MICROBIAL POPULATION DYNAMICS, ENZYME ACTIVITY AND QUANTIFICATION OF NUTRIENT RELEASE IN SOIL AMENDED WITH COMPOSTS WITH VARYING DEGREE OF MATURITY

by

Shikwambana Sydney

A MINI-DISSERTATION
Submitted in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE

in

AGRICULTURE (SOIL SCIENCE)

in the

FACULTY OF SCIENCE AND AGRICULTURE
(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO

SUPERVISOR: PROF. FR KUTU
CO-SUPERVISOR: MR. OF MADIBA

2015

DECLARATION

I declare that the Microbial population dynamics, enzyme activity and quantification of nutrient release in soil amended with composts of varying degree of maturity (mini-dissertation) hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Soil Science) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Shikwambana S **25-08-2015**

Full names **Date**

ABSTRACT

The activity and functional diversity of micro-organisms contribute to the stability and productivity of agro-ecosystems. Soil micro-organisms and enzyme activities have been suggested as potential indicators of soil quality. Hence, management practices that can enhance microbial diversity and enzyme activities are essential for improving soil health and soil fertility status. The aim of the study was to assess the effects of compost maturity age on the change in bio-quality indicators of compost and compost amended soil. Cattle manure-rich compost was prepared through thermophilic windrow composting using cattle manure and wood chips mixed at a proportion of 4:1 (w/w) to achieve a C:N ratio of 30:1. This compost was sampled at regular intervals of 30 days after the initiation of the composting process until 150 days when it was finally cured. Compost samples of varying degrees of maturity age were air-dried, pulverised and mixed with 1.2 kg surface soil at an equivalent rate of 100 kg P ha^{-1}. Each compost amended soil was transferred into well labelled plastic pots for incubation. Sampling of incubated amended soils was performed at 7 days interval until 42 days; and the samples were used for microbial count, enzyme activity, and mineralisation assessments. Data generated were analysed as factorial experiment using SYSTAT package. Treatment and interaction effects were evaluated using Fisher protected least significant difference at probability level of 5%.

Results of the chemical composition of the different composts are similar and comparable. Variation in compost maturity date, incubation time and their interaction exerted significant effects on the measured microbial counts and enzyme activities as bio-quality indicators. The content of bacteriameasured was consistently highest at each sampling date followed by the actinomycetes while fungi population count remained persistently lowest. Bacteria and β-glucosidase represent the dominant microbe and enzyme, respectively in all compost samples taken at different maturity age.The highest count of actinomycetes (6.18 CFU g^{-1}), bacterial (6.73 CFU g^{-1}) and fungi (3.06 CFU g^{-1}) were obtained during the 42-day incubation period.Of all the enzyme activities studied, β-glucosidase content was consistently highest in all compost samples across the sampling dates. Similarly, the highest concentration of ß-glucosidase (3076 mg kg^{-1} hr^{-1}), phosphatase (1480 mg kg^{-1} hr^{-1}), dehydrogenase

(120.07 µg INF g^{-1} $2hr^{-1}$) and urease (26.15 mg kg^{-1} $2hr^{-1}$) were obtained during the 42-day incubation period. The highest microbial counts and enzyme activities were reached beyond 19 days after incubation. Maximum Bray P1 (20.10 mg kg^{-1}), ammonium N (108 mg kg^{-1}) and nitrate N (189 mg kg^{-1}) were obtained at 42, 14 and 42 days after incubation, respectively. The measured temporal change in the concentrations of bio-quality parameters in the compost-amended soils were highest in compost sampled at 90 days, except for phosphatase, indicating the peak of the thermophilic process. The bio-quality parameters of these composts and the compost amended soil were influenced by compost maturity and incubation time. The uses of mature compost with desirable level of bio-quality indicators are crucial for fertility management and improved soil health.

Keywords: Compost maturity, enzyme activities, microbial count, nutrient release, soil fertility

DEDICATION

I dedicate this mini-dissertation to my beautiful wife, Amukelani Nompumelelo Shikwambana, stunning daughter Tintswalo Tiara Shikwambana, and my mother Mnene Florence Shikwambana.

ACKNOWLEDGEMENTS

Firstly, I thank the almighty God for giving me life, strength, courage and guidance; otherwise this work would not have been possible. The following people deserve special recognition. I thank my supervisor Prof FR Kutu for his enthusiasm, humour, valuable comments, constructive supervision and especially his patience throughout my study which is highly appreciated and treasured. I am also grateful to my co-supervisor, Mr OF Madiba for his valuable advices and comments. I would like to extend my special thanks to my mother Mrs Mnene Florence Shikwambana, beautiful wife Amukelani Nompumelelo Shikwambana, sisters, brothers, friends and relatives for their continued love, support and encouragement which in one way or another helped in fulfilment of this work. My sincere appreciation and thanks go to the Management of ZZ2 Berty van Zyl (Pty) Ltd, the compost production team and Dr B Nzanza for the assistance in setting up the compost heaps and collecting the compost samples. I thank Ms Tsakani Mokase and Mr Maxon Masowa for assisting in laboratory analyses. I am particularly grateful to the Department of Agriculture, Forestry and Fisheries (DAFF) for providing me with study bursary through the Professional Development Program (PDP) of Agricultural Research Council (ARC) for my postgraduate studies. Finally, I also acknowledge with thanks the additional financial support received from ARC for the implementation of my research work.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background information

Composting has been a waste management method used for the production of organic fertiliser worldwide. Organically grown foodstuffs are highly priced and considered as healthy compared to food grown with chemical fertilisers. Many types of soil-borne pathogens commonly found in soilless potting media are known to be disease-causing and also negatively affect yields (Kutsanedzie *et al*., 2015). However, compost suppresses these pathogens but the degree of suppression depends on the maturity and type of compost (Wang *et al*., 2004). Composting is differentiated from the natural decomposition of organic material since it is a process that is controlled by humans. Organic materials are recycled by nature regardless of whether or not, they are composted.

Compost maturity plays an important role in distinguishing whether it can be used as a soil conditioner for physical, chemical and biological fertility benefits or for disease suppression in soil (Kutsanedzie *et al*., 2015). Nevertheless, the chemical properties of composts often influence the severity of control of the soil-borne disease (Kutsanedzie *et al*., 2015). In mature composts, the starting raw materials are sufficiently decomposed under controlled moisture and aeration conditions leading to the formation of a stable organic product (Tiquia, 2002). The use of compost requires the production of a mature product that will prevent nutrients that are present in the soil from being tied up or immobilized. Mature compost is thus normally dark brown in colour, possesses even texture and has a pleasant and earthy aroma (Latifah *et al*., 2015).

During composting, the starting organic materials are modified by decomposition and humification through a wide range of biological and biochemical transformation processes that are actively controlled by enzymes (Rigane *et al*., 2013). From an agricultural point of view, high hydrolytic activity in composts is often beneficial. The enzymes involved during the processes promote the release of nutrients and low molecular weight organic substances that can be taken up by microbes (Tiquia, 2002). The concentration of nutrients in composts varies widely depending on the

type of compost and its maturity. The effectiveness of these nutrients in comparison to mineral fertilisers also depends on the type of compost and its maturity (Rigane *et al*., 2013).

The abundance of organisms during the composting process influence the transformation of organic materials into its final product of humic substances that are capable of retaining and releasing inorganic plant nutrients and energy in the form of heat (Latifah *et al*., 2015). These organisms are especially numerous and active during the initial phase of composting, but many remain in smaller numbers even in the finished product (Zmora-Nahum *et al*., 2005). In mature compost, enough of the original organic materials would have been consumed to prevent any substantial increase in microbial activities and therefore heat-generating capacity of the remaining materials (Sherman, 2005). This microbial stability is a prerequisite for compost maturity since stabilized compost is no longer subject to sudden chemical changes, and may therefore be safely handled, stored and/or applied (Hartley *et al*., 2009).

1.2 Problem statement

Application of unstable composts to soil has been reported to exert undesirable influences such as competition for oxygen between soil microbial biomass and seeds/plant roots thereby leading to inherent soil problems such as the production of hydrogen sulphide and nitrite, which are considered toxic (Bernal *et al*., 2009). The emission of ammonia and the presence of other phytotoxic chemical substances like phenolic compounds and ethylene oxide synthesized during the decomposition of immature compost in soil, have been reported (Marhuenda-Egea *et al.*, 2007). Another problem closely associated with the use of partially cured or unstable compost is the potential problem of soil nitrogen (N) deficit and plant starvation following application of immature compost with a high C:N ratio (Tiquia, 2002). Furthermore, the use of immature compost may immobilize native and added N and phosphorus (P) and thus suppress plant growth (Hutchison *et al*., 2005). Consequently, this study will provide information on the potential impact of varying degree of compost maturity on selected soil quality parameters.

1.3 Motivation for this study

The successful use of compost in crop production depends largely on its suitability and its potential agronomic value as influenced by its degree of maturity. Thus, this study is crucial at providing detailed information on its potential impact on soil health and farmers' profits. This will contribute to the elimination or reduction of the potential risks that may be associated with the direct use of manures such as the spread of pathogens, parasites and weed seeds (Latifah *et al*., 2015). Hence, a healthy soil under favourable crop production conditions, following the use of mature compost, will promote high crop yields and farmers' profits.

1.4 Purpose of the study

1.4.1 Aim

The aim of the study is to assess the effects of compost maturity on the temporal variation of selected bio-quality indicators and the nitrogen release characteristics of compost amended soil.

1.4.2 Objectives

The objectives of this study are to:

(i) evaluate the effect of variation in the degree of compost maturity on microbial population dynamics in compost amended soil.

(ii) evaluate the effect of variation in the degree of compost maturity on enzyme activity in compost amended soil, and

(iii) evaluate effect of variation in the degree of compost maturity on the nitrogen release characteristics of compost amended soil.

1.5 Hypotheses

This study validated the following hypotheses:

(i) Variation in the degree of compost maturity exerts no positive effects on the dynamics of microbial population in compost amended soil.

(ii) Variation in the degree of compost maturity exerts no positive effects on enzymes activity in compost amended soil.

(iii) Variation in the degree of compost maturity exerts no positive effect on the nitrogen release characteristics of compost amended soil.

CHAPTER 2

LITERATURE REVIEW

2.1 Importance of composting

Composting is defined as a biological process of transformation of organic matter under aerobic thermophilic and mesophilic conditions by which native micro-organisms produce a material that is stable, sanitized and with an important concentration of humic substances (Marhuenda-Egea *et al.*, 2007). Compost amendments are an attractive way to incorporate organic matter into the soil as it has beneficial properties, including mobilization of mineral phosphates (Wickramatilake *et al*., 2010).

Compost maturity cannot be described with a single property but it is best assessed by measuring two or more parameters. Maturity is related to the stability of the material and it also includes the potential impact of other chemical properties of the compost on plant development. Immature compost may contain high amounts of free ammonia, certain organic acids or other water-soluble compounds which can limit seed germination and root development. Any use of compost requires it to be mature and free of any potentially phytotoxic components (Bernal *et al.*, 2009).

While composting occurs naturally, efficient composting requires the control of several factors to avoid nuisance problems such as odours and dust, and also for obtain a high quality agricultural product (Hartley *et al*., 2009). The controlled conditions are basic for a composting procedure, distinguishing it from aerobic fermentation. The control of parameters such as bulk density, porosity, particle size, nutrient content, C/N ratio, temperature, pH, moisture and oxygen supply have demonstrated to be key for composting optimisation since they determine the optimal conditions for microbial development and organic material degradation (García-Ruiz *et al*., 2008).

Factors affecting the composting process can be divided into two groups, namely; those depending on the formulation of the composting mix, such as nutrient balance, pH, particle size, porosity and moisture; and those dependent on the process management, such as O_2 concentration, temperature and water content (García-Ruiz *et al*., 2008). Nutritional balance is mainly defined by the C/N ratio. Micro-

organisms require energy source (degradable organic-C) and N for their development and activity. The adequate C/N ratio required for composting is in the range 25-35, because it is considered that the micro-organisms require 30 parts of C per unit of N (Kutsanedzie *et al*., 2015). High C/N ratios make the process very slow as there is an excess of degradable substrate for the micro-organisms. However, with a low C/N ratio, there is an excess of N per degradable C and inorganic N is produced in excess and can be lost as ammonia through volatilisation or leached from the composting mass (Kutsanedzie *et al*., 2015). Hence, low C/N ratios can be corrected by adding a bulking agent to provide degradable organic-C.

A pH of 6.7-9.0 supports good microbial activity during the composting process while the optimum values are between 5.5 and 8.0 (García-Ruiz *et al*., 2008). Usually, pH is not a key factor for composting since most materials are within this pH range. However, this factor is very relevant for controlling N-losses through ammonia volatilisation, which can be particularly high at pH greater than 7.5. Elemental sulphur has been used as an amendment for avoiding excessively high pH values during composting (Mari *et al*., 2005).

2.2 Microbial dynamics during composting process

Organic material decomposition is carried out by many different groups of microbial populations (Ryckeboer *et al*., 2003). The micro-organisms involved in composting develop according to the temperature of the mass, which defines the different steps of the process (Keener *et al*., 2000). Bacteria predominate early during the composting process while fungi are present at all stages during the process but predominate at water levels below 35%; and are not active at temperatures higher than 60°C (Latifah *et al*., 2015). Actinomycetes predominate during stabilisation and curing, and together with fungi are able to degrade resistant polymers (Ryckeboer*et al*., 2003).

Aeration is a key factor for composting; proper aeration controls temperature, removes excess moisture and carbon dioxide, and provides oxygen for the biological processes. The optimum oxygen concentration is between 15 and 20% (Latifah *et al*., 2015). Controlled aeration should maintain temperatures below 60-65°C, which ensures that enough oxygen is supplied (Latifah *et al*., 2015). The optimum water

content for composting varies with the wastes to be composted, but generally the mixture should be at 50-60% (Gajalakshmi and Abbasi, 2008). When the moisture content exceeds 60%, oxygen movement is inhibited and the process tends to become anaerobic (Zmora-Nahum *et al*., 2005). During composting, a large quantity of water can evaporate so as to control temperature. As water content diminishes the rate of decomposition decreases, and rewetting is required to maintain the optimum moisture content for the microbial activity.

The reported optimum temperature range for composting is 40-65°C (García-Ruiz *et al*., 2008) while the range of 52-60°C is described as the most favourable for decomposition (Gómez-Brandón *et al*., 2007). However, temperatures above 55°C are required to kill pathogenic micro-organisms but beyond 63°C, microbial activity declines rapidly. The regulation of temperature is therefore required for controlled composting. The removal of excess heat can be achieved through controlling the size and shape of the composting mass(Zmora-Nahum *et al*., 2005).Thus, improved cooling and favourable temperature redistribution is achieved by turning operations so as to remove heat through temperature feedback-controlled ventilation (Gómez-Brandón *et al*., 2007).

In general, the composting process can be divided into two main phases namely the bio-oxidative phase and the maturing phase (Parkinson *et al.*, 2004). According to Keener *et al*. (2000), the bio-oxidative phase consists of three steps. These are, (i) an initial mesophilic phase that last 1-3 days where mesophilic bacteria and fungi degrade simple compounds such as sugars, amino acids, proteins, etc. This increases the temperature more rapidly; (ii) the thermophilic phase where thermophilic micro-organisms degrade fats, cellulose, hemicellulose and some lignin resulting in maximum degradation of the organic matter (OM); and (iii) the cooling phase, which is characterised by a decrease in temperature due to the reduction of the microbial activity associated with the depletion of degradable organic substrates. The compost mass at this stage is recolonized by mesophilic micro-organisms which are able to degrade the remaining sugars, cellulose and hemicellulose.

The residual organic matter is transformed by micro-organisms to form humic-like substances, which form complexes with extracellular enzymes stabilizing them, and preventing their degradation and denaturation (Sundberg, 2005). Additionally, the

microbial community with different physiological profiles is used as indicator of compost maturity (Tiquia, 2002). The decrease trend of microbial biomass throughout the composting process is expected, and it is associated with temperature and C/N source consumption (Tiquia, 2002).Fungal biomass decreases during the active phase and maturation stage compared to initial compost (García-Ruiz *et al*., 2008), but the bacteria continue with degradation activity, enzymatic production and the humification process (García-Ruiz *et al*., 2008).

During the active phase of the composting process, organic C decreased in the material due to decomposition of the organic materials by micro-organisms. The degradation rate decreased gradually as the composting progressed with the formation of new complexes and polymerized organic compounds (humification) during the maturation phase (Salazar *et al*., 2005). Important enzymes were involved in this biochemical process associated to C substrates. These include cellulase complex such as β-glucosidase that hydrolyses glucose, proteases and ureases that is associated with N mineralisation, and phosphatases that remove phosphate groups from organic matter (Aira *et al.*, 2007).

Mondini *et al.* (2004) reported evidence of direct influence of microbial activity as sensitive indicator of the response of ecosystem to stress factors such as drought. Similarly, a variety of microbial population develops in response to different levels of temperature, moisture, oxygen and pH within a compost pile (Wickramatilake *et al*., 2010). This microbial diversity enables the composting process to continue despite the constantly changing environmental and nutritional conditions within a pile (Hutchison *et al*., 2005). Thus, composts at different maturity dates have different microbial populations; and the presence of high microbial population helps to reduce soil borne diseases (Ryckeboer *et al*., 2003).

2.3 Dynamics of enzymatic activity during composting

Compost maturity can be evaluated through its microbial stability by determining microbial activity factors namely biomass, count, metabolic activity, and the concentration of easily biodegradable compounds (Zmora-Nahum *et al*., 2005). Aerobic respiration assesses the aerobic activity and stability since carbon derived from catabolism is attached to oxygen and produces carbon dioxide, energy and

heat under the condition. These products are high when the activity is high and the maturity is not complete (Zmora-Nahum *et al*., 2005). During the active phase of the composting process, organic C decreases in the material following decomposition by micro-organisms. The rate of degradation decreases gradually as composting progresses due to reduction of new complex and polymerized organic compounds (humification) that occur during the maturation phase (Bernal *et al.,* 2009). Thus, enzymes are associated with biochemical indicators of decomposition of organic materials, confirming to be highly sensitive variables to changes in compost maturity. Important enzymes involved in this biochemical process include β-glucosidase, which is a cellulase complex thathydrolyses β-glucosidases, proteases, and ureases during N mineralisation, and phosphatases that remove phosphate groups from organic matter (Aira *et al*., 2007). The process offers stabilized end-products that can be used as C storage and slow release fertilisers for agricultural purposes.

The presence of high content of degradable compounds in an initial mixture of composting materials may have stimulated microbial growth and enzyme synthesis that will be limited by the presence of substrate available in the biochemical reactions (Goyal *et al*., 2005). The mineralisation process of organic matter can be studied by following the dynamics of enzymes over time and correlating it with other factors such as water soluble carbon, humic and fulvic acids concentration or the presence of microbial groups (Nannipieri, 2002). β-glucosidase is an extracellular enzyme associated to hydrolysis of terminations of β-glucose chains to yield β-glucose (Nannipieri *et al.*, 2002). Enzymatic activity decreases during a composting process while high activity indicates low stabilization of the decomposing material (Castaldi *et al.*, 2008).

Phosphomonoesterases catalyze the release of inorganic phosphorus (i.e. orthophosphate) from organic phosphomonoesters and are inhibited by substrate. Due to the decrease in the size of organic materials induced by phosphatases during composting process, phosphatases also tend to decrease at maturity (Hutchison *et al*., 2005). The urease activity is related to N metabolism and it hydrolyzes urea to release NH_4^+ (Makoi and Ndakidemi, 2008). This enzyme increases at the beginning of composting due to initial high concentration of proteins (Wickramatilake *et al*., 2010). Typically, during the composting process, urease activity fluctuates due to the depletion of the substrate and the synthesis of urea by proteases resulting in urease

increases along the stabilisation phase (Castaldi *et al.,* 2008). Urease thus has a positive correlation with ammonium N but negative correlation with nitrate N (Castaldi *et al.,* 2008).

