the thermophilic phase with a lower overall prevalence compared to bacteria. Bacteria were most predominant in the compost having 28 different bacterial isolates against 17 fungal isolates. Results indicated that addition of the selected lignocellulolytic bacteria and fungi and various starter cultures significantly improved the composting process and the quality of the resultant composts. The experiment on natural composting process showed that variations in microbial population densities and diversity correspond to fluctuations in temperature within the composting materials. From the findings of this study, it is recommended that the obtained lignocelluloytic microorganisms be formulated and used for composting plant residue and other organic wastes to enhance the composting process and improve the quality of resultant bioorganic fertilizers.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Agricultural residues generated in farms are usually discarded without further processing in most parts of the world (Sabiiti, 2011). Globally, about four billion tons of crop residues are generated (Chen *et al.,* 2013). For example, the amount of crop residue produced in United States is estimated at 488 x 10^6 metric tons/ year for 21 crops (Lal, 2013). Approximately 4.2 million metric tons of agricultural residues are produced in Taiwan annually (COA, 2006) while over 500 million tons of agricultural residues are generated in India every year (Agarwal *et al.,* 2016). It is also estimated that over 60 % of all crop residues in the world are produced in low income countries with 45 % of these residues being produced in the tropics (Smil, 1999). If this waste is not properly managed, it can cause major global problems. Soil fertility, food security and environmental issues do develop due to these huge amounts of farm residues disposed off as waste. Poor disposal of farm residue causes adverse impacts on essential components of the biosphere (Sabiiti, 2011).

Rice (*Oryza sativa L*) is one of the most important staple foods for a large portion of the world's human population. Among grains, rice has the second highest worldwide production after maize (Sharif *et al.,* 2014). Besides producing seeds, rice produces waste by-products, the main one being rice straw wich is produced in large quantities of every year worldwide (Surekha *et al.,* 2003; Sidhu and Beri, 2008). In the year 2008, about 620 million tons of rice straw was produced in Asia and this quantity is increasing annually while India produces about 170 million tons of rice straw every year (Aisyah *et al.,* 2016). Although there is no documented record on the amount of rice straw produced in Mwea Rice Irrigation Scheme, the main rice producing area in Kenya, a reconnaissance survey showed that large quantities of rice straw are also generated from rice farming.

Disposal of huge amount of residue is a major challenge and hence proper management is required and the (Smil, 1999). In some places, rice straw has no commercial value and is disposed off in various ways including burning and direct incorporation into the soil (Gadde *et al.,* 2009; Rosmiza *et al.,* 2014). Burning has been traditionally used to dispose crop residues thus some farmers dispose rice straw by burning (Smil, 1999). Uncontrolled burning leads to emission of greenhouse gases such as carbon dioxide (CO_2), carbon monoxide (CO), methane (CH_4), nitrogen oxides (NOx) and sulphur dioxide (SO_2) (Venkataraman *et al.,* 2006; Minooei and Mokshapathy, 2017). Global climate is being influenced by these gases emissions from agricultural residue which is responsible for an estimated 10–12 % of total greenhouse gases emissions (Metz *et al.,* 2007). In Asia, contribution to the greenhouse gases from open burning of biomass per year was estimated to be 0.37 teragrams (Tg) of SO_2 , 2.8 Tg of NOx , 1100 Tg of CO_2, 67 Tg of CO and 3.1 Tg of methane (CH_4) (Streets *et al*., 2003). Annual contribution from burning of crop residues is estimated to be 0.10 Tg of SO_2, 0.96 Tg of NOx , 379 Tg of CO_2 , 23 Tg of CO and 0.68 Tg of CH_4 worldwide (Venkataraman *et al.,* 2006; Jain *et al.,* 2014).

Open burning has also been shown to have severe negative impacts to human health due to the harmful air pollutants released to the environment which mainly include polycyclic aromatic hydrocarbons (PAHs) polychlorinated dibenzo- p -dioxins (PCDDs) and polychlorinated dibenzofurans emitted (PCDFs) (Korenaga *et al.,* 2001; Gullett and Touati, 2003; Lin *et al.,* 2007). The pollutants are significant toxicants and potential carcinogens affecting both humans and the environment, hence impeding the economy of the world (Yokelson *et al.,* 2011).

Direct incorporation of rice straw into soil is also practised by some farmers (Surekha *et al.,* 2003**)**. Direct incorporation is where the straw is buried under a layer of soil in the farm and allowed to decompose naturally (Lal, 2013). However, due to the short time between harvest and sowing of the next crop the straw does not decompose completely. This might result into low yield from subsequent crop (Blanco-Canqui and Lal, 2009). Crop residue incorporation can enhance the transfer of disease pathogens to new plants in case the previous crops were infected (Bhatnagar *et al.,* 1983). Rice straw management through incorporation is also associated with other problems such as immobilization of plant nutrients particularly nitrogen, release of harmful organic acids, the residues impede farm preparation and contributes to reduced germination of subsequent crops (Bakht *et al.,* 2009). A study on incorporation of brassica residue carried out by Khaliq *et al.* (2011) revealed that the incorporation significantly suppressed germination traits and extended mean emergence duration of the seedlings.

In addition to burning and direct incorporation, some farmers dispose rice straw by heaping it in the farm (Surekha *et al.,* 2003**;** Sidhu and Beri, 2008). However, heaping encourages breeding of pests and rodents. Moreover, diseases from the rice plants continue spreading if the straw is left in the field without proper management (Jusoh *et al.,* 2013).

Composting can be the best alternative in rice straw management (Goyal and Sindhu, 2011). Growing concerns relating to food crisis, soil degradation, environmental pollution and loss of soil biological diversity have led to global interest in this practice (Misra and Roy, 2003; Nagavallemma *et al.,* 2006; Kumar, 2011). Composting is considered as the best approach as it eventually regulates soil fertility and minimizes the negative environmental effects ensuring environmental protection and safety (Sidhu and Beri, 2008; Goyal and Sindhu, 2011). Composting ensures the reclamation of the nutrients withinthe rice biomass to increase crop productivity and promote economic value of the byproduct (Chandra *et al.,* 2009). Production of compost from rice straw is therefore a better alternative to burning, direct incorporation of the straw into soil and heaping in rice fields. Composting is a dynamic process of rapid successive reactions that are coordinated by a number of microbial communities (Kumar, 2011). It involves the breaking down of organic matter under controlled aerobic conditions into usable products (de Bertoldi *et al.,* 1985).

Although the practice of composting is known, farmers, especially in developing countries do not make the best use of the recycling opportunities available to them (Gajalakshmi and Abbasi, 2008). This is mainly due to various hindrances in the composting process which among others include the long time span required for the compost to mature (Misra and Roy, 2003). Waste materials of plant origin are recalcitrant that is, they show great resistance to degradation. This is because they are mainly composed of lignocellulosic compounds, containing cellulose, hemicellulose and lignin (Anwar *et al.,* 2015). Lignocellulosic components are difficult to break down, thus the composting process of materials of plant origin takes a long period of time (Van Soest, 2006; Sulfab, 2013). This could have practical implications in the biodegradation of rice residues.

Rice straw from different regions displays different characteristics but it is generally known for its recalcitrant nature; resisting degradation due to its structural constituents (Van Soest, 2006). It has a unique chemical composition relative to other cereal straw. On average it consists of lignin (10–15 %), silica (75 %), 40–50 % cellulose and 9–12 % hemicelluloses, on a dry weight basis (Knauf and Moniruzzaman, 2004). All these compounds are hard to decompose rapidly under natural environmental conditions.

The composting process can be hastened through certain treatments on the organic material being composted (Insam and Klammer, 2002). Rapid composting methods make use of treatments like shredding, forced aeration, mechanical turning, balancing the C: N ratio and watering to reduce the composting period (Misra and Roy, 2003). These treatments affect the process through several mechanisms such as increased surface area for action of microorganisms and ensured heat build up within the compost pile (Patidar *et al.,* 2012).

Modern composting technologies involve inoculating organic materials with pure cultures of ligninocellulolytic microorganisms (Insam and Klammer, 2002). Compared to traditional composting methods, modern composting technologies involves higher nutrient levels, improved microbial composition in compost pile and production of enzymes to hydrolyze the materials, thus rapid decomposition (Lynd *et al.,* 2002). During composting, the consortia of microorganisms use the organic matter as a source of food to produce humus, carbon dioxide and water. Nitrate and methane may also be formed (Tuomela *et al.,* 2000). The resultant humus like product of rapid microbial composting is referred to as a bioorganic fertilizer (Das and Dkhar, 2012). In addition to hastening the composting process, microbial inoculation ensures that the bioorganic fertilizers are of high chemical, microbial and physical quality (Imbeah, 1998).

Only few microorganisms have developed mechanisms that facilitate the breaking down of the recalcitrant materials (Dashtban *et al.,* 2009; Gonzalo *et al.,* 2016). These microorganisms are capable of secreting enzymes that degrade the lignocellulosic components of the plant materials (McCaig *et al.,* 2001; Das and Singh, 2004). They produce enzymes such as cellulase, ligninolytic peroxidase and α- glucocidase. Interest in these microorganisms has increased owing to their potential commercial applications including in biodegradation (McCaig *et al.,* 2001; Lynd *et al.,* 2002).

Use of bioorganic fertilizer is an important practical measure in agriculture (Teaumroong *et al.,* 2010). Application of composted materials in soil has several merits over fresh manure, including lower numbers of viable weed seeds, less volume and particle size, hence easy application, more balanced nutrient composition, stabilized organic matter and release of nutrients at slower rates (Gajalakshmi and Abbasi, 2008; Lee, 2016). Bioorganic fertilizers are very beneficial because they contain macro and microelements required for plant growth and they enhance the physical, chemical and biological characteristics of the soil (Dadhich *et al.,* 2011). They act as soil conditioners leading to increased nutrient concentrations, high organic matter content and greater soil water holding capacity (Doran *et al.,* 1996). Increased organic matter in soil induces better water infiltration and increased capacity to retain nutrients especially preventing nitrogen losses through leaching, volatilization and denitrification (Liu *et al.,* 2014; Maeder *et al.,* 2002). Bioorganic fertilizer indirectly increases crop yields because it improves the soil structure and the amount of nutrients returned to the soil (Lohri *et al.,* 2017). Soil with high organic matter content is more productive than the same soil whose organic matter has been burnt through poor management practices and eroded by water runoff (Kaosol, 2009).

Application of bioorganic fertilizer also enhances soil fertility through increased microbial load and activity (Butterfly *et al.,* 2013). Bioorganic fertilizers enhance soil microbial activity by increasing the soil microbial biomass as the microbial inoculants used in preparation of the bioorganic fertilizer add to the soil microbial population once applied to soil (Kengo and Xu, 2001; Maeder *et al.,* 2002). Soil microbial biomass is essential in regulating nutrient availability; it potentially mobilizes large amounts of added nutrients making them available for up take by plants (Ruan *et al.,* 2004; Khan *et al.,* 2012). Moreover, as microorganisms in the soil break down the organic materials in it, a slow release of nutrients is provided over a longer period. Activities of soil microorganisms also boost crop growth and yields through restraining pests and other disease pathogens by acting as agents of biological control (Baffour-Asare, 2009).

Application of bioorganic fertilizers is also an alternative to use of farm chemical inputs, which unfortunately is the most common practice in agriculture today (Bernal *et al.,* 2009). Unlike chemical fertilizers, bioorganic fertilizers do not harm the soil components but rather improve on its quality over a long period of time which is a prerequisite for sustainable agriculture (Lal, 2013). Modern high intensity farming systems involve excessive use of synthetic fertilizers, pesticides and herbicides, most of which are not ecofriendly (Tilman, 1998). Chemical fertilizers directly enhance crop yield while on one hand, their production and use imparts various negative effects on the agricultural ecosystems such as degradation of the soil, loss of crop genetic diversity, reduction in soil microbial diversity, contamination of ground-water resources and pollution of the atmosphere (Ahmad *et al.,* 2007; Kaur *et al.,* 2008; Chaudhry *et al.,* 2009; Leite *et al.,* 2010).

Production cost of bioorganic fertilizers is generally lower than that of chemical fertilizers. High quality bioorganic fertilizers provide a lifelong solution to degraded soils and provide soil-building benefits that deal with environmental issues related to management of soil with chemical fertilizers. This makes bioorganic fertilizers a good alternative to the non renewable petrochemical fertilizers especially to smallholder farmers with financial constraints (Uyanoz, 2007; Bernal *et al.,* 2009).

Viability of soil organisms can be compromised when chemical fertilizers are applied to farming regimes (Lazcano *et al.,* 2013). Most nutrients in bioorganic fertilizers are released slowly and are less prone to leaching compared to inorganic fertilizers (Diacono and Montemurro**,** 2010). An experimental study conducted by Natsheh and Mousa (2014), demonstrated that compost can be used in replacement of chemical fertilizer since it increased soil fertility and crop yield in organic farming.

In this respect, the present work mainly focused on selecting potential microbial strains and other substances for utilization in composting rice straw. This study will contribute towards a better understanding of benefits of modern composting and microbial community structure dynamics during composting.

1.2 Statement of the problem

Condition of soil in most crop producing areas in Kenya has gradually declined due to practices that comprise continuous cultivation and nutrient extraction through crop harvest without adequate nutrient replenishment (Bebbington, 1994; Golabi *et al.,* 2004). The need to boost food production is on the increase everywhere in the world today as the world's population is increasing day by day while the supply of land is still fixed (Godfray and Garnett, 2014). With a high food demand and limited land availability, farmers regularly use synthetic farm inputs to increase crop productivity. However, there are many challenges related with the use of synthetic farm inputs including being unaffordable for smallholder farmers due to high prices, unavailability at the time they are needed by the farmers and the negative effects on beneficial soil organisms, water bodies and climate patterns (Metz *et al.,* 2007; Dadhich *et al.,* 2011).

Large amount of rice straw is produced in Mwea as residue from rice farming (Plate 2.1). However, the potential benefits of this abundant agricultural residue are presently underutilized. The abundant rice straw is also a source of environmental degradation since some farmers get rid of it through burning, direct incorporation while others just heap it in their farms; which is unsightly and a health hazard (Dobermann and Fairhurst, 2002).

Moreover, biodegradation of rice straw through ordinary composting requires three months or more for complete decomposition due to its recalcitrant nature. The time taken for composting rice straw is rather long and too slow for farmers who plant crops more than once in one year. Therefore considerable research needs to be done to investigate on possible methods of accelerating composting of recalcitrant rice straw.

There is need to develop and formulate microbial starter cultures that are adapted to local climatic conditions to ensure efficiency in the using microorganisms in composting for soil fertility and crop productivity. There is also need to utilize locally available microbial populations for identification and preservation of genetic diversity and resourcefulness.

There is also need for value addition of locally available materials in Mwea, including donkey dung and chicken droppings. The animal waste is readily available in Mwea especially the donkey dung because donkeys are the main means of transport for farm produce and inputs in this area.

1.3 Justification of the study

There is need to look for alternative ways of fertilizing soil to increase food production while at the same time preserving soil quality for sustainable agriculture (Dadhich *et al.,* 2011). Converting the rice straw into bioorganic fertilizer can be a good way of addressing issues of soil degradation, low crop yields, expensive mineral fertilizers, unavailable farm inputs and environmental degradation (Golabi *et al.,* 2004). The present research focused on utilizing locally available materials in making bioorganic fertilizer to address these challenges.

Addition of lignocellulolytic microorganisms and other starter cultures with demonstrated efficiency in increasing rate of organic matter decomposition is a possible approach of enhancing the composting process (Karnchanawong and Nissaikla, 2014). To identify such microorganisms and substances, careful and systematic selection procedures must be employed as is entailed in the present study.

The need to use locally adapted microorganisms in composting is also a factor to consider in the application of the aspect of beneficial microorganisms. This could cater for variations in application conditions that exist in different localities which impact on the effectiveness of the already commercialized effective microorganisms. Formulation of case appropriate or custom made microbial consortium is necessary to take care of the dynamism in microbial population metabolism and changes in environmental conditions (Classen *et al.,* 2015).

Composting rice straw to a bioorganic fertilizer is also an environmentally friendly means of waste disposal. The modern concept of environmental management is based on the recycling of wastes. Accordingly, composting is a safe way to treat the rice residue and reclame the nutrients contained in it (Kumar and Gopal, 2015).

Use of bioorganic fertilizers by farmers can indirectly address the issue of water shortage which has lately been very rampant in Mwea Rice Irrigation Scheme. High organic matter content in soil increases its water holding capacity. It is thus important for rice farmers to add more organic matter to their rice fields to conserve soil moisture (Doran *et al.,* 1996).

Addressing the above issues will also lead to improved economic efficiency for the smallholder farmers. Making and using bioorganic fertilizer can reduce the current high dependence of farmers on expensive synthetic fertilizers. This will also reduce the cost of farming hence improve farmers' livelihood and promote sustainable agricultural development (Fan *et al.,* 2012).

Composting donkey dung and chicken droppings instead of applying it directly for crop farming will prevent the harmful effects usually caused by direct application of animal waste into the soil. This will also promote environmental safety and economic efficiency (Bernal *et al.,* 2009).

1.4 Hypotheses

1. Microorganisms with the ability to rapidly biodegrade rice straw are morphologically, biochemically and genetically different.
2. There are significant differences in the decomposition rates of rice straw treated with different starter cultures.
3. Residual effects of the various treatments on the physicochemical properties of the resultant bioorganic fertilizers are significantly different.
4. There are significant microbial changes during natural composting process.

1.5 Objectives

1.5.1 General objective

To assess the effects of various treatments on degradation of rice straw and the quality of the finished product and describe microbial community changes during composting.

1.5.2 Specific objectives

1. To isolate and characterize microorganisms with ability to rapidly degrade rice straw.
2. To assess decomposition rates of rice straw treated with selected microorganisms, commercial effective microorganisms (EM), chicken droppings and donkey dung.
3. To determine the physicochemical quality of the resultant compost types.
4. To assess the microbial changes during natural composting process.

1.6 Significance of the study

Efficient microbial cultures which can be multiplied and used in production of bioorganic fertilizers were isolated and identified in this study. Smallholder farmers can thus use these as inoculants for fast decomposition of biodegradable materials for application into agricultural soil. Composting allows effective and efficient useof locally available organic wastes by farmers.

Application of organic fertilizer will increase soil nutrients, its cation exchange capacity, organic matter content, water retention capacity hence increased plant growth rate and yields. Improved soil conditions are very important in ensuring sustainable and increased crop production for national and global food security. It also ensures ecological health and balance due to increased soil microbiological diversity. Moreover, use of bioorganic fertilizer will reduce high dependence of farming systems on expensive, non renewable petrochemical fertilizers hence economic efficiency. Economic efficiency will improve farmers' livelihood and promote sustainable agricultural development. It will relieve farmers from having to solely depend on expensive and often unavailable chemical fertilizers. Bioorganic fertilizer is therefore an effective and affordable soil amendment that represents significant ecological and economic benefits. The results of the study will also contribute immensely to local and global knowledge.

CHAPTER TWO

LITERATURE REVIEW

2.1 Soil degradation

Agricultural intensification has increased crop yields but on the other hand created severe soil health and environmental problems (Pimentel *et al.,* 1995; Aktar *et al.,* 2009). Maintenance of soil fertility and productivity is very important because the fertility is going down due to improper use of inorganic inputs (Goyal *et al.,* 2009). Maintenance of soil quality is important for sustainable agriculture. Soil quality is an important issue due to its effects on agronomic productivity, the environment, its contribution to food security and general quality of life *(*Aktar *et al.,* 2009). A fertile soil contains elemental compounds to support plant growth and diverse, active biotic communities (Smaling *et al.,* 1997). However, the condition of soil in most agricultural areas is degraded due to practices that involve continuous cultivation and nutrient extraction through crop harvest without adequate nutrient replacement (Golabi *et al.,* 2004). Soil nutrients are gradually depleted through crop harvest, leaching and soil erosion (Angima *et al.,* 2003).

Soil fertility decline and reduced productivity is recognized as the fundamental factor causing a decrease in food security in smallholder farms of sub-Saharan Africa (Nyamangara *et al.,* 2003; Aktar *et al.,* 2009). Productivity impacts of soil degradation are due to a decline in soil fertility. Declining soil fertility is actually one of the major challenges facing crop production in Kenya today (Mutuo *et al.,* 2000). This is because farmers do not sufficiently compensate the nutrient losses by returning nutrients to the soil via crop residues (Nyamangara *et al.,* 2003). Most smallholder farmers replenish soils through addition of chemical fertilizers. Furthermore, use of synthetic fertilizers among smallholder farmers in Kenya is low due to limited affordability. For instance, in the central highlands, fertilizer use is as low as less than 20 kg nitrogen and 10 kg phosphorus per hectare due to the high cost of mineral fertilizers (Kihanda *et al.,* 2006; Kimani *et al.,* 2004).

Traditionally, smallholder farmers practised shift cultivation to replenish soil quality. Shift cultivation, however, is no longer practised as rising population densities and pressures on land use have culminated in intensive, sedentary small-scale landholding farms and extension of agriculture into marginal areas (Kaur *et al.,* 2008; Berazneva and Lee, 2012). The intensification of agriculture in small landholdings in Kenya has typically not been accompanied by sufficient inputs of nutrients to match the outputs of nutrients through harvested products and losses (Mutuo *et al.,* 2000). Furthermore, recent changes in policies; such as structural adjustment programs and abolition of subsidies on farm inputs have made them unaffordable to smallholder farmers. This has led to reduction of use of external inputs to restore soil fertility and hence widespread depletion of soil nutrients and reduction of soil fertility in smallholder agriculture in Kenya (Smaling *et al.,* 1997; Bernal *et al.,* 2009).

In Mwea, most of the paddy fields are covered with clay soils with little organic matter (vertisols). These soils are underlain by olivine basalts and phonolites. Low organic matter content is generally common in Kenya soils especially those in semi-humid to semi-arid areas (1400 mm of annual rainfall (Blokhuis, 1982). The paddy soils in Mwea have been reported to be deficient in phosphorus, nitrogen and potassium (Kondo *et al.,* 2001; Muriithi *et al.,* 2012).

Concerns about degradation of agricultural soil have thus increased the interest in sustainable agricultural strategies such as use of bioorganic fertilizers (Diacono and Montemurro**,** 2010; Dadhich *et al.,* 2011). There is evidence that use of organic amendments increases soil fertility (Goyal *et al.,* 2009). Addition of organic fertilizers has long been known as essential in increasing crop productivity (Kengo and Xu, 2001). Currently, strategies focusing on organic resources and crop residue management have been extensively recommended as fundamental to integrated soil fertility management approaches (Monforti *et al.,* 2015). Successful use of various organic residues in farming is dependent on several factors including properties of the waste, such as nutrients and content of toxic elements, availability, affordability and environmental policies (Domeno *et al.,* 2009).

The best way of examining the agronomic value of stabilized organic materials is by calculating the supply of both the organic matter and plant nutrients (Monforti *et al.,* 2015). Gradual release of these nutrients leads to increase in crop productivity in subsequent years (Kihanda *et al.,* 2006; Fan *et al.,* 2012). The organic matter strengthens soil structure by enhancing the inter-particle cohesion within aggregates and by increasing their hydrophobicity, hence decreased breakdown (Ruan *et al.,* 2004; Kaur *et al.,* 2008). In particular, high soil microbial activity as a result of addition of composted organic matter is the major factor for increased soil structural stability (Diacono and Montemurro**,** 2010). New technologies should be put in place for organic matter management to overcome current agronomic challenges.

2.2 Microbial communities during composting

Composting may appear to be an easy practice, but it is in theory very complicated (de Bertoldi *et al.,* 1983). Aerobic composting, involves pooled activities of a varied succession of diverse biological populations including bacteria, actinomycetes and fungi (de Bertoldi *et al.,* 1985). The incredible intricate interactions between these organisms in compost are responsible for this complexity (Neklyudov *et al.,* 2008). The most active biological components mediating the bioconversion process mainly include the microbial communities within the compost (Sylvia *et al.,* 2005; de Gannes *et al.,* 2013). The microorganisms perform their metabolic processes rapidly and with remarkable specificity under ambient conditions, catalyzed by their diverse enzyme-mediated reactions (de Bertoldi *et al.,* 1983). Microbial metabolic activities constantly change the physicochemical conditions of the surrounding environment, which in turn determines the ability of microorganisms to grow, metabolize and survive (McKinley and Vestal., 1985; Shiji and Padmaja, 2013).

Different microbial populations are involved. These communities exhibit successional patterns as the physicochemical conditions in the compost change due to decomposition (Ishii *et al.,* 2001). During composting, major changes take place in the nature and abundance of the microbial populations (Neklyudov *et al.,* 2008). Each microbial community is adapted to a particular set of environmental conditions (Crawford, 1983;

Partanen *et al.,* 2010). The mixed populations parallel the complex environments afforded by the composting material. Because each population is suited to a certain condition for relatively limited period of time and each is most active in decomposition of some specific type of organic matter, the actions of one group complement those of another (Golueke and Diaz, 1990; Lee, 2016). A particular microbial species will multiply rapidly initially but will decrease as the conditions change and other organisms flourish (Neklyudov *et al.,* 2008). Temperature and variations in available food mainly exert the greatest pressure in determining the species of microorganisms comprising the composting community at any one time (de Bertoldi *et al.,* 1985; Das and Singh, 2004).

There are three marked successional phases determining chemical and microbial changes during composting, as influenced by temperature. These are, mesophilic phase (moderate temperatures rising to about 40 °C), thermophilic phase (high temperatures rising to about 70 °C) and curing phase (cooling to ambient temperature) (de Bertoldi *et al.,* 1985; Ryckeboer *et al.,* 2003).

The first group of microorganisms to initiate decomposition are referred to as primary decomposers (Sylvia *et al.,* 2005). These microorganisms create an environment suitable for secondary microorganisms, which do not have the ability to utilize the initial substrates (Golueke and Diaz, 1990). Mesophilic (moderate temperature) microorganisms are characteristically predominant at the start of the process, soon ushering in thermophilic (high temperature) microbes, which colonize all parts of the compost stack with suitable temperatures. Thermophilic fungi usually appear after 5 to 10 days while actinomycetes become active in the final stages when rapid composting occur within a short period of time (Partanen *et al.,* 2010).

Metabolites produced by one group of microorganism are utilized by the other (Davis *et al.,* 1992; Golueke and Diaz, 1990). Primary decomposers are mainly mesophiles that grow best at moderate temperatures. They rapidly break down the soluble, readily degradable compounds. Fungi and actinomycetes play a critical role in the degradation of cellulose, lignin and other highly resistant compounds. These are usually confined to the outer parts of the compost and they become active only during the last stages of composting (Ryckeboer *et al.,* 2003). These resistant components are attacked after more readily decomposable materials have been utilized (Eida *et al.,* 2012). Substantial degradation of cellulose and lignin by actinomycetes and fungi can occur towards the end of the composting process when temperatures reduce and the environment is largely suitable for their growth (Ngugen *et al.,* 2007).

During the last stage, nutrients become a limiting factor and this causes a decline in microbial activity. The substrate quality declines and compounds which are not further degradable are formed (Insam and Klammer, 2002). To ascertain a suitable end product, there are fundamental factors of key importance in composting making optimization of composting conditions very important (Barrena *et al.,* 2009).

During composting, pathogens such as infectious bacteria do not persist as the temperature rises (Sylvia *et al.,* 2005; Ch'ng *et al.,* 2013). Rise in temperature provides optimal conditions to eliminate pathogens and weed seeds with rapid organic matter processing prevailing (Neklyudov *et al.,* 2008). The outer surface of the composting set up is usually cool, so mixing the outer and inner layers has to be done in order to eliminate pathogens and weed seeds, as well as ventilating it by increasing aeration (Insam and Klammer, 2002). In poorly ventilated conditions, microbes cannot survive as a result of overheating when temperature rises up. After the overheating phase, the composting materials cool down to the mesophilic state where mesophilic microorganisms return the pile to thermophilic state once again (Ryckeboer *et al.,* 2003; Oviasogie *et al.,* 2010). Eventually, the pile will deplete itself off most of the easily degradable organic substrates leaving some cellulose, most lignin and humic materials (Ch'ng *et al.,* 2013).

2.2.1 Bacteria in composting

Despite their prevalence in various organic matter, bacteria do not easily adapt to habitats with unconducive conditions due to their lack of physiological complexity (Lee, 2016). Levels of oxygen, moisture, temperature and acidity are all fundamental to growth and survival of bacteria. Aerobic bacteria normally require an oxygen level greater than 5 % and each type of bacteria have a particular range of temperature for optimal growth (Hubbe *et al.,* 2010). However, bacteria provide the most rapid and efficient composting organisms. They constitute the majority of microorganisms in composting materials compared to actinomycetes and fungi (Davis *et al.,* 1992). During composting, the bacterial populations are the one that largely cause substrate decomposition and heat generation (Oviasogie *et al.,* 2010).

Mesophilic bacteria that predominate at the beginning of the composting process are mainly rod shaped Gram-negative species, as well as rod shaped Gram-positive species (Ashraf *et al.,* 2007; Insam and Klammer, 2002). Their numbers increase exponentially during the initial stages of composting as they utilize the readily available sugars and starches (Ghazifard *et al.,* 2001). Heat is released from their metabolic activities and if conditions are suitable, compost temperatures begin to rise. When temperatures rise above 40 ºC, thermophilic species take over from the mesophilic bacteria (Partanen *et al.,* 2010). Bacteria such as *Bacillus schlegelii*, *Hydrogenobacter* spp. and species of the genus Thermus appear to be the main active microbes when temperatures get to 65 ºC and above (Riberio *et al.,* 2017). Several other *Bacillus* species have been recorded during the thermophilic stage of the composting process including the thermotolerant *Baccillus subtilis, B. polymyxa, B. pumilus, B. sphaericus, B. licheniformis*, *B. stearothermophilus* and *B. acidocaldarius* (Ghazifard *et al.,* 2001; Oviasogie *et al.,* 2010).

Several mesophilic bacteria can however be isolated from the thermophilic stages of composting (Partanen *et al.,* 2010; Riberio *et al.,* 2017). A possible reason behind mesophilic bacterial survival in the high-temperature phases of composting is the formation of microcolonies (Oviasogie *et al.,* 2010). As the activity of thermophilic bacteria decreases, the temperatures also decrease and mesophilic bacteria again predominate and are involved in compost curing and maturation. Both Gram positive and Gram negative bacteria are involved in the later mesophilic phase of composting (Davis *et al.,* 1992; Oviasogie *et al.,* 2010).

2.2.2 Fungi in composting

Among all microorganisms, fungi are the ones mainly responsible for the decomposition of the most complex plant polymers (Sylvia *et al.,* 2005). Fungi are very important in composting because they break down tough debris including cellulose and lignin (Ashraf *et al.,* 2007). They can attack organic residues that are too dry, acidic, or low in nitrogen for bacterial decomposition. Fungi break down initial debris, allowing bacteria to proceed with decomposition process without the complex components (Insam and Klammer, 2002). They are therefore known as the most important primary lignocelluloses degraders that degrade complex polymeric substrates (Lee, 2016). Fungi are present in higher numbers when compost temperatures are moderate and moisture levels are lower (Ashraf *et al.,* 2007). Nonetheless, mesophilic fungi have been observed during initial stages of composting (de Bertoldi*et al.,* 1983; de Bertoldi *et al.,* 1985). During the final stages of composting where temperatures between 40 and 60 ºC occur, a high diversity of thermotolerant fungi including *Thermomyces* spp., *Penicillium duponti, Geotrichum candidum, Cladosporium, Aspergillus, Mucor, Rhizopus* and *Absidia* spp. have been observed (Ghazifard *et al.,* 2001). The optimum range for the survival of thermotolerant fungi is between 45 and 50 ºC (Partanen *et al.,* 2010), while the disappearance of viable fungi in composts occurs before temperatures reach 60 ºC (Hassen *et al.,* 2002).

At the late mesophilic stage where temperatures decrease and the activity of thermophilic fungi also decrease, mesophilic fungi again begin to recolonize (Ch'ng *et al.,* 2013). Rawat *et al.* (2013) observed that diverse populations of mesophilic fungi existed from the start to the end of the composting process. Several studies on fungal communities during the later stages of composting reported that species of *Alternaria, Aspergillus,* *Bipolaris, Fusarium, Mucor, Rhizopus, Peziza, Phoma* and *Trichoderma* dominate (Ryckeboer *et al.,* 2003).

2.2.3 Actinomycetes in composting

Actinomycetes are considered a higher form of bacteria that are partly closely related to bacteria and partly closely related to fungi (Hassen *et al.,* 2002). Actinomycetes are essentially strict aerobic saprophytes which form chains or filaments and are common in many environments. They also tolerate higher temperatures and pH than fungi (Barka *et al.,* 2016). Actinomycetes have ability to slightly break down cellulose and lignin making them key agents in decomposition of lignocellulose although their ability is less than that of fungi (Tuomella *et al.,* 2000; Ashraf *et al.,* 2007).

Actinomycetes usually occur at the bottom of the compost pile since they cannot coexist with bacteria due to the antibiotics they produce; they usually stay far away from bacteria in grayish clusters (Ch'ng *et al.,* 2013; Chavan *et al.,* 2015). They have the ability to decompose the more resistant components and thus play an important role in degrading cellulose, lignin, chitin and proteins (Hassen *et al.,* 2002; Eida *et al.,* 2012). When doing so, they release carbon, nitrogen and ammonia which are attributable to the earthy smell of compost (Chavan *et al.,* 2015).

Actinomycetes have been found to be ineffective competitors when nutrient levels are high because of their slow development compared to bacteria and fungi making them better competitors at low nutrient levels (Royckerboer, 2003; Partanen *et al.,* 2010). They are not very active during the initial stages of composting but become active later on through enzymes that enable them to chemically break down resistant debris that is relatively unavailable to bacteria and fungi (Tuomella *et al.,* 2000). Certain species of actinomycetes are thermotolerant and are found to be increasingly active at low nutrient levels and temperatures of up to 60 ºC (Royckerboer, 2003; Ashraf *et al.,* 2007). These species of actinomycetes such as *Thermoactinomycetes* and *Saccharomonospora* spp. appear during the thermophilic phase (Sylvia *et al.,* 2005). The most commonly occurring actinomycetes found in the end of the composting process form long, threadlike branched filaments that resemble grey spider webs (Guatam *et al.,* 2012; Barka *et al.,* 2016).

2.2.4 Protozoa in composting

Protozoa are only a small proportion of the huge microbial biomass in compost (Lee, 2016). Protozoa normally dwell in water droplets within compost and they feed on the bacteria and fungi (Ch'ng *et al.,* 2013). In composting, protozoa have important responsibilities related to disease suppression and nutrient cycling (Nester *et al.,* 2007). By feeding on bacteria which have high nitrogen contents, the protozoa make a significant impact on the nitrogen cycle in the compost (Chavan *et al.,* 2015).

2.3 Lignocellulolysis

Lignocellulose of plant biomass is chemically complex consisting of major polymers such as cellulose, hemicellulose, pectin and lignin (Sorek *et al.,* 2014). The polymers consist of insoluble, crystalline microfibrils which are highly resistant to degradation by enzymatic hydrolysis. Lignocellulose of plant biomass is thus generally resistant to decomposition (Fernandes *et al.,* 2011). The abundance of lignocellulosic waste such as agricultural residue, industrial and forest waste, and the need for their biodegradation and bioconversion has increased the demand for more effective lignocellulolytic microorganisms (Ashita *et al.,* 2000; Sorek *et al.,* 2014). Lignocellulolytic microorganisms are those capable of degrading and utilizing lignin, cellulose and hemicellulose as carbon and energy sources (Dashtban *et al.,* 2009). Lignocellulolytic microorganisms carry out a critical role of recycling this abundant plant biomass in the biosphere (Zhang *et al.,* 2006; Hubbe *et al.,* 2010).

Efficient degradation of lignocellulosic biomass involves a cooperative action of various microorganisms producing multiple enzymes that act specifically and synergistically (Saini *et al.,* 2015). During composting, the capacity of microorganisms to degrade the composting feedstock depends on their ability to produce the appropriate enzymes (Dashtban *et al.,* 2009). All microorganisms that are known to effectively degrade lignocellulosic materials produce ligninase and cellulase enzymes with different specificities (Zhang *et al.,* 2006; Fernandes *et al.,* 2011).

Both fungi and bacteria have been heavily exploited for their abilities to produce a wide variety of ligninase and cellulase enzymes (Hubbe *et al.,* 2010). However, most emphasis has been placed on the use of fungi because of their capability to produce copious amounts of these enzymes than bacteria (Berlin *et al.,* 2005). Most fungi secrete large amounts of extracellular ligninase and cellulases that are very efficient in depolymerising lignocellulosic substrates (Hubbe *et al.,* 2010; Dashtban *et al.,* 2015).

Cellulases are a consortium of free enzymes comprised of endoglucanases, exoglucanases cellobiohydrolase and cellobiases (Siddiqui *et al.,* 2000). Study of cellulases at molecular level has revealed some of the features that contribute to their activity. However, despite the considerable diversity, sequence comparisons show that the catalytic cores of cellulases belong to a restricted number of families (Dashtban *et al.,* 2010; Gatuam *et al.,* 2012). Within each family, the various enzymes share a common folding pattern, the same catalytic residues and the same reaction mechanism (Zhang *et al.,* 2006).

In most lignocellulolytic microbes, cellulase synthesis is repressed in the presence of easily metabolized, soluble carbon sources and induced in the presence of cellulose (Hubbe *et al.,* 2010). Induction of cellulases appears to be effected by soluble products generated from cellulose by cellulolytic enzymes synthesized constitutively at a low level. These products are presumably converted into true inducers by transglycosylation reactions (Gatuam *et al.,* 2012).

2.4 Molecular characterization of lignocellulolytic microorganisms

Microbial diversity during composting can be investigated using phenotypic, physiological, biochemical and genotyping techniques (Overeas, 2000; Bohannan and Hughes, 2003). Phenotypic methods utilize cultural and morphological properties including growth rate and colony characteristics on culture media (Li *et al.,* 2016). Molecular gene typing techniques complement conventional methods that are still indispensable for the complete study of microorganisms as they provide pure cultures that can be used for additional characterization (Ryckeboer *et al.,* 2003). However, genotyping techniques are more advanced because they can effectively differentiate genera, species and strains of microorganisms being studied (Bohannan and Hughes, 2003). Molecular techniques have overcome the major disadvantage associated with the impossibility of detecting non-cultivable microorganisms with the development of new systems (Sohier *et al.,* 2014). Currently there are a wide range of molecular methods available mainly relying on extraction of DNA, RNA or proteins (Bartlett and Stirling, 2003).

Sanger sequencing is one of several approaches used to sequence DNA products for molecular characterization of organisms (Tyson *et al.,* 2004). It is among the first methods to be applied in molecular studies (Hugenholtz *et al.,* 1998). Although more advanced techniques such as the next generation technology (NGS) are becoming more popular, Sanger sequencing is still very useful, providing results that are still reliable for genotyping (Tyson *et al.,* 2004; Rastogi and Sani, 2011).

Sanger sequencing requires the extraction of DNA from test organisms (Bartlett and Stirling, 2003). The DNA extracted should be in sufficient amounts and of high quality for subsequent downstream processing (Rastogi and Sani, 2011). Different DNA extraction methods yield different quantity and purity of extracted DNA (Bartlett and Stirling, 2003). Successful extraction of both high quality and high molecular weight genomic DNA is actually the key challenge for downstream applications (Bag *et al.,* 2016).

The nature of the cell wall of the microbial cells should be considered when choosing DNA extraction methods. This is because; the structure of the cell wall determines the effectiveness in lysing the cells in order to access the DNA (Relman, 1999; Venter *et al.,* 2004). To obtain sufficient amount of DNA from microbial cells with known resistant cell walls, it is important to pretreat the samples before applying the lysing reagents. However, harsh sample treatment can affect DNA quality, while mild processing can result in partial lysis especially for microorganisms having thick peptidoglycan layers in their cell walls (Bartlett and Stirling, 2003; Bag *et al.,* 2016). Therefore, cell lysis methods should be optimized in order to obtain genomic DNA of the required quality and quantity (Brooks *et al.,* 2015). Lysis of microbial cells exposes their genomic DNA to diverse cellular and extracellular molecules including different types of nucleases (Virgin and Todd, 2011). Despite its inert nature, the double stranded DNA molecule is physically fragile and highly susceptible to active forms of exo and endonucleases. Therefore, inactivation of all the nucleases in lysis solutions is necessary. This is normally attained by incorporating strong denaturing agents or chemicals that chelate residual metallic ions from the suspension (Venter *et al.,* 2004; Bag *et al.,* 2016).

Several commercial kits are available for use in extracting genomic DNA from samples. Most of these kits use silica-based column in which DNA adsorbs selectively to a stationary solid phase at high pH and high salt concentration (Brooks *et al.,* 2015). Different laboratories use varied techniques for DNA extraction depending on the type of samples and cost implications (Virgin and Todd, 2011).

DNA sequencing targets either whole genomes or selected genes such as 16S rRNA (ribosomal RNA) and Internal transcribed spacer (ITS) region, for bacteria and fungi, respectively (Clarridge, 2004). Before sequencing is done, the target region/ genome in the extracted DNA is amplified via polymerase chain reaction (PCR) (Hugenholtz *et al.,* 1998). After sequencing is completed, classification of the organisms is done by estimating the phylogenetic relatedness to known organisms based on the homology of 16S rRNA or ITS sequences. The closest affiliation of a new organism or gene sequence is then assigned (Tyson *et al.,* 2004; Ghebremedhin *et al.,* 2008).

The 16S rRNA gene sequence has 1500 base pairs in length on average and comprises of variable and conserved regions. This gene is large enough with adequate interspecific polymorphisms to offer different and statistically valid analysis (Virgin and Todd, 2011). Primers are usually used to sequence the gene as complementary to the conserved regions and the sequence of the variable regions (Relman, 1999).

The ITS region is the most widely sequenced DNA region in molecular study of fungi and has been recommended as the universal fungal barcode sequence (Peay *et al.,* 2008; *Schoch et al.,* 2012). It has primarily been most applicable for molecular classification at the species level as well as within species (White *et al.,* 1990). The complete ITS region in fungi has an average length of between 500 and 700 base pairs across all fungal lineages (Porter and Golding, 2011). Among different regions of genes, the internal transcribed spacer (ITS) region has been shown to have the highest chances for successful identification of the highest number of fungi (Schoch *et al.,* 2012).

2.5 Factors that affect composting process

During composting, a favourable environment must be provided in order to promote growth of microorganisms as a way of increasing decomposition of the organic matter.

These conditions relate to temperature, pH, oxygen concentration, moisture content and nutrients availability (Insam and Klammer, 2002; Umsakul *et al.,* 2010). Changes in these parameters are critical to the composting process.

2.5.1 Inoculation

Inoculation can be a useful tool to accelerate the breaking down of lignocellulose components in composting materials. Inoculation as a strategy of enhancing lignocellulose degradation can be done using lignocellulolytic microorganisms (Lim *et al.,* 2015). The inoculation can increase the composting rate and the product quality because progress of composting process and suitability of compost as a soil conditioner are determined by microbial activity (Goyal *et al.,* 2009; Zeng *et al.,* 2011; Lim *et al.,* 2015). The application of these microbes to compost could also increase the efficacy of compost towards the control of different soil-borne plant pathogens (Gautam *et al.,* 2012).

Previous studies have proved that addition of microbial inocula leads to higher degradation of lignocellulose than in non-inoculated materials (Zeng *et al.,* 2011). A higher efficiency was observed for lignin, when three microorganisms; *Bacillus shackletonni, Streptomyces thermovulgaris* together with *Ureibacillus thermosphaericus* were assessed as inoculants during composting (Peters *et al.,* 2000). In a study by Wei *et al.* (2007), process inoculation with *Bacillus casei, Lactobacillus buchneri, Candida rugopelliculosa* and *Trichoderma* spp enhanced the degree of compost humification with a high scope of aromatization of humic acids than in the control with no inoculation.

Studies on compost enrichment with microbes also suggests the role of inoculation with mixed cultures in converting organic waste into multi-functional bio-fertilizers for bioresource recycling and sustainable agriculture applications (Lim *et al.,* 2015). The inoculation of these functionally active and pathogen suppressing microbes into less effective composts could improve the quality of composts for their disease suppressiveness and plant growth promoting qualities (Vida *et al.,* 2016).

2.5.2 Temperature

Temperature changes in composting influences the rate at which most biochemical reactions occur (Insam and Klammer, 2002; Pan and Sen, 2013). Temperature is an indicator of bioavailability of nutrients and the setting in of potential limiting factors. It is also correlated with the capacity of the composting process to eliminate pathogens (Bustamante *et al.,* 2008).

Temperature rises during composting because the materials being composted acts as a self-insulating matrix that retains heat produced through microbial activity (Ranalli *et al.,* 2001). This heat generation is fundamental for the succession of microbial communities throughout the bioprocess. Temperature affects the metabolic activity of microbial decomposers which in turn determines how well they break down organic matter (Insam and Klammer, 2002). High temperatures characterize aerobic composting and serve as a sign of rigorous microbiological activities. The ideal temperature for initial decomposition is between 20 and 40 °C, but it increases to above 50 at subsequent stages (Ngugen *et al.,* 2007).

During the composting process, temperatures vary within mesophilic and thermophilic ranges, subdividing the composting process into three different phases, comprising of mesophilic, thermophilic and cooling and maturation phase (Ranalli *et al.,* 2001). During the mesophilic state, bacteria, fungi and actinomycetes that grow best in warm temperatures are dominant and as they carry out cellular metabolic activities heat is released and the temperature rises above 40 °C. The rapid increase in temperature involves a transition from a mesophilic to a thermophilic microbial flora (Ryckeboer *et al.,* 2003). As temperatures continue to rise, pathogens and weed seeds within the compost pile are destroyed (Insam and Klammer, 2002). This phase is therefore regarded as being very critical for sanitization of compost. Generally, the higher the temperature, the more efficient will be the destruction of pathogens (Ryckeboer *et al.,* 2003). However, too high temperatures deactivate mesophilic microbes whereas a longer duration of high temperatures accelerates nitrogen loss as ammonia through volatilization (WERL, 2005). However, temperatures higher than 70 ºC, deactivate even most of the thermophilic bacteria (Ryckeboer *et al.,* 2003). Finally, the temperature falls initiating a cooling phase, mesophilic microbes re-colonize the substrate and initiate compost maturation (Peters *et al.,* 2000). Mechanical turning and aeration is used to regulate temperatures within a compost pile (Drechsel and Kunze, 2001).

2.5.3 Moisture

Water is a very critical aspect of a composting system (Arvanitoyannis *et al.,* 2006). Water functions as the solvent for substrates, a medium for heat storage and as temperature adjusting substances through evaporation. Moreover, microorganisms that drive degradation of composting materials have a physiological need for water (Drechsel and Kunze, 2001; Umsakul *et al.,* 2010).

Theoretically, the amount of water in composting materials could go up to 100 % without causing detrimental effects itself. However, as the water level rises, the rate of oxygen diffusion goes down. The oxygen becomes inadequate to supply the metabolic requirements of the composting process and it slows down becoming anaerobic (Ngugen *et al.,* 2007). The anaerobic environment lowers the rate of organic matter degradation causing secretion and subsequent accumulation of organic acids coupled with denitrification (Jusoh *et al.,* 2013).

The actual maximum water content for successful aerobic composting however varies with feedstock used. For tough fibrous materials, the maximum moisture quantity can be much higher without destroying structural properties or making the materials to become soggy and compact (Kiyasudeen *et al.,* 2016). Kim *et al.* (2015) recommended optimum moisture content in compost to be 40– 60 % on dry mass basis for effective decomposition of organic materials. Beyond this range, microbial activities of aerobic microorganisms decrease considerably, since too dry or water-logged conditions result in decreased supply of oxygen. Moisture content is also important because it provides a medium for transporting dissolved nutrients needed for metabolic and physiological activities of microorganisms (Umaskal *et al.,* 2010).

Although water is normally added to the composting materials externally, it is also generated internally as the organic matter in the compost is mineralized (Butlera *et al.,* 2001). Approximately 0.5- 0.6 ml of water is produced per every gram of organic matter mineralized. However, net loss of water occurs through heat and convective transport of moisture during forced aeration and turning. This could reduce the moisture content to less than 40 % except if water is added externally (Umaskal *et al.,* 2010).

A dry compost pile does not decompose efficiently (Hubbe *et al* 2010; Partanen *et al.,* 2010). Optimum moisture levels required for the composting process can be maintained through regular addition of water to the composting pile (Drechsel and Kunze, 2001).

2.5.4 Oxygen

Biodegradation can take place in both aerobic and anaerobic conditions. However, composting occurs optimally in aerobic conditions (Kumar, 2011). Aeration is therefore regarded as a very essential factor that determines a successful composting process (Guo *et al.,* 2012). Inadequate aeration can cause anaerobic conditions due to insufficient oxygen, while excessive aeration could reduce the rate of composting via loss of heat, water and ammonia (Insam and Klammer, 2002). Aerobic composting needs massive amount of oxygen particularly at the initial stages. If oxygen is in short supply, growth of the aerobic microbes is interfered with, resulting in lower decomposition rates (Arvanitoyannis *et al.,* 2006). Optimal aeration depends on the composition of the raw materials and aeration methods (Guo *et al.,* 2012).

Under anaerobic conditions, some microbes function without oxygen (Kumar, 2011). However, anaerobic respiration is known to be less energy efficient due to the fact that it utilizes chemical elements such as sulfur, nitrates and carbon dioxide as electron acceptors unlike aerobic respiration that uses molecular oxygen (Hao *et al.,* 2001). Anaerobic oxidation releases ammonia, hydrogen sulphide, amines, methane, acetates, lactates and reduced metal ions. All these products are unpleasant due to their odour and toxic nature (Bustamante *et al.,* 2008). Undesirable odour during composting is thus characteristic of anaerobic conditions. To prevent such undesirable environment from developing, compost is best carried out under aerobic conditions (Guo *et al.,* 2012).

At the onset of decomposition, oxygen concentration within the composting material is about 15–20 % (almost similar to the normal concentration in the atmosphere) while carbon dioxide concentration varies from 0.5 to 5 % (Bustamante *et al.,* 2008). In addition, there is an oxygen gradient in the compost matrix and anoxic conditions may develop deep inside the compost pile, especially in passively aerated composting systems (Hao *et al.,* 2001). In such complex environments, microbial communities differ significantly between the outer surface and the inner sections of the compost matrix (Maeda *et al.,* 2010).

Microorganisms in compost oxidize carbon for energy using up oxygen and producing carbon dioxide (Insam and Klammer, 2002). As biological activity progresses, oxygen concentration decreases and carbon dioxide concentration increases. If the minimum oxygen concentration within the pile falls below 5 %, the activity of aerobic microbes would become ineffective. Oxygen levels higher than 10 % are considered optimal for driving aerobic composting (Arvanitoyannis *et al.,* 2006). Moreover, aeration removes excessive heat, water vapour and other gases trapped in the compost pile. It is particularly very important to remove any excess heat especially in very warm climates as the risk of overheating or fire is high. Aeration is thus indispensable for aerobic composting (Drechsel and Kunze, 2001). Oxygen is supplied through passive or forced aeration or by mechanically turning the compost materials.

2.5.5 C: N Ratio

C: N ratio of organic matter refers to the quantity of carbon relative to the amount of nitrogen present. All organic matter contains a certain amount of carbon combined with a lesser amount of nitrogen (Umsakul *et al.,* 2010). The standard recommended value for C: N ratio at the start of composting process is approximately 30: 1. However, this may vary depending on the materials being used with maximum decomposition occurring when there is adequate amount of carbon, nitrogen, phosphorus and other essential nutrients in the substrate (Insam and Klammer, 2002; Pourzamani and Ghavi, 2016).

C: N ratio is one of the parameters often used to evaluate the rate of decomposition during composting because it indicates the maturity of compost (Drechsel and Kunze, 2001). The C: N ratio is important because it signifies carbon and nitrogen sources for microbes taking part in the composting process. C: N value is also critical because of its effects on plants when organic matter is added to soil (Partanen *et al.,* 2010; Lee, 2016). A complete carbon turnover can be attained during aerobic decomposition of plant residues. As the composting process progresses, the C: N ratio is gradually reduced from

30:1 to about 10-18:1 in the resultant compost. Compost with a C: N ratio of less than 20 is considered mature and can be utilized without causing any negative effects on the plants or the soil (Surekha *et al.,* 2003).

Biological activity is reduced if the compost pile has too much carbon relative to nitrogen (Pourzamani and Ghavi, 2016).Several cycles of organisms are needed to burn the excess carbon via complex chemical processes. When organisms die, their cellular nitrogen and carbon become available for the next cycle of organisms. The new group of organisms develops new cells which again require nitrogen to burn excess carbon and they release carbon dioxide (Lee, 2016; Fourti *et al.,* 2013). When this happens, the amount of carbon reduces and the limited amount of nitrogen is recycled. Eventually, when the ratio of available carbon to available nitrogen becomes significantly low, nitrogen is released as ammonia (Surekha *et al.,* 2003). Under suitable conditions, some ammonia may be converted to nitrates. Other nutrients such as phosphorus, potassium and micronutrients necessary for microbial growth are normally available in more than required amounts in compostable materials (Zhao *et al.,* 2012).

2.5.6 pH

pH affects composting by influencing growth of microorganisms by determining the solubility of nutrients within growth medium hence nutrient availability (Pan and Sen, 2013). During decomposition, pH generally varies between 5.5 and 8.5. The initial pH is influenced by the nature of the substrate being decomposed (Pourzamani and Ghavi, 2016). When the initial pH is between 6.0 and 7.0, the pH of the composting pile may drop slightly during the first 2 or 3 days of aerobic decomposition, or due to the formation of organic acids. If the pH is between 5.0 and 5.5, there will be insignificant changes during this period. After about four days of composting, the pH usually starts to rise and levels off at between 8.0 and 9.0 towards the end of composting (Goyal *et al.,* 2005; Pan and Sen, 2013).

Maintenance of pH during composting does not require special adjustment if the material is kept aerobic (Surekha *et al.,* 2003). However, during the early stages of composting, large amounts of organic acids are usually generated during anaerobic decomposition. The organic acids accumulate within the substrate and this causes a reduction in pH which accelerates the growth of fungi (fungi grow best in acidic conditions) (Insam and Klammer, 2002; Ashraf *et al.,* 2007). Usually, the organic acids disintegrate into other compounds as decomposition progresses, and the pH rises (Ch'ng *et al.,* 2013). Rise in pH is also attributable to volatilization of the organic acids and release of ammonia by microbes as they break down proteins and other nitrogenous substances (Umsakul *et al.,* 2010). Later in the composting process, the pH tends to become neutral as the ammonia is either lost to the atmosphere or incorporated into new microbial cells. Mature compost generally has a pH between 6.7 and 8 (Insam and Klammer, 2002; Goyal *et al.,* 2005).

2.6 Compost maturity and stability

Before using compost as soil fertilizer and conditioner, it is necessary to assess its sanitary, maturity and stability levels (Bazrafshan *et al.,* 2016). One of the most important properties that determine the suitability of compost for various agricultural applications is the degree of maturity and stability (Kiyasudeen *et al.,* 2016). Maturity and stability levels of compost indicate compost quality (Arvanitoyannis *et al.,* 2006).

Compost maturity means the degree of decomposition of phytotoxic organic compounds produced during active composting stages (Hogg *et al.,* 2002), and the proportion of stable humus produced as a result of the tranformation of organic matter (Wu *et al.,* 2000). An optimum degree of compost maturity is attained when the compost is stable but still active enough to support microbial activity, especially when considering its use as a biological control or suppressive agent (Barral and Paradelo, 2011).

A stable compost is one whose organic matter is at an advanced state of degradation, which is resistant to further decomposition (Arvanitoyannis *et al.,* 2006). This is the principal requirement for safe application of compost to soil, as it implies a stable organic matter content and absence of microbial activity. The physicochemical stability of compost determines its shelf-life and the suitability of the compost for various agricultural uses (Hogg *et al.,* 2002; Bazrafshan *et al.,* 2015).

Mature composts are ready for agricultural applications and they contain negligible or acceptable amounts of phytotoxic elements like NH_3 or short chain organic acids (Stoffella and Kahn, 2001). However, some phytotoxic properties of compost such as soluble salts and presence of persistent herbicides are mainly associated with feedstockquality (Wichuk and McCartney, 2010).

Immature composts inhibit plant growth and they can cause continuous evolution of phytotoxic gases that can even harm the environment (Villar *et al.,* 2016). If immature compost is applied to soil, it can cause anaerobic conditions as the microorganisms utilize oxygen in the soil pore spaces to break down the materials (Mahmoodi and Zazouli, 2015). These effects occur because the compost activates high microbial activity (which reduces oxygen concentration in the soil) and immobilizes the existing soil available nitrogen (Arvanitoyannis *et al.,* 2006). Another problem caused by immature compost is the phytotoxicity related to presence of organic acids formed during the early stages of the composting process (He *et al.,* 1995).