2.4 Nutrient release characteristics during and after composting

The process of composting of three different animal slurry with straw, ammonia emission decreased in the order of poultry (77%) > pig (54%) > cattle (47%) (Magdoff and Wiel, 2004). Similarly, Ryckeboer *et al*. (2003) reported that the concentration of mineral elements within a compost heap increases during a composting process provided leaching losses are reduced. Contrarily, Boulter-Bitzer *et al*. (2000) reported that the concentration of ammonium N was highest during the thermophilic phase but decline rapidly as the composting process progresses. Also, other documented research reports suggest that organic material degradation and aeration demand are at the maximum during the thermophilic stage (Tiquia *et al*., 2002). A low NH_4-N/NO_3-N ratio in mature compost has been documented at compost maturity due to high nitrification process (Parkinson *et al.*, 2004).Thus, nutrient concentration and quality of the final product are strongly influenced by N losses during the composting process (Mondini *et al.*, 2004). Preventing ammonium N losses at the beginning of a composting process is therefore critical for nitrate N formation during compost maturity (Parkinson *et al*., 2004). Therefore, high N-losses occur in manure composting due to the high initial ammonium N concentration and the presence of easily mineralisable compounds such as uric acid in poultry manure and slurry (Bernal *et al.,* 2009).

Mondini *et al.* (2004) investigated the relationship between enzymatic activity and quality of OM and reported that the humic-enzyme complexes should be considered as a process directly related to compost stability. During compost maturity phase, carbon dioxide evolution, fulvic acid and NH_4 concentrations are reported to decrease while nitrification process and the concentration of humic increase with a nitrification index of less than 0.16 (Tiquia *et al.,* 2002).

2.5 Role of microbes and enzyme activity in soil fertility management

In a general solid waste stream, the overall efficiency of organic material break down depends on microbes and their activities. Micro-organisms through different kinds of substrate based hydrolytic enzymes promote the degradation of organic materials (Marschner *et al*., 2003). The enzymes released by the micro-organisms during composting breakdown several organic compounds characterized by a complex structure, finally leading to the solubilisation of simple water soluble compounds (Avis *et al.,* 2008). The degradation of the labile substrates contained in organic materials can be followed by studying specific hydrolases, which are relatively easy to determine, and specific to the substrate (Marschner *et al*., 2003). Various hydrolytic enzymes are believed to control the rate at which various substrates are degraded (Avis *et al.,* 2008). Important enzymes involved in the composting process include: cellulases, which depolymerise cellulose, B-glucosidases which hydrolyse glucosides, and urease involved in N-mineralisation, phosphatases and arylsulphatase that remove phosphate and sulphate groups from organic compounds (Mondini *et al*., 2004).

Soil enzymes have also been suggested as potential indicators of soil quality because of their essential role in soil management. Soil enzyme activities are both early and sensitive indicator of different management practices, inducing changes in soil fertility (Melero *et al*., 2009). Numerous authors have reported that the addition of organic amendments activates micro-organisms to produce enzymes related with the cycle of the most important nutrients (Madejón *et al*., 2001; Marschner *et al*., 2003).

2.6 Impact of compost maturity on compost quality attributes

Compost application stimulates biological activity through mineralisation thereby increasing the levels of soil available N and P and of some micro elements, depending on the maturity of compost (Salazar *et al*., 2005). The quality of mature compost in terms of biological activity in soil includes an increment of soil microbial biomass, soil respiration, and enzyme activities such as phospho-, mono- and di-estereases, dehydrogenases, β-glucosidases, arylsuphatases, deaminases, ureases and proteases (Hargreaves *et al.*, 2008). However, in some cases, a decrease on

protease, urease and deaminase activities is observed due to a toxic effect caused by the presence of trace elements (Hargreaves *et al.*, 2008).

Sources of C such as cellulose, lignin, starch, and the presence of N as proteins, organic P and other nutrients in mature compost stimulate biological activity and soil mineralisation processes. The main C sources in humic extract are polyphenolic compounds with low availability and mineralisation rate, although water soluble C can also be an important C source for soil microorganisms (Hao *et al.,* 2001). Soils with permanent availability of organic or inorganic sources of NH_4, reduce significantly their urease activity; the use of urea as fertiliser in agricultural soils can cause interference on the urease laboratory determination (García-Gómez*et al.,* 2003).

The addition of organic materials into the soil enhances microbial diversity as well as its biomass (Hargreaves *et al.,* 2008). Several authors have reported a general increase in functional groups such as mycorrhizal fungi and beneficial rhizosphere bacteria, in an agroecosystem through various farmers' practices that include the addition of organic materials into the soil. Salazar *et al*. (2005) found that the presence of humic acids at concentration of up to 30 mg L^{-1} from the addition of organic materials resulted in increased numbers of soil active microbes. Similar findings were reported by Kutsanedzie *et al*. (2015), García-Gómez *et al.* (2003) and Hao *et al.* (2001). Organic matter content is an important factor influencing microbial population, particularly the labile and organic sources of C, P, and N; from them, soil microbes construct aggregates and can proliferate within the soil ecosystem (García-Gómez *et al.,* 2003). The benefit associated with microbial diversity include plant growth promotion, due to several factors such as direct phytohormone production, or indirectly by mineralisation of organic matter or improvement of soil conditions; there is another important indirect way which contributes to promoting the plant growth: the suppression of plant pathogens and the systemic induced resistance that protect the plants of potential pathologies and pests (García-Gómez *et al.,* 2003).

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Description of the study sites

The study consists essentially of two major activities namely compost production and laboratory incubation study. The compost was prepared at ZZ2 Berty van Zyl (Pty) Ltd composting site (23°38'11.09"S and 30°20'44.33"E) near Mooketsi, which is 100 km North of Polokwane. The laboratory incubation work for N and P release and bio-quality indices assessment was done in the Soil Science laboratory at the School of Agricultural and Environmental Sciences, University of Limpopo. The bio-quality indices assessment, which involves microbial count and the measurement of enzyme activity in the incubated compost amended soil was done in the Soil Microbiology laboratory of the Agricultural Research Council, Grain Crops Institute (ARC-GCI), Potchefstroom.

3.2 Compost preparation and chemical characterisation

3.2.1 Compost preparation and sampling

The composition of the compost was a ratio of 20% fibrous plant materials (bailed hay/grass, wood chips and saw dust) to 80% cattle manure. The compost was made after determination of chemical composition of each raw material and the exact volumes of each of the raw materials was calculated to get the C:N ratio of 30:1 in the compost heap. The compost was turned every two weeks on activation with water and addition of Effective Micro-organisms (EM) to provide satisfactory aeration and enhanced microbial activities. The moisture content was measured daily and so as to maintain the moist at between 50 and 60% during the first 6-8 weeks. Follow-up turns were done according to the moist content and the temperature was measured daily. Temperatures above 63°C and low moisture content requires turning with water. Compost sampling was done at 30, 60, 90, 120, and 150 days after the commencement/preparation of the compost.

3.2.2 Chemical characterisation of the compost samples

3.2.2.1 pH measurement

The pH of each compost sample was determined in a 1:5 (mass/vol) ratio using a pH meter as described by Camoes (2009).

3.2.2.2 Electrical conductivity measurement

Electrical conductivity (EC), which is a measure of salt concentration in a compost-water mix (1:5 mass/volume ratio), was measured with a conductivity meter as described by Camoes (2009).

3.2.2.3 Total carbon and nitrogen measurement

Approximately 10 g of the compost samples (powder form) was used for total C and N determinations using a Carlo Erba NA 1500 C/N/S Analyzeras described by Okalebo *et al*. (2002).

3.2.2.4 Percent moisture, total solids, ash and organic matter

Duplicate 5 g sub-samples of each compost sample were used for percent moisture, total solid, and ash determination using standard analytical procedures (Okalebo *et al*., 2002). The percent organic matter content was calculated as shown in the equation: Organic matter = total solids - ash.

3.2.2.5 Determination of trace elements concentration

The determination of trace elements, namely Pb, Cd, As and Sb in the compost samples were performed following the procedure described by Mustafa (2003) while Zn, Cu, Fe and Mn were extracted following nitric acid digestion as described by Hseu (2004). The concentration of these elements was measured on an Inductively Coupled Plasma Optical Emission Spectrometric (ICP-OES).

3.2.2.6 Total elemental composition

Total macronutrient (P, K, Ca, Mg and Na) content in the compost samples was determined as described by Okalebo *et al*. (2002).

3.3 Description of the incubation study and accompanying laboratory determinations

The composts with different maturity dates were each weighed and thoroughly mixed with 1.2 kg surface soil with no recent history of organic amendment at an equivalent rate of 100 kg P ha^{-1} soil (an average of 462 g of dry compost equivalent in 1.2 kg of soil. Prior to the mixing, the soil was passed through a 2 mm sieve so as to remove all foreign materials including stones. Thereafter, the soil was transferred into labelled small plastic pots for incubation in the laboratory under a controlled temperature of between 15 and 25°C. Additional 1.2 kg soil without organic amendment was also incubated as control for the purpose of comparison.

The soil used was collected from experimental farm of School of Agriculture and Environmental Sciences, Syferkuil (23°50'36.86"S and 29°40'54.99"E), University of Limpopo and was sandy loam in texture. Initial soil analysis revealed the following: 11% clay, 6.35 mg kg^{-1} Bray P1, 0.48% organic carbon and pH (H_2O) of 7.6. Prior to the placement of the compost-amended soils in the incubation chamber, calculated amount of water was added just to keep the amended soil moist. This was maintained throughout the incubation period by checking the weight of the pots on a weekly basis and adding water as needed to adjust the weight back to the original level. The pots were, however, left opened to allow for free air exchange while the incubation process lasted for 42 days. Soil sampling for N, P, microbial counts, and enzyme activities determination was done at 7, 14, 21, 28, 35 and 42 days after incubation (DAI). During each sampling date, about 250 g soils was scooped out from each treatment, thoroughly mixed, labelled and used for the various determinations.

3.4 Laboratory determinations on incubated compost amended soil samples

3.4.1 Bray P1 extraction and determination

Available Bray P1 in 6.67g triplicate sub-samples from each compost amended soil was carried out as described by Bray and Kurtz (1945). Bray-P1 solution was prepared by mixing 30 ml of NH_4F solution (37 g of NH_4F dissolved in 1litre distilled water) with 50 ml of HCI in a 1000 ml volumetric flask; and then it was filled to the mark using deionised water. The weighed soil was then transferred into a 250 ml Erlenmeyer flask and 50 ml of Bray P1 solution added. The mixture was shaken

manually for 60 seconds then the extract was filtered through 42 mm Watchman filter paper.

The preparation of reagent A was achieved by first dissolving 12 g of ammonium molybdate in 250 ml of distilled water. Similarly, 0.2908 g of antimony potassium tartrate was dissolved in a separate 100 ml of distilled water. Both reagents were mixed with 2.5N sulphuric acid (148 ml conc. Sulphuric acid to 1l) in a 2l volumetric flask and distilled water was added to make the mark. Preparation of Reagent B was done by dissolving 1.056 g of ascorbic acid into 200 ml of reagent A.

A P-stock solution of 250 ppm was prepared using 0.549 g KH_2PO_4 in a 500 ml volumetric flask and distilled water added to the mark. Standard solutions containing various P concentrations (0, 0.5, 1, 2.5, 5, 10 and 12.5 ppm) were prepared using the stock solution. Approximately 100 ml Bray-1 solution was added to 0, 1, 2, 5, 10, 20, and 25 ml of the standard P stock solution and made to 500 ml mark with distilled water. Colour development in standard P solutions and in samples were done by mixing 5 ml of each of the standard solutions and sample extract, 3 ml distilled water and 2 ml reagent B in test tubes. The resulting solution was thoroughly mixed and left to stand for 30 minutes so as to allow for the full development of the molybdenum blue colour and the absorbance read on T60 UV-visible spectrophotometer at wavelength of 882 nm.

3.4.2 Mineral N extraction and quantification

Determination of nitrate (NO_3^-) and ammonium (NH_4^+) N in fresh compost samples were carried out following 0.5M K_2SO_4 extraction procedure described by Maynard and Kalra (2001). The concentration of NO_3 and NH_4 in the extract was measured calorimetrically using UV-Visible Spectrophotometer at 410 and 655nm wavelengths, respectively.

3.4.3 Microbial population count

Standard microbiological procedures described by Bloem *et al*. (2006) were employed for the isolation and enumeration of microbial groups. Different microbial growth media designed to be selective for heterotrophic microbes, actinomycetes and filamentous fungi were used for the microbial analyses. These microbial

populations were subjected to the physiological ability of microbes to grow on each of the selective media.

General heterotrophic plate counts were done on nutrient agar (NA). Actinomycete was isolated and enumerated on actinomycete isolation agar (Sigma-Aldrich, South Africa). To obtain filamentous fungal counts, malt extract agar (MEA), was used supplemented with 30ppm chloramphenicol and 50ppm streptomycin and prepared according to manufacturer's recommendations.

These various media were all sterilized at 121°C for 15 minutes and made into pour plates each consisting of a Petri dish (90mm diameter) containing an isolation medium. A dilution series ranging from 10^{-1} (using 1g of soil in 9ml of saline solution) to 10^{-5} was prepared in triplicate and a 100µL aliquot of each dilution was spread on the isolation plates. The various isolation plates were incubated at room temperature and enumerated after 3 days for the bacteria, and 7 days for the actinomycetes and fungi. The various counts, expressed as coliform unit per gram (CFU g^{-1}), were transformed into logarithm base 10 (i.e. Log_{10}).

3.4.4 Enzyme activity determination

Enzyme activities were determined calorimetrically using enzyme-specific procedures as described below. Once the colour was developed, a micro-plate spectrophotometer reader was used in determining the absorbance at the specified wavelength. Compost samples were passed through a 2 mm sieve and kept at 4°C. Enzyme assays were done on duplicate samples, with a control for each sample. Although dehydrogenase analysis is the only assay that is mandatory to be carried out using moist samples, this was however, done for all the three assays for standardization of results (Tabatabai and Dick, 2002). Hence, all data generated were corrected for moisture content during the data analysis.

β-glucosidase was determined by adapting the procedure described by Hartley *et al*. (2009). One gram of moist compost sample was incubated at 37°C for one hour with toluene, modified universal buffer pH 6.0, and *p*-nitrophenol-β-D-glucosidase (ρNG); and then shaken for 30 minutes with calcium chloride and tris(hydroxymethyl) amino methane (THAM) before filtering through a Whatman no. 2v filter. The absorbance of

released *p*-nitrophenol (ρNP) was tested with a micro-plate reader at 405 nm immediately.

The methodology adopted for the determination of urease enzyme was the non-buffered method described by Nannipieri *et al*. (2002). Five gram moist compost sample was incubated at 37°C for two hours with a urea solution and then shaken with potassium chloride. The resulting suspension was filtered through Whatman no. 2v filter paper. Prior to measuring the absorbance in a micro-plate reader at 690 nm, the filtrate was prepared with sodium salicylate or sodium hydroxide solution and sodium dichloroisocyanide solution for the colour development for 30 minutes.

The assay for acid phosphatase was adapted from Tabatabai (1994). One gram of moist compost sample was incubated at 37°C for one hour with toluene, modified universal buffer (MUB, pH 6.5 for acid phosphatase), and ρ-nitrophenol (ρNP). Thereafter, calcium chloride and sodium hydroxide was added and the mixture immediately filtered through Whatman no. 2v filters. The absorbance of ρNP was measured immediately with a micro-plate reader at 405 nm.

The dehydrogenase assay came from Schinner and Von Mersi (1990). One gram of field-moist soil was mixed with THAM and iodonitrotetrazolium violet-formazan (INT) solution. This enzyme assay required incubation at 40°C in the dark for two hours. Then the samples were mixed with an extraction solution and kept in the dark for another thirty minutes. Absorbance of the reaction product INT was read in a glass cuvette on the spectrophotometer at 464 nm immediately.

3.5 Data analysis

The data generated were analysed as factorial experiment consisting of compost maturity date (sample ID) and soil sampling dates after incubation (Sdate). The main effects and interactions were evaluated using Fisher protected least significant difference, LSD, at probability level of 5% following SYSTAT statistical computing package (SYSTAT Version 9.0). The number of days after incubation was plotted against the measured parameters (i.e. microbial activities, enzyme activities and mineralisation), and the data modelled using a second order polynomial equation:

$$Y = aX^2 + bX + c.$$

where: Y and X in the equation implies microbial population count or enzyme activity or concentration of nutrient released and maturity dates or days after incubation, respectively; “a” and “b’ represent the coefficients in the polynomial equation while ‘c’ connotes the intercept for the plot. The optimum content (Y) of each measured parameter at date (X) from the incubation data was estimated using the equation $Y = c-b^2/4a$ and $X = [-b \pm \sqrt{(b^2-4ac)}]/2a$, respectively as described by Angel and Runde (2010).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Chemical characteristics of the composts

The chemical composition of the different compost samples collected are shown in Table 1. An increase in pH from 8.97 to 9.45 was recorded during the active phase, suggesting the alkalinisation of the manure as a consequence of the release of ammonia from the degradation and mineralisation of organic compounds (Smith and Hughes, 2002). The increase in pH was comparable with similar observations by Smith and Hughes (2002), which attributed it to the microbial decomposition of the acids accompanied by the release of alkali and alkali earth metals that were previously bound in organic matter. Another reason of the increase in pH could be the formation of NH_4^+as a consequence of the mineralisation of proteins (Sánchez-Monedero *et al*., 2001). In this process, the acidity contributed by the small molecule organic acids and CO_2, produced from carbohydrates under the function of microbiology, could not be completely neutralized by alkalinity from the dissolved ammonia nitrogen resulting in the gradual increase of pH and final stabilization at a relatively high level (Yang *et al*., 2008). The pH values reached during the maturation are rather high and this fact could have important implications on the fertility and productivity of soils subjected to compost amendment, as well as on the development of pH-sensitive plants (Boulter-Bitzer *et al*., 2000).

The electrical conductivity (EC) value of compost reflects its degree of salinity and suitability for crop growth. The measured value decreased after the active phase of the composting process probably due to reduced release of soluble salts like ammonium and phosphate resulting from the decomposition of the easily biodegradable organic substrate in the compost heap (Ranalli *et al*., 2005).

The moisture content of the compost samples varied from 3.41 to 15.31%. Except for sample collected at 150 days after the initiation of the composting process, the moisture content was found to be relatively high compared to the recommended standard (EPA, 1995). The decline in moisture content percentage during the thermophilic phase of composting may be due to the high evaporating rates recorded (Islam *et al*., 2004). High moisture content promotes fermentative metabolism and

results in production of incompletely decomposed products like organic acids (Smith and Hughes, 2002). The increased moisture content during decomposition reduces EC values. This point indicates that the high water content during initial stages reduces the rate of decomposition and facilitate the release of ions like Fe^{3+} and Mn^{2+}, resulting in decreased EC (Niwagaba *et al*., 2009).

The OM content of the compost samples varied from 22.5 to 32.8%. These values are within the normal standard range reported by CCQC (2001). High quality compost materials will usually have a minimum of 50% organic content based on dry weight but decreased with compost maturity due to microorganisms feeding on organic carbon (Sánchez-Monedero *et al*., 2001). Maturity is also related to the humic acid fraction (responsible for regulating the carbon cycle and acts as a stimulant for the proliferation of microflora) of compost generated towards the last stage of composting. At the initial stage of composting, organic matter is mineralized and respiration rate is high. However, during maturity, as most of the OM is stabilized, and respiration rate slows down. This showed that compost was mature within 90 days of decomposition.

Total N of the compost samples ranged between 1.32 and 2.12% with values similar to those reported by Ranalli *et al*. (2005) for composted distillery residues. The decrease in total N concentration during composting was attributed to an increase in ammonia emission that resulted in N losses (Hao *et al.,* 2001). The total P content of the compost samples on the other hand, varied between 0.345 and 0.430% with the highest value obtained in compost sample taken at 150 days. Only the total P content in the final cured compost was comparable to the recommended standards value of 0.4-1.1% as prescribed for high quality compost (Canada Composting Council, 2008).