Standard indicators of compost maturity and stability consist rate of germination and the amount of soluble organic matter (humic and fulvic acids), which denote the degree of humification (Wu *et al.,* 2000). A stable and mature compost also has stable values of several indicators such as respiration rates, microbial load and biomass, organic matter content and C: N ratio (Wichuk and McCartney, 2010). Other indicators of compost maturity include colour, oduor, volatile solids reduction, cation exchange capacity, C: N ratio and inorganic nitrogen values (Tiquia, 2010).

Among these, germination index (GI) and plant growth index (PGI) are the most common techniques used in compost phytotoxicity tests (Kapanen and Itavaara, 2001). Germination index test is a good method for rapid evaluating of phytotoxicity, while plant growth index test can give a better estimation of compost impact on plant growth for a longer time (Gomez-Brandon *et al.,* 2008). Thus use of GI or PGI tests can thus be determined from practical needs and time requirement (Ko *et al.,* 2008).

2.6.1 Germination index (GI)

Germination indexwas first introduced by Zucconi *et al.* (1981). It is used to evaluate toxicity of compost to seedlings to establish if the compost is mature (Zucconi *et al.,* 1981a; Lasaridi *et al.,* 2006). It measures time taken for seeds to germinate and roots to elongate (Barral and Paradelo, 2011). Germination indexallow for evaluation of both low levels of toxicity, that affect root growth, as well as high levels of toxicity, which influences seed germination (Zucconi *et al.,* 1981). It is based on a relatively simple to perform germination bioassay that quantifies seed germination and root elongation after treating the seeds with compost water extracts (Lasaridi *et al.,* 2006). Compost extracts are filtered products of compost mixed with any solvent, usually water, but not fermented or brewed.Immature compost may contain phytotoxins that in most cases end up killing seed embryos. Seeds grown using immature compost will normally not sprout or may die immediately after sprouting (Fuentes *et al.,* 2004; Araujo and Monteiro, 2005). Compared to root elongation, seed germination bioassay has relatively low sensitivity. The seed is less sensitive to many toxic substances, because many chemicals may not be absorbed by seeds and the embryonic plant draws its nutritional requirements internally from seed stored materials (Paradelo *et al.,* 2008). However, the roots are responsible for absorption and accumulation of elements from the growth medium so the root lengths are more affected by the concentration of the compost (Oncel *et al.,* 2000; Araujo and Monteiro, 2005).

The seed phytotoxicity test computes GI as the percentage of germination and root elongation of selected seeds compared to a control, usually being deionized or distilled water (Araujo and Monteiro, 2005). There are large variations among various types of seeds that have been used in compost phytotoxicity studies (Tiquia and Tam, 1998). Cress (*Lepidium sativum*) seemingly is the most commonly used (Lasaridi *et al.,* 2006). Other seeds that are commonly used include tomato (*Solanum lycopersicum* L), Chinese cabbage (*Brassica campestris*) and radish (*Raphanus raphanistrum*) (Fuchs, 2002; Doncean *et al.,* 2013).

2.6.2 Plant growth index (PGI)

Use of aqueous extracts of the compost provides the relevant information but does not offer a complete description of toxicity that should not only take into account the fraction of contaminants dissolved in the water at that time but also the fraction associated with the solid matrix (Oleszcuzuk, 2008). Direct growth tests allow overcoming this problem. Although the seed germination test has been widely used, it should be noted that this stage of plant growth is mainly insensitive to many toxic chemical because the embryo is isolate from the environment and many chemicals are not absorbed by the seed which supplies nutrients to the embryo (Araujo and Monterio, 2005; Barral and Paradelo, 2011). Being a more sensitive method for phytotoxicity assessment, outcomes of PGI are very critical in predicting plant growth response upon application of composts (Baffour-Asare, 2009).

2.6.3 Humification index (HI)

During the composting process, the organic material undergoes humification resulting in the conversion of the material into humus like substance (Das and Dkhar, 2012). The content of humic materials is a key indicator of the compost quality and its efficacy in nutrient supply, especially as a soil conditioner for improving water retention and soil fertility (Baran, 2002).

Humus is different from other non humic substances such as carbohydrates, fats, waxes, alkanes, peptides, amino acids, proteins, lipids and organic acids (Komilis and Kanellos, 2012). Most of the non humic substances are quickly broken down by microorganisms while as the soil humus is slowly degraded under natural conditions. When in combination with other soil minerals, humus can survive in the soil for several hundred years (Kaiser and Guggenberger, 2007).

Humic substances (HS) are consisted of three major components: humic acid (HA), fulvic acid (FA) and humin. Humic acid (HA) and fulvic acid (FA) are major fractions of humic substances and represent a significant component of compost (Baran*,* 2002). Now that the content of HA in the compost is a result of breakdown of cellulose and lignin and to the polymerization of phenolic compounds (Eusterhues *et al.,* 2003)**,** the content of HA in compost is associated to degradation of the organic matter. Furthermore, because there is a steadily rising prevalence of HA relative to FA during composting, the ratio between them signifies an important index of compost maturity (de Bertoldi *et al.* 1983).

Humic acids comprise the greatest of the three fractions, which make the most basic ingredients of fertile soils due to their intense direct and indirect means of action on soil chemical, physical and biological attributes (Tan, 2003; Canellasand Facanha, 2017). Humic acids can significantly lower the rate of water evaporation and promote its use by plants. Consequently, the humic acids are responsible for increased water holding capacity of soils (Turan *et al.,* 2011). Humic acids assist in remediating plant chlorosis, increase the permeability of the plant membranes and strengthen enzyme systems of plants (Barral and Paradelo, 2011). They accelerate cell division, induce greater root development and contract effects of stress. Presence of humic acids causes plants to grow stronger and resist diseases better (Trevisan *et al.,* 2010).

Another is the direct and positive influence on their bioavailability. It can also detoxify the soil of heavy metals (Tan, 2003). Research has shown that heavy metals can be "locked up" with the addition of humic acid. Humic acids are capable of interacting with contaminants and can be applied to environmental remediation (Zhang *et al.,* 2015).

In order to decide on appropriate environmental and agricultural applications of humic substances (HSs) in compost, assessment of the compost quality based on HS content is necessary. Humification index (HI), which refers to the ratio of HSs to non-HSs, is recognized as a useful parameter for estimating the HS content of compost (Fukushima *et al.,* 2009).

Both the HS quality and the HS content are determined by compost maturity. Factors such as atomic ratios of carbon to hydrogen, oxygen to carbon and carbon to nitrogen, acidic functional group content, molecular weight and spectroscopic parameters influence the degree of humification and also impact on the HS quality (Tan, 2003; Canellasand Facanha, 2017). Composts containing high amounts of humic acid are effective soil amendments, especially for increasing and sequestering soil carbon to remediate contaminated soils and to control nutrient runoff (Conte *et al.,* 2005). Information on the ratios of humic acids (HA) to fulvic acids (FA) is useful in predicting possible reactions of the organic matter portion with soil components since FAs are more soluble and reactive compared to HAs (Komilis and Kanellos, 2012; Canellas and Facanha, 2017). During humification, chemical properties such as total exchangeable acidity, carboxyl group content and N-containing functional group content increase (Kaiser and Guggenberger, 2007).

2.6.4 Respiration index

Measurement of microbial respiration rates is often used to monitor the composting process and assess compost maturity (Wu *et al.,* 2000; Boulter-Bitzer *et al.,* 2006; Scaglia *et al.,* 2007; Komilis and Kanellos, 2012). Oxygen (O_2) uptake rate, carbon dioxide (CO_2) production rate or the heat liberated change as a result of microbial activity (Iannotti *et al.,* 1994).

Tests for evaluating compost stability through latent metabolism include respiration activity and heat production, both of which are indicative of the amount of degradable organic matter still present and which is inversely related to stabilization. Such tests generally provide near optimum conditions for microbial respiration (Boulter-Bitzer *et al.,* 2006). Respiration is directly related to the metabolic activity of a microbial population rate (Scaglia *et al.,* 2007). Microorganisms respire at higher rates in the presence of large amounts of bioavailable organic matter while respiration rate is slower if the material is scarce (Barrena *et al.,* 2006).

Respiration is estimated by rate of CO_2 evolution or O_2 uptake (Iannotti *et al.,* 1994). A decreasing respiration rate signifies a reduction in biodegradable carbon and increasing carbon stability (Lasaridi *et al.,* 2006). Respirometric studies usually determine O_2 consumption or CO_2 production caused by mineralization of the compost's organic matter. Such studies can be performed using pure composts or compost mixed with soil in a ratio relevant to a particular agricultural use (Iannotti *et al.,* 1994; Brinton, 2001). Composts which are not mature induce a high demand for O_2 with generation of a lot of CO_2 due to rapid proliferation of microorganisms caused by abundance of readily biodegradable substances in the raw materials. Therefore, O_2 consumption or CO_2 production are reflective of compost stability and maturity (Boulter-Bitzer *et al.,* 2006).

Respirometric tests are determined by a number of factors such as temperature, humidity and both incubation and preincubation conditions (Barrena *et al.,* 2006). Sayara *et al.* (2010) evaluated the quality of composts with different stability (as denoted by respiration rates) in remediating soil intentionally contaminated with polyaromatic hydrocarbons (PAHs). The results showed that stable composts biodegraded up to 98 % of the PAHs after 30 days, where as the most unstable (poorly composted) compost attained only 40 % degradation of the PAHs.

It should however be noted that, a low respiration rate does not always necessarily indicated that phytotoxicity will not occur (Lasaridi *et al.,* 2006). This is especially true when microbial activity is obstructed by high metal concentration. Wu *et al.* (2000) discovered that compost samples from a composting facility demonstrated phytotoxicity despite having a low rate of evolution of carbon dioxide. Respiration index is intense during the early stages of composting, when easily degradable organic substances cause rapid proliferation of microbes but diminishes with time (Barrena *et al.,* 2006).

2.6.5 Heavy metal concentration in compost

Toxicity of compost is associated with the physiological availability of heavy metals (Smith, 2009). Regular and extensive application of toxic compost will result in accumulation of heavy metals in soil and an increase in toxicity (Zennaro *et al.,* 2005). However, in some cases, composts added to soils may act as a sink for heavy metals (Zang *et al.,* 2006). Heavy metal concentrations in composts are of great concern mainly due to their toxic effects on organisms and their potential for long term accumulation in food chains (Smith, 2009; Wong and Selvam, 2006). Heavy metals can alter growth, morphology and metabolism of soil organisms, consequently reducing soil fertility (Fuentes *et al.,* 2004). Presence of heavy metals in soil leads to their uptake by plants and successive accumulation in animal tissues. Bio magnifications occur along the food chain and this can adversely affect health of organisms and the environment (Chibuike and Obiora, 2014). Composting can aid in promoting complexation of heavy metals especially metals whose mobility and availability decrease with decreasing toxicity (Anwar *et al.,* 2015).

Presence and persistence of heavy metals in compost could result in contamination of the receiving soils (Faustman and Omenn, 2001). Heavy metals inputs cannot be compensated by plant uptake or through leaching. This is particularly true for Pb, Cr, Ni and Cd and to a less extent for Hg, Cu und Zn (Himanen and Hanninen, 2011). The negative effect can be minimized by use of high quality composts with low heavy metal concentration (Zennaro *et al.,* 2005).

Bioavailability of heavy metals can vary depending on the type and maturity of the compost (Faustma and Omenn, 2001). It has been shown that heavy metals cause a notable delay in seed germination and that they can impede plant growth severely (Munzuroglu and Geckil, 2002). Results of a study by Ingelmo *et al.* (2012) on bioavailability of heavy metals (Zn, Pb, Cu, Ni and Cd) in a relatively low stability compost (C: N ratio of 22) show that metal ions (except Pb) become more bioavailable in the compost than in the original feedstock.

Unlike other organic contaminants such as ammonia which disappear during the composting process, most of heavy metals tend to remain in the final product (Ingelmo *et al.,* 2012). This constitutes a very important problem from an environmental and agricultural standpoint (Fuentes *et al.,* 2004). Consequently, it is necessary to evaluate the concentration and phytotoxic effects of heavy metals in compost (Bazrafshan *et al.,* 2015). Because composting is a biologically mediated process, it is important to know if high microbial activity during the active degradation phase could occasion degradation of heavy metal contaminants (Barker and Bryson, 2002).

2.7 Physicochemical properties of the compost

2.7.1 pH of compost

Organic matter mineralization results in the formation of organic and inorganic acids that provide H^+ to the compost thus altering the pH within the compost (Sun *et al.,* 2003).

Basically, a neutral (pH ± 7) favours biological activity and the transformation of organic matter. Within the typical pH range of between pH 5.5 and 7.5 there is no direct relation between pH and organic matter degradation that is, pH increase normally does not result in a decrease of organic matter) (Garcia-Gil *et al.,* 2003).

The most important effect of pH on the compost is on ion solubility, which in correspondingly influences microbial and plant growth due to the effect on plant nutrients. A pH range of 6.0 to 6.8 is ideal for most crops because it corresponds to optimum solubility state for the most essential plant elements (Zhang *et al.,* 2006). Minor elements such as iron and most heavy metals are more readily soluble at lower pH (Wuana and Okieimen, 2011).

pH levels for optimal availability for many nutrients are known. Phosphorus is highly available within a pH range of between 6 and 7, macronutrients especially N, K, Ca, Mg and S are readily available within a pH of between 6.5 and 8, while most micronutrients such as B, Cu, Fe, Mn, Ni and Zn are highly available in pH between 5 and 7 (Pourzamani and Ghavi, 2016). Beyond these optimal ranges, nutrients are available to plants at reduced amounts.With the exception of molybdenum (Mo), availability of micronutrients generally decreases as soil pH values approach 8 (Singer and Ewing, 2000). Metals such as copper, iron, nickel and zinc are tightly attached to the soil particles at high pH and are thus more available at low pH levels than high pH levels. This possesses potential metal toxicities risks for crops in acid soils. Conversely, basic cations such as calcium, potassium and magnesium are more weakly bound to the soil and are prone to leaching at low pH (Pourzamani and Ghavi, 2016; Fageria and Zimmermann, 1998).

Activities of soil microorganisms also are greatest near neutral conditions, but optimal pH ranges vary for each type of microorganism. Specifically, very acid soils (less than 5) cause microbial activity and their population densities to be substantially lower than in neutral soils (Garcia-Gil *et al.,* 2003). Studies have shown that certain 'specialized' microorganisms, such as nitrifying bacteria and nitrogen-fixing bacteria associated with legumes, generally perform poorly when soil pH is below 6 (Pourzamani and Ghavi, 2016; Sun *et al.,* 2003).

The impact of addition of compost to the pH of the soil or any other growth medium depends on the pH of the compost and the amount added. Each specific plant species requires a specific pH range (Bennett *et al.,* 2014). Therefore, to estimate the effect of compost, which in turn will affect soil parameters and consequently plant growth and yield, pH is a necessary parameter of which to be aware. Most compost has a pH of between 6 and 8 (Fischer and Glaser, 2012).

2.7.2 Cation Exchange capacity (CEC)

Cation exchange capacity of compost is an estimate of the quantity of positive charges per unit weight of compost, or the measure of cations in exchangeable state a given compost sample contains (Brady and Weil, 1999). As composting progresses, aromaticity, alkyl C and carboxyl groups increase while concentration of polysaccharides and aliphatic chain declines (Kopittke and Menzies, 2007). This results into an increase in cation exchange capacity of the compost. A high cation exchange capacity is essential in for plant growth during the use of compost as a soil conditioner or in potting mix (Butterfly *et al.,* 2013). The more the organic matter content, the higher the CEC, as such, different types of organic materials have different levels of CEC (Astera, 2014). Fine textured composts usually have a greater cation exchange capacity than coarse ones (Kopittke and Menzies, 2007).

Humus, the finished product of decomposed organic matter, has the highest CEC level because organic matter colloids have high numbers of negative charges (Butterfly *et al.,* 2013). Humus has CEC levels two to five times higher than montmorillonite clay and up to 30 times more than kaolinite clay making it very essential for promoting soil fertility. Cation exchange capacity is therefore very important for retention and supply of plant nutrients and for adsorption of contaminants (Brady and Weil, 1999).

Cation exchange capacity shows the compost's ability to supply nutrients to a plant (Astera, 2014). Depending on the CEC level, the exchanged nutrients are either absorbed by the plant, get leached or lost through erosion (Delgado, 2002). Composts with high CEC are able to bind more cations such as Ca^{2}+ and K+ to the exchange sites of organic matter particle surfaces. Such composts will also have a higher buffering capacity which enhances the soil's ability to resist changes in pH (Kopittke and Menzies, 2007). Compost with high amounts of organic matter normally has higher CEC and buffering capacities than those with less organic matter (Butterfly *et al.,* 2013). Low pH generally causes lower CEC, because the higher concentration of H+ ions in solution will neutralize the negative charges on organic matter (Butterfly *et al.,* 2013).

2.8 Impact of addition of compost into soil

2.8.1 Role of compost in suppressing disease causing microorganisms

Compost application to agricultural fields is an excellent natural approach used to fight against plant diseases through suppression of soil-borne plant pathogens (Aviles *et al.,* 2011). The role of composts in disease suppression was first suggested by Hoitink *et al.* (1975). It was shown that addition of organic matter to soils directly or indirectly suppresses disease causing microorganisms. The soil becomes more microbially active and more suppressive to soil borne pathogens (Dirk and Muntean, 2013). Soils managed with organic inputs generally have a more active microbial population than those managed with inorganic fertilizers, hence more resistant to soil borne plant diseases (Badr Eldin *et al.,* 2000).

Composts suppress plant diseases via the combined effects of physiochemical and biological actions (Postma *et al.,* 2003). Physiochemical components comprise any physical or chemical aspects of compost that lower disease severity by directly or indirectly influencing the pathogen or host capacity for growth (Aviles *et al.,* 2011). Such aspects encompass nutrient levels, organic matter, moisture and pH. Biological attributes involve compost inhibiting microbial communities by competing for nutrients with pathogens, production of antibiotic, production of lytic and other extra cellular enzymes, parasitism and predation, induction of host mediated resistance in plants and any other process that hinders disease development (Jeanine *et al.,* 2002; Blaya *et al.,* 2015).

The act of composting affects pathogenic populations that are present in organic waste (Aviles *et al.,* 2011). The main factors controlling eradication of pathogens during composting include exposure to high-temperatures, formation of toxic compounds during or after the self-heating process and microbial antagonism (Hoitink and Boehm, 1999; Blaya *et al.,* 2015). These factors operate in succession or at the same time. Heat is however the best basis for evaluating sanitation because toxins and microbial antagonism are more difficult to monitor (Postma *et al.,* 2003; Aviles *et al.,* 2011). Conditions prevailing in composts are generally extremely detrimental to most plant pathogens, although sensitivity to these conditions differs amongst pathogenic microorganisms (Hoitink *et al.,* 1996; Bonanomi *et al.,* 2007). According to Pugliese (2010), three objectives should be pursued during composting with respect to pathogenic microbes namely; prevention of growth and dissemination of pathogens, destruction of pathogens originally present and rendering the compost inhospitable for their regrowth.

The microorganisms present in compost constitute the major factor responsible for plant pathogen suppressiveness of composts (Noble and Coventry, 2005). In order to achieve consistent suppression effects a controlled inoculation has to be done (Postma *et al.,* 2003; Pan *et al.,* 2012). It is important to prepare tailor-made suppressive composts using appropriate inoculants and suitable raw materials in a controlled process to produce compost for the intended use (Bonanomi *et al.,* 2007).

Suppressive effects of composts are also dependent on compost maturity, with very young composts mostly showing low suppression (Erhartand Burian*,* 1997). Addition of well stabilized compost in soil produces better results in disease suppression than fresh organic matter (Bernal *et al.,* 2009). Excessive nutrient and energy contents of fresh organic material can suppress the production of essential enzymes of antagonists and thus distinctly impact their effectiveness (Noble and Coventry, 2005). Furthermore, pathogens are spawning in immature composts but are suppressed in mature composts (Bonanomi *et al.,* 2007). During maturation, the suppression potential of the compost increases in general (Erhartand Burian*,* 1997; Postma *et al.,* 2003). However, extremely stabilized organic material does not support the activity of biological control materials (Hoitink and Boehm, 1999). If maturity passes a certain stage, the organic matter is highly stabilized, whereas the microbiological activity becomes less and consequently the compost ceases to be disease suppressive (Hoitink and Boehm, 1999; Bonanomi *et al.,* 2007).

Only in some cases, fresh composts perform more efficient than mature composts (Bernal *et al.,* 2009). The microbial populations settling in composts change continuously during the decomposition process. Therefore, it follows that not all pathogens react in the same way on the maturity degree of composts (Noble and Coventry, 2005). For some pathogens, it is still not known whether the stage of maturity of compost plays a specific role (Postma *et al.,* 2003). According to Chef *et al.* (1983) chrysanthemum and hemp becomes more efficiently protected against a *Fusarium oxysporum* attack if treated with mature compost instead of fresh compost. However, Chefetz *et al.* (1996) could not find a correlation between the age of mixed waste compost and the protection of cotton against *Fusarium oxysporum f.sp. vasinfectum.*

Depending on the factor responsible for the action against pathogens, two different mechanisms can be differentiated as general and specific suppressiveness (Bonanomi *et al.,* 2007). The first one is associated to the activity of the whole compost microbiota and the competitive effect of antagonistic microorganisms, with no major intervention of specific microorganisms. This kind of suppressive action is determined by the amount of available decomposable organic matter, which in turn is depending on the dosage amended (Noble and Coventry, 2005; Yogev *et al.,* 2010). The other suppressive mechanism implies the action of specific microorganisms. Since the efficacy of such mechanism depends on the presence in compost of the active microorganism against the pathogen to be suppressed, variability is quite higher in these cases (Hoitink and Boehm, 1999; Yogev *et al.,* 2010). A possible solution for this drawback is the enrichment of compost with specific biocontrol agents by means of external supplementation (Hoitink *et al.,* 1996; Noble and Coventry, 2005).Therefore, specific disease suppressive effects of composts can only be guaranteed when composts are colonized by specific antagonists during composting. Therefore, for suppression of a particular disease with compost, inoculation of a particular microorganism (s) into compost must be done (Hoitink *et al.,* 2001).

2.8.2 Role of compost in bioremediation of contaminated soils

Soils that are contaminated with hazardous chemicals require remediation in order to restore them (Atlas and Bragg, 2009). Bioremediation has been shown to be an efficient, reliable and ecofriendly approach to the restoration of such soils (Barker and Bryson, 2002). In recent times, composting and compost addition to soil have been realised to be very successful soil bioremediation options (Kastner and Miltner, 2016). Due to the extensive and diverse metabolic power of microbes taking part in composting, a highly advanced metabolic diversity is established as a 'metabolic memory' within the composting materials (Aviles *et al.,* 2011). Compost addition to soil can thus be viewed as super-bioaugmentation' with a complex natural mixture of degrading microbes, coupled with a 'biostimulation' through the nutrients contained in organic substrates (Barker and Bryson, 2002; Kastner and Miltner, 2016). The compost also improves the abiotic soil conditions, thus enhancing soil microbiological aspects in general (Atlas and Bragg, 2009).

Bioremediation through composting can be achieved by mixing contaminated soils with fresh, high-energy feedstock or finished compost to the contaminated soils (Barker and Bryson, 2002). Composting and compost addition are thus important strategies that also act as stimulants of natural attenuation (Nilanjana and Chandran, 2011).

With respect to organic pollutants in soils, compost application can have various effects including adsorption (Kiyasudeen *et al.,* 2016). Compost bioremediation due to its adsorption capacity works by reducing the mobility of the toxic compounds (Buyuksonmez, 1999). In adsorption, positively or negatively charged organic molecules bind with their opposite charged counterparts in organic matter (Barker and Bryson, 2002). This way, toxic molecules can be bound and become less bioavailable (Anwar *et* *al.,* 2015). Adsorption of organic contaminants is therefore influenced by their surface charge and their solubility, both of which are affected by pH (Wuana and Okieimen, 2011).

Further, compost induced improvement of soil microbial activity contributes to an oxidative decay of pollutants (Kaur *et al.,* 2005). Higher microbial activity accelerates the degradation of pesticides and other synthetic organic compounds (Beesley, 2010). Microorganisms in compost are known to minimize the bioavailability of heavy metals, a fundamental aspect in the remediation of contaminated soil (Barker and Bryson, 2002; Beesley, 2010).

Composting has also been demonstrated as a useful approach to the remediation of soils contaminated with explosives, with a degradation rate of up to 99.7 percent 2,4,6-trinitrotoluene, 99.8 percent of hexahydro-1,3,5-trinitro-1,3,5-triazine and 96.8 percent octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine at the Umatilla Army Depot (Panz and Miksch, 2012). Six-month old compost mixed with petroleum contaminated soils was observed to degrade petroleum at a rate eight times faster than in natural conditions (Adebusoye *et al.,* 2007).

Plate 2.1: Rice straw produced in large amounts

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

Rice straw for use in laboratory, greenhouse and field experiments was obtained from Mwea, the main rice producing area in Kenya. Field experiments were carried out in Mwea at Horticultural Crop Directorate grounds while laboratory and greenhouse experiments were carried out in Department of Microbiology, Kenyatta University, in Nairobi, Kenya.

Mwea is situated in Kirinyaga County, about 100 Km South East of Nairobi (Fig. 3.1). Mwea has a Rice Irrigation Scheme at 0° 41' 1.98" S and 37° 21' 31.26" E, the largest rice growing scheme in East and Central Africa. The scheme consists of about 12,285 hectares of land and about 7,022 farm households. Both aromatic and non aromatic rice varieties are grown in this area, with Basmati 370 being the most common variety. About 95 per cent of rice is under irrigation while the remaining five per cent is rain fed. The average unit of production under irrigation is 5.5 tonnes per hectare for the aromatic variety and seven tonnes for non-aromatic varieties. With such huge rice farms, rice straw is generated in large quantities at the scheme among other rice residues.

In addition to rice farming, tomato, maize and french beans farming are major agricultural activities in Mwea. Moreover, donkeys are very common in Mwea because most farmers use them as a means of transport especially in their farming activities.

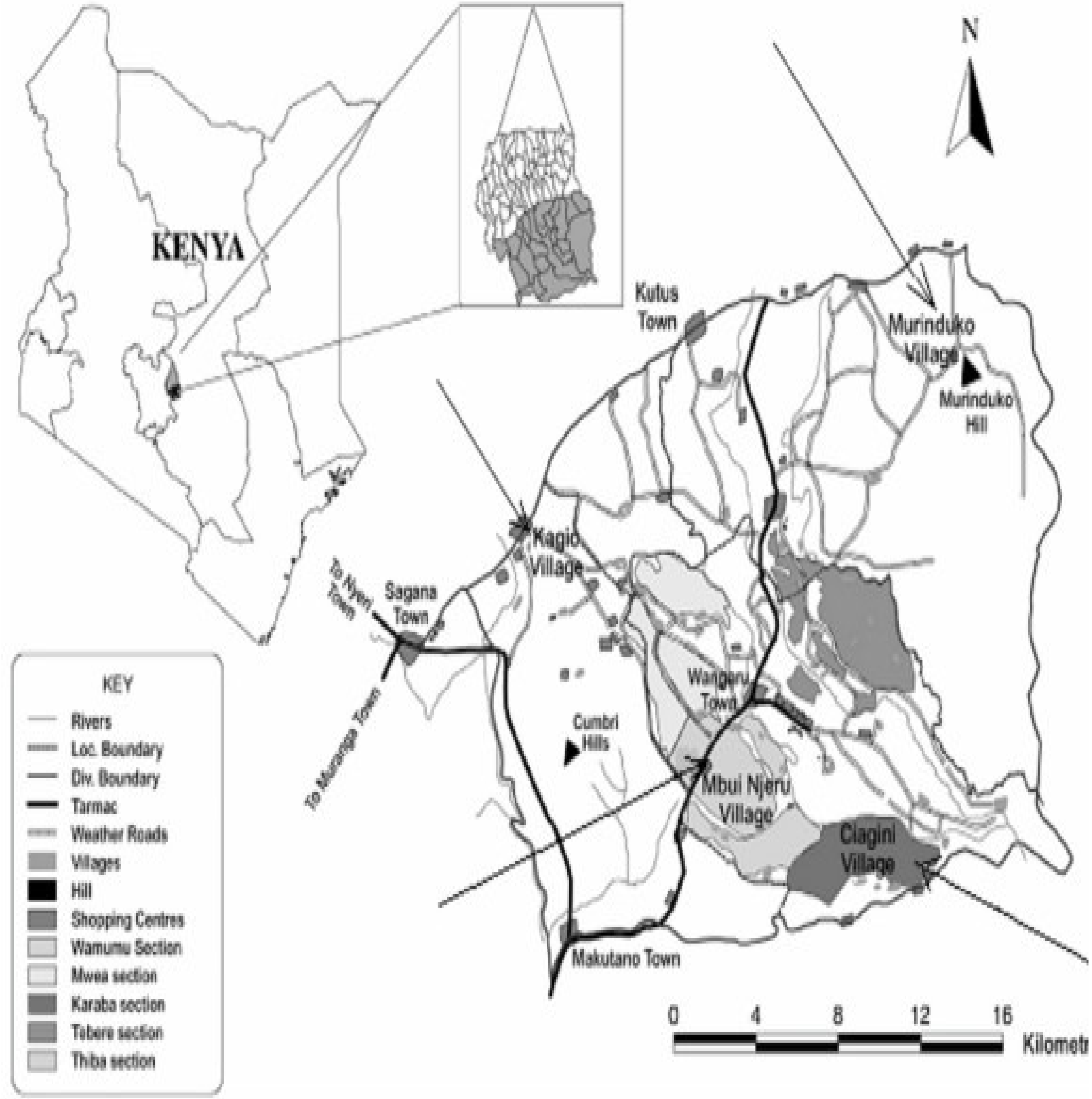

Figure 3.1: Map indicating the study site (Mwea) in Kirinyaga County, Kenya (Ng'ang'a *et al.,* 2008).

3.2 Research design

The study involved four main treatments consisting of rice straw treated with chicken droppings, commercial EM, locally constituted microorganisms and donkey dung. In addition to the four, a control was included. The product from the control experiment during composting eventually became the fifth treatment in subsequent tests. Field and greenhouse experiments were arranged in rows based on completely randomized design with three replications each.

3.3 Isolation of microbial inoculants

Two kilograms of straw of partly decomposed rice straw was was randomly collected from four rice fields in Mwea. Microorganisms to be used as a starter culture for composting experiments were isolated from the straw. Inocula for the isolation tests were prepared by shaking 10 grams of the partly decomposed rice straw in 90 ml 0.9 % sterile normal saline/ NaCl. Appropriate dilutions were prepared in the normal saline and inoculated onto Nutrient agar for bacteria and actinomycetes and Potato Dextrose Agar (PDA) for fungi in Petri plates. Inoculation onto the plates was done by spread plate method. The plates were then incubated at 37 °C for 24 to 48 hours for bacteria and actinomycetes; and at 28 °C for between 3 to 7 days for fungi. Colonies obtained were then purified by sub-culturing on appropriate culture media. The colonies were then categorized into several groups based on colony colour, shape, margin and elevation. The isolates were then subjected to biochemical and microscopic tests.

3.4 Ability to grow on rice straw

The microbial isolates obtained were evaluated for their ability to grow on rice straw without any other source of carbon. Rice Straw Agar (RSA) was prepared by constituting 20 g (dry weight) of straw (milled and sieved to 1.5 mm size particle) mixed with 1.5 g of gelatin, 20 g of Agar and 1000 ml of distilled water. The medium was autoclaved at 121 °C for 18 minutes and then dispensed into Petri dishes. A colony of each test strain was streaked on the RSA agar and another on Nutrient agar plate as a control for bacteria and actinomycetes. For fungi, a disk of mycelium was placed on the RSA plate and in PDA plate to act as a control. The plates were then incubated at 37 °C for 24 to 48 hours and at 28 °C for between 3 to 7 days for bacteria, actinomycetes and fungi, respectively. The plates were examined daily to assess microbial growth and growth expressed in terms of relative comparison against the control (Pointing, 1999).

3.5 Lignocellulose degrading ability

Cellulose degrading ability was tested using filter paper degradation and Carboxymethyl cellulose (CMC) agar colonization while lignin degrading ability was tested using tannic acid, Phenol Red, Methyelene Blue and Azure II tests.

3.5.1 Filter paper degradation

A loopful of fresh bacterial/ actinomycete culture or a 5 mm disk of fungal mycelium for all the isolates was inoculated in triplicate on Nutrient agar and PDA agar Petri dishes for bacteria/ actinomycetes and fungi, respectively. After inoculation, a sterile filter paper was laid aseptically in each Petri dish. The plates were then incubated at 37 °C and at 28 °C (for bacteria, actinomycetes and fungi respectively) for between 3 to 7 days. Microbial growth was examined every day. Filter paper colonization indicates cellulolysis (Thorn, 1993).

3.5.2 Carboxymethyl cellulose agar test

A loopful of fresh bacterial culture or a 5 mm disk of fungal mycelium for all the isolates was inoculated in triplicates on 1 % CCM agar Petri dishes The plates were then incubated at 28 °C for 10 days and microbial growth examined daily. The plates were then flooded with an aqueous solution of Congo red (1 % in distilled water) and allowed to stand for 15 minutes. Excess Congo red was poured off and the plates were further flooded with 1 N NaCl for 15 minutes. Development of a clear zone indicates cellulolysis (Thorn, 1993).

3.5.3 Methylene Blue, Azure II and Phenol Red degradation

To select microorganisms capable of producing ligninolytic enzymes (laccase, lignin peroxidase and manganese peroxidase), a selection protocol based on their ability to decolourize synthetic dyes Azure II, Phenol Red and Methylene Blue was used. These dyes are structurally similar to lignin (Pointing, 1999). The dyes were filter sterilized and added to autoclaved culture media under aseptic conditions. Nutrient agar and PDA agar plates containing 0.2 g/ 100 ml Azure II (Archibald, 1992), 0.1 g/ 100 ml Phenol Red and 0.2 g/ 100 ml Methylene Blue (Manji and Ishihara, 2004) were streaked with test isolates and incubated at 37 °C and 28 °C for 2- 5 days for bacteria/ actinomycetes and fungi respectively. The plates were observed for zone of decolourization after the appropriate incubation period. Decolourized zone that appeared around the colony indicated the presence of ligninolytic enzyme activity in the isolate; thus, isolates which decolourized any of the dyes were considered as positive for lignin degradation and selected for use in composting experiments (Pointing, 1999).

3.5.4 Tannic acid test

Phenoloxidase was detected by inoculation of test isolates on nutrient agar and PDA agar supplemented with 0.5 % (w/v) filter sterilized tannic acid. After 2- 5 days of culturing various colonies, the plates were flooded with $FeCl_3$ solution (0.01 M $FeCl_3$ in 0.01 N HCl) and kept for 10 minutes at room temperature. Brown/ reddish colour around growth area indicated positive result (Pointing, 1999).

3.6 Thermotolerance test

A single colony of each microbial isolate was picked from a fresh culture and streaked on Nutrient agar for bacteria and actinomycetes and PDA agar for fungi. The plates for the control tests were incubated at 28 °C and those for bacteria and actinomycetes at 55 °C for 72 hours or at 55 °C for a week for fungi. To confirm the vitality of the isolates, all plates without microbial growth after the incubation period at 55 °C were further subjected to another incubation period at 28 °C. Growth of the microorganisms was checked daily. All tests were performed in triplicate. Stock cultures of the isolates positive for the above tests were prepared in Petri dishes and in agar slants using NA and PDA for bacterial and fungi respectively and then stored at 4 ºC. They were later used for biochemical and molecular tests and then formulated into starter culture for composting experiments.

3.7 Microscopic and biochemical characterization of microbial isolates

3.7.1 Microscopic examination

Growth characteristics, colony morphology and cell features of the isolates were examined both visually and by use of a compound light microscope. Fungal microscopy was done by placing a small portion of mycelia and spores on a microscope slide onto which a drop of Lactophenol cotton blue stain had been previously placed. The hyphal and reproductive structures of various fungal isolates were then observed under the x40 objective lenses and the images were photographed and recorded. Gram staining procedure was carried out on the bacterial and actinomycetes isolates to determine their Gram reaction status and shape of the cell.

3.7.2 Biochemical characterization

The isolates were then subjected to a series of biochemical tests to further confirm their identity. The tests were carried out using their appropriate reagents and culture media by following their respective recommended procedure (Breed *et al.,* 1957). Reaction out comes were observed within the recommended time period and the results tabulated. These tests included motility, catalase, oxidase, citrate, mannitol, nitrate reduction and methyl red tests.

3.8 Molecular characterization

3.8.1 DNA extraction from bacteria and actinomycetes

Isolates from stock cultures were freshly grown on nutrient agar at 37 °C for 24 to 48 hours. Individual pure colonies were scooped from the fresh culture plates and placed into 1.5 ml appropriately labeled Eppendorf tubes for DNA extraction. The DNA of the bacterial isolates was extracted using Zymo Research Quick DNA kit according to the manufacturer's instructions. Into each Eppendorf tube, 200 µl of phosphate buffered saline was added and pulse vortexed to suspend the cells. Then, 20 µl of proteinase-K and additional 200 µl of lysis buffer were added and the tubes pulse vortexed for 15 seconds. Following this, the sample mixtures were incubated at 56 ºC for 10 minutes. Pulse centrifugation was done to collect droplets at the top of the tubes. Into the tubes, 200 µl of absolute ethanol was added and the mixture pulse vortexed for 15 seconds before being transferred into Zymo spin columns (in 2 ml collection tubes). The transfer was done carefully without wetting the tube rims, then cups of the columns closed and the assembly centrifuged at 8000 rpm for 1 minute at room temperature. The spin columns were again placed into clean collection tubes and the tubes containing filtrates were discarded. Initial 500 µl of DNA prewash buffer was added to the spin columns and centrifuged again at 8000 rpm for 1 minute and the collection tubes contained the filtrate was discarded. This was followed by another careful addition of 500 µl of g- DNA wash buffer into the columns and centrifugation at maximum speed (14,000 rpm) for

3minuites. A final centrifugation step was carried out again at maximum speed for 1minute to ascertain complete removal of residual buffer g- DNA wash bufferwhich could cause problems in downstream applications. The spin columns were then placed in clean 1.5 ml centrifuge tubes, 100 µl elution buffer was added directly onto the resin at the base of the column and incubated at room temperature (25 ºC) for 1 minute and then centrifuged at 8000 rpm for 1minute to elute DNA. The above steps were carried out at room temperature. The DNA was then quantified using agarose gel electrophoresis (Wade *et al.,* 2003). The excess DNA was stored in the fridge at 4 ºC for use in downstream processes.

3.8.2 DNA extraction from fungi

Fresh fungal colonies were prepared from stock cultures by sub cultivation on PDA agar plates at 28 ºC for 4 days. Normal saline (500 µl) was put in 1.5 ml Eppendorf tubes. Five loopfuls of fungal mycelia for each sample were added into their respective well labelled Eppendorf tubes and vortexed at maximum speed for 30 seconds. The tubes were then centrifuged at 13000 rpm for 10 minutes at room temperature to decant the normal saline. CTAB lysis buffer (400 µl) was added and the mixture vortexed until the cells were uniformly mixed. The samples were then incubated in a water bath at 65 °C for 2 hours with intermittent inversions. After the two hours, chilled Phenol: Chloroform: Isoamyl (24:24:1) was added, mixed gently and then incubated at -20 °C for 20 minutes. The samples were then mixed again gently by inversions and later centrifuged at 13000 rpm for 10 minutes. The supernatant was transferred to a new sterile 1.5 ml Eppendorf tube. Chilled absolute ethanol (400 µl) was then added and mixed gently. The samples were incubated again for 20 minutes at -20 °C and later centrifuged at 13000 rpm for 8 minutes. The liquid phase was then carefully decanted and 400 µl chilled 70 % ethanol added and mixed gently. Centrifugation at 13000 rpm for 2 minutes at room temperature was then done and the liquid phase gently decanted. The tubes were then inverted on a sterile paper towels used to dry the DNA pellet. After drying, the DNA pellet was eluted by adding 50 µl of PCR water to dissolve the pellet by gentle tapping. The DNA was then quantified using agarose gel electrophoresis (Wade *et al.,* 2003). The excess DNA was stored in the fridge at 4 ºC for use in downstream processes.

3.8.3 Polymerase Chain Reaction (PCR) for bacteria

Fragments of 16S rRNA of the DNA extracted from the bacteria were amplified using Techgene thermocycler FTGENE5D model (Techne- UK). Amplification of the genomic DNA was carried out according to the procedure outlined in Wade *et al.* (2003). The primers used to amplify the 16S rRNA region were 27F (5'AGAGTTTGATCMTGGCTCAG'3 and 1492R (5'TACGGYTACCTTGTTACGACTT'3) forward and reverse primers, respectively. PCR was performed in a 30 µl reaction volume containing 0.3 µl of 10 µM primer 27F, 0.3 µl of 10 µM primer 1492R, 15.00 µl Taq master mix and 13.40 µl PCR water. The mixture was thoroughly mixed by vortexing before 1 µl of DNA template were added into each reaction tube for PCR reactions. A tube containing 1 µl PCR water was included in the reaction as a negative control and 1 µl DNA template of positive control in one other reaction tube. The samples were then loaded onto the PCR machine and amplification of the 16S rRNA gene done at 35 cycles of denaturation at 94 °C for 1 minute, annealing at 55 °C for 30 seconds and extension at 72 °C for 45 seconds. A final extension step of 5 minutes at 72 °C was done to ensure complete amplification.

3.8.4 Polymerase Chain Reaction (PCR) for fungi

These reactions were also performed in Techgene thermocycler FTGENE5D model (Techne- UK) following the procedure outlined in Wade *et al.* (2003). Universal primers ITS1 (5'TCCGTAGGTGAACCTGCGG'3) and ITS4 (5'TCCTCCGCTTATTGATATGC'3) forward and reverse, respectively, were used for amplification. The PCR was performed in a 25 µl reaction volume containing 1.25 µl of 10 µM primer ITS1, 1.25 µl of 10 µM primer ITS4, 12.50 µl Taq master mix and 9.00 µl PCR water. The mixture was thoroughly mixed by vortexing before 1 µl of DNA template were added into each reaction tubes for PCR. A tube containing 5 µl PCR water was included in the reaction as a negative control and 1 µl DNA template of positive control in one other reaction tube. The samples were then loaded onto the PCR machine and amplification of the ITS region done under the following conditions: initial denaturing step at 94 °C for 5 minutes, followed by 35 cycles (denaturing at 94 °C for 60 seconds, annealing for 60 seconds at 55 °C extension for 90 seconds at 72 °C) and a final extension step of 5 minutes at 72 °C to ensure complete amplification.

3.8.5 Gel electrophoresis of the PCR products

Confirmation of amplification of the DNA fragments (bacteria, actinomycetes and fungi) was carried out using agarose gel electrophoresis. Five (5) µl of the PCR products were mixed with SYBR green loading dye (2 µl) and loaded onto agarose gel containing 1 % (w/v) agarose gel and 0.5X TBE buffer. Separation by gel electrophoresis occurred at 80 V within 30 minutes. One hundred base pairs (100 bp) ladder was used for estimation of the molecular sizes of the bands. The PCR products bands were visualized with UV trans-illuminator lamp and photographed.

3.8.6 Purification of PCR products

Positive PCR products were purified using Zymo Research purification kit (Zymo USA) according to the manufacturer's instructions as follows: 5 volumes of buffer PB was added to 1 volume of PCR samples in Zymo spin columns. The spin columns were placed into 2 ml collection tubes and centrifuged for 30- 60 seconds to bind DNA onto membrane base. The flow through was discarded and the columns were centrifuged again for 30- 60 seconds. Addition of 750 µl of buffer PE to the spin columns was done and then re-centrifuged for another 30- 60 seconds to wash. The flow through was then discarded and the columns replaced into the same collection tubes and centrifugation done for 1 minute at maximum speed to remove residual ethanol in the buffer PE. The columns were then placed in a clean 1.5 µl centrifuge tubes and 50 µl elution buffer EB (10mM Tris HCl, pH 8.5) was added, incubated for 1 minute and then centrifuged for another 1 minute. Five (5) µl of the purified products were loaded onto gel tank and run at the same conditions as above to confirm the presence of the purified products. These products were stored at -20 °C.

3.8.7 Sequencing of 16S rRNA genes

The purified PCR products were sequenced using Big DyeTM Terminator V3.1 cycle sequencing kits; an ABI 3130xl automated capillary Genetic Analyzer (Applied BioSystems) to obtain 16S rRNA partial sequences. The universal forward and reverse primers used were as thosed in the PCR reaction (section 3.8.3). Each pair of the partial sequences obtained was further aligned to enable the designing of the internal primers for complete 16S rRNA gene sequencing.

3.8.8 Sequencing of ITS genes

The purified PCR products were sequenced using Big DyeTM Terminator V3.1 cycle sequencing kits; an ABI 3130xl automated capillary Genetic Analyzer (Applied BioSystems) to obtain ITS partial sequences. The universal forward and reverse primers as used in the PCR reaction were employed in the initial partial sequencing. Each pair of the partial sequences obtained were further aligned to enable the designing of the internal primers for complete ITS gene sequencing.

3.8.9 Sequences analysis

The preliminary partial sequences were compared with those in the GenBank database using the Basic Local Alignment Search Tool (BLAST) program for the initial identification of the isolates. Identified isolates were grouped according to their BLAST identities and aligned using CLUSTAL W (1.6) multiple sequence alignment program to give the pair wise percentage identity. This was done for distinguishing a limited number of varied organisms whose 16S rRNA or ITS genes would be subjected to complete sequencing. The organisms with high percentage similarities were considered similar or closely related. One of these was picked for complete sequencing along with those which had low percentage similarities. The distinctly varied individual 16S rRNA or ITS partial genes were then fully sequenced using the designed synthesized internal primers to ascertain their closest relatives. The major resource used in further identification and characterization of the organisms was GenBank (Maidak *et al.,* 1999; Cole *et al.,* 2003). A value of one hundred was used as the bootstrap value for assessing phylogenetic relationships among microorganisms through maximum likelihood method. Evolutionary analyses of the nucleotide sequences were conducted in MEGA7 software.

3.9 Composting process

Composting experiments were carried out at the Horticultural Crop Directorate grounds, Mwea, in Kirinyaga County. Microbial starter cultures of selected microorganisms obtained from the partly decomposed rice straw (section 3.3), were prepared by harvesting microbial cells and/ or spores and formulating them using sterile carrier materials. Bacterial cultures were prepared using 10^7 CFU/ ml cells while fungal mycelium and spores having 10^4 spores/ ml of distilled water were used. These were then mixed together and inoculated in one composting set up as the starter culture (Sutripta *et al.,* 2010).

An open structure was constructed and fitted with wire mesh benches onto which the composting experiments were set up. The structure was made in such a way as to allow maximum aeration and avoid direct effects of rainfall and sunlight on the composting materials. Gunny bags were used as vessels for holding the composting materials. The experiment involved four treatments (T1, T2, T3 and T4) and a control (T0). T1 consisted of rice straw mixed with chicken droppings in the ratio of 10: 1 (w/w), T2 contained rice straw mixed with commercial effective microorganisms, mixed according to manufacturer's instructions, T3 had rice straw treated with microorganisms previously isolated and selected in preliminary experimentswhile T4 consisted of rice straw mixed with donkey dung in the ratio of 10: 1.

Dry rice straw was obtained from different rice farmers in Mwea and transported to the study site for the experiments. The rice straw was chopped to moderate length of about 3-5 centimeters and mixed thoroughly. Ten (10) kilograms of the rice straw were put into each gunny bag with three replications for every treatment. The rice straw was watered and inoculated with the respective starter cultures. The mixture was thoroughly mixed, arranged in gunny bags and the bags closed to maintain and avoid loss of moisture during composting. Further watering was done whenever necessary to prevent the composting content from drying. The compost piles were also turned regularly.

3.10 Physicochemical analysis during composting

Temperature, pH and electrical conductivity (EC) readings were taken on daily basis throughout the composting period. Temperature readings were measured by inserting a thermometer into the compost pile. Three readings were taken in every compost pile; the first one close to the surface, second one at the center and the third one close to the bottom of the composting pile. An average value was later calculated.

For pH and electrical conductivity tests, 10 of grams compost sample was collected into a sterile plastic bag. Each sample was put into a 250 ml glass beaker containing 90 ml distilled water and the mixture stirred for 20 minutes. It was then allowed to settle and pH and electrical conductivity readings taken using a digital electrode pH and EC multimeter. The electrode probes were thoroughly washed and rinsed with distilled water before and after use. The meters were also calibrated regularly using appropriate standards to ensure high levels of accuracy.

3.11 Characterization of mature compost

3.11.1 Analysis for physicochemical properties

Compost obtained from the composting experiment was analyzed for various physicochemical properties. Levels total nitrogen and available phosphorus were detwrmined using Kjeldahl and Oslen methods respectively. Phosphorus and magnesium were measured in Milton Roy Spectronic 100, a digital UV-Visible spectrophotometer at 880 nm wavelength. Potassium, sodium and calcium were analyzed by flame photometer method, in AA500 pg model instruments. The content of organic carbon was analyzed by Walkley- Black method (Weaver, 1994). Carbon-nitrogen (C: N) ratio was calculated using the carbon and nitrogen contents. pH and electrical conductivity was measured using digital electrode pH and EC meters, Metrohm 632 and Bridge model 31 respectively. Cation exchange capacity was tested using ammonium acetate method (Chapman, 1965). The equipment used were calibrated with known and unchangeable content of these elements to control any anomalies.

3.11.2 Humification Index

To evaluate the degree of humification (DH %) and the humification index (HI) of the compost samples, total extractable carbon (TEC %) and humic and fulvic acid carbon (C(HA+FA)%) of each were determined. For the analysis of the total extractable carbon, 2g of the composts were treated with 100 ml of a 0.1N NaOH/ $Na_4P_2O_7$ solution for 48 hours at 65 ºC. After centrifugation at 2500 rpm for 20 minutes, the supernatant solution was filtered through a 0.45 µm Millipore filter and then stored at 4 ºC (Ciavatta *et al.,* 1990). The humic and fulvic acids were fractionated through acidification of 25 ml of the extract with 50 % H_2SO_4, separating the humic acids HA (precipitated) from the fulvic acids FA (in solution); the FA were purified on a polyvinylpyrrolidone (PVP) column, resolubilized with NaOH 0.5N and then added to the humic portion (Ciavatta *et al.,* 1990). The combined HA+FA fractions were quantitatively transferred into a calibrated 50 ml volumetric flask and topped up to volume with NaOH 0.5N and stored at 4 ºC.

Ash content was determined by combustion of 10 grams of compost sample from every treatment at 650 ºC in a furnace, until a constant weight of samples was reached. Total organic carbon (TOC %) was determined by mineralization of the organic matter to CO_2. One hundred (100) mg of compost was mineralized with 20 ml of 1/3M Potassium dichromate ($K_2Cr_2O_7$) and 26 ml of 96 % H_2SO_4 for 10 minutes at 160 ºC (Springer and Klee, 1954). The organic carbon content was estimated through back-titration of the samples with a solution of 0.2N $FeSO_4$. The determination of extractable organic carbon (TEC %) was carried out by mineralization of 5 ml of the extracts with 5 ml of 1/3M $K_2Cr_2O_7$ and 20 ml of 96 % H_2SO_4 for 10 minutes at 160 ºC (Ciavatta *et al.,* 1990). The content of humic and fulvic acid carbon C (HA+FA) % was obtained by mineralization as described above on 2 ml of the humic extracts. The Degree of Humification (DH %) and Index of Humification (HI) of the composts was calculated according to Ciavatta *et al.* (1990), as follows: **DH %=** C (HA+FA) 100/ TEC; **HI**= NH/ C (HA+FA).

Where, NH = TEC- C (HA+FA), corresponding to the amount of non humified carbon extracted with 0.1N NaOH/ $Na_4P_2O_7$ solution. DH is the percent of humified carbon in the extract while HI represents the ratio between non humified and humified extracted carbon.

After completion of the sample extraction, the quantity of extractable carbon present in the compost samples was determined using a calorimeter model G10s UV-vis (Thermo Fisher Scientific). Quantitation was done by measuring colour intensity that resulted from the presence of Cr^{3+} in the solution. The calorimeter was set to measure light absorbance through the samples at a wavelength of 601 nm. The actual concentration was then calculated by comparison of the readings obtained against a standard curve with known TEC concentrations (Nelson and Sommers, 1996).

3.11.3 Germination index

Germination index (GI) was measured by growing tomato seeds in a 1:2 (w/v) water soluble compost extract. The extract was obtained by centrifuging a mixture of 10 g compost and 20 ml distilled water at 3200 rpm for 30 minutes followed by filtration through filter paper. Extraction was performed under vacuum using 0.45 µm filter papers. The resultant solution was mixed with distilled water in the proportion of 100 %, 75 % and 50 %, respectively with 100 % of distilled water as control in the experiment. Two ml of each mixture was added into a Petri dish (9 cm) with filter paper laid previously, and 10 seeds of tomato were spread on the filter paper. All the seeds were pre-wetted for 2 hours prior to the initiation of the experiment to enhance germination. All the Petri dishes were then incubated in the dark at temperature of 25 ± 1 °C for 24 hours, 48 hours, 72 hours and 14 days. The number of germinated seeds and root length were measured after the above time intervals and the GI calculated according to Equation: **GI** = G100 % x R100 % + G75 % x R75 % + G50 % x R50 % / G0 % x R 0 % x3.

Where: G=Total number of seeds germinated multiplied by average root length; R= Root length of various treatments; G0 and R0 are values obtained using distilled water (control) (Tiquia and Tam, 1998).

3.11.4 Plant growth index (PGI)

Plant growth index (PGI) determination was conducted using a mixture of compost and vermiculite at different concentrations of compost of 0 %, 20 %, 40 %, 60 %, 80 % and

100 % (v/v). The mixture was put into two liter plastic pots. Each mixture was irrigated with distilled water, 20 tomato (*Solanum lycopersicum* L) seeds spread on the surface of each pot and then covered with a small amount of vermiculite. The pots were placed in the greenhouse at a temperature of 25 ± 1 ºC and 12/12 of light/dark cycle for 55 days. The pots were arranged in a completely randomized design with three replications per treatment.

The plants were watered regularly during the growth period. On the day of termination of the experiment, plant height and the number of leaves in four plants from every treatment were measured. The seedlings were then carefully uprooted by washing off the growth media with water to avoid damaging the roots. Total fresh plant weight of four representative plants randomly picked from each of the treatments was measured using a digital weighing balance. The roots and the shoot were then separated by cutting at the base of each plant using a scalpel, dried at 105 °C and their separate and collective weights measured and recorded. The PGI was then calculated and expressed as the ratio of average dry weight of the treatments (20 %, 40 %, 60 %, 80 % and 100 % of compost) to the weight of the control samples (0 % of compost) (Woods End Research, 2002). Root: shoot ratios were also calculated to determine the mode of allocation of nutrients during growth. Phosphorus, nitrogen and potassium concentrations in the tomato plants biomass were analyzed using the methods described previously (section 3.11.1).

3.11.5 Heavy metal extraction and analysis

One gram of each compost type was placed in a 250 ml digestion tube and 50 ml of concentrated nitric acid added (Zeng, 2004). The sample was then heated for 45 minutes at 90 °C and the temperature increased to 150 °C at which the sample was boiled until a clear solution was obtained. A few drops on hydrogen peroxide were added followed by addition of a concentrated nitric acid (5 ml added three times). Digestion was left to take place until the volume reduced to about 5 ml. The inside of the walls of the tube were rinsed with distilled water and the tube swirled throughout the digestion to prevent loss of the sample. The sample was then allowed to cool and 5 ml of 1 % nitric acid was added to the sample. The solution was filtered with Whatman No. 42 filter paper and a 0.45 µm Millipore filter paper. The solution was quantitatively transferred to a 50 ml volumetric flask by adding distilled water ready for analysis (Pollack and Favoino, 2004).

Concentration of total heavy metals was determined using Buck Scientific 210VGP Atomic Absorption Spectrophotometer. The operating parameters of the machine were set according to the manufacturers specifications (Buck Scientific Manual, 2003). Samples were mixed mixed by shaking vigorously before aspiration into Atomic Absorption Spectrophotometer for specific metal concentration determination. Values were expressed in mg/ kg.