Table 1: Characteristics of the various composts studied

Parameters	UNITS	SD30	SD60	SD90	SD120	SD150
pH (H_2O)		8.97	9.45	9.22	9.31	8.87
Elec. conductivity	mS/m	622	437	461	456	508
Moisture	%	3.4	5.5	13.0	15.3	5.2
Total solids	%	96.6	94.5	87.0	84.7	94.8
Ash	%	63.8	64.6	60.4	62.2	68.9
Organic Matter	%	32.8	29.9	26.6	22.5	25.9
Total C	%	28.1	24.5	21.3	20.1	17.5
Total N	%	2.12	1.82	1.73	1.80	1.39
Total P	%	0.35	0.39	0.39	0.38	0.43
C/N ratio		13.3	13.4	12.3	11.1	12.6
C/P ratio		6.1	4.7	4.4	4.8	3.2
Calcium	%	1.91	1.92	1.78	1.80	2.22
Magnesium	%	0.66	0.71	0.74	0.72	0.70
Potassium	%	2.60	2.44	2.37	2.10	1.98
Sodium	%	0.30	0.29	0.28	0.24	0.21
Zinc	mg/kg	51	71	72	75	69
Copper	mg/kg	18	21	23	27	22
Manganese	mg/kg	291	334	365	399	324
Iron	mg/kg	1533	1559	1728	1775	1561
Arsenic	mg/kg	0.2-0.7	0.2-0.6	0.5-0.8	0	0
Cadmium	mg/kg	0.02	0.03	0.03	0.03	0.02
Lead	mg/kg	1.89	2.37	2.23	3.41	3.13

SD30, 60, 90, 120 & 150 refer to the different compost sampling/maturity dates

At the end of composting, total organic carbon (OC) content and C:N and C:P ratios decreased, whereas total P concentrations increased (Table 1). The increased in P may be related to high humic acid. Humic acid formation prevents the formation of insoluble Ca-P (Hartley *et al*., 2009). The decrease of total organic C concentration in the composts resulted from degradation of the organic materials during the composting process. The maximum decomposition of organic waste materials was observed when the substrates contained the optimum available C, N, P and S ration. The C/N ratio of all the compost samples ranged from 11.1 to 13.3. The ratio decreased mainly during the bio-oxidative phase, which is prior to 90 days of composting due to the high decomposition of the organic materials (Tiquia, 2002). Pansua and Thuriés (2003) reported that the value of C/N ratio exerts an influence on microbial activities. The ratio in the 150-day cured compost though greater than 12, was within the acceptable limit of 8.5 to 12.0 for high quality compost as reported by Bernal *et al*. (2009). The decrease in C/N ratio as the compost matures is indicative of complete degradation of organic substrate by micro-organisms. Fuchs (2002) indicated that C/N ratio narrows down as nitrogen remains in the system, while some of the carbon is released as CO_2. Total OC of the compost samples varied from 17.5 to 28.1%; and it decreased at all the stages of compost sampling. Mondini *et al*. (2003) similarly found that the percentage of OC decreased as a result of decomposition by microbial population leading in part to CO_2 evolution and microbial biomass assimilation (Ranalli *et al*., 2005). Pansua and Thuriés (2003) reported that the C loss accounted for the initial total C during the composting process.

The concentration of Na, Ca and Mg in the compost samples decreased with compost maturity. Calcium concentration was normal in the different compost samples while Mg concentration was high relative to the recommended standard value of 1.0-4.0% and 0.2-0.4%, respectively prescribed for high quality compost by CCQC (2001). Findings from earlier studies revealed that the Na content in wastes are generally highly soluble during decomposition and readily gets leached (García-Ruiz *et al*., 2008; Tiquia *et al*., 2002). Hence, moderate Na concentration in composts is reported not to have any adverse effect on soil and plants (Ranalli *et al*., 2005) but high concentration could exert potential negative effects on soil structure

and permeability thereby resulting in alkaline salts that may become toxic and corrosive to plants (Tiquia *et al*., 2002). The K concentration of the compost samples ranged between 1.98 and 2.597% (Table1). The decrease in K as with sodium is probably because of the high solubility and its readily leached nature (Islam *et al*., 2004). Interestingly, K represents an essential nutritional element required for plant growth and not known to have any harmful or toxic effect on humans (Islam *et al*., 2004).

The measured trace or heavy metal content of the composts represents a crucial component of compost quality parameter due to their roles on some bacteria in the food borne diseases and consequently, their effect on human health (Islam *et al*., 2004). The concentration of lead measured was low compared to the recommended standard value of 12-100 mg kg^{-1}as prescribed for high quality compost by Compost Council Quality of California-CCQC (2001). The concentration of arsenic detected in the first three samples were too low for accurate quantification due to Fe interference during measurement on the atomic absorption spectrophotometry used (Hartley *et al*., 2009). The concentration of other trace metals namely Fe, Mn, Cu and Zn measured in the different composts are also low (CCQC, 2001). These results are in agreement with the findings of Hutchison *et al*. (2005). The low concentration trace metals improved soil physical characteristics such as water holding capacity, improved chemical characteristics such as nutrient retention capacity, and stimulation of microbial activity that can improve plant growth and decrease the leaching of pollutants into water suppliers. The low trace metal limit harm to plants by tying up trace pollutants and toxic organic compounds (Marschner *et al*., 2003).

4.2 Microbial population count in compost samples and compost amended soils following incubation

The measured population count for the three microbial species studied following incubation of compost amended soil was significant ($P \leq 0.05$) affected by compost maturity or age (Ct),soil sampling dates (Ip) and Ct x Ip interaction effect (Table 2). The compost ID which is maturity or age and soil sampling dates has the influence in bacteria, fungi and actinomycetes. The interaction of the compost maturity or age (Ct) and soil sampling dates (Ip) was significant different (Table 2). Bacteria represent the highest microbes count as the ages of compost increases followed by

the actinomycetes while fungi count was the least in all treatments. Compost maturity influenced microbial population and proliferates within the soil ecosystem upon soil application (García-Gómez *et al.,* 2003). Micro-organisms through different kinds of substrate based enzymes promote the degradation of organic materials (Hao *et al*., 2001). Numerous authors have reported that the addition of mature compost activates micro-organisms to produce enzymes that are related to the cycling of most important nutrients (Marschner *et al*., 2003). The highest population count of bacteria, fungi and actinomycetes were 6.93 CFU g^{-1}, 3.41 CFU g^{-1} and 6.66 CFU g^{-1}, respectively. The bacteria, fungi and actinomycetes were recorded highest in 90 days old compost (SD90). Microbial community is used as indicator of compost maturity (USDA., 2000). The availability of easily usable organic substances in the composting heap promotes increased population of the fastest-growing bacteria hence, the dominance of mesophilic bacteria followed by actinomycete. The changing microbial population count in the compost samples taken at different compost age is in agreement with earlier works (Islam *et al*., 2004). This is associated with changing temperatures as well as the consumption of C and N during the composting process (Kutsanedzie *et al*., 2015).

The highest bacterial population was 6.83 CFU g^{-1} at 14 days after incubation. Fuchs (2002) also stated that populations of bacteria increased gradually and were found to be the largest at 14 days after incubation, and then decreased gradually until the end of the incubation period. However, the tendency of decrease from 21 days was quite weak compared to the tendency of increase within the initial14 days after incubation. The highest amount of fungal population was 3.18 CFU g^{-1} at 14 days after incubation. The fungal population also peaked at 14 days ad increased gradually and then remained at a relatively stable level until the end of the composting. The highest amount of actinomycetes population was 6.39 CFU g^{-1} at 21 days after incubation (Table 2).

A unique feature of many extracellular enzymes common in fungi is that they are capable of breaking down a wide range of compounds (Klamer and Baath, 1998). An increase in the bacteria count was observed during the first 30 days in incubation followed by a steady decline phase for the 60-day and 90-day old compost samples (Figure 1). The 30-day, 120-day and 150-day compost samples generally decreased

from 7 to 42 day during the incubation, which may have been due to the reduction of microbial activity associated with the depletion of degraded organic substrate (Keener *et al*., 2000). The 90-day compost gave the highest amount of bacteria count throughout the incubation period. Although, fungi grow and reproduce much slowly than bacteria when food is readily available, the former are well suited for exploiting an environment rich in complex recalcitrant organic compounds such as found in the compost during the curing stage (García-Ruiz *et al*., 2008). Actinomycetes predominate during the stabilisation and curing phases due to its ability to degrade resistant polymers (Ryckeboer *et al*., 2003).

Table 3 contains the polynomial regression equations, P-values and the estimated optimum content (Y) of each parameter measured at time or date (X) in the different compost samples and during the composting period as well as the 42-day incubation period. Based on the regression model, the maximum bacterial population count of 6.73 CFU g^{-1} will be obtained in 98-day (three months and 8 days) old compost (Figure 4). The gradual fall in bacteria count between week 12 to week 14 (90-day to 105-day) indicate the depletion of nutrients and the process approaching stability (Goyal *et al*., 2005) Also, the model estimated the maximum fungal population value of 3.06 CFU g^{-1} in 163-day (five months and 13 days) old compost (Figure 5). While the maximum actinomycetes count of 6.18 CFU g^{-1} will be obtained in 107-day (three months and 17 days) old compost (Figure 6). However, following the mixing of the various compost samples with soil and incubation, the regression model estimated the highest bacteria count of 6.93 CFU g^{-1} in 18 days after incubation from compost amended soil (Figure 7). The estimated maximum fungi population count of 3.42 CFU g^{-1} was obtained in 15 days after incubation (Figure 8). The bacteria and fungi counts measured in the compost amended soil samples were high at the beginning of incubation period, increased steadily but dropped at 14 days after incubation and again declined towards the end of the incubation period. The highest actinomycete count of 6.67 CFU g^{-1} was obtained in 21 days after incubation (Figure 9). Similar results were obtained by Fuchs (2002) who reported that the population of actinomycetes increased quickly, and attained its peak during the first 21 days after incubation.

Table 2: Microbial population count and enzymes activities measured in compost samples and compost-soil mix at different sampling times after incubation

Treatments	Microbial counts (CFU / g^{-1})		
	Bacteria	Fungi	Actinomycete
Compost ID			
SD30	6.66	2.90	5.82
SD60	6.56	2.97	5.88
SD90	6.83	3.18	6.39
SD120	6.67	2.99	6.08
SD150	6.58	3.05	6.01
Incubation period (DAI)			
7	6.75	3.36	5.79
14	6.93	3.41	6.35
21	6.81	3.21	6.66
28	6.86	3.23	6.59
35	6.50	2.75	5.70
42	6.00	2.14	4.74
Significance(p-values)			
Compost ID (Ct)	<0.001	<0.001	<0.001
Incubation period (Ip)	<0.001	<0.001	<0.001
Ct x Ip interaction	<0.001	<0.001	<0.001

SD implies compost sampling/maturity date; DAI implies days after incubation

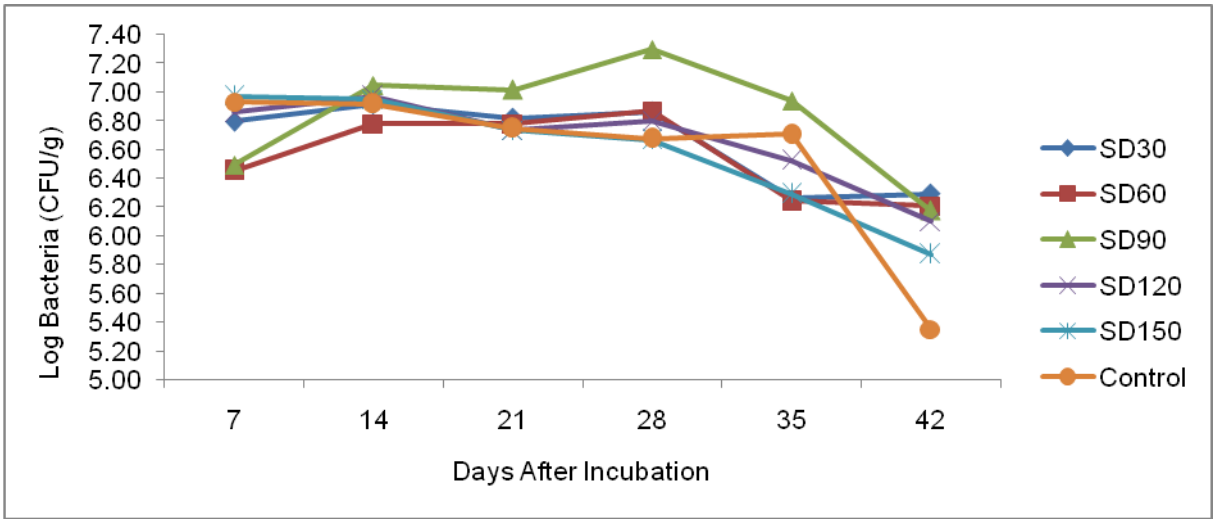

Figure 1: Mean values of bacterial population count (CFU g^{-1}) measured from the different compost samples over the 42-day incubation period.

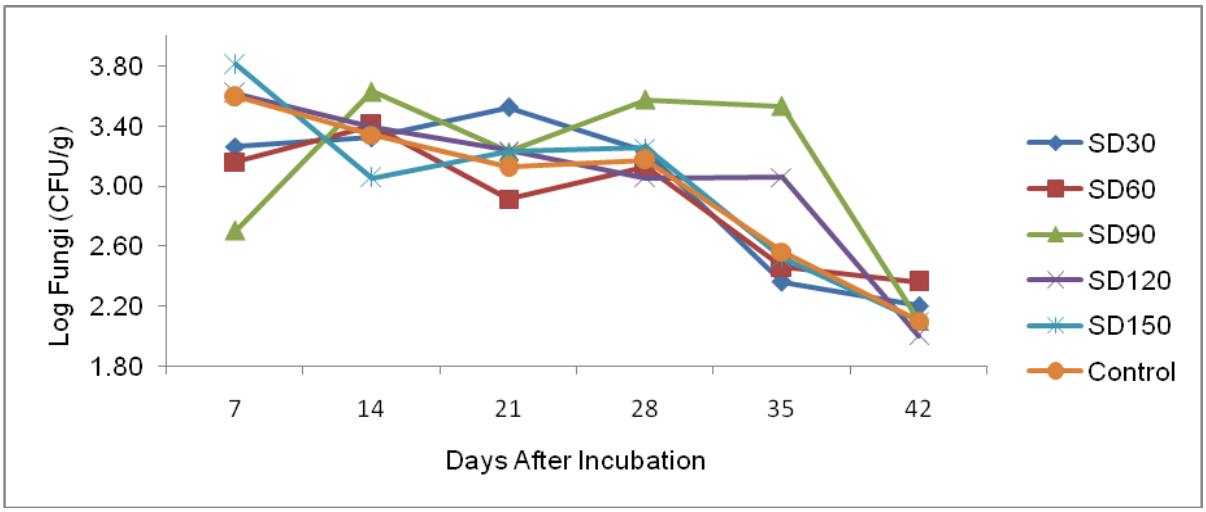

Figure 2: Mean values of fungal population count (CFU g^{-1}) measured from the different compost samples over the 42-day incubation period.

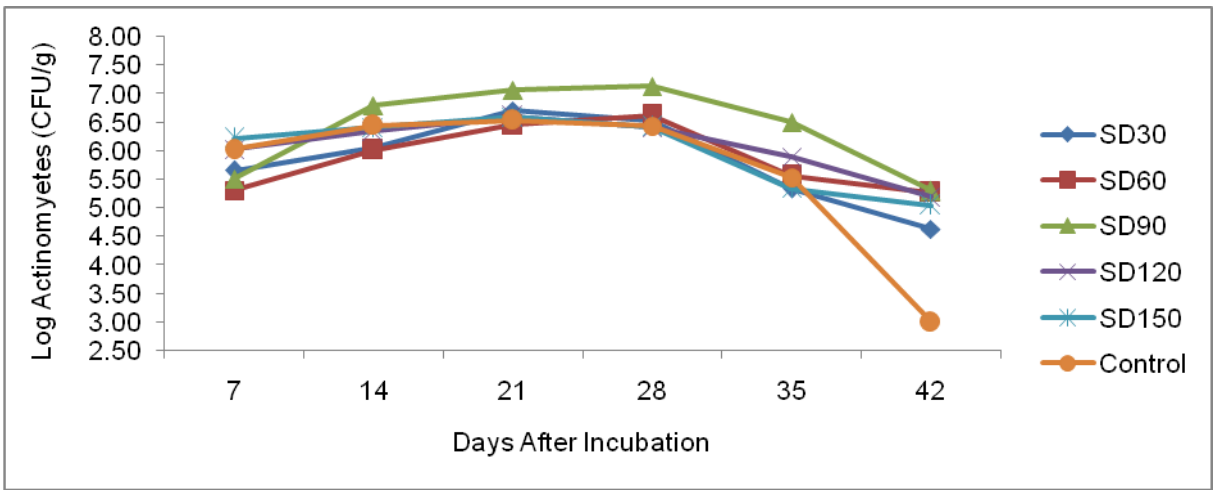

Figure 3: Mean values of actinomycetes population count (CFU g^{-1}) measured from the different compost samples over the 42-day incubation period.

Table 3: Polynomial equations of the various response parameters with the zinc rates as independent variable and the corresponding R^2 values of the equation

Treatments	Parameters	Regression equation	p- values	Y- values	X- value
Compost maturity (age)	Bacteria	$y= -2E-05x^2 + 0.0039x + 6.5407$	<0.001	6.73	97.5
	Fungi	$y= -4E-06x^2 + 0.0013x + 2.9571$	<0.001	3.06	162.5
	Actinomycete	$y= -5E-05x^2 + 0.0107x + 5.6035$	<0.001	6.18	107.0
Incubation period	Bacteria	$y= -0.0016x^2 + 0.0.0571x + 6.4186$	<0.001	6.73	17.8
	Fungi	$y= -0.0016x^2 + 0.0466x + 3.0785$	<0.001	3.42	14.6
	Actinomycete	$y= -0.0045x^2 + 0.1917x + 4.6327$	<0.001	6.67	21.3

P = significant value; R^2= measured response

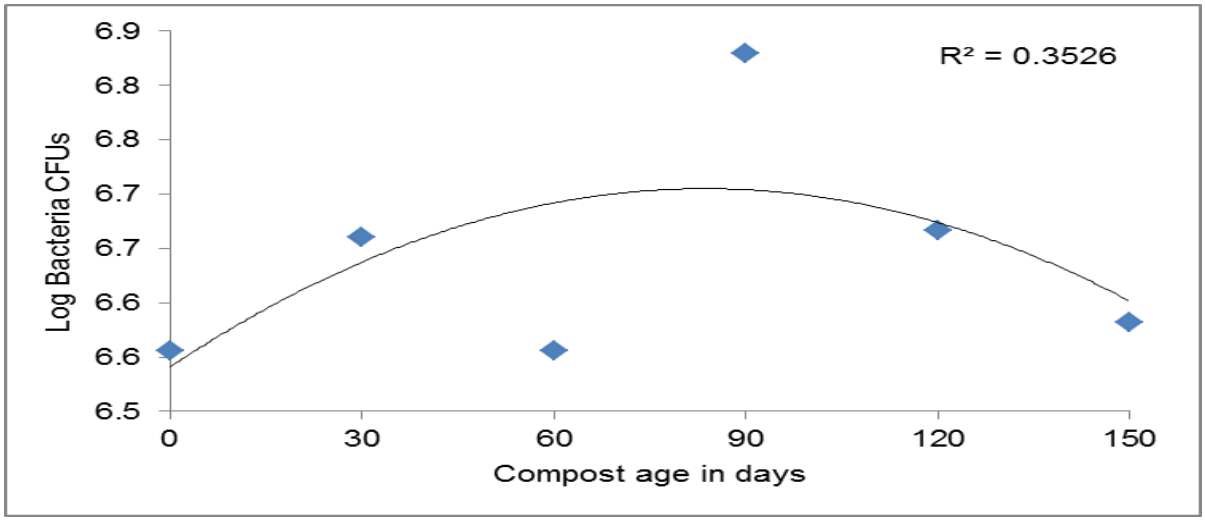

Figure 4: Polynomial regression of the mean bacterial population count (CFU g^{-1}) over the 150-day composting period.