3.12 Evaluation of microbial community changes during composting

A natural composting experiment was set up within a greenhouse at the Department of Microbiology, Kenyatta University. Dry rice straw obtained and chopped to moderate length of about 3-5 centimeters, watered and mixed thoroughly. Twelve (12) kilograms of rice straw was put in four plastic bags each (several holes were pierced on the bags for aeration) and arranged on a wire mesh bench to allow for composting. The compost piles were turned regularly for aeration and watered whenever necessary. Temperature readings were taken on daily basis throughout the composting period while 10 g compost samples taken from each bag for microbiological analysis on daily basis for the first seven days and after every 7 days thereafter.

Each of the 10 g compost sample was serially diluted to varying dilutions in sterile normal saline. The dilutions varied at different times of the experimental period, with different dilutions being chosen as appropriate at various composting phases. An inoculum of 0.1 ml was always taken and inoculated onto Nutrient agar for bacteria and actinomycetes and on PDA agar for fungi by spread plate method. The inoculated plates were incubated at 37 °C for bacteria and actinomycetes and at 28 ºC for fungi. Phenotypically different colonies were selected and purified by repeated sub-culturing. The colonies were later subjected to biochemical and molecular tests for further identification as described in section 3.7.2 and 3.8 above. Changes in microbial load within the compost at different phases of composting were estimated by total plate count technique. Weight loss due to decomposition was determined on weekly basis by measuring the weight of each set up using a spring balance.

3.13 Data analysis

All data collected was tested for homogeneity of variance by Bartlett test before analyses. The data was then transformed wherever necessary to fulfill the assumptions of ANOVA. The data was then analyzed by one-way analysis of variance (ANOVA). In order to test for any compounding effects of any changes among days, the data was subjected to analysis of covariance (ANCOVA). Wherever applicable, post hoc test was performed using Tukey's Honest Significant Difference (HSD) test ($P < 0.05$). Pearson's correlation coefficient was also performed to establish the relationship among various parameters. All statistical analyses were performed using SPSS version 16 software while molecular data was analyzed using Mega7 software.

CHAPTER FOUR

RESULTS

4.1 Phenotypic diversity of microbial cultures

4.1.1 Morphological characteristics

Diverse microorganisms were isolated from the partly decomposed rice straw. The isolates consisted of 49 bacterial colonies and 20 fungi. Screening tests on these isolates led to the elimination of 29 of the bacterial and 9 fungal colonies on the basis that they did not demonstrate lignocellulolytic potential. After the screening procedures, 20 bacteria and 11 fungi were selected and used as a starter culture for the controlled composting experiment.

The extra bacterial (8) and fungal (6) colonies whose characterization results have been included in this section are those that were later isolated from the experiment on the study of microbial population changes during a natural composting experiment. All the bacterial and fungal isolates isolated from the partly decomposed rice straw were however all represented in isolates obtained from compost during the experiment on microbial dynamics of composting.

The bacteria were diverse in relation to their colony size, elevation, margin, colony shape and pigmentation (Table 4.1). The pigmentation of most of the bacterial colonies varied from whitish to cream. Several colonies however had very outstanding colony colours including bright orange, bright yellow and shiny red (Plate 4.1). The shiny red coloured isolate (AB21) was very predominant during the natural composting experiment. Based on its cultural and biochemical characteristics, this isolate was identified as *Serratia marcescens* (Table 4.1, Table 4.2). Most of the colonies were, flat, whitish in colour and irregular in shape. It was also observed that several colonies were highly mucoid while others hada dry and crusty appearance. Several colonies suspected to be actinomycetes exhibited rhizoidal like growth, with some showing highly branched mycelia with regular or irregular patterns (Plate 4.1). Cultural tests revealed that some colonies were non motile while the highest proportion of about 54 %, were motile. According to the morphological characteristics observed, a preliminary classification showed that the isolates belong to genera *Bacillus, Serratia, Pseudomonas* and *Streptomycetes*.

Table 4.1: Colony characteristics of bacterial isolates

Colony	Pigmentation	Shape	Margin	Elevation	Motility
AB4	Yellow-orange	Round	Entire	Raised	+
AB6	Creamish	Irregular	Serrated	Flat	+
AB13	Green-yellow	Round	Entire	Raised	-
AB14	Gray	Regular	Entire	Raised	-
AB15	Translucent	Mucoid	Entire	Flat	+
AB16	Yellow	Regular	Entire	Raised	-
AB19	Yellow-orange	Regular	Entire	Concave	-
AB21	Red	Regular	Entire	Convex	+

AB4- AB21- Test bacterial isolates. Key: + positive; - negative

The fungal colonies selected from the screening tests and composting experiment were all moulds. The moulds were observed to vary widely in terms of colour of mycelium, reverse pigmentation, colony texture, hyphal structure and asexual spores produced (Table 4.1; Plate 4.2; Plate 4.3). The colour of mycelia of most of the fungal moulds changed with age. Most of the fungi would show meaningful colony formation on PDA media at least starting from the third day after inoculation.

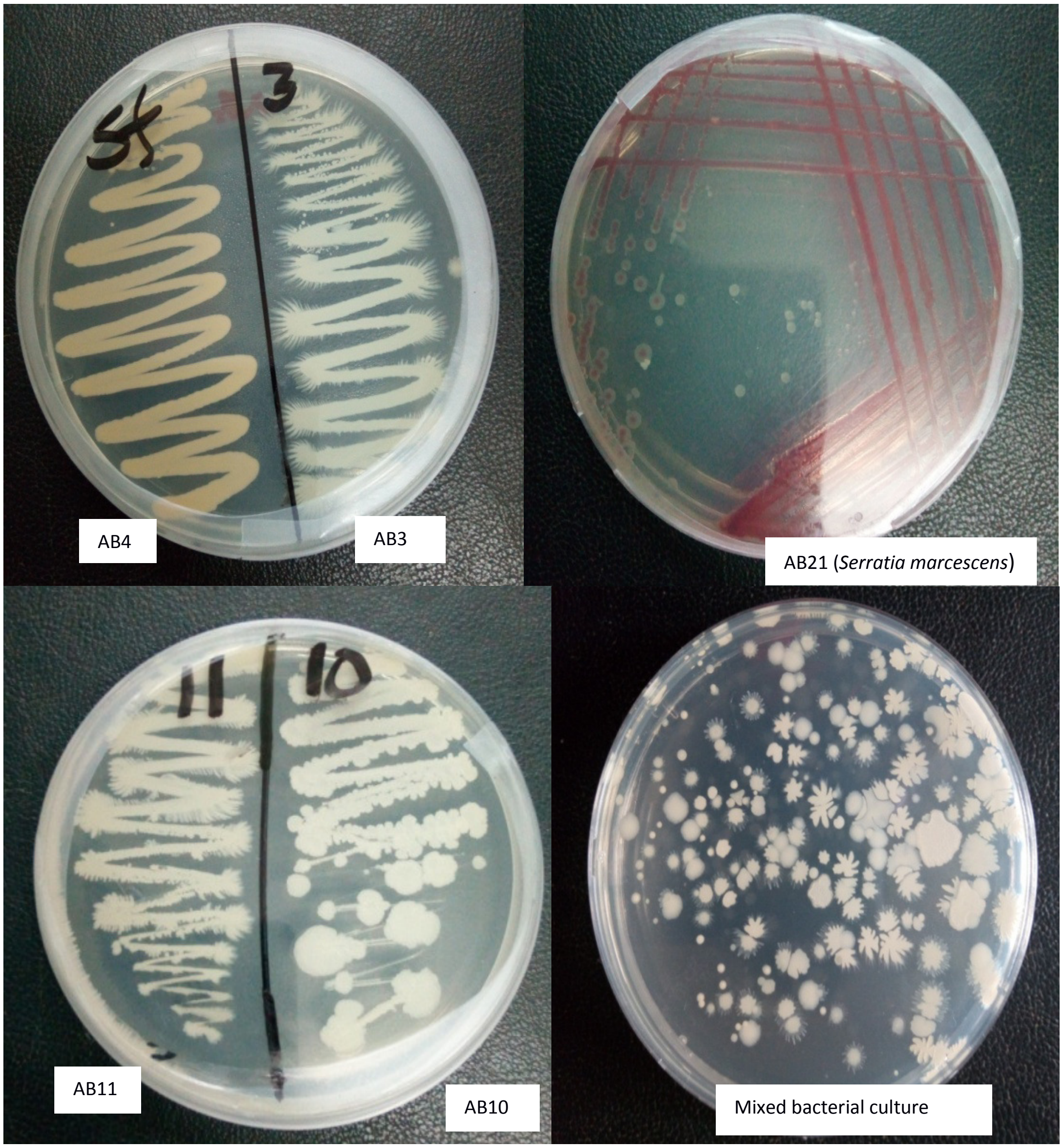

Plate 4.1: Morphological features of bacterial colonies
AB3- AB21- Test bacterial isolates.

For most colonies, especially those later classified as *Trichoderma* and *Aspergillus*, they demonstrated very drastic shifts in mycelial colour, acquiring intense pigmentation as they advanced in age. Mycelial pigmentation shifts were also accompanied by colour change on the reverse of the colony. Bright pigmentation on the reverse colony was especially observed in isolates later identified as *Fusarium* species.

Microscopic observations of fungal isolates revealed that most of the fungi had septate hyphae with a few being coenocytic. The examination showed that most of fungi formed conidiospores, most of which were actually microconidiospores while a few produced sporangiospores (Plate 4.3, Table 4.2).

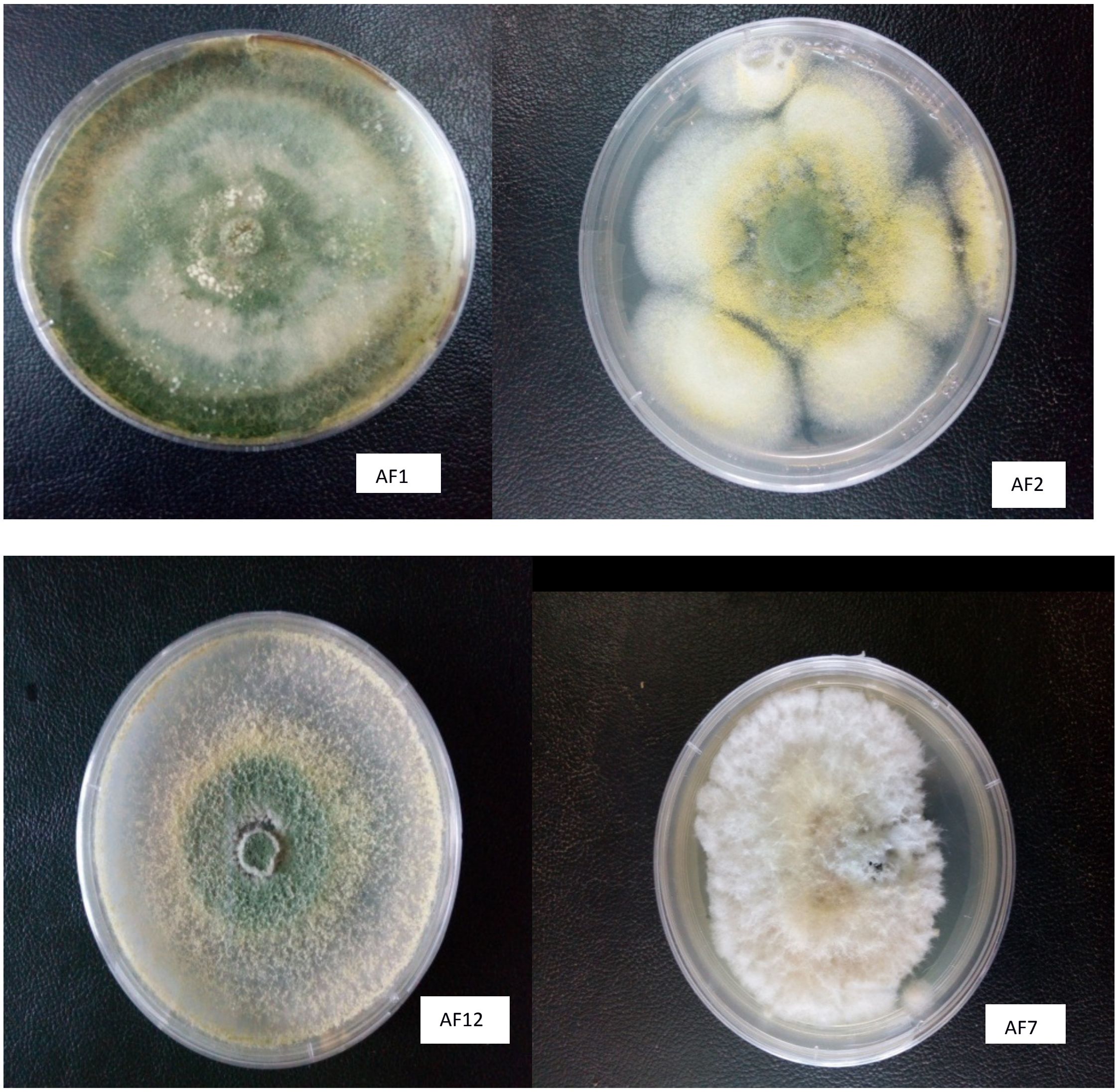

Plate 4.2: Morphological characteristics of fungal colonies
AF1- AF12- Test fungal isolates.

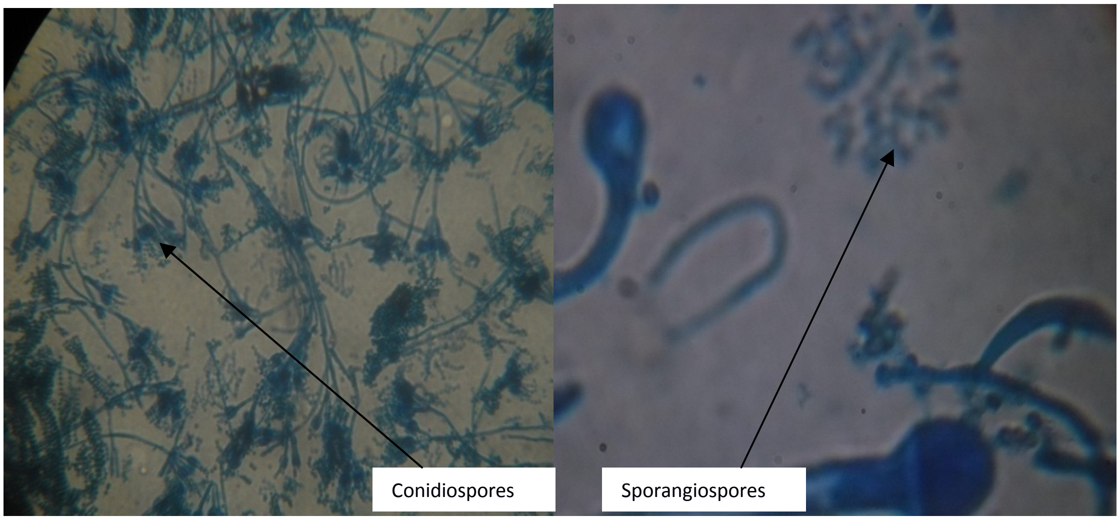

Plate 4.3: Reproductive structures in fungal isolates as seen under the light microscope

Table 4.2: Morphological characteristics of fungal isolates on PDA culture medium

Isolate	Colour	Texture	Hyphae	Spores
AF1	Green	Concentric rings	Septate	Conidiospores
AF2	Yellow-green	Powdery	Septate	Conidiospores
AF3	Greenish	Powdery	Aseptate	Sporangiospores
AF4	Dark green	Fluffy	Septate	Chlamydospores
AF5	Blue-green	Fluffy	Septate	Conidiospores
AF6	Dark Green	Lobed	Sepatate	Conidiospores
AF7	White	Fluffy	Aseptate	Conidiospores
AF10	Gray	Fluffy	Aseptate	Conidiospores

AF1- AF10- Test fungal isolates.

4.1.2 Biochemical characteristics and Gram status of bacterial isolates

Colony isolates obtained from the partly decomposed rice straw and from the composting experiment, gave varied results on the biochemical tests that they were subjected to. Gram staining test showed that most bacteria were Gram positive with only a few being Gram negative (Table 4.3). Most were rod in shape indicating that a higher number of the isolates belonged to Genus *Bacillus.* Cocci and filamentous cells were also observed. Microscopic observations showed that a few of the isolates were spore formers. Results on oxidase, catalase, mannitol, citrate and nitrate utilization are shown in table 4.3. Tests on nitrate reduction showed that almost all the isolates had the ability to utilize nitrates while a few were variable for nitrate utilization (Plate 4.4, Table 4.3).

Plate 4.4: Selected biochemical tests of bacterial isolates

Table 4.3: Biochemical characteristics of the bacterial isolates

Colony	Gram status	Citrate	Nitrate utilization	Oxidase	Mannitol	Catalase
AB4	+	-	V	+	+	+
AB6	+	+	+	-	v	+
AB13	-	-	+	+	+	+
AB14	-	-	-	+	+	+
AB15	-	v	+	+	+	-
AB16	+	+	+	-	-	v
AB19	+	+	-	-	+	+
AB21	-	+	+	-	+	-

AB4- AB21- Test bacterial isolates; + positive; - negative; v variable.

4.2 Lignocellulolytic properties of the microbial isolates

Fity four percent (54 %) of the isolated microorganisms demonstrated a strong ability to degrade rice straw. This was demonstrated by the luxurious growth exhibited by these colonies while growing on the RSA agar (Plate 4.5). However, the isolates took a longer period of time to show any signs of growth on the Rice Straw medium than the time taken in the control (Nutrient agar and PDA agar). Seven percent (7 %) of the colonies with the ability to grow on rice straw however gave negative results when tested for filter paper degradation ability and they were therefore omitted in subsequent stages of the selection procedure.

Cellulose degradation ability of the microorganisms was shown by their ability to colonize the filter paper that was laid on their growth medium. The level of colonization generally varied between bacteria and fungi with most fungi showing more luxourious growth on the filter papers than the bacteria (Plate 4.5). Ability to grow on Carboxymethyl cellulose agar with decolourization of Congo Red dye around colonies was also demonstrated across various test colonies. Most (55 %) of the isolated fungal colonies demonstrated positive ligninolytic properties as they were able to decolourize the test dyes (Methylene Blue, Azure II and Phenol Red) with the formation of a clear zone around the colonies (Plate 4.5). Fifty nine per cent (59 %) of the bacterial isolates were not able to decolourize these dyes and were therefore omitted during further screening stages.

Tests on thermotolerance ability of the microorganisms revealed that only 5 of the bacterial isolates were able to grow normally at a temperature of 55 ºC. A few others showed slight signs of growth but the colonies died within a short time, while the rest of the isolates did not grow at all. On the other hand, none of the fungal isolates were positive for the test on thermotolerance. Isolates that did not demonstrate thermotolerance ability were however included among those selected for use in composting as far as they were positive for the other tests.

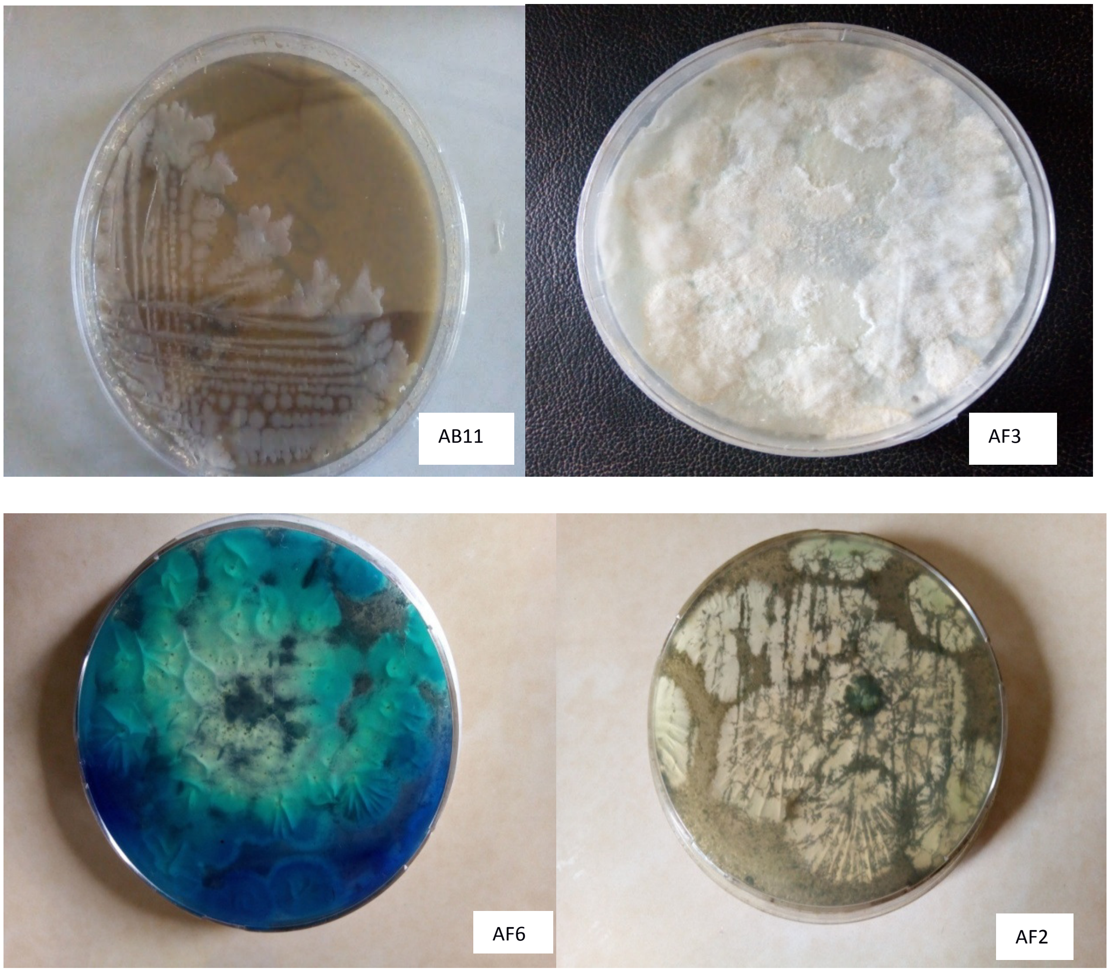

Plate 4.5: Lignocellulolytic properties of bacterial and fungal isolates
AB11- Growth on Rice Straw agar; AF3- Filter paper degradation; AF6- Methylene Blue decourilization; AF2- Azure II declourization.

4.3 Molecular characteristics of the microbial isolates

All isolates from natural composting experiment (section 3.12) that were morphologically and biochemically similar with those selected for the starter culture (section 3.3) were not included during molecular DNA analysis. Only DNA from colonies that appeared morphologically and biochemically different were characterized molecularly.

4.3.1 Verification of PCR products of amplified DNA by gel electrophoresis

DNA extraction procedures applied in this study yielded genomic DNA of a standard quality and quantity suitable for further molecular downstream processing. The PCR amplification of the genomic DNA was very successful in yielding required sizes and concentration of targeted regions of both the 16S rRNA and the ITS sections in the DNA of bacteria and fungi, respectively. Bands of the PCR products were produced at 1 kb mark on the gel electrophoresis using 1 kb plus DNA ladder from Invitrogen and are as shown in Plate 4.6 and Plate 4.7 for bacteria and fungi, respectively.

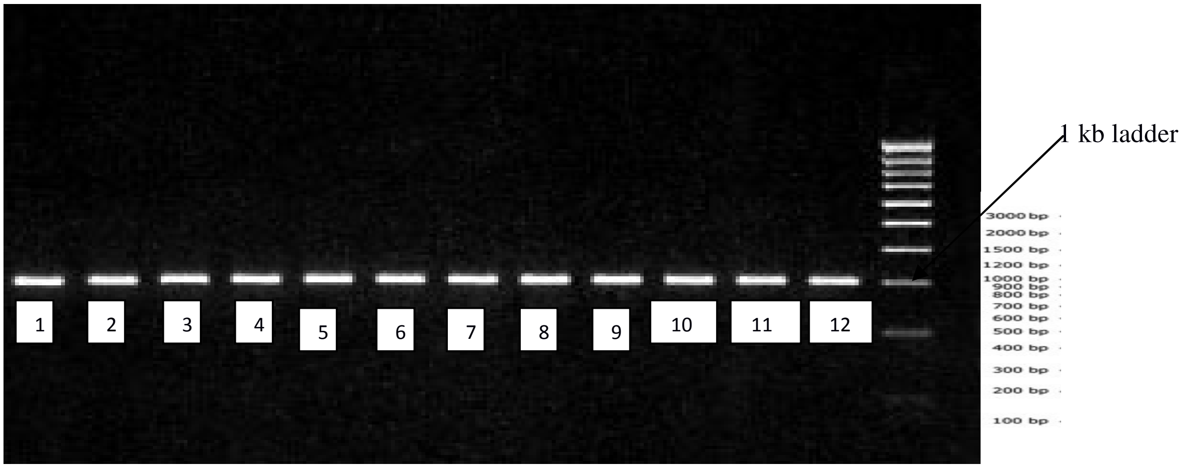

Plate 4.6: Image of PCR products of 16S rRNA region of bacterial isolates on agarose gel

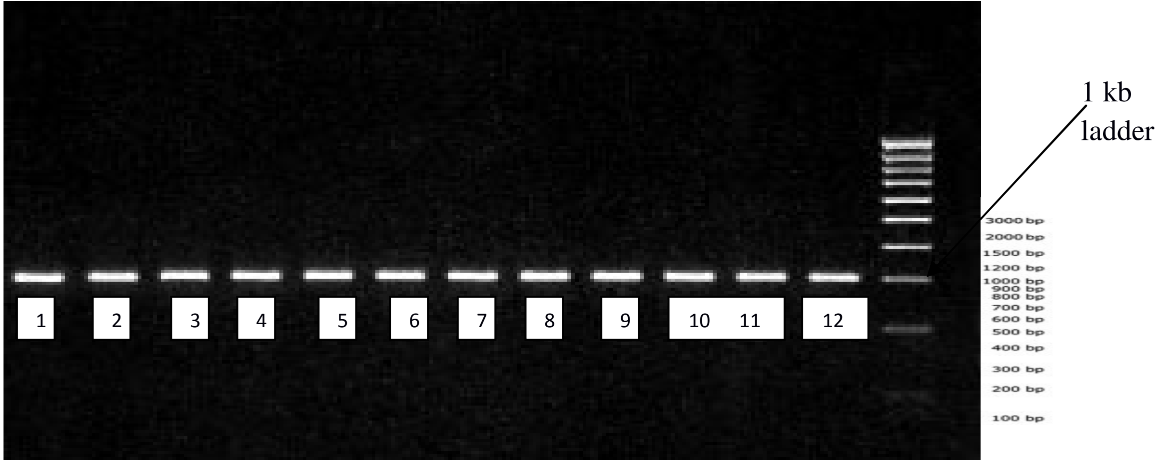

Plate 4.7: Image of PCR products of the ITS region of fungal isolates on agarose gel

4.3.2 Sequencing of the 16S rRNA and BLAST results

Sequencing of the 16S rRNA of the bacterial isolates on the 5' and the 3' ends using the 27F and 1492R primers, respectively yielded sequences that showed marked difference in length. The sequences varied in length ranging between 622 and 1163 base pairs (bp), with an average length of 1038 bp. The sequences were analyzed appropriately and the final sequences put in BLAST in National Centre for Biotechnology Information (NCBI) data base for identification. All the sequence lengths were long enough for BLAST search. Most of the BLAST results gave percentage similarities within the range of 98–100 %, with 80 % level of similarity being the lowest. Identity of one of the strains matched with an uncultured *Bacillus* spp which have never been cultivated on culture media but have only been detected by molecular techniques.

Using the accession numbers, information on the properties of the isolates was obtained. The properties included the organisms' general description, ecological distribution and potential biotechnological application. The information showed that 64 % of the isolates were alkaliphiles and spore formers. The isolates have also been reported to have potential in biodegradation and other biotechnological applications.

4.3.3 Sequencing of the ITS genes and BLAST results

Sequencing of the ITS genes of the fungal isolates done on the 5' and the 3' ends using ITS1 and ITS4 primers also yielded sequences of varied length. The shortest sequence had 322 bp while the longest had 630 bp. The average length for most of the PCR sequences was about 610 bp. The sequences were analyzed appropriately and the final sequences searched against Basic Local Alignment Search Tool (BLAST) data base in

National Centre for Biotechnology Information (NCBI) data base for identification. Most of the BLAST results gave percentage similarities within the range of 98– 100 %. Further information on the properties of the isolates was also obtained from literature through the accession numbers. This information was used to affirm the identity of the organisms.