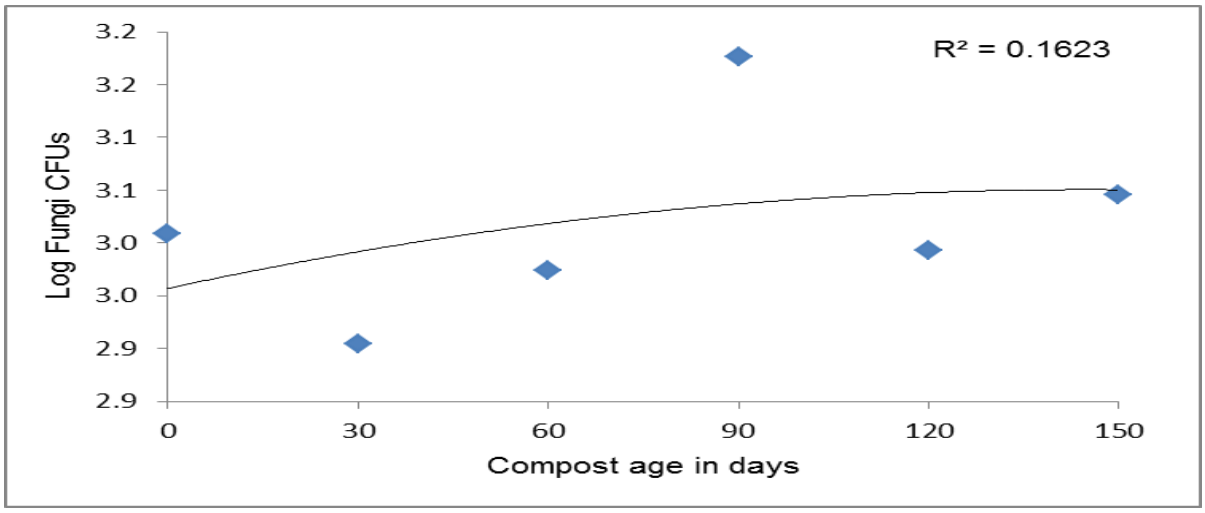

Figure 5: Polynomial regression of the mean fungal population count (CFU g^{-1}) overthe 150-day composting period.

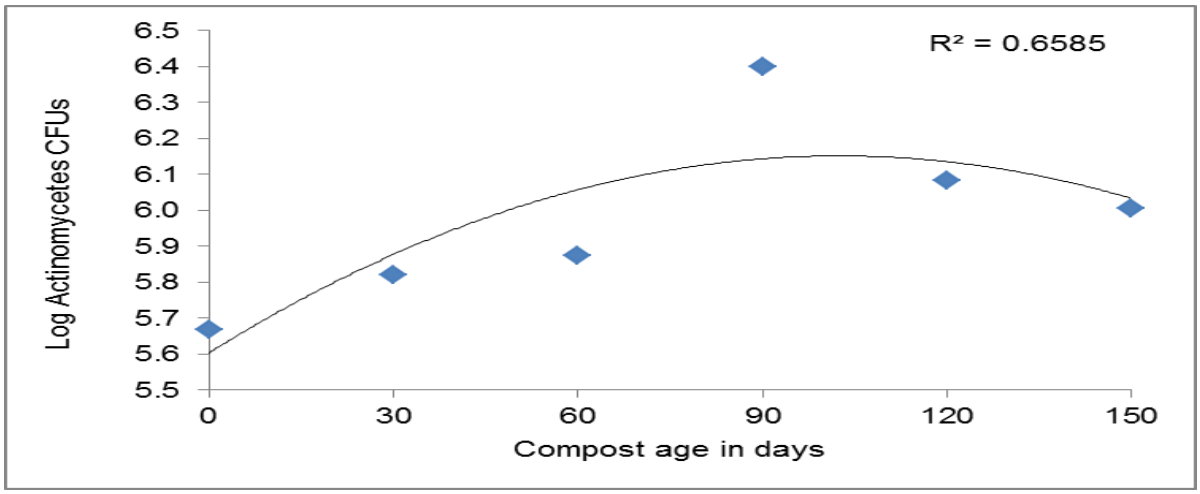

Figure 6: Polynomial regression of the mean actinomycetes population count (CFU g^{-1}) over the 150-day composting period.

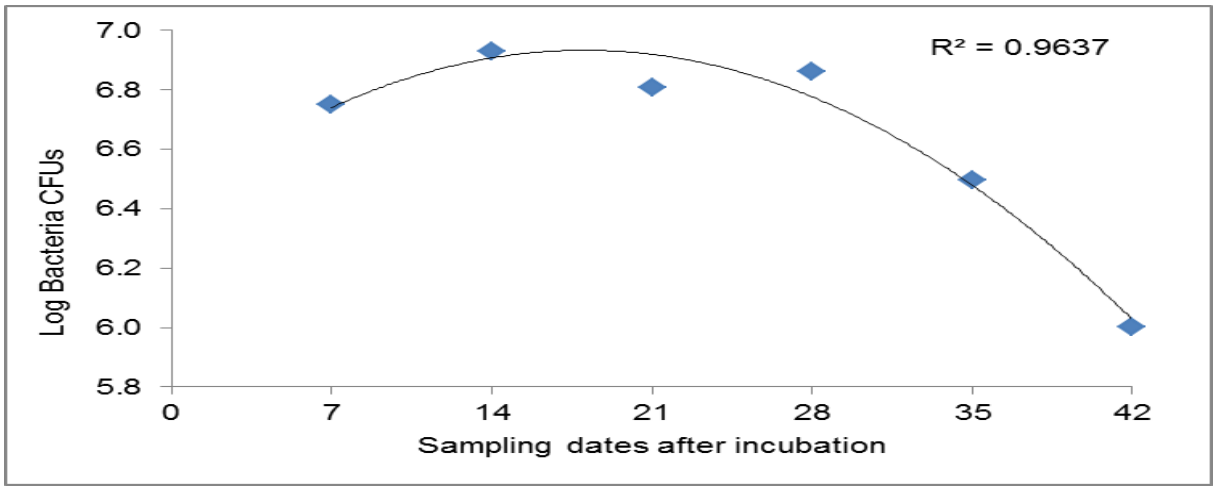

Figure 7: Polynomial regression of the mean bacterial count (CFU g^{-1}) measured during the 42-day incubation period.

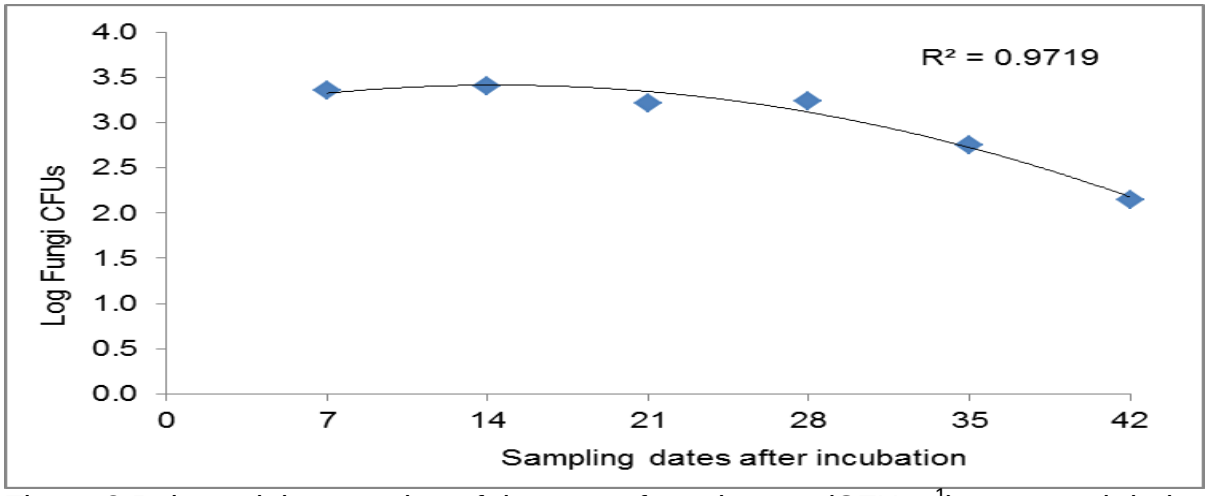

Figure 8:Polynomial regression of the mean fungal count (CFU g^{-1}) measured during the 42-day incubation period.

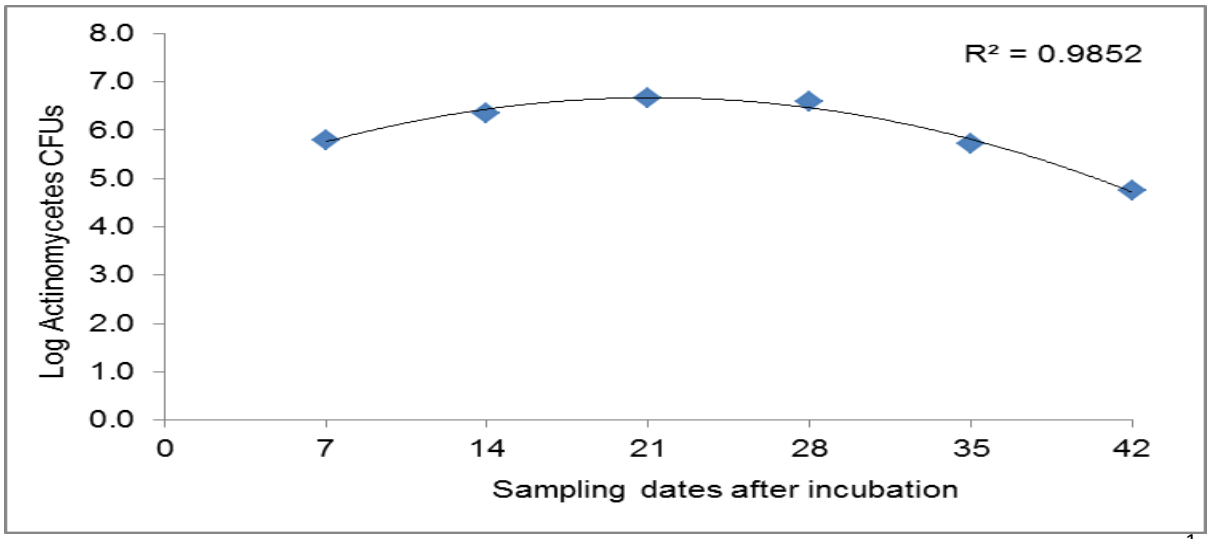

Figure 9: Polynomial regression of the mean actinomycetes count (CFU g^{-1}) measured during the 42-day incubation period.

A significant compost maturity x incubation period interaction effect on the three microbial population species counts was obtained (Table 4) suggesting that the measured count at the different sampling dates during the incubation period was dependent on the age of sampling (maturity) of the composts. This is in agreement with earlier findings by Garland (2006) who reported that the sampling date is a most contributing factor in composting. The mean bacteria (7.30 CFU g^{-1}) and actinomycete (7.14 CFU g^{-1}) counts were significantly highest in soil amended with the 90-day old compost (SD90) at 28 DAI while the highest mean fungi count (3.81CFU g^{-1}) was obtained in soil amended with 150-day old compost (SD150) at 7 DAI. The fungal population is highest only within seven days of 42, then these organisms will release nutrients really early within which might be good for the use by crops early in their seedling stage. The bacteria and actinomycetes are only active on day 28 when crops will still have need for the nutrients that are released by composts. Most crops have peak demand for nutrients and water at 42 days (six weeks) after plantings (Fuchs, 2002).

Table 4: Effect of compost maturity x incubation period interaction on microbial population counts(CFU / g^{-1})

Treatments		Bacteria(CFU / g^{-1})	Fungi(CFU / g^{-1})	Actinomycete (CFU / g^{-1})
Compost ID	**Incubation period**			
Control	7	6.93^{bcdef}	3.59^{abc}	6.02^{hij}
Control	14	6.92^{bcdef}	3.39^{bcde}	6.45^{cde}
Control	21	6.75^{efgh}	3.24^{cdef}	6.53^{cde}
Control	28	6.68^{hij}	3.17^{def}	6.43^{cde}
Control	35	6.71^{ghi}	2.56^{hij}	5.57^{klm}
Control	42	5.35^{p}	2.10^{kl}	3.00^{p}
SD30	7	6.80^{defgh}	3.26^{bcdef}	5.66^{jkl}
SD30	14	6.92^{bcdefg}	3.33^{bcde}	6.05^{fghi}
SD30	21	6.82^{cdefgh}	3.05^{efg}	6.70^{bcd}
SD30	28	$6.87^{bcdefgh}$	3.23^{cdef}	6.53^{cde}
SD30	35	6.26^{mn}	2.36^{ijkl}	5.34^{lmn}
SD30	42	6.30^{lmn}	2.20^{jkl}	4.63^{o}
SD60	7	6.46^{klm}	3.16^{def}	5.31^{lmn}
SD60	14	6.78^{defgh}	3.40^{bcde}	6.02^{hij}
SD60	21	6.78^{defgh}	3.34^{bcde}	6.45^{cde}
SD60	28	$6.87^{bcdefgh}$	3.12^{ef}	6.63^{cd}
SD60	35	6.24^{n}	2.46^{ijk}	5.57^{klm}
SD60	42	6.21^{n}	2.36^{ijkl}	5.28^{mn}
SD90	7	6.49^{jkl}	2.70^{ghi}	5.51^{lm}
SD90	14	7.05^{b}	3.63^{ab}	6.80^{abc}
SD90	21	7.02^{bc}	3.52^{abcd}	7.07^{ab}
SD90	28	7.30^{a}	3.57^{abc}	7.14^{a}
SD90	35	6.94^{bcdef}	3.53^{abcd}	6.50^{cde}
SD90	42	6.18^{n}	2.10^{kl}	5.30^{lmn}
SD120	7	$6.86^{bcdefgh}$	3.62^{ab}	6.03^{ghij}
SD120	14	6.97^{bcd}	3.32^{bcde}	6.34^{defgh}
SD120	21	6.74^{fgh}	2.91^{fgh}	6.61^{cd}
SD120	28	6.80^{defgh}	3.05^{efg}	6.42^{cdef}
SD120	35	6.53^{ijk}	3.05^{efg}	5.90^{ijk}
SD120	42	6.10^{n}	2.00^{l}	5.19^{mn}
SD150	7	6.97^{bcd}	3.81^{a}	6.23^{efghi}
SD150	14	6.95^{bcde}	3.37^{bcde}	6.41^{defg}
SD150	21	6.74^{fgh}	3.23^{cdef}	6.60^{cde}
SD150	28	6.67^{hij}	3.25^{bcdef}	6.42^{cdef}
SD150	35	6.29^{lmn}	2.52^{ij}	5.34^{lmn}
SD150	42	5.87^{p}	2.10^{kl}	5.04^{n}
LSD		**0.21**	**0.38**	**0.38**

SD implies compost sampling/maturity date; LSD Implies Least Significant Different

4.3 Enzyme activities in compost samples and compost amended soils

The activities of the various enzymes measured were significantly ($p<0.001$) affected by compost maturity or age (Ct), incubation period (Ip) as well as Ct x Ip interaction effect (Table 5). The activity of β-glucosidase was highest in each compost treatment while dehydrogenase activity was the lowest. Madejón *et al*. (2001) reported that the addition of mature compost activates micro-organisms to produce enzymes related with the cycle of the most important nutrients. Soil enzyme activities are both early and sensitive indicator of different management practices, inducing changes in soil fertility (Benítez *et al*., 1999). The activity of both β-glucosidase and phosphatase were strongly influenced by compost maturity with higher concentration obtained as the age of compost increases. Acid phosphatase activity plays a key role in organic P mineralisation and is generally activated when P availability is low (Nannipieri *et al*., 2002). The interaction between compost maturity or age (Ct) and soil sampling dates (Ip) on the three measured enzyme activities in all the compost samples was significant (Table 5). The implication is that the amount of each enzyme measured was dependent on both the compost age or maturity and compost sampling dates.

The highest concentration of β-glucosidase (3188 mg kg^{-1} hr^{-1}) and phosphatase (1623 mg kg^{-1} hr^{-1}) in compost samples obtained during composting were both obtained in 60-day (SD60) old compost. The phosphatase activity decreased slightly during the active phase probably due to the feedback inhibition of this enzyme by organic phosphate (Mondini *et al*., 2003). The lowest concentration of β-glucosidase (1742 mg kg^{-1} hr^{-1}) and phosphatase (1111 mg kg^{-1} hr^{-1}) groups were both obtained in 30-day (SD30) old compost. β-glucosidase is regarded as a key enzyme involved in the degradation of polysaccharides through hydrolysis of the terminal β-D-glucose chains (Castaldi *et al*., 2008). Therefore, the change in β-glucosidase activity could be an indication of the low content of polysaccharides following hydrolysis in the compost as supported by Castaldi et al. (2008). The highest concentration of urease (30 mg kg^{-1} $2hr^{-1}$) was obtained in both 30-day (SD30) and150-day (SD150) old composts. The highest concentration of dehydrogenase (121 µg INF g^{-1} $2hr^{-1}$) was obtained in 150-day (SD150) old compost. The lowest concentration of urease (23 mg kg^{-1} $2hr^{-1}$) and dehydrogenase (105 µg INF g^{-1} $2hr^{-1}$) were obtained in 90 and 120-day old compost, respectively. The enzymes, urease activity, tend to increase

during the active thermophilic phase of composting because of the initial high concentration of protein that induces the protease activity thereby releasing urea, and consequently induce the urease enzymes (Mon *et al*., 2007).

The highest mean concentrations of β-glucosidase (8277 mg kg^{-1} hr^{-1}), phosphatase (3823 mg kg^{-1} hr^{-1}) and urease (33 mg kg^{-1} $2hr^{-1}$) in compost amended soils were obtained at 42 days after incubation in 60-day old compost for β-glucosidase and phosphatase, and 150-day old compost for urease (Table 5). The β-glucosidase is an extracellular enzyme associated with glucose chains hydrolysis to yield β-glucose (Castaldi *et al.*, 2008). Its concentration decreases during the composting process indicating the use of polysaccharides and possible attainment of stabilization phase (Castaldi *et al.*, 2008). A steady increase in β-glucosidase concentration was measured from the different composts following soil amendment during the first 35 days of incubation; but increased drastically beyond 35 DAI except for the 30-day old compost (Figure 10). This probably indicates the use of polysaccharides and incomplete stabilization for that compost (Castaldi *et al.*, 2008). The lowest enzyme activities of 1539 mg kg^{-1} hr^{-1} obtained at 7DAI for β-glucosidase, 980 mg kg^{-1} hr^{-1} obtained at 21DAI for phosphatase and 22 mg kg^{-1} $2hr^{-1}$ obtained for urease were at 7, 21 and 35 DAI in 30-day old compost for β-glucosidase, 150-day old compost for phosphatase, and 90-day old compost for urease.

The measured phosphatase activity in compost amended soils was comparable to soil which is not amended with compost (control) except at 21 DAI where SD60 compost gave quantitatively higher concentration and 42 DAI where SD30 compost was far much lower (Figure 11). This could be due to the formation of an enzyme-humus complex, which will make this enzyme more resistant to denaturation (Mondini *et al*., 2003). The pattern of distribution of the measured urease activity in the compost amended soil is highly variable (Figure 12). Urease activity induces protease activity at the early stage of compost maturity releasing urea (Makoi and Ndakidemi, 2008). During compost maturity process, urease activity presents fluctuations due to repeated substrate depletion and synthesis of urea (Makoi and Ndakidemi, 2008). The highest value of dehydrogenase (155 µg INF g^{-1} $2hr^{-1}$) was obtained at 7 DAI in 150-days old compost, while the lowest value (91 µg INF g^{-1} $2hr^{-1}$) was obtained at 42 DAI in 90-day old compost.