4.3.4 Identity of the microbial isolates

Details of representative bacterial and fungal identification obtained through sequencing of 16S rRNA and ITS and their homology match search of gene banks including the isolate number (query name), the percentage identity, GenBank accession numbers and the BLAST hit descriptions of the identified organisms are shown in Table 4.4 and Table 4.5, respectively.

The sequences enabled the identification of the organisms to genus and a few up to species levels. The bacteria belonged to Phylum Firmicutes (*Exiguobacterium, Pseudomonas, Serratia* and *Bacillus*), Proteobacter (*Ochrobactrum*) and Actinobacteria (*Cellulosimicrobium*). The *Bacillus* species included the *B. licheniformis, B. subtilis, B. cereus, B. thuringiensi, B. anthracis, B. horikoshii, B. safensis, B. pumilus, B. mycoides* and *B. amyloliquefaciens*. Fungi were mainly Ascomycetes comprising of Genus *Aspergillus, Fusarium, Trichoderma, Penicillium and Cladosporium*. Several of the isolates identified as *Trichoderma* through preliminary tests later were shown to belong to teleomorph of *Trichoderma*, the *Hypocreceae.* Such species were however referred to by their assigned *Trichoderma* identity.

4.3.5 Phylogeny of the organisms

Results of the bacterial and fungal sequences (16S rRNA and ITS) respectively are shown in Table 4.4 and Table 4.5, respectively. Low percentage identity rates indicated low similarity of 16S rRNA/ ITS genes of the organisms signifying that they were not closely related while sequences with high percentage similarities were considered to be of organisms that are very closely related.

Table: 4.4: BLAST output from 16S rRNA sequences of bacterial isolates

Isolate code	Sequence length	Percentage Identity	GenBank Accession No	Hit description (Best match)
AB1	1155	98 %	MG491523.1	*Bacillus anthracis*
AB2	1163	99 %	KX129842.1	*Bacillus velensis*
AB4	1100	98 %	KX609731.1	*Exiguobacterium* spp
AB9	1146	99 %	KX364922.1	*Bacillus safensis*
AB13	1144	99 %	DQ178220.1	*Pseudomonas mendocina*
AB14	1114	98 %	KJ150725.1	*Psychrobacter* spp
AB15	1161	80 %	KM975676.1	*Ochrobactrum* spp
AB16	1110	99 %	KC581673.1	*Cellulosimicrobium* spp

AB1- AB16- Test bacterial isolates.

Table: 4.5: BLAST output from fungal ITS sequences

Isolate Code	Sequence length	Percentage Identity	GenBank Accession No	Hit description (Best match)
AF1	1414	100 %	KF494177.1	*Trichoderma harzianum*
AF2	638	100 %	KX067855.1	*Aspergilus flavus*
AF5	362	95 %	KM278047.1	*Penicillium* spp
AF6	540	98 %	KM977776.1	*Cladosporium* spp
AF7	554	100 %	KF998978.1	*Fusarium chlamydosporum*
AF10	551	99 %	KX965656.1	*Fusarium* spp
AF14	609	99 %	JQ082503.1	*Penicillium citrinum*
AF15	408	99 %	KR709055.1	*Fusarium equisetum*

AF1- AF15- Test fungal isolates.

The bacteria clustered into seven distinct groups using *Trichoderma harzianum* strain KF494177 as out-group (Fig. 4.1). Strains of organisms in closely related genera were clustered together into one clade on the phylogenetic tree but there were some discrepancies observed where some organisms identified in one genus were clustered separately in the branches of the phylogenetic tree. Phylogenetic analysis of the fungal isolates using *Bacillus thuringiensis* HQ917120 as an out group yield a dendogram with two main clusters. It was observed that organisms from different genera were clustered together in the same cluster. *Fusarium incarnatum* was placed at the lowest point in relation to time in the genetic tree (Fig. 4.2).

The aligned 16S rRNA and ITS sequences revealed the evolutionary history of the test organisms as inferred by Maximum Likelihood method based on the Tamura-Nei model. Phylogenetic trees with the highest log likelihood (-14393.74) for 16S rRNA sequences (Figure 4.1) and (-6261.36) for ITS sequences (Figure 4.2) were obtained. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value.

The trees are drawn to scale, with branch lengths measured in the number of genetic changes per site. For 16S rRNA sequences, the analysis involved 27 nucleotide sequences with 1st+2nd+3rd+Noncoding codon positions being included. All positions containing gaps and missing data were eliminated giving a total of 501 positions in the final dataset.

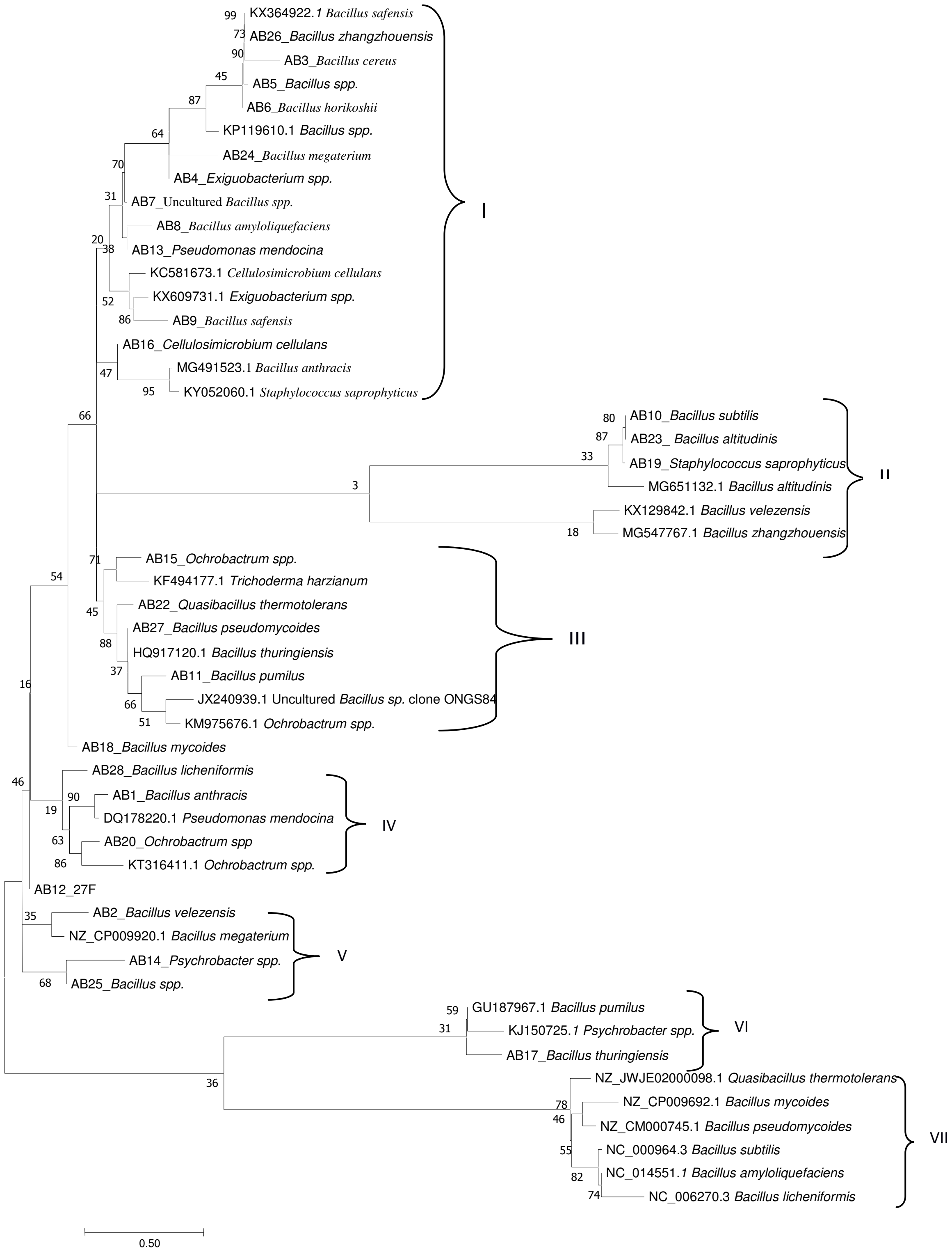

Figure 4.1: Phylogenetic tree of the bacterial strains based on their 16S rRNA gene sequences; AB1- AB28- Test bacterial isolates; the rest are the reference strains.

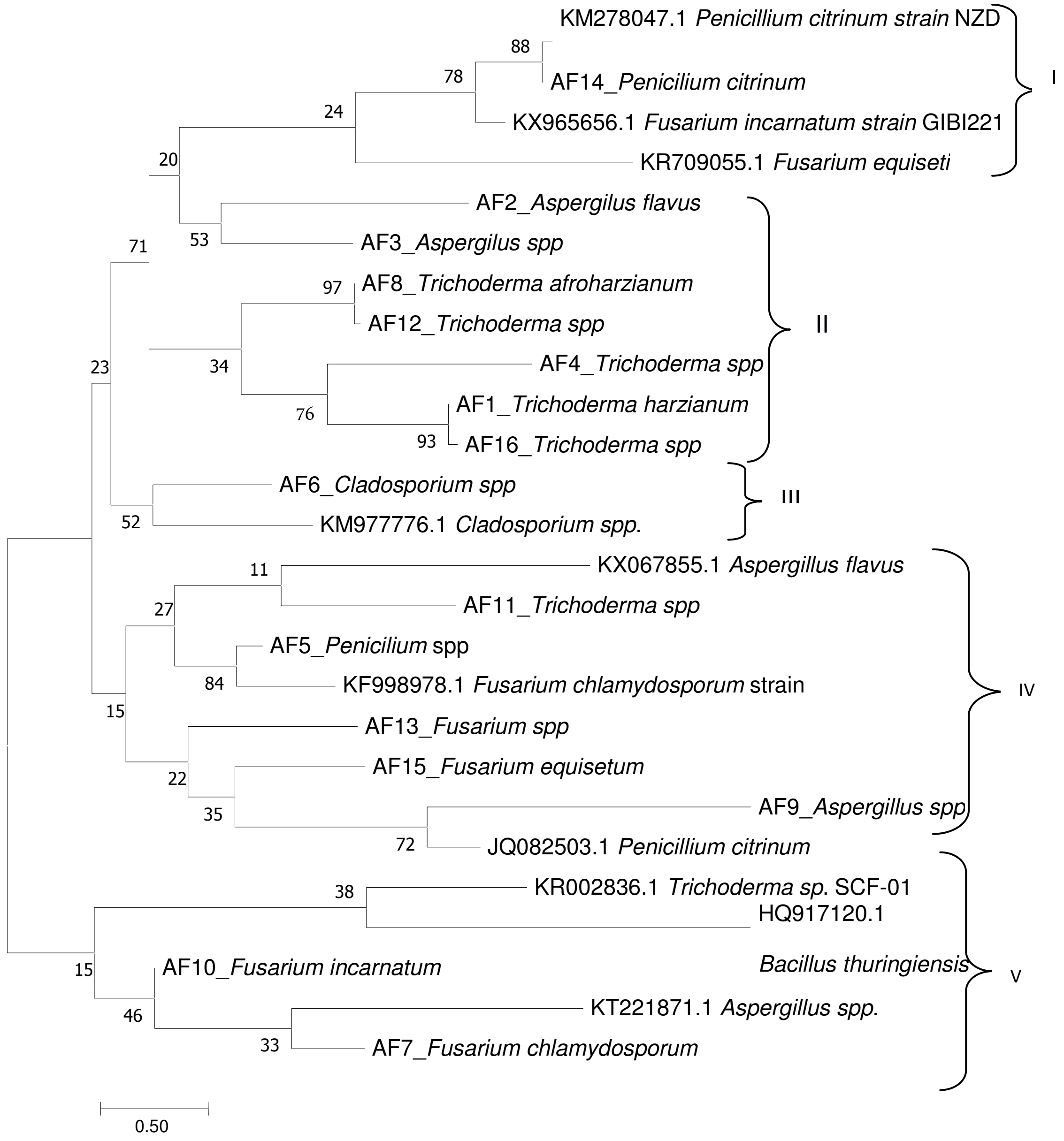

Figure 4.2: Phylogenetic tree of the fungal strains based on their ITS gene sequences AF1- AF16- Test fungal isolates; the rest are the reference strains.

For ITS sequences, analysis involved 16 nucleotide sequences with all positions containing gaps and missing data also being eliminated giving a total of 323 positions in the final dataset.

4.4 Microbial community changes during composting

The natural composting process proceeded gradually over a time period of 90 days. There was an initial rapid rise in temperature up to the 9th day, after which the temperature started to decline gradually. The temperature readings observed during the composting process ranged between 22 ºC and 47 ºC (Fig. 4.3). The composting period largely exhibited mesophilic temperatures with a short lived thermophilic phase. After the thermophilic phase, the temperature readings became stable having very minimal fluctuations among days (Fig. 4.3). The temperature values recorded during the thermophilic phase were significantly different with those of the mesophilic and the curing phases of the composting experiment ($p < 0.001$).

Number of bacteria increased from 8.7 x 10^5 CFU/ g to 2.1 x 10^6 CFU/ g at the initial stage of the composting process. However, the microbial load decreased during the thermophilic phase to 1.30 x 10^6 CFU/ g. This decline in bacterial cells was accompanied by an increase in the number of actinomycetes from 1.2 x 10^5 CFU/ g to 2.3 x 10^7 CFU/ g

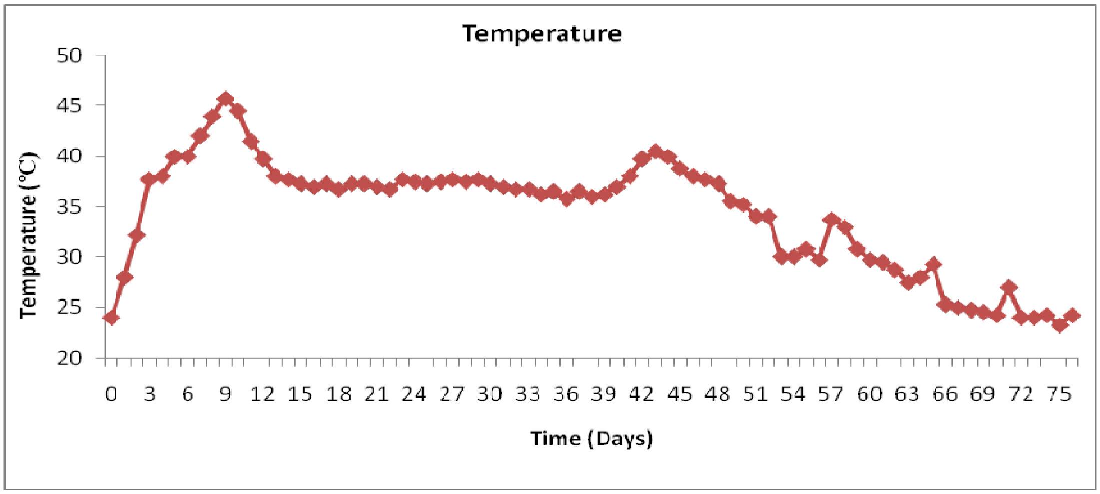

Figure 4.3: Temperature changes during the natural composting process of rice straw

towards the end of the composting period. Fungi were also isolated from the composting setup and their diversity fluctuated among different phases of the composting period. There were less fungal cultures in the samples taken during the first few days of the experiment. Further, a decreased fungal diversity in the compost samples analyzed corresponded with the phase during which the compost piles recorded high temperatures. Over all, the diversity of the fungi isolated from the composting experiment was less compared with that of bacteria (Table 4.6).

The most dominant bacterial strains were mainly *Bacillus* species because they were present in every sample analyzed, giving this genus a 100 % prevalence rate in the compost. The *Bacillus* species included *Bacillus cereus, B. horikoshii, B. subtillis and B. licheniformis* (as confirmed through molecular techniques). *Serratia marcescens* was also very predominant during the composting process, having 62.5 % rate of prevalence. Members of genera *Aspergillus* and *Trichoderma* were the most common fungi obtained, having 37.5 % and 50 % rates of prevalence, respectively (Fig. 4.4). *Aspergillus niger* and *Trichoderma harzianum* comprised the most predominant species in these genera.

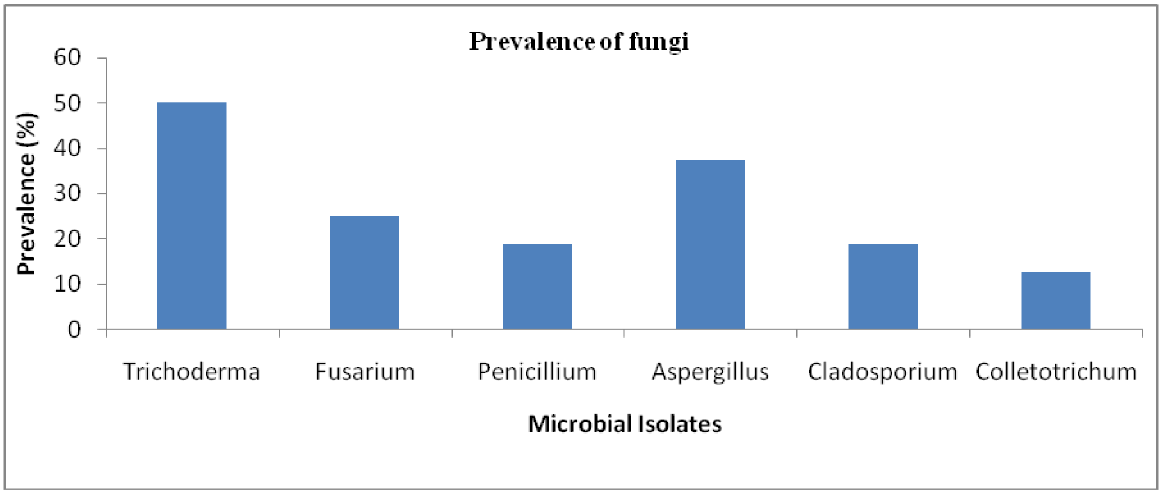

Figure 4.4: Prevalence of fungal isolates during the composting process of rice straw

Bacterial isolates obtained demonstrated a more diverse structure than the fungal isolates. The structure of some of the most predominant bacterial and fungal species is shown in Table 4.6. The bacterial species diversity declined greatly post mesophilic phase while the fungal isolate were fewer at the initial stages of the composting process.

Changes in temperature readings correlated positively with changes in the number of colony forming units (CFU) obtained from the compost samples tested. The colony forming units were highest during the mesophilic phase. Progress in the bioprocess was accompanied by a gradual reduction in weight and volume of the composting materials. One way ANOVA revealed a significant difference between the initial weight of the compost materials and the final weight on the day of termination of the composting experiment ($p < 0.001$) at 5 % significance level. There was however no significant weight loss during the first three weeks of the composting period (Fig. 4.5).

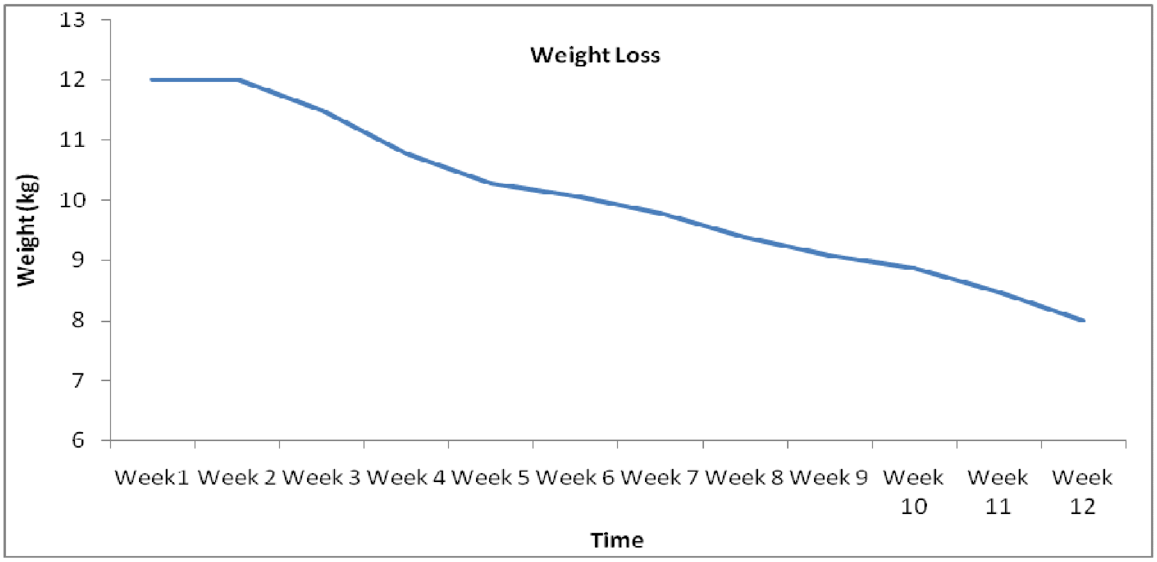

Figure 4.5: Changes in weight of rice straw during composting period

Table 4.6: Diversity of bacteria and fungi at different phases of composting rice straw

Phase of composting	**Bacterial isolates**	**Fungal isolates**
Mesophilic (24- 40 °C)	*Bacillus horikoshii, B. pseudomycoides, B. cereus, B. thuringiensis, B. subtilis, Pseudomonas* spp, *Cellulosimicrobium cellulans, Serratia marcescens, Staphylococcus saprophyticus, Psychrobacter* spp, *Ochrabactrum* spp.	*Aspergillus niger, Trichoderma harzianum Fusarium equisetum*
Thermophilic (42- 46 °C)	*B. amyloliquefaciens, B. velensis, B. anthracis, B. Subtilis, B. pumilus, Ochrabactrum* spp, , *B. licheniformis, B. megaterium, B. thuringiensis Quasibacillus thermotolerans*	*Penicillium citrinum, Trichoderma harzianum, Aspergillus flavus, Aspergillus niger, Fusarium* spp, *Colletotrichum* spp
Cooling (36- 24 °C)	*B. cereus, B. pumilus, B. safensi, B. subtilis, B. thuringiensis, Serratia marcescens.*	*Cladosporium* spp, *Penicilium* spp, *Trichoderma harzianum, Aspergillus flavus, Aspergillus niger.*

4.5 Physicochemical parameters during composting

Active decomposition was accompanied by several physical and chemical changes on the feed stock materials. During the active decomposition, a pleasant earthy smell was noted in all treatments when the composting materials were being turned. There was a gradual consistent reduction in the volume of the composting materials as decomposition proceeded. By 62nd day of composting, all the compost materials showed stability in temperature, pH and electrical conductivity levels.

4.5.1 Temperature

Temperature readings of the composting experiment, taken daily showed very wide variations among days, within the range of between 23 to 56 ºC. The highest temperature (56 ºC) and the lowest (23 ºC.) were observed in treatments T1 and Treatment T3, respectively (Fig. 4.6). Mean temperature values varied narrowly among treatments (Table 4.7). One way analysis of variance (ANOVA) of the temperature readings indicated significant differences among the treatments, with T1 and T3 having the highest mean values which were not statistically different. Mean temperature value of the control (T0) was significantly lower compared to all other treatments ($p = 0.001$) at 5 % level of significance.

4.5.2 pH

The composting process started at generally low pH levels in all the treatments of the study. The pH then rose gradually as decomposition of materials set in. Initiation of active decomposition of materials led to a slight decline in pH with the lowest pH of 6.56 being recorded in treatment T1 (Table 4.7). The pH then increased steadily attaining alkaline conditions followed by a decline to almost neutral conditions at the end of the composting period. There were however some random fluctuations among neutral, acidic and alkaline pH at different times among the treatments. The pH generally stabilized at 7.50– 8.50 but peaks were detected at 10.46 on day 23 in T1 and at 10.28 on day 22 in T3.

The pH readings observed in this study were closely related among all the treatments for the most part of the composting period. By 21st day a pronounced difference in the pattern of the pH was observed. This was followed by a sharp increase in pH on 33rd day till close to the end of the composting process when the pH decreased gradually. For treatment T2, notable variations of pH were observed, with the readings being slightly higher than in other treatments for most part of the composting period. Mean pH readings in the control were significantly lower compared to all other treatments ($p < 0.001$) (Table 4.7). Analysis of covariance (ANCOVA) indicated that pH values recorded on day 8 were significantly different from those made on 13th, 14th, 15th, 16th, 19th, 20th and 27th days. pH changes recorded from this study revealed a significant positive correlation with the temperature readings ($r= 0.717$, $p = 0.01$).

Table 4.7: Mean levels of physicochemical parameters throughout the composting process

Treatment	**Temperature (ºC)**	**EC** (µS/ L)	**pH**
T0	33.25±5.98c	682.59±245.55c	7.89±0.47c
T1	36.19±8.32a	1266.20±533.81a	8.03±0.61ab
T2	34.57±7.58ab	1028.26±414.53b	7.99±0.65b
T3	35.54±7.31a	1171.42±456.59a	8.17±0.66a
T4	34.44±6.04ab	990.37±466.33b	8.12±0.70ab
P value	0.001	0.001	< 0.001

Values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level.
T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

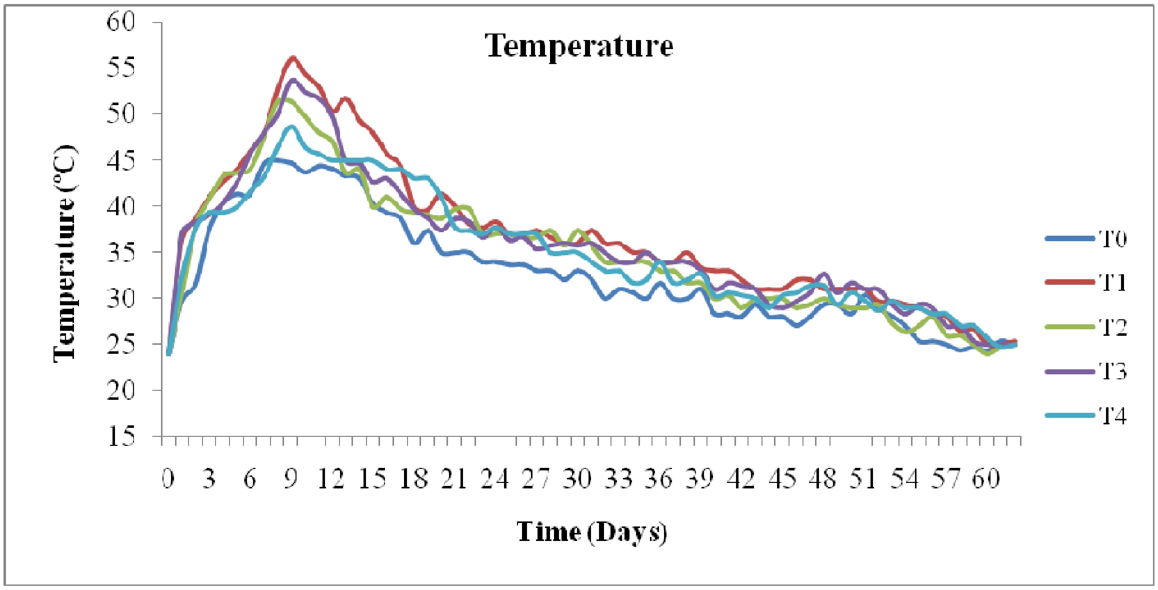

Figure 4.6: Temperature readings throughout rice straw composting period
T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

4.5.3 Electrical conductivity (µS/ L)

Inoculation demonstrated higher efficiency in affecting the electrical conductivity (EC) of the amended rice straw compared to the control throughout the process. During the study period, EC values of the composting materials were within the range of 217- 2147 µS/ L. The lowest and the highest EC reading occurred in treatment T0 and T1 respectively. Mean EC values of treatment T1 and T3 were significantly higher than those of the other treatments ($p < 0.001$) (Table 4.7).

The electrical conductivity of the compost increased gradually during the composting period with a sharp decrease occurring betweenday 14th and day 18th in treatment T3.

Treatment T1 recorded the highest EC readings in most of the days while T0 usually had the lowest EC readings (Fig. 4.7). Analysis of covariance (ANCOVA) revealed that the EC readings recorded on 8th day of the composting period were significantly different from those of all other days except the 18th, 11th and 15thdays (p = 0.997, p = 0.479 and p = 0.101, , respectively) at 5 % level of significance. There was also a significant negative correlation between EC values with the temperature (r= - 0.592, p = 0.01).