Table 5: Enzyme activities measured in compost samples and compost-soil mix at different sampling times after incubation

Treatments	Enzyme activities			
	β-glucosidase (mg kg^{-1} hr^{-1})	Phosphatase (mg kg^{-1} hr^{-1})	Urease (mg kg^{-1} $2hr^{-1}$)	Dehydrogenase (µg INF g^{-1} $2hr^{-1}$)
Compost ID				
SD30	1742	1111	30	109
SD60	3188	1623	29	111
SD90	2885	1531	23	111
SD120	3018	1599	28	105
SD150	2878	1456	30	121
Incubation period (DAI)				
7	1539	983	29	155
14	1570	1105	27	100
21	1608	980	29	105
28	1938	1046	28	98
35	1597	1066	22	103
42	8277	3823	33	91
Significance (p-values)				
Compost ID (Ct)	<0.0001	<0.0001	<0.0001	<0.0001
Incubation period (Ip)	<0.0001	<0.0001	<0.0001	<0.0001
Ct x Ip interaction	<0.0001	<0.0001	<0.0001	<0.0001

SD implies compost sampling/maturity date; DAI implies days after incubation

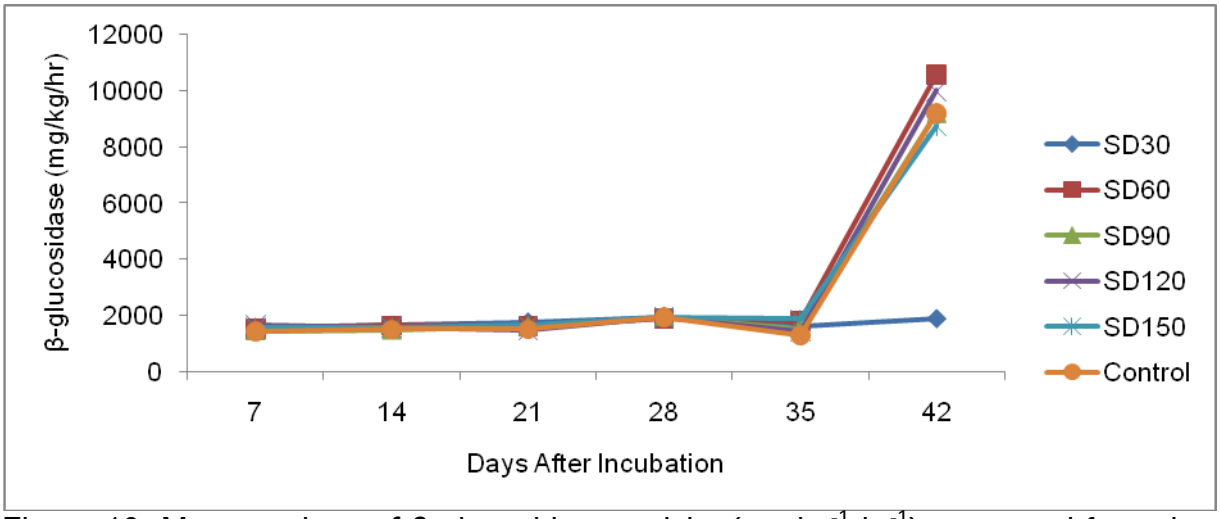

Figure 10: Means values of β-glucosidase activity (mg kg^{-1} hr^{-1}) measured from the different compost samples over the 42-day incubation period.

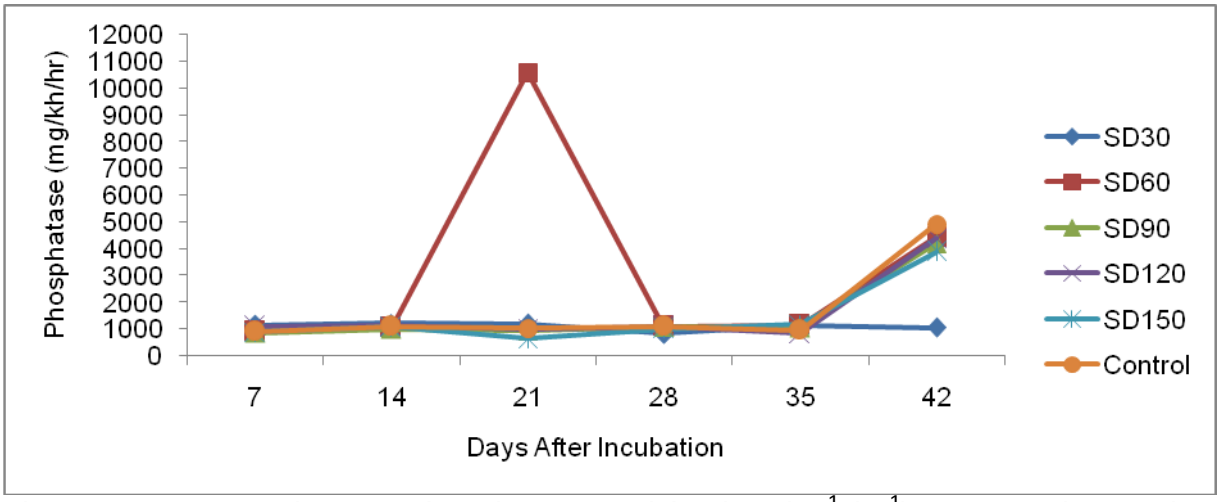

Figure 11: Mean values of phosphatase activity (mg kg^{-1} hr^{-1}) measured from the different compost samples over the 42-day incubation period.

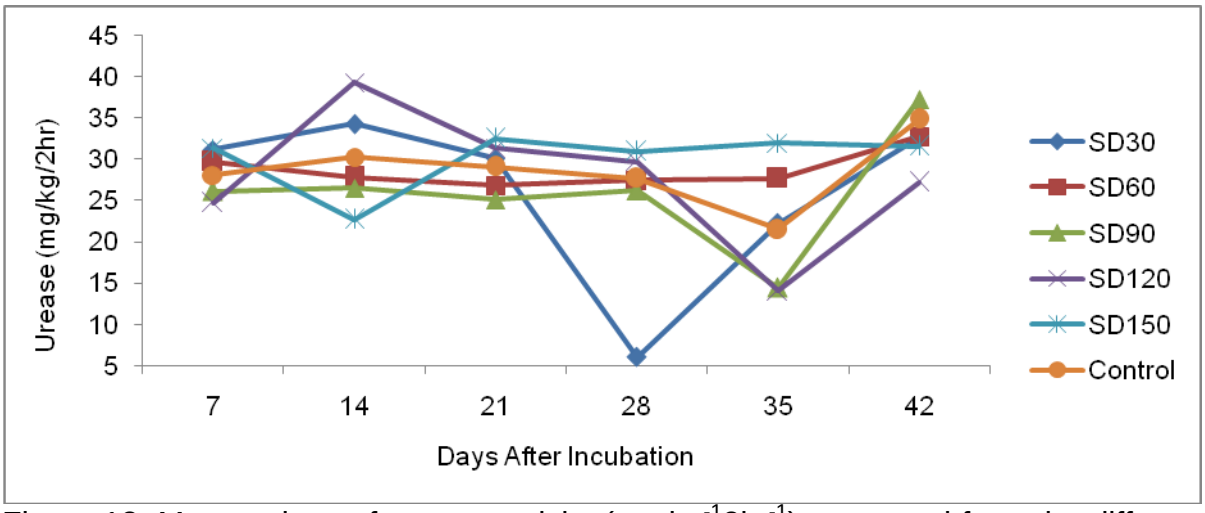

Figure 12: Mean values of urease activity (mg $kg^{-1}2hr^{-1}$) measured from the different compost samples over the 42-day incubation period.

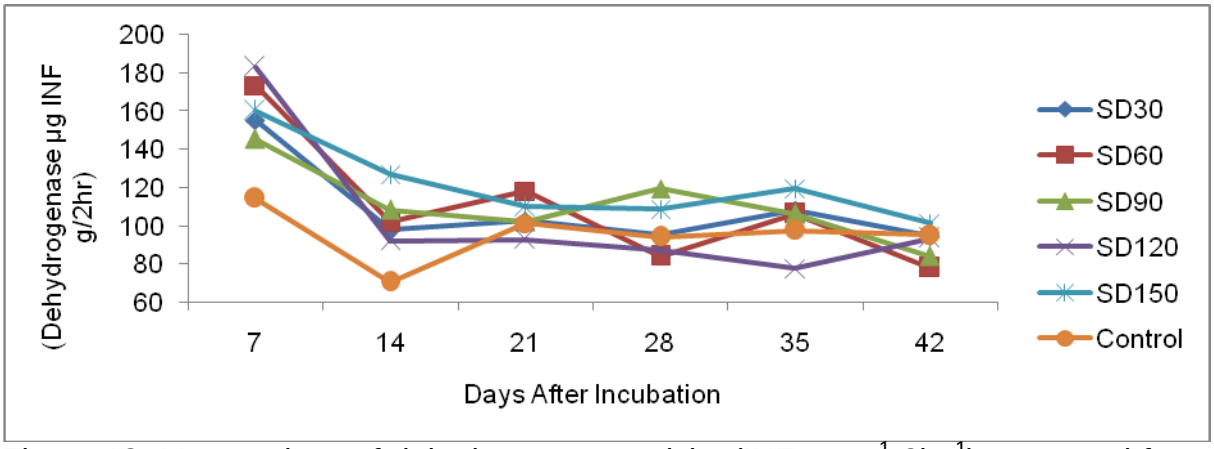

Figure 13: Mean values of dehydrogenase activity (INF µg g^{-1} $2hr^{-1}$) measured from the different compost samples over the 42-day incubation period.

Table 6 contains the polynomial equations, R^2-value and the estimated optimum content (Y) measured at date (X) for the various enzyme activities for the different compost samples and incubation period. Based on the regression model, the maximum β-glucosidase activity (3076.40 mg kg^{-1} hr^{-1}) is predicted in 235 days (seven months and 25 days) old compost (Figure 14), which is far beyond our own composting period. β-glucosidase have been reported to be the key enzyme involved in the degradation of polysaccharides, in which polysaccharides were degraded by hydrolyzing the reducing terminals of the β-D-glucose chains (Castaldi *et al*., 2008). However, the maximum concentration of phosphatase activity (1479.60 mg kg^{-1} hr^{-1}) was estimated in 59 days (one month and 29 days) old compost (Figure 15) while that of urease (26.15 mg kg^{-1} $2hr^{-1}$) was in 81 days (two months and 21 days) old compost (Figure 16). β-glucosidase activity played important roles in the degradation of cellulose. The polynomial model revealed that the maximum dehydrogenase activity (120.07 µg INF g^{-1} $2hr^{-1}$) was obtained in 262-day (8 months and 22 days) old compost (Figure 17), which is beyond our own composting period. This implies that the application of mature compost automatically induces the synthesis and growth of enzymes without necessarily affecting the overall microbial activity (Tiquia *et al*., 2002).

The activity of β-glucosidase measured in compost amended soil was highest (683.07 mg kg^{-1} hr^{-1}) in 18 days after incubation (Figure 18) while phosphatase was highest activity (617.60 mg kg^{-1} hr^{-1}) at 19 days after incubation (Figure 19). The activity of urease measured in compost amended soil reach the highest level (26.44 mg kg^{-1} $2hr^{-1}$) in 23 days after incubation (Figure 20) while dehydrogenase activity was highest (91.99 µg INF g^{-1} $2hr^{-1}$) at 33 days after incubation (Figure 21). Dehydrogenase, which is used as a measure of overall microbial activity, is important for all microorganisms because it is involved in the respiratory chain (Castaldi *et al*., 2008). Dehydrogenase activity concentration increased and reached the peak at 30 days of composting and it progressively decreased until the end of composting. Dehydrogenase activity set upright for the overall population of heterotrophic microorganisms, it should be positively related to all microorganisms.

Table 6: Polynomial equations of the various response parameters with the zinc rates as independent variable and the corresponding R^2 values of the equation

Treatments	Parameters	Regression equation	p- values	Y- values	X- value
Compost maturity (age)	β-glucosidase	$y= -0.0114x^2 + 5.3565x + 2447.2$	<0.0001	3076.00	234.9
	Phosphatase	$y= 0.0072x^2 - 0.8479x + 1504.6$	<0.0001	1479.60	58.9
	Urease	$y= 0.0006x^2 - 0.0966x + 30.033$	<0.0001	26.15	80.5
	Dehydrogenase	$y= -0.0003x^2 + 0.1572x + 99.474$	<0.0001	120.07	262.0
Incubation period	β-glucosidase	$y= 0.0797x^2 - 5.2056x + 176.99$	<0.0001	683.07	18.5
	Phosphatase	$y= 0.0102x^2 - 0.4784x + 32.047$	<0.0001	617.60	18.7
	Urease	$y= 5.0134x^2 - 187.9x + 2378.2$	<0.001	26.40	23.5
	Dehydrogenase	$y= 11.564x^2 - 427.46x + 4633.3$	<0.0001	91.89	32.7

P= significant value; R^2= measured response

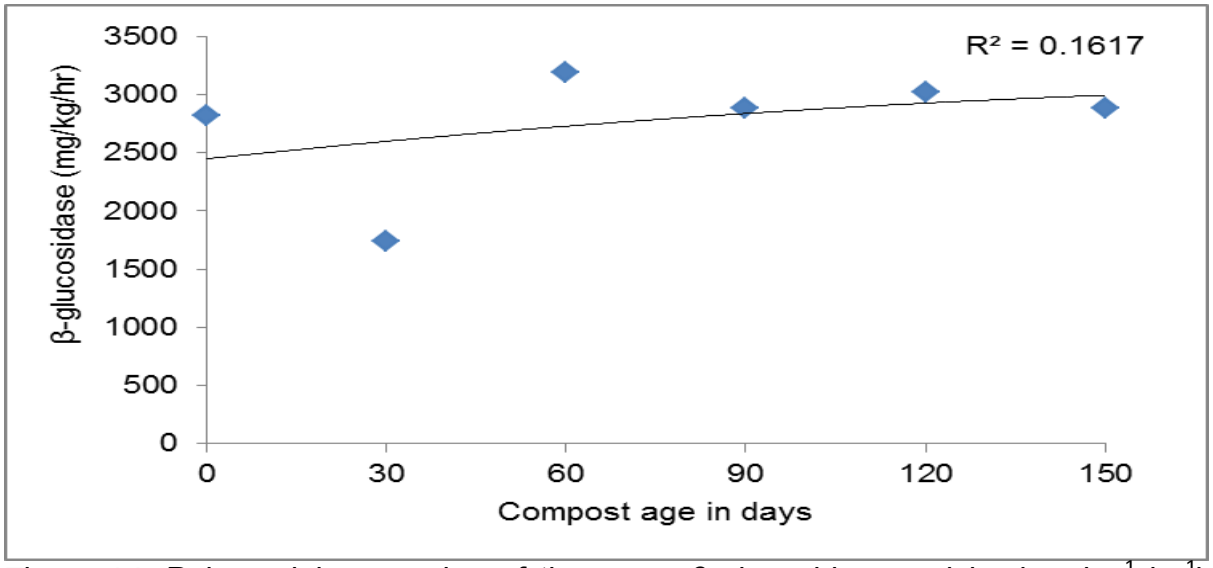

Figure 14: Polynomial regression of the mean β-glucosidase activity (mg kg^{-1} hr^{-1}) over the 150-day composting period.

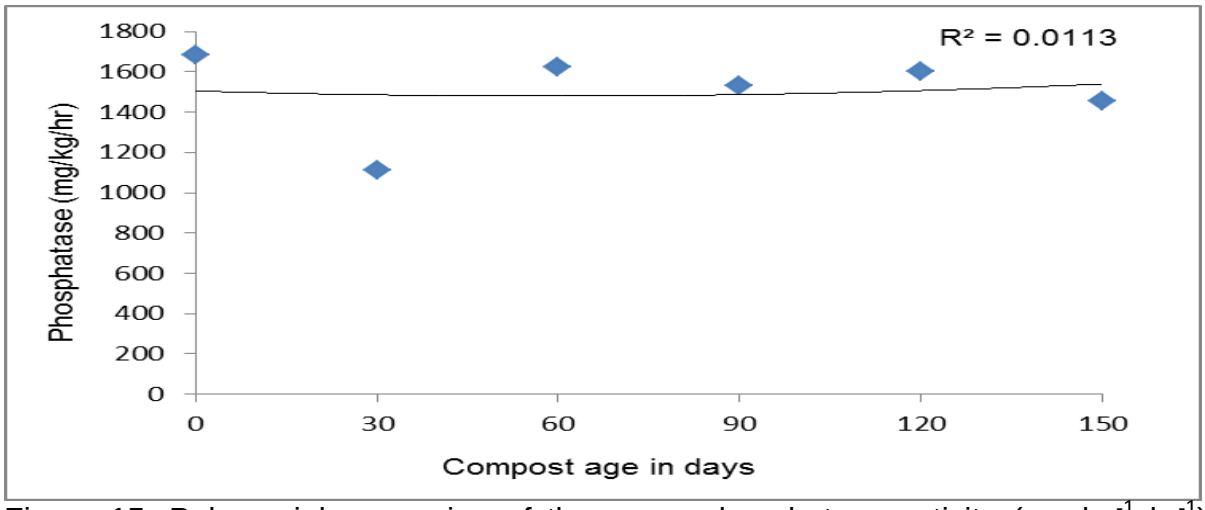

Figure 15: Polynomial regression of the mean phosphatase activity (mg kg^{-1} hr^{-1}) over the 150-day composting period.

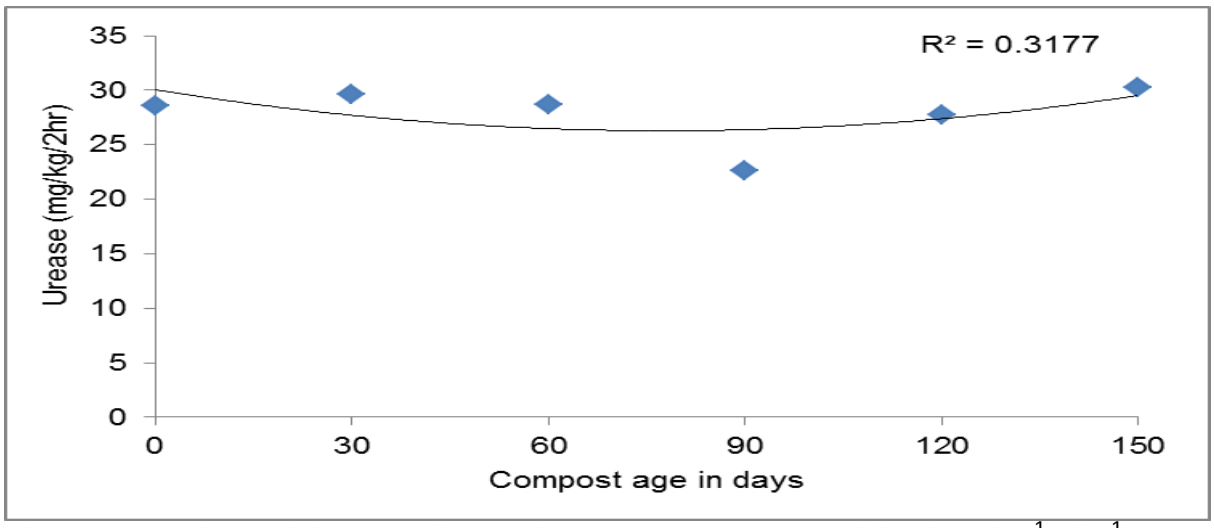

Figure 16: Polynomial regression of the mean a urease activity (mg kg^{-1} $2hr^{-1}$) over the 150-day composting period.