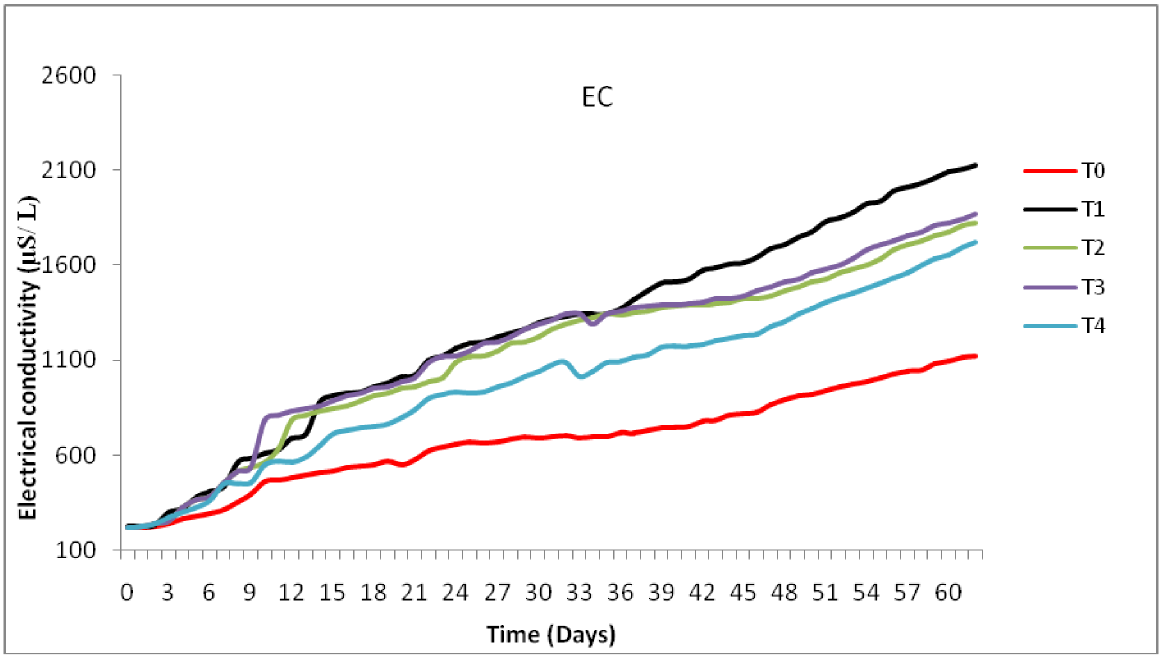

Figure 4.7: Electrical conductivity readings during rice straw composting process
T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

4.6 Physicochemical properties of the final composts

Eventual completion of the composting process was accompanied by several physical and chemical changes on the feedstock materials. There were visible changes in the appearance of the composting materials in terms of colour and texture. By 62nd day of composting, all the compost materials showed fine texture, much darker brown or black colours and homogeneity of materials (Plate 4.8).

Plate 4.8: Physical properties of compost samples from resultant rice straw composts
T1– compost made from rice straw mixed with chicken droppings; T3- compost made from rice straw treated with microorganisms isolated in preliminary experiments of this study.

4.6.1 pH in final rice straw composts

pH values of the final composts obtained in various treatments did not show any significant differences (Table 4.8). All the composts had final pH values close to neutrality (7.16- 7.56). Pearson's correlation coefficient revealed a negative significant relationship between pH and nitrogen content of the composts ($r = -0.527$, $p = 0.05$).

4.6.2 Electrical conductivity (EC) (dS/ m)in final rice straw composts

Electrical conductivity levels of the final compost ranged from 1.85 to 2.13 dS/ m. The lowest value was recorded in T2 while the highest EC reading was reported in compost T0. Except in T1, the electrical conductivity of the resultant composts was higher than that of the feedstock.

4.6.3 Cation exchange capacity (CEC) (Cmol/ kg) in final rice straw composts

The cation exchange capacity of the final compost was affected significantly by the treatments of the study. The highest mean CEC reading (25.25±2.90) was observed in set up treated with chicken droppings (Table 4.8). This value was significantly higher tcompared to that of the control (p = 0.046). Pearson's correlation coefficient revealed a positive significant relationship between CEC values with phosphorus concentrations (r= 0.730, p = 0.01). A similar relationship existed between CEC and nitrogen, but it was not statistically significant (r= 0.464).

Table 4.8*:* Levels of physicochemical properties of various rice straw composts

Treatment	**pH**	**CEC (Cmol/ kg)**
T0	7.57±0.30	16.96±4.70b
T1	7.16±0.48	25.25±2.90a
T2	7.68±0.26	24.09±3.06ab
T3	7.95±0.33	21.22±1.12ab
T4	7.32±0.21	21.70±1.60ab
P value	0.410	0.004

Where P is significant, values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level. T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

4.6.4 C: N ratio in final rice straw composts

Organic carbon content of the final compost was significantly affected by the treatments used in this study. Organic carbon content in T0 was significantly higher compared to that of T1 and T2 (p = 0 .035 and p = 0.048, respectively). A huge reduction in the organic carbon from the initial carbon is seen in their C: N ratios. The C: N ratio values showed significant differences (p = 0.001) (Table 4.9). Treatment T0 produced compost with the highest C: N ratio while treatment T1 produced compost with the lowest C: N ratio (Table 4.9).

Table 4.9: C: N ratio of the starting substrates and of the final composts

Treatment	**Initial**	**Final**
T0	60.33±3.51a	23.75±2.70a
T1	58.67 ±1.53a	10.84±1.28b
T2	38.33±0.58c	12.22±2.17b
T3	38.31 ±1.16c	10.99±0.45b
T4	52.00±2.00b	11.32±2.08b
P value	< 0.001	< 0.001

Values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level. T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

4.6.5 Available phosphorus (mg/ kg) in final rice straw composts

Concentration of available phosphorus in the resultant composts varied widely among the various treatments of the study with lowest concentration being obtained in the control (T0) and the highest in T1 respectively (Table 4.10). One way ANOVA revealed significant differences among treatments T0, T3 and T4 ($p < 0.001$). Available phosphorus concentrations in T2 were significantly higher than in T3 ($p < 0.001$). The phosphorus values correlated negatively with EC values (r= - 0.724, p = 0.01). Pearson’s correlation coefficient also revealed a strong negative significance relationship between phosphorus concentrations with carbon content (r= 0.710, p = 0.01).

4.6.6 Percentage nitrogen in final rice straw composts

Total nitrogen levels in final rice straw composts of all the treated rice straw were significantly higher than in the control (T0) ($p = 0.008$). The nitrogen content however did not differ significantly among the other four treatments; T1, T2, T3 & T4 at 5 % level of significance (Table 4.10). Nitrogen readings recorded among all the compost types were within a narrow range of between 1.04 and 2.24 %. Pearson's correlation coefficient revealed a strong negative significant relationship between nitrogen concentrations and carbon content ($r= - 0.704$, $p = 0.01$).

4.6.7 Potassium and Calcium in final rice straw composts (Cmol/ kg)

Statistical analysis of the potassium content mean values by one way ANOVA indicated no significant differences among treatments. The levels of potassium ranged narrowly from 7.74 to 8.35 Cmol/ kg. The highest content of potassium was obtained in treatment T3 while the lowest was obtained in T0 (control). Calcium content varied within a wide range of between 23.42 and 31.64 Cmol/ kg. However, these values were not statistically different (Table 4.10).

Table 4.10*:* Chemical properties of the final rice straw composts

Treatment	**Available P (mg/ kg)**	**Nitrogen %**	**Potassium (Cmol/ kg)**	**Ca (Cmol/ kg)**
T0	733.73±19.62c	1.12±0.09b	8.17±0.84	30.17
T1	1094.50±65.39a	1.87±0.38a	7.90±0.86	23.42
T2	1090.33±24.37a	1.94±0.14a	7.94±0.31	27.24
T3	829.17±36.08c	2.10±0.00a	8.35±0.80	31.64
T4	842.50±17.32b	1.87±0.40a	7.74±0.80	27.98
P value	< 0.001	< 0.001	0.668	0.812

Values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level. T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

4.6.9 Heavy metal concentrations in final rice straw composts

Various heavy metals tested in the compost (cadmium, lead, copper and zinc) were all at detectable and varying concentrations among the five compost types. One way ANOVA indicated that the concentration of the heavy metals differed significantly among treatments. The amount of cadmium detected in T3 was significantly higher compared to that in T1, T2 and T4 ($p < 0.001$). Lead concentration value in the control was significantly higher compared to all other samples ($p < 0.001$) (Table 4.11). Copper levels in T0, T1, T2 and T3 were all lower than those in uncomposted rice straw (sampled before the composting process (Table 4.12). Zinc mean levels ranged from 3.80 to 6.50 mg/ kg which were detected in T2 and T1 respectively.A negative significant correlation was revealed between germination index (GI %) values and levels of copper and zinc in the compost types ($r= - 0.788$, $p = 0.01$ and $r= - 0.858$, $p= 0.01$, respectively).

Table 4.11: Concentrations of heavy metals in various compost types (mg/ kg)

Treatment	**Cadmium**	**Copper**	**Lead**	**Zinc**
T0	0.07±0.0179ab	1.12±0.06b	3.18±0.21a	6.30±0.13a
T1	0.0435±0.0037b	1.01±0.11bc	1.54±0.37b	6.50±0.14a
T2	0.0434±0.0110b	0.66±0.45d	2.00±0.14b	3.80±0.13c
T3	0.0801±0.0048a	0.95±0.10c	1.68±0.11b	4.24±0.14c
T4	0.0396±0.0037bc	1.35±0.03a	1.82±0.31b	5.44±0.39b
P value	0.004	< 0.001	< 0.001	< 0.001

Values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level.
T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

Table 4.12: Heavy metals' content in feedstock materials before composting (mg/ kg)

Substrate	Cadmium	Copper	Lead	Zinc
Chicken droppings	0.0637	2.5469	3.5048	13.7738
Donkey dung	0.0252	1.5505	2.1923	6.7571
Untreated rice straw	0.0483	1.1462	1.351	0.9093

4.6.10 Bioassays on compost stability, maturity and phytotoxicity

4.6.10.1 Humification properties

Humification index (HI) in all the five compost types was generally high ranging from 2.57 to 11.05 % (Table 4.13). A relatively lower HI value in T3 positively correlated with the highest DH of 31.66 % in the same compost. HI values of the five composts revealed positive significant correlation with GI values ($r = 0.556$, $p = 0.05$). The positive effect of HI on tomato growth was indicated by a positive significant correlation between HI and root length ($r = 0.553$, $p = 0.05$)

Table 4.13: Mean values of humification properties of various rice straw composts

Treatment	HI %	DH %	C(HA+FA) %
T0	6.77±0.0001b	13.78±0.0128b	0.0037±0.0000
T1	6.80±0.0003b	10.71±0.0066c	0.0035±0.0000
T2	11.05±0.0014a	8.95±0.0872d	0.0028±0.0000
T3	2.57±0.0000c	31.66±0.6099a	0.0045±0.0000
T4	3.04±0.000c	13.55±0.2954b	0.0034±0.0000
P value	< 0.001	< 0.001	

Values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level. T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

4.6.10.2 Germination Index (GI)

Germination index of the composts tested in the present study varied from 182.33±4.63 to 275.67±5.49 %. Compost T2 had the highest germination index, followed by T3, while T1 had the lowest germination index (Fig. 4.8). The germination indices of compost T2 and T3 (prepared using commercial EM and isolated microbial inoculum, respectively) were higher relative to other treatments. Pearson's correlation coefficient revealed a significant negative relationship between levels of lead (r= - 0.788, p= 0.01) with the germination index of the compost.

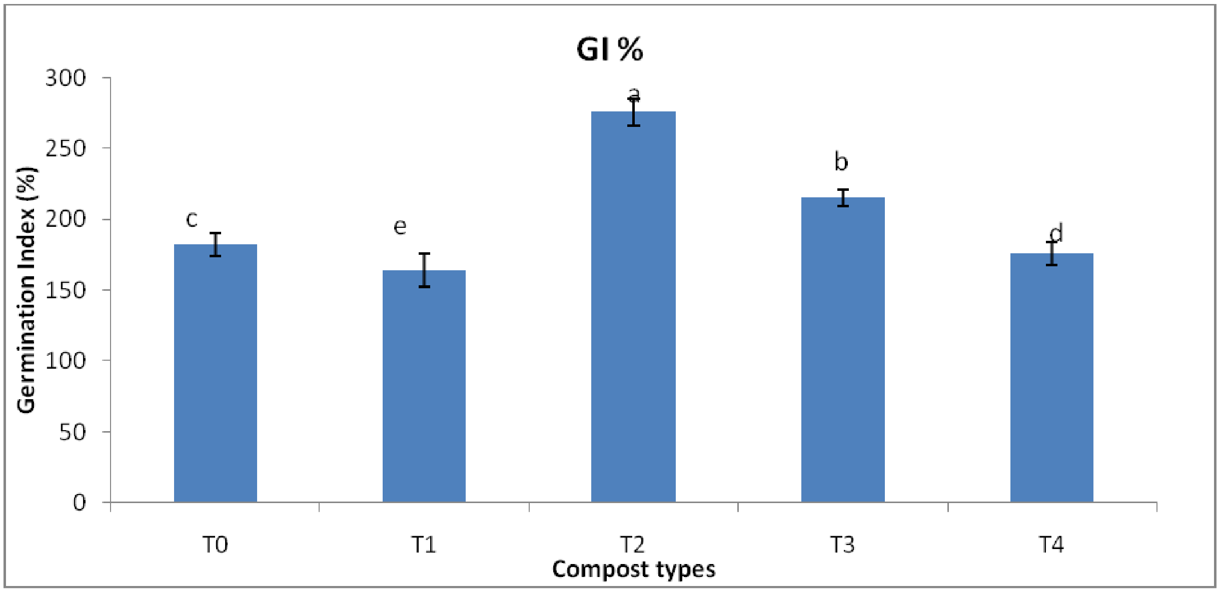

Figure 4.8: Germination index values of various rice straw composts
T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung. Letters a- d represent the standard errors among the significantly different GI values.

4.6.10.2.1 Number of tomato seeds germinated

Rate of seed germination in different concentrations of compost extracts varied from one treatment to the other. Average number of seeds germinated after 72 hours, in 50 %

compost extract of compost T4, was more than those germinated in 100 % compost extract of the same treatment. On the other hand, for T3, the average number of seeds germinated after 72 hours, in 50 % compost extract was less than those germinated in a 100 % compost extract. Extracts of compost prepared using microorganisms isolated during the study (T3) showed the highest rate of seed germination (80 %). The lowest rate of seed germination was observed in compost extracts of treatment T0. Over all, results from this experiment showed that the compost extract used favoured higher seed germination when it in the diluted forms (75 % and 50 %) than at 100 % concentration (Table 4.14, Table 4.15).

Table 4.14: Number of tomato seeds germinated in 100 % of rice straw compost extracts

Time	T0	T1	T2	T3	T4
24 Hours	0	0	0	0	0
48 Hours	1	5	5	2	1
72 Hours	3	7	6	8	6
Average %	30 %	70 %	60 %	80 %	60 %

T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

4.6.10.2.2 Root elongation (after 14 days) (mm)

Tomato seeds treated with 50 % compost extract had relatively higher root length compared to those treated with 75 % and 100 % compost extract. At 50 % compost extract concentration, the seeds in T2 had a significantly higher mean root length value than those of T0, T1, T3 and T4 ($p < 0.05$) There were no significant differences in the root length among various compost types both at 70 % and 100 % compost extract concentration (Table 4.16).

Table 4.15: Number of tomato seeds germinated in 50 % of rice straw compost extracts

Time	T0	T1	T2	T3	T4	Control
24 Hours	3	0	0	2	0	0
48 Hours	7	5	4	4	5	6
72 Hours	7	8	6	7	7	7
Average %	70%	80%	60%	70%	70%	70%

T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

Table 4.16: Root length (mm) of tomato grown in rice straw composts extracts

Treatment	100 %	75 %	50 %
T0	10.39±0.15	13.49±0.43b	17.04±0.16b
T1	12.30±0.07	10.17±0.09c	13.12±0.11c
T2	12.83±0.04	19.83±0.06a	20.17±0.19a
T3	11.50±0.16	12.57±0.28b	16.79±0.41b
T4	11.26±0.24	10.91±0.21c	12.47±0.55c
P value		< 0.001	0.001

Values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level.
T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

4.6.10.3 Plant growth index (PGI)

Seed germination occurred within 7 days after planting, in all compost types at all compost concentrations (100, 80, 60, 40 & 20) except for T0 at 80 %, T2 and T3 at 100 % while no seeds germinated in the treatment without compost (0 % or 100 % vermiculite), which later occurred at the 12th day.

Results indicate that the rate of seed germination was lowest in compost type T2 and in the control. For all the compost types, the 80 % compost concentration had the highest values (16.92±8.57, 42.80±12.20, 39.74±6.04) for fresh weight per plant, number of leaves and plant height respectively (Table 4.18).

Flower buds in the tomato plants first appeared on the 47th day of the growth period. This was observed in tomato plants in treatments, T0 100 %, T0 80 %, T0 60 %, T1 100 %, T1 80 %, T3 40 %, T4 80 % and T4 60 %. By the 55th day (day of termination of the experiment), none of the plants under treatment of composts T2 had produced any flower bud. Treatment T1 had the highest rate of flower bud formation as it occurred in all concentrations of compost T1 except at 40 %. By the 52nd day of growth the flowers bud in treatment T1 80 % opened completely to indicate anthesis. The highest (26.28 g/ plant) and the lowest (1.58 g/ plant) fresh weight per plant was observed in 60 % compost of treatment T4 and in 40 % of treatment T2, respectively, after 55 days of growth. Whole plant dry weight per plant ranged from 0.20 to 3.96 g/ plant in T2 and T4 respectively. The lowest PGI value (93.05 %) and the highest (241.88 %) were obtained in T2 and T3 respectively (Table 4.17). Analysis of variance did not reveal any significant differences in the different PGI mean values at 5 % level of confidence.

Root: shoot ratio was generally low with the lowest ratio being observed in the plants grown in 100 % concentration of compost T0. The highest root: shoot ratio was recorded in tomato plants grown in 40 % concentration of compost type T0 (Table 4.18). In this study, it was shown that most tomato plants grown at 20 % concentration of compost showed signs of chlorosis especially in treatment T2.

Table 4.17: Plant growth indices of the compost types and dry weight of tomato plants

Treatment	PGI %	Dry weight/ plant
T0	240.45	2.04
T1	237.92	2.35
T2	93.05	0.69
T3	241.88	1.81
T4	210.62	1.79
P value	0.084	0.084

Values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level. T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

Table 4.18: Tomato growth parameters at different concentrations of rice straw compost

Concentration	Wet weight/plant	Leaves/plant	Height/plant
100 %	13.86±7.65ab	40.25±10.93a	36.55±8.18a
80 %	16.92±8.57a	42.80±12.20a	39.74±6.04a
60 %	13.62±6.14ab	36.05±7.78ab	39.06±6.24a
40 %	10.12±5.82b	31.35±6.07b	38.30±5.01a
20 %	6.36b±2.56c	20.80±7.52c	28.59±5.70b
P value	0.043	< 0.001	< 0.001

Values followed by the same letters within the columns are not significantly different from each other according to Tukey`s Honest Significant Difference (HSD) at 5 % level.

T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

Table 4.19: Root: shoot ratio of tomato plants grown in rice straw composts extracts

Concentration	100 %	80 %	60 %	40 %	20 %
Treatment					
T0	0.14	0.23	0.35	0.44	0.26
T1	0.16	0.29	0.24	0.18	0.21
T2	0.15	0.15	0.18	0.22	0.23
T3	0.15	0.23	0.31	0.20	0.23
T4	0.20	0.17	0.35	0.21	0.26

T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung.

4.6.11 Nutrient levels in tomato plants

Nitrogen, phosphorus, potassium, calcium and magnesium (N, P, K, Ca and Mg) content in the tomato plants analyzed were all higher than those in the composts. Concentration (%) ranges for P, K, Ca and Mg were from 0.26 to 0.54, 0.11 to 0.7, 0.45 to 2.08 and 0.65 to 1.95, respectively. Analysis of variance showed that there were no significant differences in the mean values of all these macro and micronutrients analyzed among various treatments (Figure 4.10). However, copper content (25.97±3.15) (mg/ kg) in tomato plants under treatment T4 were significantly lower than those in tomato plants in all other treatments ($p < 0.001$). Similarly, tomato crops in treatment T0 had significantly lower zinc content (43.37±11.74) (mg/ kg) than all other treatments ($p < 0.001$). There were no significant differences in the variation between compost and tomato nitrogen, phosphorus, potassium, calcium and magnesium concentrations among treatments. Copper concentration in the tomato plants were significantly higher than those in the composts used to grow the crops ($p< 0.001$).

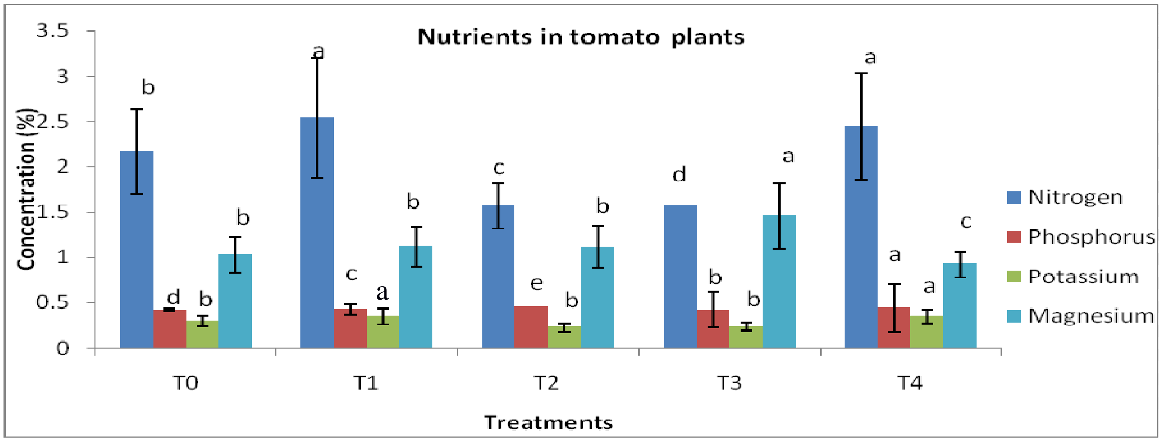

Figure 4.9: Levels of nutrients in tomato plants grown in different rice straw composts T0- Control; T1- rice straw treated with chicken droppings; T2- rice straw treated with commercial EM; T3- rice straw treated with microorganisms isolated in preliminary experiments of this study; T4- rice straw treated with donkey dung. Letters a- d represent the standard errors among the significantly different values.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Activities of isolated lignocellulolytic microbial cultures

High number of bacterial isolates (49) than fungi (20) obtained from the screening experiment can be explained by the fact that bacteria are more cosmopolitan and less metabolically complex than fungi (Partanen *et al.,* 2010). Bacteria are able to depend on simple sugars that are usually readily available on organic matter (Cohan, 2002). However, higher rate (55 %) of lignocellulolytic potential was observed for fungal isolates than the bacteria (41 %). Despite the bacteria having been more than the fungi, most proved that they do not possess lignocellulolytic ability. Bacterial colonies selected however demonstrated to be strong producers of the relevant enzymes. Among the selected fungi, lignocellulolytic potential was very well demonstrated as shown by the formation of large and visible zones of decolourization around the colonies on the test dyes (Plate 4.5). These results support the fact that fungi are better ligninase and cellulase producers compared to bacteria (Berlin *et al.,* 2005).

The dyes used (Azure II, Phenol Red and Methylene Blue) are structurally similar to lignin. Ability to decolourize the dyes indicates that the cells have degraded the dyes, most probably by secreting enzyme lignase (Pointing, 1999). Presence of filter paper on the culture media was quickly overcome by the fungal culture as reflected by their luxurious growth. Ability of the cultures to colonize the filter paper which is mainly made of cellulose meant that the organisms were secreting enzymes that enabled them to breakdown the filter papers. The results observed on the bacterial colonies suggest that the bacteria have relatively low levels of lignocellulolytic ability than fungi.

The negative results on thermotolerance tests on all fungi evaluated, supports the fact that most fungi are mesophilic with microbial thermophily being more established in bacteria (Ashraf *et al.,* 2007). Absence of thermotolerance ability meant that the isolates would be most active during the mesophilic phase of composting while the positive isolates would be involved in driving composting during high temperatures. The results obtained during the study of microbial population changes in composting attest to this. It is expected that the microorganisms would work synergistically in degrading the lignocellulolytic rice straw after inoculation. The survival of the microbial cells after inoculation dependens on availability of nutrients and other factors as provided within the composting materials (Maki *et al.,* 2009).

Ability to form spores would also be important in enhancing the survival of the cells during periods of limited activity. *Bacillus* species, which were the most predominant among the bacterial isolates, are known spore formers (Paul and Clark, 1989). Persistent presence of these microbial cultures within the compost would be of importance especially for those isolates known to be agents of biological control such as the *Trichoderma* species (Ashraf *et al.,* 2007). *Trichoderma* species were also the most predominant among the fungal isolates. This suggests that the resultant compost produced from treatment T3 was very rich and would impact positively on the microbial load, diversity and activity of recipient soil. Such organisms are known for their role in increasing crop yields and controlling plant diseases (Blaya *et al.,* 2015). They impact on plant growth by facilitating nutrient cycling and supplying biologically active substances to plants. They also suppress pathogens by producing antagonistic substances which act on the pathogens (Somers *et al*., 2004). Presence of such organisms in the final compost will certainly be beneficial to agricultural crops.

The benefits of microorganisms in compost have been demonstrated by the ability of the resultant compost types to support growth of the tomato plants in the present study. This confirms that the bioorganic fertilizers were of a balanced composition that would improve soil properties to support plant growth.

The growth of the tomato plants in the present study solely depended on the bioorganic fertilizers, which were only mixed with varying concentrations of vermiculite, a nutrient deficient growth medium. This confirms the beneficial effects of the bioorganic fertilizers which is further demonstrated by the fact that the tomato plants did not show symptoms of any pathogenic infection. This means that the composts were of high sanitary standards. This is likely to have been achieved during the thermophilic stage of the composting process. High temperatures during this phase normally suppress any pathogens that may be present in the feedstock materials. This demonstrates that the bioorganic fertilizers can effectively be applied to increase crop yields for better food security.

The ability of composting to significantly reduce the weight and volume of the rice residue as observed in the present study attests to the role of composting in reduction of waste materials from the environment. Their removal from the environment would control breeding of harmful rodents and pests among other environmentally oriented benefits.

5.1.2 Diverse properties of the lignocellulolytic cultures

The features used in classification of the bacterial isolates used in this research correspond with those described in Bergey's Manual of determinative Bacteriology (Skerman *et al.,* 1989), while classification of fungal isolates correspond with the guidelines in "Systematics of Fungi" by Onofri and Belisario (1998).

Results obtained by visual and microscopic observation of the fungal colonies were mostly reliable for classifying the isolates only up to the genus level. However, for a few of them such as in Genus *Aspergillus*, morphological characterization could identify the isolates to species level. For example, *A. niger* could be easily identified by its production of characteristic black spores. All colonies suspected to be *A. niger* were therefore not subjected to molecular tests. In the most part, preliminary identification results for both bacteria and fungi corresponded with those obtained through molecular analysis. Preliminary classification indicated that the fungi belonged to five genera including *Aspergillus, Trichoderma, Fusarium, Penicilium* and *Mucor.*

Results of preliminary identification of bacterial isolates based on morphological and biochemical tests gave slight variations after further characterization using the extracted genomic DNA. This is in regard to the fact that a few of the colonies that appeared filamentous and having highly branched mycelia had been suspected to be actinomycetes. This however was not supported by analysis of their DNA sequences, which indicated that they were bacteria. This can be explained by the fact that some bacteria are filamentous. Further, bacteria tend to exhibit varied growth characteristics on different culture media and under varying environmental conditions (Cohan, 2002).

Some bacteria showed varied responses to some biochemical tests. This was observed in a few of the colonies studied, whereby a colony would be positive/ negative for a particular test but would give different results on a repeat test (Table 4.3). This is actually a known property among certain bacteria, including *Bacillus* (Sullivan *et al.,* 1987). Notwithstanding, the results obtained from the biochemical tests were still useful for preliminary classification of the organisms. Isolate AB21 was conclusively identified as *Serratia marcescens* through morphological and biochemical analysis.