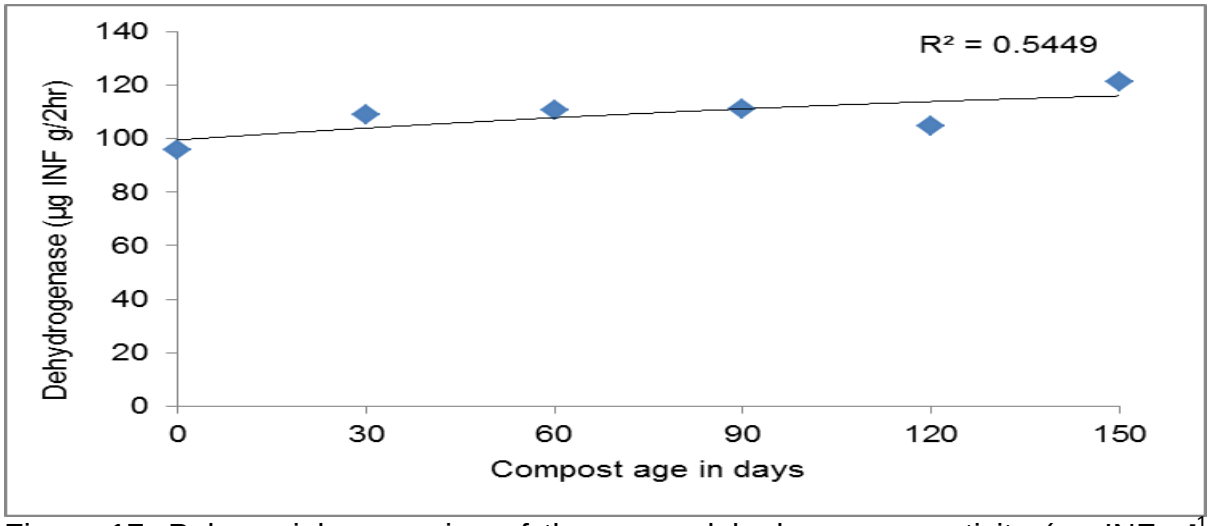

Figure 17: Polynomial regression of the mean dehydrogenase activity (µg INF g^{-1} $2hr^{-1}$) over the 150-day composting period.

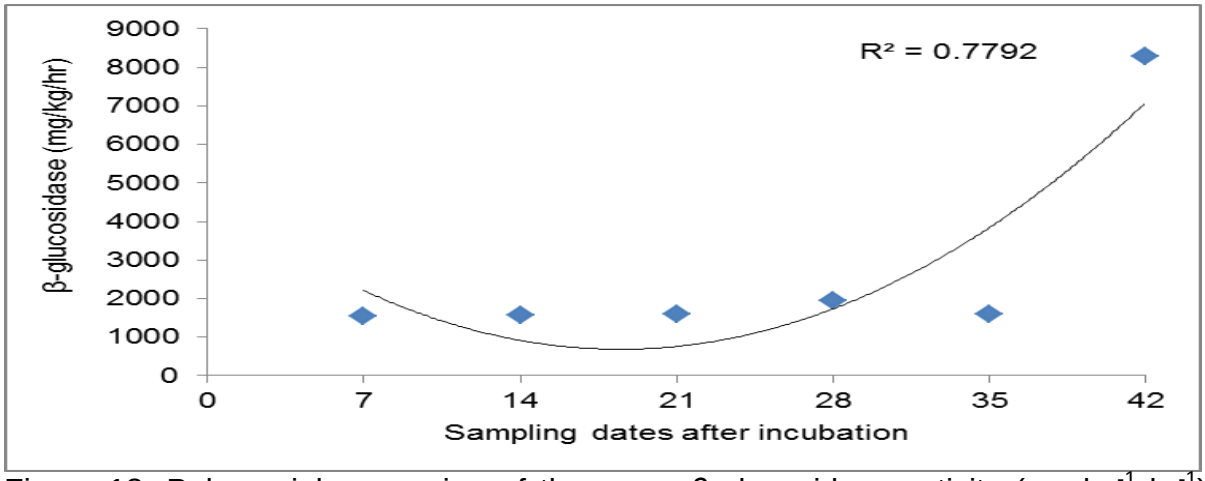

Figure 18: Polynomial regression of the mean β-glucosidase activity (mg kg^{-1} hr^{-1}) measured during the 42-day incubation period.

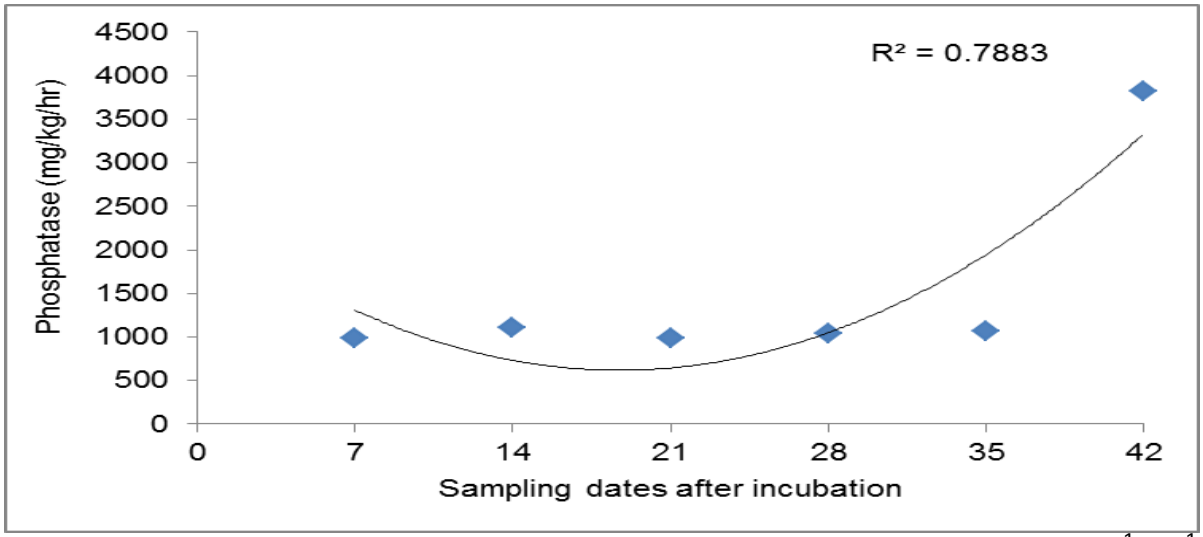

Figure 19: Polynomial regression of the mean phosphatase activity (mg kg^{-1} hr^{-1}) measured during the 42-day incubation period.

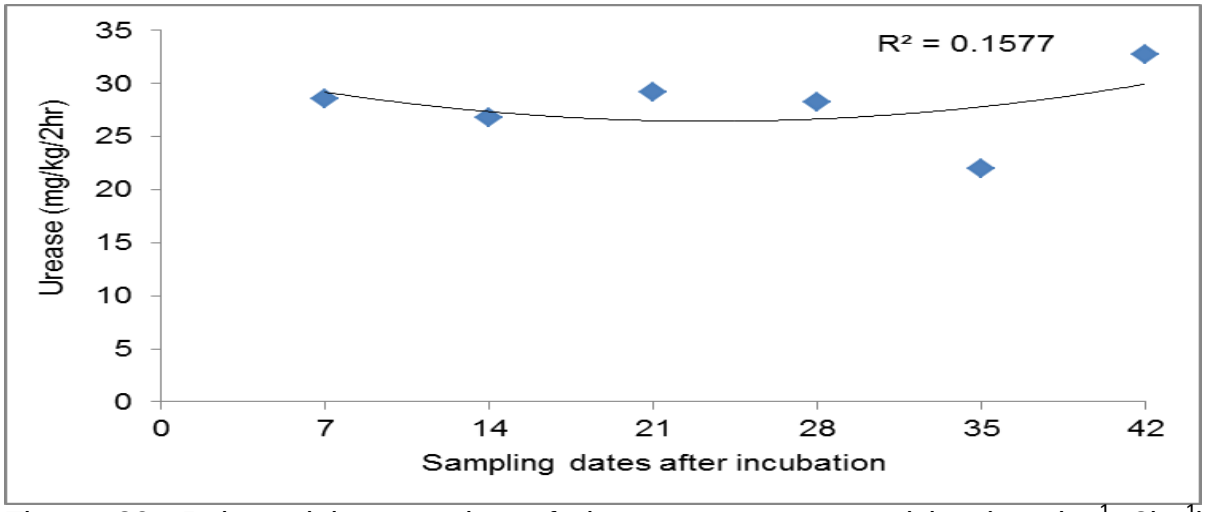

Figure 20: Polynomial regression of the mean urease activity (mg kg^{-1} $2hr^{-1}$) measured during the 42-day incubation period.

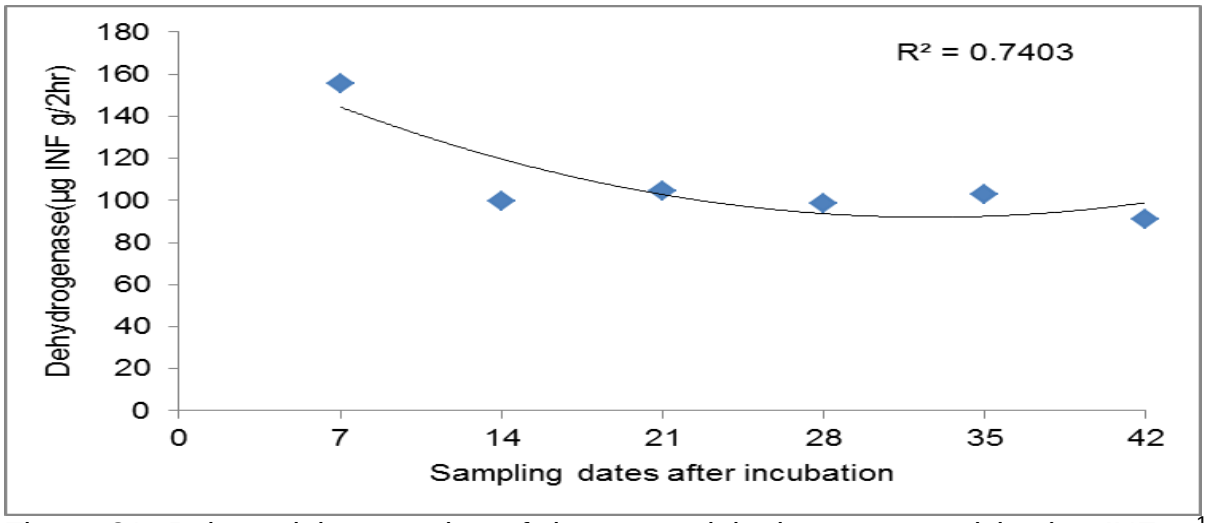

Figure 21: Polynomial regression of the mean dehydrogenase activity (µg INF g^{-1} $2hr^{-1}$) measured during the 42-day incubation period.

A significant compost maturity x incubation period interaction effect on the three microbial population species counts was obtained (Table 7). This suggests that the measured count at the different sampling dates during the incubation period was dependent on the age of sampling (maturity) of the composts. This is in agreement with earlier findings reported by Garland (2006). β-glucosidase activity was highest (10585 mg kg^{-1} hr^{-1}) in compost amended soil with the 60-day old compost (SD60) at 42 DAI while the highest phosphatase activity (4935.3 mg kg^{-1} hr^{-1}) was similarly obtained at 42 DAI but in soil which is not amended with compost. This result is in agreement with earlier work reported by Castaldi *et al*. (2008). Enzymes are a useful tool to quantify the dynamics of biodegradation process and provide valuable information about the stability and maturity of the compost.The β-glucosidase and

phosphatase activities were observed to be active from day 28 when most crops will probably still have to use nutrients from applied compost. Most crops have peak demand for nutrients and water at 42 days (six weeks) after planting. The highest concentration of urease (39.35 mg kg^{-1} $2hr^{-1}$) and dehydrogenase (183.25 µg INF g^{-1} $2hr^{-1}$) activity in soil amended with 120-day old compost (SD120) was obtained at 14 and 7DAI, respectively. Urease activity is highest at 14 days, which might be good for the microbial population (Castaldi *et al*., 2008). The dehydrogenase activity is highest at 7 days, which is much early activity and might be good for increase microbial population (Castaldi *et al*., 2008).

Table 7: Effect of compost maturity x incubation period interaction on enzyme activities

Treatments		β-glucosidase	Phosphatase	Urease	Dehydrogenase
Compost ID	**Incubation period**	**(mg kg^{-1} hr^{-1})**	**(mg kg^{-1} hr^{-1})**	**(mg kg^{-1} 2 hr^{-1})**	**(µg INF g^{-1} 2 hr^{-1})**
Control	7	1442^{d}	927.30^{c}	28.10^{abcd}	114.45^{efg}
Control	14	1519^{d}	1108.60^{c}	30.30^{abcd}	70.65^{k}
Control	21	1510^{d}	1032.10^{c}	29.10^{abcd}	101.35^{efghij}
Control	28	1933^{d}	1111.30^{c}	27.70^{abcd}	94.25^{fghijk}
Control	35	1300^{d}	977.30^{c}	21.50^{bcde}	97.65^{efghijk}
Control	42	9201^{bc}	4935.3^{a}	34.85^{ab}	95.20^{fghijk}
SD30	7	1552^{d}	1170.30^{c}	31.20^{abcd}	155.35^{abc}
SD30	14	1655^{d}	1234.80^{c}	34.30^{ab}	97.95^{efghijk}
SD30	21	1775^{d}	1199.50^{c}	30.10^{abcd}	102.80^{efghij}
SD30	28	1960^{d}	837.20^{c}	27.20^{abcd}	95.20^{fghijk}
SD30	35	1617^{d}	1168.40^{c}	22.20^{abcde}	108.00^{efghij}
SD30	42	1896^{d}	1055.30^{c}	32.60^{ab}	94.55^{fghijk}
SD60	7	1531^{d}	911.50^{c}	29.70^{abcd}	173.55^{ab}
SD60	14	1655^{d}	1057.60^{c}	27.85^{abcd}	102.15^{efghij}
SD60	21	1627^{d}	1058.50^{c}	26.85^{abcd}	118.15^{def}
SD60	28	1905^{d}	1107.20^{c}	27.45^{abcd}	84.30^{ghijk}
SD60	35	1822^{d}	1168.90^{c}	27.70^{abcd}	106.55^{efghij}
SD60	42	10585^{a}	4435.3^{ab}	32.75^{ab}	78.30^{ijk}
SD90	7	1457^{d}	865.60^{c}	26.15^{abcd}	145.50^{bcd}
SD90	14	1481^{d}	1012.10^{c}	6.50^{e}	108.50^{efghi}
SD90	21	1617^{d}	952.80^{c}	25.10^{abcd}	102.50^{efghij}
SD90	28	1974^{d}	1089.6^{c}	26.25^{abcd}	119.60^{def}
SD90	35	1558^{d}	1058.10^{c}	14.40^{cde}	106.30^{efghij}
SD90	42	9225^{bc}	4208.00^{ab}	37.30^{ab}	84.10^{hijk}
SD120	7	1677^{d}	1119.30^{c}	24.75^{abcd}	183.25^{a}
SD120	14	1559^{d}	1137.30^{c}	39.35^{a}	92.25^{fghijk}
SD120	21	1483^{d}	982.00^{c}	31.40^{abcd}	93.00^{fghijk}
SD120	28	1942^{d}	1107.2^{c}	29.70^{abcd}	87.60^{ghijk}
SD120	35	1430^{d}	840.50^{c}	14.00^{de}	77.85^{jk}
SD120	42	10016^{d}	4409.40^{ab}	27.25^{abcd}	93.50^{fghijk}
SD150	7	1574^{d}	903.60^{c}	31.40^{abcd}	160.45^{ab}
SD150	14	1552^{d}	1077.90^{c}	22.70^{abcde}	127.05^{cde}
SD150	21	1634^{d}	655.90^{c}	32.50^{ab}	110.00^{efgh}
SD150	28	1913^{d}	1022.30^{c}	31.00^{abcd}	108.50^{efgh}
SD150	35	1852^{d}	1179.60^{c}	32.00^{abc}	119.65^{def}
SD150	42	8741^{d}	3896.30^{b}	31.60^{abcd}	101.30^{efghij}
LSD		**900**	**828**	**17.7**	**30.2**

SD implies compost sampling/maturity date; LSD Implies Least Significant Different

4.4 Nitrogen and P mineralisation in compost samples and compost amended soils

The concentration of nitrate N and Bray P1 released from the compost amended soil during the incubation period was significantly ($P \leq 0.05$) affected by compost maturity or age (Ct), incubation period (Ip) and Ct x Ip interaction effect (Table 8). None of the compost age (Ct), incubation period (Ip) or the Ct x Ip interaction effect exerted any significant effect on the mean concentration of ammonium N released from the compost amended soil during the incubation. Although the concentration of ammonium N in the compost amended soils did not exceed the upper limit value of 400 mg kg^{-1} prescribed for stable composts (Canada Composting Council, 2008), the measured nitrate N concentrations were lower than 200 mg kg^{-1} (Table 8). The mean concentration of nitrate N mineralised from the compost amended soils was the highest while the concentration of Bray P1 mineralised was the lowest. The highest mean Bray P1 (20.50 mg kg^{-1}) and nitrate N (192 mg kg^{-1}) concentrations mineralised were both obtained in the 150-day old compost. The highest mean of Bray P1 (20.10 mg kg^{-1}) and nitrate N (189 mg kg^{-1}) concentrations were obtained at 42 and 21 DAI, respectively. Higher concentrations of nitrate N than ammonium N were obtained from all compost types and at all incubation period. This finding is in agreement with the findings by Bernal *et al.* (2009) who reported that the release of nitrate N was always more than ammonium N probably due to insufficient oxygen that may cause gaseous loss by denitrification and higher pH that inhibit nitrifying microorganisms. Thus, nitrate N concentration at the end of a composting process is often higher than ammonium N due to adequate aeration (Wang *et al*., 2004).

The concentration of extractable P content gradually increased as the compost matures and the concentration of P released also increased with incubation time increases (Figure 22). This increase P concentration with maturity and incubation could be because of its mineralisation. The concentration of nitrate N increased as the compost mature and also with incubation time (Figure 23). The rise in N level during maturation phase in 120-day old compost could possibly be due to the concentration effect caused by strong degradation of labile organic carbon compounds which reduces the weight of composting materials (Bernal *et al.,* 2009). At the end of composting process the content of NO_3^- should be higher than that of NH_4^- indicating adequate aeration conditions (Salazar *et al*., 2005). The compost

samples collected at different maturity dates did not exceed the threshold limit of 400 mg kg^{-1} suggested by Canada Composting Council (2008) for NH_4^-concentration in stable composts but the levels of NO_3^- were lower than the expected during the maturation phase (Figure 23 & 24).

Table 9 below shows the polynomial equations, p-values and the estimated optimum content (Y) measured at date (X) for the various nutrients mineralised from the different composts during the incubation period. The regression model estimated the maximum concentration of Bray P1 (28.90 mg kg^{-1}) mineralised in 541-day (17 months and 21 days) compost (Figure 25). The model also estimated the highest ammonium N concentration (95.61 mg kg^{-1}) mineralised in 89-day old compost (Figure 26) while the highest nitrate N (184.27 mg kg^{-1}) concentration mineralised in 64-day compost (Figure 27). From the regression model, the highest estimated mean Bray P1 concentration of 19.98 mg kg^{-1} mineralised from the compost amended soil was obtained at approximately 42 days (one month and 12 days) after incubation (Figure 28). The highest estimated mean nitrate N concentration (185.39 mg kg^{-1}) mineralised was obtained at 21 days after incubation (Figure 29) while the highest estimate of ammonium N concentration (93.56 mg kg^{-1}) mineralised from the compost amended soil was obtained in 7 days after incubation (Figure 30). Composting and incubation did not influence ammonium N mineralisation, with an overall mean of 97 mg kg^{-1} (Table 8). The second order polynomial model used in this study suggests that the R^2-value for ammonium N measured from the 150-day old compost was lower than 37% (Figure 26) while during the 42-day incubation period, the value was less than 14% (Figure 29).

Table 8: Bray P1 and mineral N concentrations measured from compost-soil mix at different sampling times after incubation

Treatments	Amount of nutrients released (mg kg^{-1})		
	Bray P1	NH_4-N	NO_3-N
Compost ID			
SD30	14.38	97	182
SD60	16.44	99	183
SD90	15.01	92	185
SD120	17.42	98	185
SD150	20.50	96	192
Incubation period (DAI)			
7	8.65	98	187
14	12.37	108	181
21	16.00	90	189
28	18.17	92	187
35	18.84	102	183
42	20.10	92	186
Significance (p-value)			
Compost ID (Ct)	<0.0001	0.8627	<0.0001
Incubation period (Ip)	<0.0001	0.0920	<0.0001
Ct x Ip interaction	<0.0001	0.7767	<0.0001

SD implies compost sampling/maturity date; DAI implies days after incubation

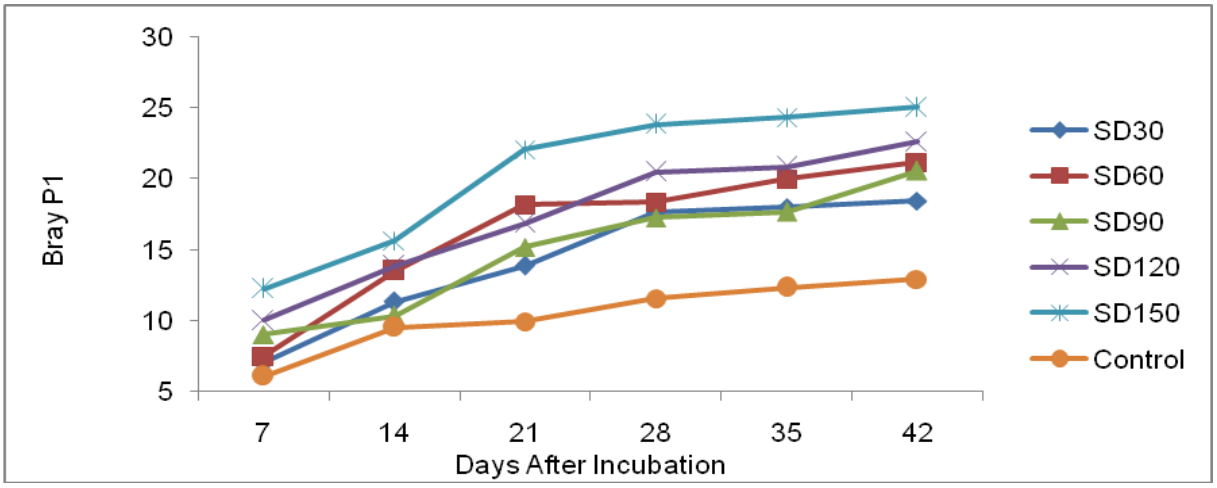

Figure 22: Means values of Bray P1 concentration (mg kg^{-1} measured from the different compost samples over the 42-day incubation period.

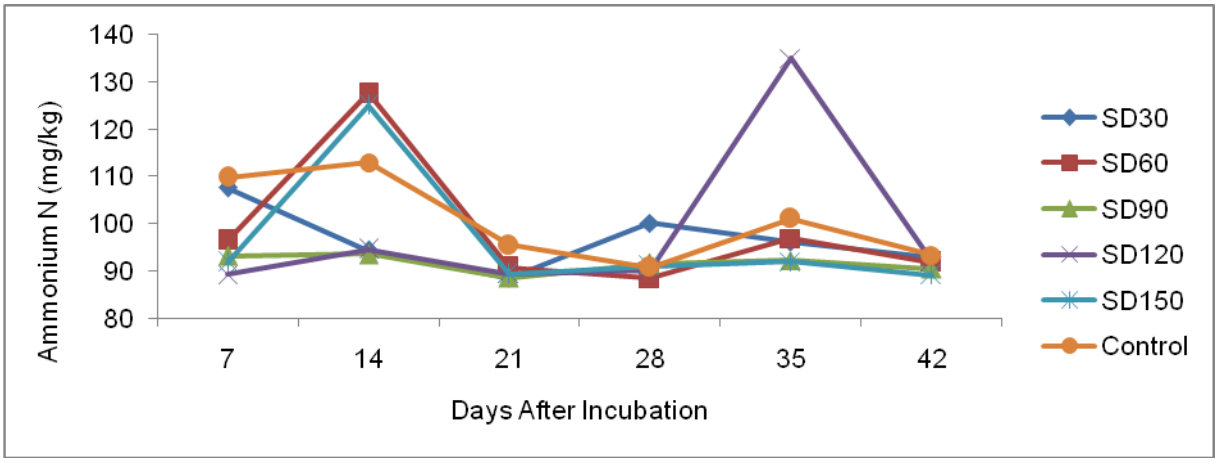

Figure 23: Mean values of ammonium N concentration (mg kg^{-1}) measured from the different compost samples over the 42-day incubation period.

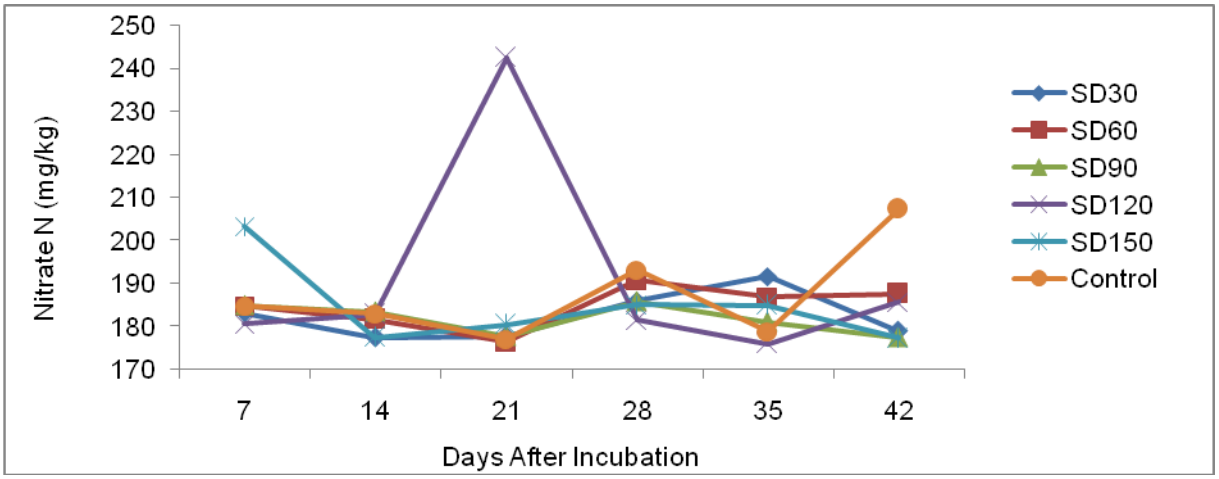

Figure 24: Means values of nitrate N concentration (mg kg^{-1}) measured from the different compost samples over the 42-day incubation period.

Table 9: Polynomial equations of the various response parameters with the zinc rates as independent variable and the corresponding R^2 values of the equation

Treatments	Parameters	Regression equation	p-values	Y- values	X- value
Compost maturity (age)	Bray P1	$y= -6E-05x^2 + 0.0649x + 11.339$	<0.0001	28.89	541.0
	Ammonium N	$y= 0.0006x^2 - 0.1067x + 100.35$	0.8627	95.61	88.9
	Nitrate N	$y= 0.0004x^2 - 0.0509x + 185.89$	<0.0001	184.27	63.6
Incubation period	Bray P1	$y= -0.0088x^2+0.7527x + 3.7851$	<0.0001	19.88	42.4
	Ammonium N	$y= 0.0031x^2-0.3459x + 103.21$	0.0920	93.56	55.8
	Nitrate N	$y= 0.0006x^2-0.0088x + 185.42$	<0.0004	185.39	7.3

P= significant value; R^2= measured response

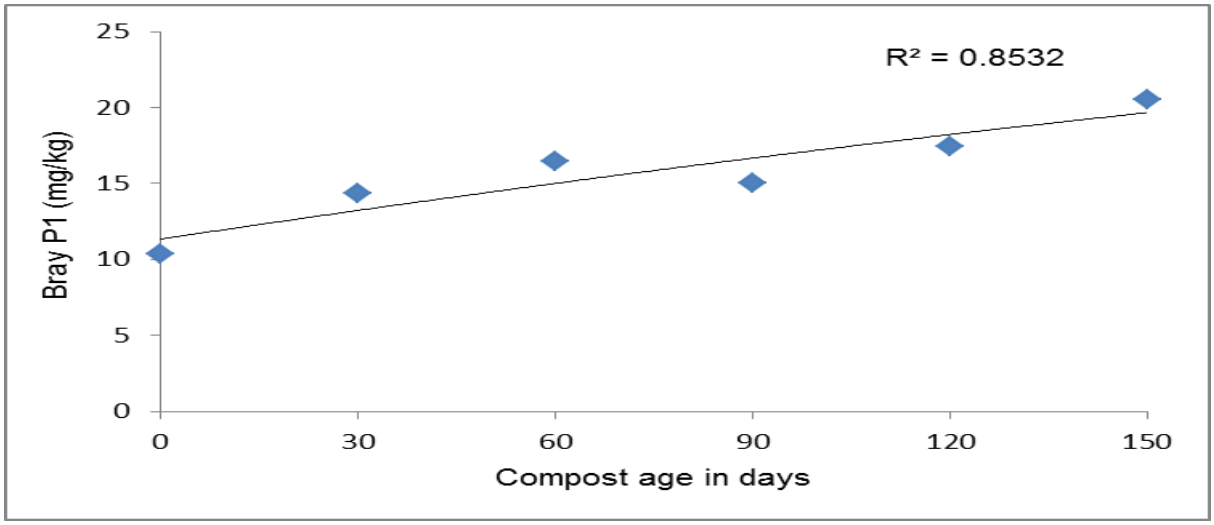

Figure 25: Polynomial regression of the mean extractable Bray P1 concentration (mg kg^{-1}) over the 150-day compost age.

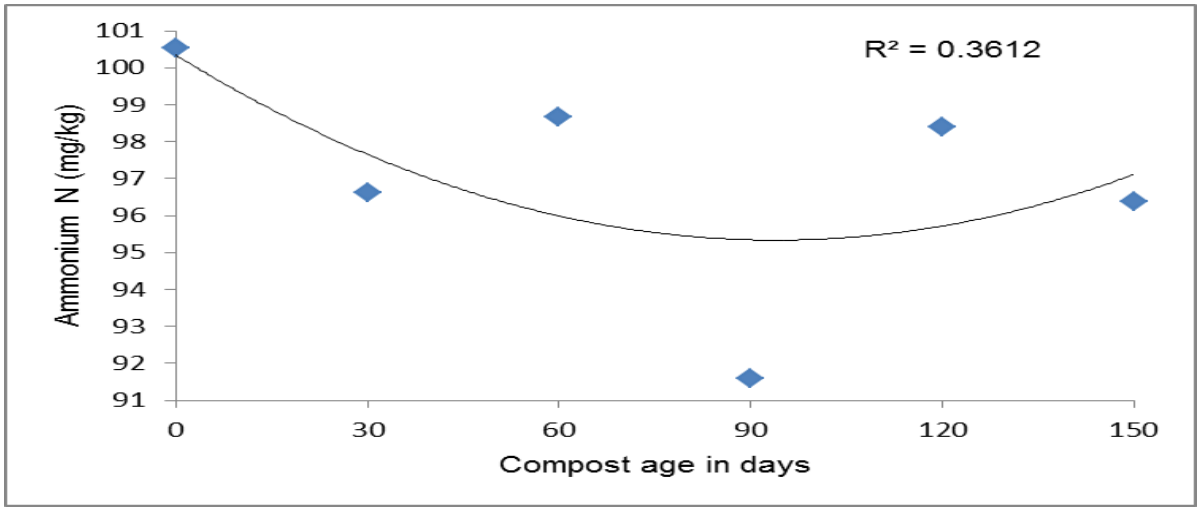

Figure 26: Polynomial regression of the mean ammonium N concentration (mg kg^{-1}) over the 150-day compost age.

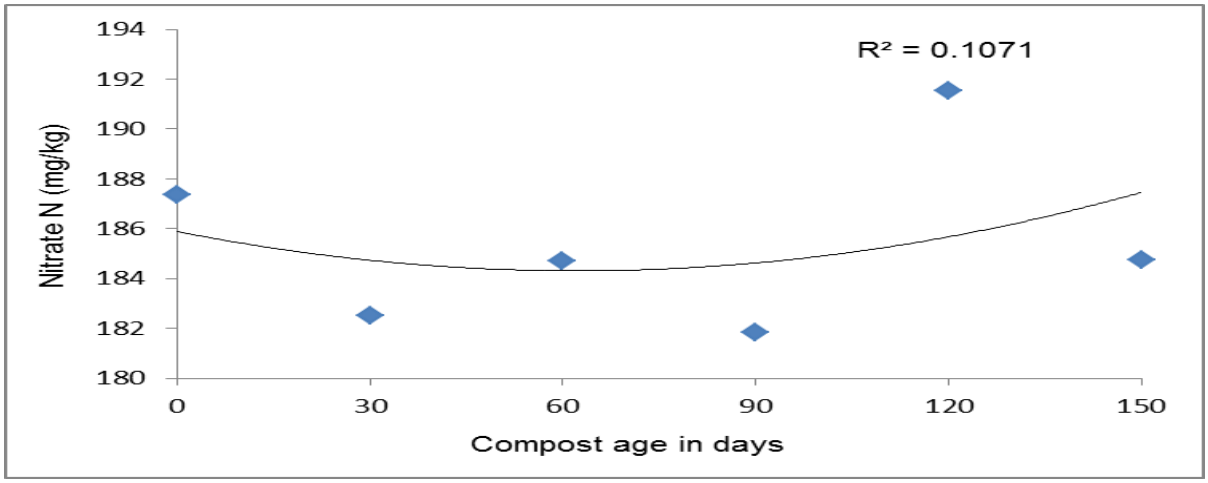

Figure 27: Polynomial regression of the mean nitrate N concentration (mg kg^{-1}) over the 150-day compost age.

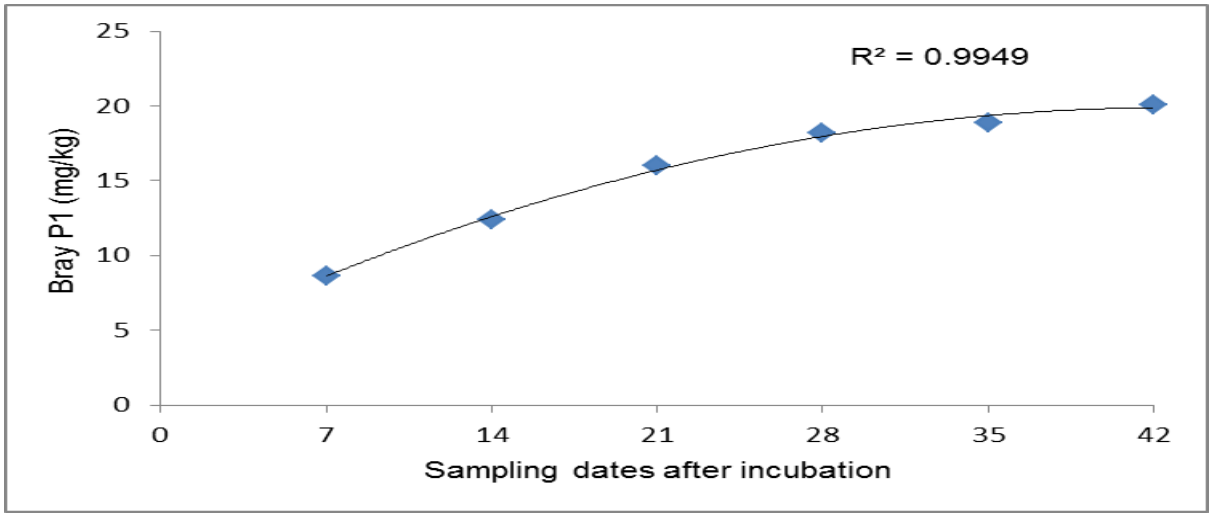

Figure 28: Polynomial regression of the mean extractable Bray P1 concentration (mg kg^{-1}) measured during the 42-day incubation period.

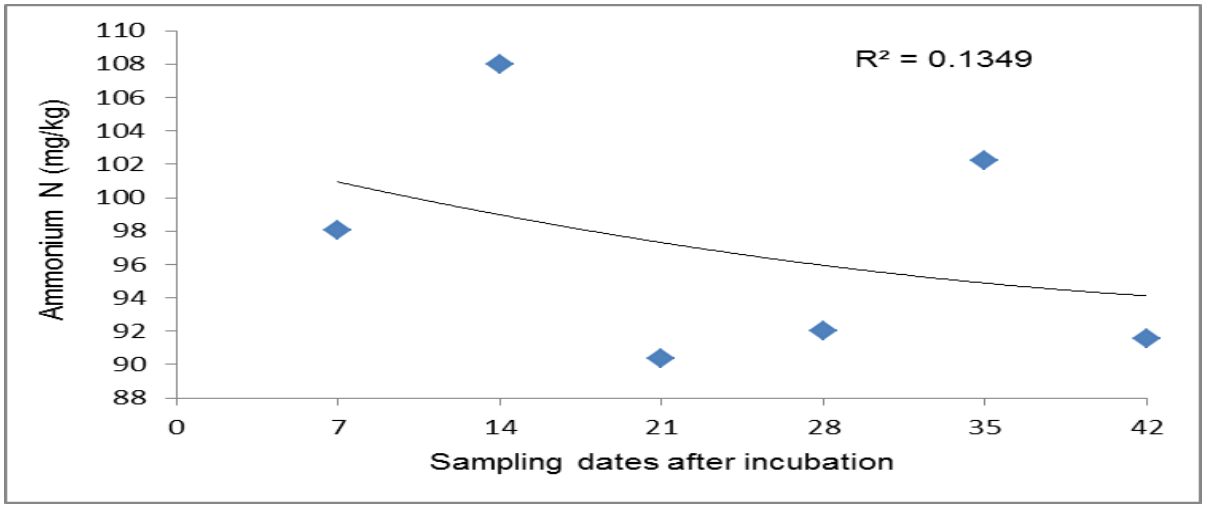

Figure 29: Polynomial regression of the mean ammonium N concentration (mg kg^{-1}) measured during the 42-day incubation period.

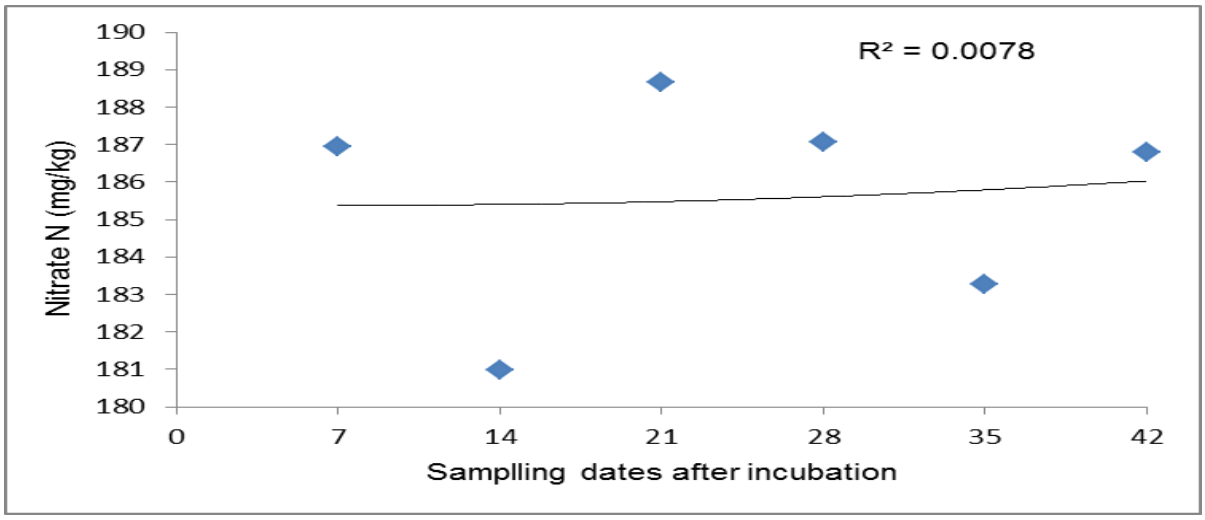

Figure 30: Polynomial regression of the mean nitrate N concentration (mg kg^{-1}) measured during the 42-day incubation period.