Molecular analysis of targeted sections of DNA (16S rRNA and ITS) of both the microorganisms used as starter culture for composting and those isolated from the composting experiment confirmed that the isolates were genetically different. Sanger sequencing of the genes at 16S rRNA and ITS region in the DNA of the cells successfully confirmed the identity of all the bacterial and fungal isolates, respectively. The identity obtained from the DNA sequences largely corresponded with the preliminary characterization results.

The percent identity levels (80- 99 % and 95- 99 % for both bacteria and fungi respectively) obtained after searching for similarity of the DNA sequences in BLAST were generally reliable for conclusive identification of the organisms. Further, the length of the sequences obtained from the Sanger sequencing were also within the expected length of about 500 bp for fungi and 1500 bp for bacteria. The successful identification and the high levels of similarity of the DNA sequences with those in genBank reflects on the effectiveness of molecular identification through the use of DNA sequences rather than other molecular approaches such as genes expression. Unlike gene expression, use of sequences is a more accurate approach in molecular characterization.

Results from molecular characterization confirmed that lignocellulolytic ability is widely distributed among members of both Kingdom Monera and Fungi. According to the results, lignocellulolytic potential is very common among members of Division Ascomycota, in Kingdom Fungi, while members of Phylum Firmicutes especially those in Genus *Bacillus* are also lignocellulolytic. Members in Genus *Bacillus* can be said to have demonstrated their high speciation rates and the ability to have ecological specialization within compost due to their high prevalence rate of 100 % (Papke and Ward, 2004).

Different clusters generated from phylogenetic analysis of the DNA sequences of the microbial isolates as shown in the dendograms reflect on the genetical similarities and differences among the microbes. Microorganisms which have evolved along the same line of evolution appear in the same cluster while those that have evolved along different lines appear in different clusters. It has been shown that closely related organisms may show ecotype variations depending on the different ecological functions brought about by their varied ecological roles (Acinas *et al., 2004;* Case and Boucher, 2011). It has further been established that different organisms may adapt to similar ecological environments making them functionally similar (Hunt *et al*., 2008).

The clustering of bacterial and fungal isolates as shown by their respective dendograms suggests that the bacterial isolates have closer evolutionary relationship with each other than the fungi. Different bacteria are at different positions in the evolutionary lineage which may have also arisen from genetic changes such as deletions substitutions or insertions through mutations, in addition to the speciation caused by ecological roles (Brown and O'Neill, 2010). For example bacteria AB5 seems to have arisen through several events of genetical changes within a relatively short period of time compared to other clusters.

The position of bacteria AB14 identified as *Psychrobacter* spp is a true reflection of the current classification system that classifies extremophiles as ancient bacteria. *Psychrobacter* are largely psychrophillic in nature, growing optimally within a temperature range of between -10 to 40 ºC.This is also true for isolate AB9 (*Bacillus* *safensis*) whose position suggests that it is an ancient form of bacteria. *Bacillus safensis* is also a known extremophile. Species of *Bacillus safensis* inhabitate varied places, some having being found to live in the gut of warm blooded animals. The position of bacteria AB8 in the evolution lineage relates with the fact that the bacteria *Bacillus amyloliquefaciens* was confirmed as a novel organism not so long ago (in the year 1987) (Priest *et al.,* 1987).

Isolate AF6 was the only *Cladosporium* species obtained during the study; the evolutionary history of the organism as illustrated by the dendogram, shows that it is not very closely related with the other fungi. The results also suggest that it is likely to be among the the oldest organisms in evolution lineage among the fungi studied. Evolutionary position of fungi AF14, as shown by the results of the study suggests that it is among the most recent organisms in its lineage. The organism was identified as *Penicillium citrinum*, a telomorph in Genus *Penicillium* whose novelty was not established too long ago.

Although culture-independent techniques have an indispensable usefulness for the characterization of compost microflora, culture-dependent methods, which permit the isolation and physiological characterization of microorganisms, represent a valuable approach enabling the assessment of their role within the microbial community. However, molecular characterization is generally known to be more rapid and more accurate. Unlike phenotypic identification, 16S rRNA and ITS sequencing provides accurate identification of isolates with atypical phenotypic characteristics.

5.1.3 Microbial population changes during composting

Temperature readings observed in the composting experiment were a reflection of changes in metabolic actions of organisms within the composting materials. The initial low temperatures depict the phase during which the microorganisms were adapting to the environment having limited nutrients available for utilization (Insam and Klammer, 2002). The rise in temperature that followed this phase depicts increased metabolism by the microorganisms as nutrients became more available, hence more heat was generated (Peters *et al.,* 2000). The notable peaks in temperature levels represent the thermophilic phases of the bioprocess, with peaks noted during advanced stages of composting being lower than the initial one and more short lived, suggesting reduced microbial activity and vitality as the compost matured (Kiyasudeen *et al.,* 2016; Lee, 2016).

The significant differences in weight loss observed indicates that the composting process was able to successfully bioconvert and transform the rice straw (Pan *et al.,* 2012; Lim *et al.,* 2015). Possible low bioconversion activities at the initial stages of the composting process are further suggested by the low weight reduction rate at the start of the experiment. The positive relationship between temperature changes and weight loss is in support of the importance of temperature during bioconversion of organic matter (Rawat and Johri, 2013).

Changes in physicochemical parameters during the composting process were accompanied by changes in microbial populations and their diversity. An initial increase in the number of bacterial isolates observed in this study was due to the ability of these organisms to use simple nutrients such as sugars that were readily available in composting materials (Ghazifard *et al.,* 2001). Availability of nutrients and the temperature regimes highly favoured growth of mesophilic bacteria most of which belonged to Genus *Bacillus.*

The gradual increase in microbial diversity during the composting reflected on further microbial adaptation to the composting environment and their rapid multiplication to become the most populated species (Ghazifard *et al.,* 2001; Insam and Klammer, 2002). Rise in population densities was later followed by decline in the microbial load. The decline observed from time to time during the composting process indicates that the microorganisms could not cope with the environmental conditions prevailing in the compost piles leading to their death (Ryckeboer *et al.,* 2003). The number of bacteria recorded during the thermophilic phase 1.30 x 10^6 CFU/ g were comparatively lower than 3.1 x 10^8 CFU/ g, obtained in a similar study by Abu-Bakar and Ibrahim (2013).

The high prevalence rate by members of Genus *Bacillus* and *Serratia* corresponds to their established cellulolytic ability mostly reflected in their importance as decomposers of solid materials. *Bacillus* species are ubiquitous soil and compost borne saprotrophytes, which has been exploited in the commercial production of industrial enzymes (Grimont and Grimont, 2006). Theyare involved in decomposing cellulosic materials due to their known cellulolytic activities and common presence in plant litter and such related wastes (Maki *et al.,* 2009). Different species of bacterial populations have been detected at varying stages of waste degradations from previous studies (Ryckeboer *et al.,* 2003).

Takaku *et al*. (2006) isolated a high number of bacterial strains (87) in a study on microbial communities in rice hull composting using culture independent methods. This confirms the limiting nature of culture dependent methods. It is expected that culture independent approach would yield more abundant bacteria from the organic materials. The fungi isolated from the compost were mainly *Asperigillus niger* and *Trichoderma harzianum*. Results of the present research relate with Anastasi *et al*. (2005) who reported the highest load and number of species of *Aspergillus* in addition to *Penicillium* in a study on two composts.

5.1.4 Effects of inoculation on the composting process

Using the starter cultures of the study in composting the rice straw successfully hastened the bioprocess which was completed within 62 days. This is a remarkable improvement from the traditional period of three months and above that is generally taken by materials of plant origin to decompose (Romero-Olivare *et al.,* 2017).

Variations in temperature, pH and EC levels among treatments of the present study are a reflection of the differences in the biodegradation activities within the compost materials as affected by treatment of the rice straw with different starter cultures. These changes are as a result of biochemical conversions within the compost. The physical and chemical environment within the composting pile and the parameters usually fluctuate depending on the type, number and the activities of the organisms within the composting materials (deBertoldi *et al.,* 1983).

Statistically significant differences revealed in temperature readings among treatments of this study are due to variations in microbial biochemical activities in the compost as affected by the treatments on the rice straw (Davis *et al.,* 1992; Lee, 2016). Low mean temperature value (33.25) in the control relative to other treatments confirms this inference. Three phases (mesophilic, thermophilic and cooling) of composting in relation to temperature changes were observed in all the treatments of the present study. Thermophilic phase was first attained in treatment T1 and lastly in T4. This can be explained by the fact that chicken dropping is a nutrient rich medium compared to rice straw and donkey dung, hence it activated a more rapid microbial degradation. A prolonged thermophilic phase in the experiments of this study is indicative of the high initial C: N ratio of the rice straw (Eiland *et al.,* 2001). Umsakul and Srimuang (2010) conducted a similar experiment using water hyacinth but the highest temperature reached was 40 ºC, which is attributable to the low initial C: N ratio of the water hyacinth. The decline in temperature at the end of composting (curing phase), indicates that most of the degradable components had already been converted into stable compounds and the materials were well stabilized (Keener *et al*., 2000).

The high temperature levels observed in T1 may have led to production of high quality compost, as reflected by the high plant growth index values recorded in compost from the same treatment. High temperatures play an important role in sanitization of the compost hence the high growth rate of tomato plants. This makes composting an effective approach for suppressing disease causing pathogens and for removal of weeds that may be present in feedstock materials (Postma, 2003).

The relatively high electrical conductivity (EC) levels in the rice straw treated with chicken droppings (T1) indicate that the manure had the highest degradative effect on the rice straw leading to a high release of free ions as compared to other treatments of the study (Smith and Doran, 1996). Chicken dropping is known to harbour high numbers of microorganisms suggesting that the manure can be exploited as a possible source of lignocellulolytic microorganisms for industrial applications (Devi *et al.,* 2012). Moreover, chicken dropping has readily available nutrients that can enhance the microbial populations in compost through biostimulation (Das and Chandran, 2011).

Treatment T1 was closely followed by T3 in respect to EC. Treatment T3 starter culture consisted of a high population of known microorganisms that were carefully formulated and had been shown to be in their active form. The nature and state of this inoculum therefore explains why T3 showed higher biochemical activity compared to T2, T4 and the Control (T0) as suggested by the high EC. The highest EC value recorded from this study (1.266 mS/ L) was lower compared to 3.85 mS/ L obtained by Pan and Sen (2013) in a similar experiment using wheat straw. This might be due to the fact that rice straw is more recalcitrant than wheat straw hence lower in dissolution into ions (Pan and Sen, 2013). EC is determined by the amount of soluble salts within a sample and it is an indicator of the composting status (Saidi *et al.,* 2008).

The slight decline in pH readings at the early stages of composting might have been due to release of organic acids into the composting medium. Alkaline conditions observed in this study as shown by high pH during the later stages of the experiment might have been due to volatilization and degradation of the organic acids by microorganisms and the subsequent release of ammonia through mineralization (Nolan *et al.,* 2011). The peaks detected at 10.46 on day 23 for T1 and at 10.28 on day 22 for T3 also suggest high production of ammonia. During the final stages of the composting experiment, the pH levels stabilized to be within the recommended range of between 6 and 8) (Troy *et al.,* 2012). The pH variation profiles among treatments of the present study are similar to those obtained by Zhu (2007) and Li *et al.* (2008) who did co-composting of animal manure with rice straw.

The pleasant earthy smell detected during the composting process is an indicator of proper aeration and absence of anaerobic conditions. Completion of the composting process was also signaled by the visual changes in colour and texture of the compost (Lee, 2016). Changes in the physicochemical parameters greatly determine the progress and the outcome of every composting process (Lim *et al.,* 2015).

5.1.5 Effects of inoculation on resultant composts

Organic nutrients in the composting materials are used by microorganisms during decomposition of organic materials transforming them into inorganic forms which can be utilized by plants (Eklind and Kirchmann, 2000). Consequently, the rate at which these nutrients are converted helps to estimate the extent of decomposition achieved during composting as well as quality of the final compost with regard to plant available nutrients.

C: N ratio values of the composts produced by the treatments of this study were all within the recommended range of between 10- 18 except in the control (T0). High C: N ratio in compost indicates presence of unutilized complex nitrogen and carbon while complete breakdown of these materials is indicated by low a C: N ratios (Dobermann and Fairhurst, 2002). The C: N ratio in treatments T1, T2, T3 and T4 were all lower than 17.6: 1, the value in compost produced by inoculating rice straw with a microbial consortium in a research carried out by Pan and Sen (2013). Results from this study indicated that C: N ratio had a significant effect on total nitrogen and organic carbon ($p < 0.05$) of the final compost. Organic carbon content of final composts were significantly reduced compared to those recorded by (Baffour-Asare, 2009; Prempeh, 2010).

All the compost types produced by the present study attained the recommended pH level of between 6 and 8 (Troy *et al.,* 2012), with the values being close to neutrality (7.16-7.56). A suitable pH in compost is very critical because it is the greatest factor that influences nutrient availability for uptake by plants. Acidic pH (< 5) is detrimental to plant growth due to imbalances in nutrient levels (Smith and Doran, 1996). Nutrients such as phosphates, nitrogen, potassium and calcium are poorly available in acidic pH while aluminium and manganese become available up to toxic levels for the plants. A pH greater than 8 implies possible high levels of exchangeable sodium and magnesium (Fageria and Zimmermann, 1998; Butterfly *et al.,* 2013).

The positive correlation between cation exchange capacity (CEC) of the composts with phosphorus and nitrogen contents recorded in the present study ($r= 0.730$, $r= 0.464$, respectively) supports the fact that humic acids, the main components of compost bind the positively charged multivalent ions (Pedra *et al.,* 2008). Composts generally have high cation exchange capacity levels which increase the soil CEC when added into soil (Fuchs, 2002).

Nitrogen content in composts from all the treated rice straw in the present study was significantly higher than that in the control. Results from the present study demonstrated that percent nitrogen in all the composts types increased compared with that of the initial substrates. This is a clear reflection of the role of microorganisms in nutrient cycling. Nitrogen is a crucial component of proteins, nucleic acids and amino acids necessary for microbial growth and function (Uyanoz, 2007). The highest compost percent nitrogen value of 2.10 % recorded in this study was only slightly higher than 2.08 %, obtained by Kausar *et al.* (2014) in an experiment on composting rice straw. Nitrogen concentration in compost is very critical since too high or too low amount in compost products can lead to phytotoxicity effect on crops due to release of ammonia and organic acids by microorganisms in the soil (Eklind and Kirchmann, 2000).

Compost produced by inoculating rice straw with the isolated lignocellulolytic microorganisms (T3) had the highest levels of nitrogen (Table 4.8). This indicates that inoculation of rice straw with the selected microorganisms may have played a significant role in producing good quality bioorganic fertilizer. However, mean phosphorus level in this compost was significantly lower than those in T1, T2 and T4 (Table 4.8). All the composts produced in this research had phosphorus concentrations within the recommended levels of between 800- 2500 mg/ kg (Hogg *et al.,* 2002), except for the compost from the control experiment, T0 (733 mg/ kg). Compost quality is directly related to the microbial communities involved in composting (Peters *et al*., 2000; Taiwo and Oso, 2004).

Addition of effective microorganisms (EM) in treatment T2 significantly increased the phosphorus content (1090.33) compared to other treatments ($p < 0.05$). Positive effects of EM on composting rice straw are also demonstrated by the significant high levels of cation exchange capacity (CEC) of the resultant compost in this treatment (24.09 Cmol/ kg, $p = 0.004$) (Table 4.8). This suggests that EM is suitable for increasing mineralization of organic matter during composting. Properties of this compost type (T2) indicate that it was within the category of well matured composts and can be used without any restriction (Hogg *et al.,* 2002). Available phosphorus concentrations were also significantly affected by the chicken droppings used to treat the rice straw.

Potassium content levels of resultant composts of the present study also indicated that the composts were well matured. Potassium concentration mean value of 1.65 observed by Li-li *et al.* (2013) in a study on composting rice straw using rabbit manure was lower compared to 7.74, obtained from compost T4 prepared usingdonkey dung in the present study. Similar to a study by Kausar *et al.* (2014), results of the present research demonstrated that inoculation of rice straw is able to increase the levels of organic compounds in the final compost.

5.1.6 Effects of composting on heavy metals bioavailability

Presence of heavy metals in compost raises serious concerns about the adverse ecological effects of application of such compost to agricultural land (Pollack and Favoino, 2004). Excessive accumulation of heavy metals in the soil may eventually pollute plant, microbial, human and animal food chains (Paradelo *et al.,* 2008).

Levels of the heavy metals (Zn, Pb, Cu and Cd) analyzed in the present study were all below limits in the guidelines for countries with set standards for compost utilization (Table 5.1, Vander Derf *et al.,* 2002). However, low levels of heavy metals in compost can still cause negative impacts to seed germination as indicated by the negative significant correlation revealed between germination index (GI) values and levels of copper in the compost types. The results of this study generally indicate that the concentration of some heavy metals in the final product increased while the levels decreased for other heavy metals for various treatments compared to the concentrations in the untreated rice straw. This can be explained by the fact that when microorganisms interact with chemical elements in their environment, the end result is either the creation of a less toxic or a more toxic environment (Chibuike and Obiora, 2014).

The significantly high lead concentrations in T0 (control) compared to all the other treatments indicates that the amendments in the other treatments significantly contributed towards reducing lead from the compost materials during composting. The extra microbial populations present in the four treatments through their biological additives might have played a role in lowering the lead concentrations (Barker and Bryson, 2002). The relatively high zinc levels detected in T1 are likely to have originated from poultry feeds. Animal feeds are usually fortified with zinc among other essential minerals in order to keep the animals in good health (Tiquia, 2010; Duian *et al.,* 2014). The feeds might have found their way into the chicken droppings used in this treatment, hence the high concentration. The concentrations of the heavy metals (Zn, Pb, Cu and Cd) in all the compost types of this study were significantly lower compared to those reported by Prempeh (2010).

Table 5.1: Limits of heavy metals (mg/ kg) for countries with compost standards

Element **Country**	**Cadmium**	**Copper**	**Lead**	**Zinc**
Austria (Class 1)	4	400	500	200
Belgium (Agr)	5	100	600	1200
Switzerland	3	150	150	2000
Denmark	1.2	-	120	5000
France	3	-	800	-
Germany (Class 2)	1.5	100	150	400
Italy	10	600	500	2500
Netherlands (Class A)	2	300	140	1200
Spain	10	450	300	1100
Canada	3	100	150	14000

Key: Class 1versus class 2or class A versus Class AA are calculated on 30 % organic matter basis; Agr- agricultural use (Vander Derf *et al.,* 2002).

5.1.7 Effects of composting process on compost maturity and stability

Levels of humification, germination potential and plant growth potential of the composts types of this study were evaluated to test their phytotoxicity, maturity and stability. Compost obtained from treatment T3 had the lowest humification index (HI) value and the highest degree of humification (DH) (Table 4.11). Based on this, compost type T3 was the most matured among all the composts tested. A low level of maturation was observed in compost T2 as suggested by its having the lowest DH % of 8.95 and the highest HI level of 11.05 (Table 4.11). However, HI values in the five compost types were all higher than the recommended limit of < 0. 5 % (Ciavatta *et al.,* 1990). The high levels may have been due to the recalcitrant nature of the rice straw feedstock used in this experiment (Rosmiza *et al.,* 2014). The high carbon content in the rice straw may have also contributed to the high HI index level observed in this study (Das and Dkhar, 2012).

Germination indices of the compost types studied were all above 100 % (Fig. 4.8) the recommended threshold for testing phytotoxicity in compost. It has been suggested that a germination index of 50 % indicates the disappearance of phytotoxicity (Zucconi *et al.,* 1981; Wong *et al.,* 2001), while increased GI is indicative of decreased phytotoxicity and hence a more matured product (Tiquia *et al.,* 1998).

The relatively high germination indices of compost T2 and T3 further confirms the beneficial effects of use of microorganisms as composting starter cultures. Activities of the microorganism may have played a role in removing phytotoxic substances that might have been in the compost piles (Tiqua, 2010). On the other hand, low germination index value of compost prepared using chicken droppings (T1) compared to T2 and T3 suggests that this compost had relatively more phytotoxic substances (Tiqua, 2010). Such substances might have originated from antibiotics and other feed additives normally used in raring chicken. These eventually find their way into the chicken droppings (Zhang *et al.,* 2012). However, high rate of seed germination and root elongation at 50 % compost concentration than at 100 % for the same compost type suggests that successful use of such composts in crop farming can be achieved by mixing the compost with other growth medium and through watering (Keener *et al.,* 2002; Barral and Paradelo, 2011).

GI generally increased with increase in the dilution of the extract suggesting that there were no phytotoxic organic compounds in the mature compost. This is because phytotoxic substances normally produce inhibitory effects on seed germination, even in low concentration (Estaun *et al.,* 1985; Jodice, 1989). Compost germination index test results reported by Doncean *et al.* (2013) using tomato (*Solanum lycopersicum* L) seeds were within the range (176.00 to 275.67) obtained in the present study (Fig. 4.8). Similarly, Sesay (1997) recorded high germination rates for tomato and attributed it to high tolerance of electrical conductivity of tomato seeds. This means that the inhibitory potential on the tomato seeds used in this study may also have influenced the results obtained.

Plant growth index (PGI) values of between 93.05 % and 241.88 % (Table 4.15), obtained in the present study reflect a high level of maturity and stability in the compost types tested (Hogg *et al.,* 2002; Barral and Paradelo, 2011). These levels meet the requirement for PGI of compost according to the standards for countries with compost quality guidelines (Hogg *et al.,* 2002). The results showed that the type of compost and the concentration of the compost affected the rate of growth of the tomato plants. The positive relationship between PGI values and phosphorus content of the composts studied supports the fact that the plant growth is directly affected by compost quality (Brinton, 2001). Based on the PGI test, the order of the quality of the compost types investigated was T3> T0> T1> T4> T2. Presence of heavy metals in the compost influenced the PGI and the characteristics of the tomato crop as suggested by several observations made during the study.

5.1.8 Effects of the resultant compost types on tomato growth

The composts types used to grow the tomato crops in the present study were of varied physicochemical conditions. Levels, quality and quantity of the nutrients such as P, N, K, Mg and other exchangeable ions (CEC, pH and EC), determine the plant growth process, its resistance to pests and pathogens and net productivity (Bakht *et al.,* 2009).

The variations in physicochemical quality of the compost observed may have caused the differences in the growth properties of the tomato plants studied in this research. The significantly high phosphorus content in T1 corresponded with the generally high PGI in the same treatment at all concentrations of the compost. Negative relationships between phosphorus, nitrogen and the shoot and root fresh weight, respectively, confirms the fact that plants respond to nitrogen and phosphorus availability by changing their root: shoot ratios (Agren and Franklin, 2003). The low root: shoot ratio observed in all the tomato plants in this experiment suggests that the compost types did not have nutrient limitations (Table 4.17) (Ericsson, 1995). A decrease in soil fertility commonly corresponds to a rise in the root: shoot ratio, meaning that shoot growth increases more in weight than root growth (Yeh *et al.,* 2000). Plants' response to the environment to optimize their resource use is indicated in the allocation between shoots and roots depending on nutrient availability (Bloom *et al*., 1985). Nitrogen stimulates shoot growth at the expense of root growth while phosphorus usually stimulates root growth (Agren and Franklin, 2003). Generally, when nutrient availability increases, plants allocate relatively less to their roots, which is consistent with a resource optimization theory as increasing nutrient availability means that less effort is required to acquire this resource (Ericsson, 1995). Plants growing in phosphorus deficient soil allocate a greater proportion of assimilates to root growth (Schreeg *et al.,* 2014).

Composting organic materials is effective in suppressing disease pathogens and weeds that may be present in feedstock materials. This fact might have led to the tomato plants showing no signs of infection despite the fact that they were not treated with conventional pesticides. Some microorganisms may act as biological control agents leading to disease suppression. The extra microbial load present may also act as a strategy of biostimulation which might be remedy for contaminated soils. The study has demonstrated that tomato crops can be grown organically by use of bioorganic fertilizers.

5.1.9 Nutrient levels in tomato plant tissues

Bioavailability of nutrients in the composts of this study is shown by the positive correlation between the nutrient content in the composts with that in tomato biomass.Bioavailabilityof nutrients for plant uptake is determined by several factors making the relatioship between nutrients and plant systems complicated and interdependent (Malvi, 2011; Gibson *et al.,*2006).

Low levels of nitrogen, phosphorus and potassium (0.93 to 3.5, 0.26 to 0.54, 0.11 to 0.7 %) in the tomato plants indicates that the plants were not able to obtain sufficient amounts of these nutrients from the growth medium. Extremely low phosphorus content in the tomato plants does correspond with the levels recorded in most of the compost types studied (Table 4.10, Fig. 4.9). Conversely, calcium and magnesium levels in the tomato plants (0.45 to 2.08 and 0.65 to 1.95 %) were within the recommended sufficiency limits for tomato crops (Reuter and Robinson, 1986; Mills and Jones, 1996; Hogg *et al.,* 2002).

The increase in nutrient levels in the tomato plants compared to those in the composts, observed in this research, is a reflection of the outcome of the process of natural assimilation. The comparatively higher levels of copper and zinc metals in the tomato plants demonstrate the occurence of bioaccumulation of toxic elements along the food chain (Pollack and Favoino, 2004). Higher copper content in the tomato plants compared to that in the composts used to grow the tomato crops is an indicator of bioaccumulation. Biomagnification and the transfer of nutrients usually occur along the food chain (Pollack and Favoino, 2004). Presence of copper in the tomato plants also reflects on the bioavailability status of the heavy metals in the composts studied (Yashim *et al.,* 2014).

5.2 Conclusions

According to results of the present study, the following conclusions have been drawn:

i) Rice straw is inhabited by lignocellulolytic bacteria and fungi mainly belonging to Phyllum Firmicutes and Division Ascomycota respectively.

ii) Addition of lignocellulolytic bacteria and fungi and the starter cultures of this study significantly enhanced the composting processof rice straw

iii) Addition of lignocellulolytic bacteria and fungi and the starter cultures of this study significantly improved the quality of rice straw compost.

iv) Microbial changes during composting largely correspond with changes in physicochemical parameters within the composting materials.

v) Composting is an effective approach for reducing the amount of organic wastes in the environment.

vi) Bioorganic fertilizers have the ability to support and promote plant growth.

5.3 Recommendations

i) Rice straw and animal waste should be exploited as sources of lignocellulolytic microorganisms for use in composting.

ii) The lignocellulolytic microorganisms should be formulated into a composting starter culture at commercial scale.

iii) Composting processes should be enhanced by use of both commercially available biological products as well as locally available materials.

iv) Use of bioorganic fertilizers in crop farming should be encouraged to supplement or complement chemical fertilizers.

v) Composting should be employed as an environmental conservation and protection strategy.

vi) Future studies should focus on analyzing the enzymes secreted by these lignocellulolytic microorganisms including the rate of secretion.

vii) Survival and persistence of exogenous microbes with potential biogical control ability in compost should be evaluated to ascertain their presence in the final compost.

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APPENDICES

DNA sequences of 16S rRNA region of test bacteria

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GTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGC
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GAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAG
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DNA sequences of ITS region of test fungi

>AF1_*Trichoderma harzianum*

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>AF2_*Aspergilus flavus*

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>AF3_*Aspergilus* spp

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>AF4_*Trichoderma* spp

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>AF5_*Penicilium* spp

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AACGGATCTCTTGGTTCCGGCATCGATGAAGAATTCAACGAAATGCGATAAC
TAATGTGAATTGCAAAATTCAGTGAATCATCCAGTCTTTGAACGCACATTGCG
CCCTCTGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCCAG
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>AF6_*Cladosporium* spp

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AGAAA

>AF7_*Fusarium chlamydosporum*

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>AF8_*Trichoderma afroharzianum*

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CTGTCCGAGCGTCATTTCAACCCTCGAACCCCTCCGGGGGGTCGGCGTTGGG
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>AF9_*Aspergillus* spp

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>AF10_*Fusarium incarnatum*

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>AF11_*Trichoderma* spp

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>AF12_*Trichoderma* spp

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>AF13_*Fusarium* spp

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>AF14_*Penicilium citrinum*

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CAAGCCCGGCTTGTGTGTTGGGCCCCGTCCCCCCCGCCGGGGGGACGGGCCC
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>AF15_*Fusarium equisetum*

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>AF16_*Trichoderma* spp

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