A significant compost maturity x incubation period interaction effect on the Bray P1 and nitrate N was obtained (Table 10). This suggests that the measured count at the different sampling dates during the incubation period was dependent on the age (maturity) of the composts. Sánchez-Monedero *et al.* (2001) found that cured compost contain higher amounts of Bray P1. They ascribed this to the fact that earthworms take up non-soluble P as a nutrient in their bodies for syntheses and release the remaining P in a mineralised form and concluded that mature compost might be an effective method producing a product with better plant nutritional value. The larger available N content is related with the fact that organic N from compost is mineralized gradually, offering constant availability of this element, because organic fractions are broken down by microorganisms, constituting an important N source for plant nutrition (Magdoff and Wiel, 2004). The highest mean Bray P1 concentration (25.00 mg kg^{-1}) obtained was in soil amended with the 150-day old compost measured at 42 DAI while the highest nitrate N (242.39 mg kg^{-1}) was in the 120-day old compost measured at 21 DAI. The steady increase in Bray P1 concentration mineralised, and the peak obtained at 42 DAI is considered fairly satisfactory for crops since P is made available for use by crops during both the seedling and vegetative stages of plant growth. Previous studies suggest that most crops have peak nutrients and water demand at 42 days after planting (Wang *et al*., 2004). The nitrate N released from the 120-day old compost in this study attained its peak at 21 DAI when crops will still have use for the nutrient. Salazar *et al*. (2005) similarly reported the successful use of 120-day old compost can to increase soil N and reduce inorganic N fertilizer in Thompson seedless grapes.

Table 10: Effect of compost maturity x incubation period interaction on nutrient mineralisation (mg kg^{-1})

Treatments			
Compost ID	**Incubation period**	**Bray P1(mg kg^{-1})**	**NO_3N(mg kg^{-1})**
Control	7	6.08^{s}	184.76^{d}
Control	14	9.52^{pqrs}	183.06^{d}
Control	21	9.95^{opqr}	177.07^{d}
Control	28	11.56^{mnopq}	193.16^{bcd}
Control	35	12.31^{lmnopq}	178.89^{d}
Control	42	12.90^{lmnop}	207.32^{b}
SD30	7	7.03^{rs}	183.17^{d}
SD30	14	11.33^{nopq}	177.51^{d}
SD30	21	13.88^{jklmn}	177.73^{d}
SD30	28	17.60^{fghi}	186.03^{d}
SD30	35	17.99^{fghi}	191.67^{bcd}
SD30	42	18.44^{efghi}	179.05^{d}
SD60	7	7.50^{rs}	184.87^{d}
SD60	14	13.51^{klmno}	181.63^{d}
SD60	21	18.14^{fghi}	176.47^{d}
SD60	28	18.36^{efghi}	190.69^{bcd}
SD60	35	19.97^{defgh}	186.90^{cd}
SD60	42	21.14^{bcdef}	187.56^{cd}
SD90	7	9.05^{qrs}	184.98^{d}
SD90	14	10.36^{nopqr}	183.39^{d}
SD90	21	15.16^{ijklm}	177.84^{d}
SD90	28	17.27^{ghij}	185.81^{d}
SD90	35	17.68^{fghi}	181.09^{d}
SD90	42	20.55^{cdefg}	177.79^{d}
SD120	7	10.01^{opqr}	180.59^{d}
SD120	14	13.87^{jklmn}	182.84^{d}
SD120	21	16.84^{hijk}	242.39^{a}
SD120	28	20.45^{cdefgh}	181.53^{d}
SD120	35	20.81^{bcdefg}	176.08^{d}
SD120	42	22.56^{abcd}	185.70^{d}
SD150	7	12.25^{lmnopq}	203.27^{bc}
SD150	14	15.64^{ijkl}	177.40^{d}
SD150	21	22.03^{abcde}	180.43^{d}
SD150	28	23.79^{abc}	185.15^{d}
SD150	35	24.29^{ab}	184.99^{d}
SD150	42	25.00^{a}	177.29^{d}
LSD		**3.68**	**17.23**

SD implies compost sampling/maturity date; LSD Implies Least Significant Different

4.5 Correlation analyses of the enzyme activities, microbial population counts and nutrients mineralised during incubation period

Table 11 shows the correlation matrix for the various soil quality indicators measured during the 42-days incubation period after soil amendments. There was a significant and positive relationship ($p<0.05$) between β-glucosidase and urease ($r^2 = 0.287$*) as well as alkaline phosphatase ($r^2 = 0.978$***); but negative correlation between nitrate and ammonium N ($r^2 = -0.239$*). Contrary to the findings by Castaldi *et al.* (2008) who reported a positive correlation between urease and NH_4^+ but negative correlation with NO_3, the results of my study revealed the opposite (i.e. positive correlation between urease and NO_3, and negative correlation between urease and NH_4^+). The positive effect of compost on enzyme activities is probably a combined effect of a higher degree of stabilization of enzymes to humic substances and an increase in microbial population count with increased soil C concentration (Zmora-Nahum*et al*., 2005). This is evident in my results with the observed strong negative correlation between phosphatase and urease with microbial population count. The fungi count in the composts showed a significantly positive correlation between bacteria and actinomycetes, with negative (though significant) correlation with Bray P1, urease, alkaline phosphatase, β-glucosidase and dehydrogenase. This may be related to the stimulatory effect of compost addition on microbiological populations and improving enzymatic activity (Madejón *et al*., 2001).

The concentration of dehydrogenase measured in the composts showed negative but significant correlation with alkaline phosphatase and β-glucosidase activity. Extractable Bray P1 showed positive correlation with alkaline phosphatase and β-glucosidase activity, but negative correlation with dehydrogenase activity. The positive correlation between Bray P1 and alkaline phosphate obtained in this study contradicts earlier findings by Madejón *et al*. (2001) who reported a negative correlation between available Bray P and alkaline phosphatase. The change in microbial population count during the composting period could possibly explain the lack of correlation between dehydrogenase and microbial counts. Rigane *et al*. (2013) found that microbial composition depends on carbon microbial biomass and that under normal condition only about 15% of the microorganisms are active, whereas most of them are inactive or not viable (Kutsanedzie *et al*., 2015). The

results further showed a positive and significant ($p \leq 0.05$) correlation between actinomycetes and bacteria, but significant and negative correlation with Bray P1, urease, alkaline phosphatase and β-glucosidase. In my study, the positive effect of OM could be more important than the inhibitor effect of the high available P content.

Table 11: Pearson correlation matrix between enzyme activities, microbial population counts and nutrientsmineralised from the compost amended soil samples during the 42-days incubation period

	Urease	Phosphatase	B-glucosidase	Dhgenase!	NH_4^+- N	NO_3^--N	Bray P1	LogNA	LogACT	LogMEA
Urease	-									
Phosphatase	0.309**	-								
B-glucosidase	0.287*	0.978***	-							
Dhgenase!	-0.0420	-0.288*	-0.293*	-						
NH_4^+- N	-0.1197	-0.131	-0.1637	-0.1064	-					
NO_3^--N	0.096	0.068	0.055	-0.074	-0.239*	-				
Bray P1	0.1011	0.326**	0.385***	-0.451***	-0.182	0.058	-			
LogNA	-0.268*	-0.718***	-0.695***	0.173	0.151	-0.135	-0.358	-		
LogACT	-0.273*	-0.653***	-0.602***	-0.008	0.0346	-0.055	-0.0610	0.888***	-	
LogMEA	-0.272*	-0.713***	-0.714***	0.340**	0.1171	-0.073	-0.477***	0.872***	0.746***	-

Dhgenase! Implies dehydrogenase; NA, ACT and MEA implies bacteria, actinomycete and fungi, respectively. *, **, *** are significant at 0.05, 0.01 and 0.001 level of probability, respectively.

CHAPTER 5

SUMMARY, CONCLUSION AND RECOMMENDATIONS

Composting represents one of the versatile and remunerative strategies for not only managing biodegradable wastes, but also promoting nutrients recycling. The age of compost (i.e. compost maturity) has significant influence on compost quality while incubation period upon mixing of such compost with soil will also exert influence on soil bio-quality parameters. Since microbes are the heart of soil, they provide the substratum for soil health and thus, soil quality. Compost maturity is beginning to be more recognized as a significant parameter to evaluate compost. The reason is that immature and poorly stabilized composts pose well-known problems during storage, marketing and use. In storage, immature composts may become anaerobic which often leads to odours and/or the development of toxic compounds, as well as bag swelling and bursting. Immature composts may heat up in pallets during shipment. Continued active decomposition when these composts are added to soil or used as growth media may exert negative impacts on plant growth due to reduced oxygen in the soil-root zone, reduced available nitrogen, or the presence of phytotoxic compounds. For the survival and growth of soil microbes, organic carbon source is vital; and serves as the source of carbon for the microbes.

The results of this study suggest that there was an increase of quantity and activity of microbial population count, aspect of great importance in organic matter turnover and nutrients availability in compost amended soil. In terms of the decomposition of organic matter during composting, enzymatic activities such as β-glucosidase and alkaline phosphatase are a useful tool to monitor the dynamics of biodegradation process and provide valuable information about the stability and maturity of the compost. The higher level of dehydrogenase activity was observed in soil treated with 150 days mature compost. Composting and incubation time had significant influence on microbial population counts, enzyme activities and the nitrate N and available P release characteristics of the composts. The implication is that, the contents of these various microbial groups and enzyme activities studies varied remarkably depending on both compost age and incubation period. These microbes and enzymes play vital role in the biochemical reactions and recycling of the N and P contents of the composts. The higher concentrations of the different microbial groups

and the enzyme activities are vital enablers that promote nutrients release following compost addition to the soil. Based on the results of this study, the use of 120-day old or more compost is recommended when such composts are targeted to supply the needed N and P for crop use in order to safe on fertiliser costs. Further studies are needed to evaluate the long term effect of compost maturity and the length of storage on the different crops grown under field condition in different agro-ecology.

LIST OF REFERENCES

Aira, M., Monroy, F., and J. Dominguez. 2007. Earthworms strongly modify microbial biomass and activity triggering enzymatic activities during vermin-composting independently of the application rates of pig slurry. Science of the Total Environment 385 (3): 252-261.

Aislabie, J., and J.R. Deslippe 2013. Soil microbes and their contribution to soil services. In*:* Dymond JR (ed.), Ecosystem services in New Zealand – conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand, 143-161pp.

Angel, R.A., and D.C. Runde. 2010. Elementary and intermediate algebra for college students, fourth Edition, ISBN: 978-0-321-62092-7.

Avis, T.J., Gravel, V., Antoun, H., and R. Tweddell. 2008. Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biology and Biochemistry 40: 1733-1740.

Bernal, M., Alburquerque, J., and R. Moral. 2009. Composting of animal manures and chemical criteria for compost maturity assessment: A review. Bioresource Technology 100: 5444-5453.

Bloem, J., Hopkins, D.W., and A. Benedetti. 2006. Microbiological methods for assessing soil quality. USA: CABI publishing.

Boulter-Bitzer, J.I., Boland, G.J., and J.T. Trevors. 2000. Compost: a study of the development process and end-product potential for suppression of turfgrass disease. World Journal of Microbiology and Biotechnology 16: 115-134.

Bray, R.H., and L.T. Kurtz. 1945. Determination of total, organic and available forms of phosphorus in soils. Soil Science 59: 39-45.

Camoes, M.F. 2009. The quality of pH measurements 100 years after its definition. Accreditation and Quality Assurance 14(10):521-523.

Canada Composting Council. 2008. A Summary of Compost Standards in Canada. Available at: www.compost.org/ standard.html.

Castaldi, P., Garau, G., and P. Melis. 2008. Maturity assessment of compost from municipal solid. Waste Management 28 (3): 534-540.

Chen, Y., Inbar, Y., Chefetz, B., and Y. Hadar. 1997. Compost and recycling of organic wastes. In: Rosen, D., Tel-Or, E., Hadar, Y., Chen, Y. (eds.), Modern Agriculture and the Environment. Kluwer Academic Publishers, Dordrecht, The Netherlands, 341-362pp.

Compost Council Quality of California-CCQC. 2001. *Compost Maturity, Index*. Technical Report. Available online from http://www.ccqc.org.

Fuchs, J.G. 2002. Practical use of quality compost for plant health and viability improvement. In: Insam, H., Riddech, N., Klammer, S. (eds.), Microbiology of Composting. Springer Verlag, Heidelberg, 435-444pp.

Gajalakshmi, S., and S.A. Abbasi. 2008. Solid waste management by composting: State of the Art. Critical Reviews in Environmental Science and Technology 38 (5): 311-400.

García-Gómez, A., M.P., Bernal, and A. Roig. 2003. Carbon mineralisation and plant growth in soil amended with compost samples at different degrees of maturity. Waste Management Research 21: 161-171.

García-Ruiz, J.M., Regüés, D,Alvera, B., Lana-Renault, N., Serranomuela, P., Nadal-Romero, E., Navas, A., Latron, J. and C. Martí-Bono. 2008. Plant cover, flood generation and sediment transport at catchment scale: a gradient of experimental catchments in the central Pyrenees. Journal of Hydrology 356: 245-260.

Garland, T. 2006. Selection experiments: an underutilized tool in biomechanics and organismal biology. BIOS Scientific publishers, Oxford. U.K, 23-56pp.

Goyal, S., Dhull, S., and K. Kapoor. 2005. Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. Bioresource Technology 40: 121-127.

Gómez-Brandón, M., Lazcano, C., and J. Domínguez. 2007. The evaluation of stability and maturity during the composting of cattle manure. Chemosphere70: 436-444.

Hao, X.Y., Chang, C., Larney, F.J., and G.R. Travis. 2001. Green gas emissions during cattle feedlot manure composting. Journal of Environmental Quality 30:376-386.

Hartley, W., N.M., Dickson, P. Riby, and N.W. Lepp. 2009. Arsenic mobility in brown field soils amended with green waste compost or biochar and planted with *Miscanthus*. Environmental Pollution 157: 2654-2662.

Hargreaves, J., M., Adl, and P. Warman. 2008. A review of the use of composted municipal solid waste in agriculture. Agriculture, Ecosystems and Environment 123: 1-14.

Hseu, Z.Y. 2004. Evaluating heavy metals content in nine compost using four digestion methods. Bioresource Technology 95: 53-59.

Hutchison, M.L., Walters, L.D., Avery, S.M., Munro, F., and A. Moore. 2005. Analyses of livestock production, waste storage, and pathogenlevels and prevalences in farm manures. Applied Environmental Microbiology 71: 1231-1236.

Islam, M., Morgan, J., Doyle, M., Phatak, S., Millner, P., and X. Jiang. 2004. Fate of *Salmonella enterica* and *Serovar typhimurium* on carrots and radishes grown in fields treated with contaminated manure compost or irrigation water. Applied Environmental Microbiology 70 (4): 2407-2502.

Keener, H.M., Dick, W.A., and H.A.J. Hoitink. 2000. Composting and beneficial utilization of composted by-product materials. In: Dick, W.A. (ed.), Land application of agricultural, industrial, and municipal by-products. Soil Science Society of America, Inc., Madison, 315-341pp.

Kutsanedzie, F., Ofori, V., and K.S. Diaba. 2015. Maturity and safety of compost processed in HV and TW composting systems. International Journal of Science, Technology and Society 3: 232-239.

Latifah, O., Osumanu, H.A., and M.A Nik. 2015. Improving ammonium and nitrate release from urea using clinoptilolite zeolite and compost produced from agricultural wastes. The Scientific World Journal 2015: 1-12.

Madejón, P., Murillo, J.M., Marañón, T., Cabrera, F., and R. López. 2001. Bioaccumulation of As, Cd, Cu, Fe and Pb in wild grasses affected by the Aznalcóllar mine spill (SW Spain). Science of Total Environment Journal 290: 105-120.

Magdoff, F., and R. Weil. 2004. Soil organic matter in sustainable agriculture. CRC Press. USA, 295-327pp.

Makoi, J., and P. Ndakidemi. 2008. Selected soil enzymes: Examples of their potential roles in the ecosystem. African Journal of Biotechnology 7 (3): 181-191.

Marhuenda-Egea, F.C., Martínez-Sabater, E., Jordá, J., Moral, R., Bustamante, M.A., Paredes, C., and M.D. Pérez-Murcia. 2007. Dissolved organic matter fractions formed during composting of winery and distillery residues: evaluation of the process by fluorescence excitation–emission matrix. Chemosphere 68: 301-309.

Mari, I., Ehaliotis, C., Kotsou, M., Chatzipavlidis, I., and D. Georgakakis. 2005. Use of sulfur to control pH in composts derived from olive processing by-products. Compost Science and Utilization 13: 281-287.

Marschner, P, Fu Q.L, and Z. Rengel. 2003. Manganese availability and microbial populations in the rhizosphere of wheat genotypes differing in tolerance to Mn deficiency. Journal of Plant Nutrition and Soil Science 166: 712-718.

Maynard, D.G., and Y. P. Kalra. 2001. Nitrate and Exchangeable Ammonium Nitrogen. Forestry Canada Edmonton, Alberta, Canada, 25-37pp.

Melero S, López-Garrido R, Madejon E, Murillo JM, Vanderlinden K, Ordóñez R, and F. Moreno. 2009. Long-term effects of conservation tillage on organic fractions in two soils in Southwest of Spain. Agriculture, Ecosystem and Environment 133: 68-74.

Mon, R. Irurtia C., Botta G.F., Pozzolo O., Bellora F., Rivero D., Bomben M. 2007. Effects of supplementary irrigation on chemical and physical soil properties in the rolling pampa region of Argentina. Cienc. Inv. Agriculture. 34 (3):187-194

Mondini, C., Dell'Abate M.T., Leita, L., and A. Beneditti. 2003. An integrated chemical, thermal and microbiological approach to compost stability evaluation. Journal of Environmental Quality 32: 2379-2386.

Mondini, C., Sa´nchez-Monedero, M.A., Sinicco, T., and L. Leita. 2004. Evaluation of extracted organic carbon and microbial biomass as stability parameters in ligno-cellulosic waste composts. Journal of Environmental Quality 35: 2313-2320.

Mustafa, T. 2003. Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry. Microchemical Journal 74: 289-297.

Niwagaba, C., Nalubega, M., Vinnerås, B., Sundberg, C., and H. Jönsson. 2009. Substrate composition and moisture in composting source-separated human faecesand food waste. Environmental Technology14: 487- 497.

Nannipieri, E., Kandeler, E., and P. Ruggiero. 2002. Enzyme activities and microbiological and biochemical processes in soil. New York, 1-33pp.

Okalebo, J.R., Gathua, K.W., and P.L. Woomer. 2002. Laboratory methods of soil and plant analysis: A working manual. TSBF: Nairobi, Kenya, 85-107pp.

Pansua, A.M. and L. Thuriés. 2003. Kinetics of C and N mineralisation, N immobilization and N volatilization of organic inputs in soil. Soil Biology and Biochemistry 35 (1): 37-48.

Parkinson, R., Gibbs, P., Burchett, S., and T. Misselbrook. 2004. Effect of turning regime and seasonal weather conditions on nitrogen and phosphorus losses during aerobic composting of cattle manure. Bioresource Technology 91: 171-178.

Ranalli, A., Malfatti, A., Lucera, L., Conteto, S., and E. Sotiriou. 2005. Effects of processing techniques on the natural colourings and the other functional constituents in virgin olive oil. Food Research International 38: 8-9.

Rigane, M.K., Michel, J.C., Medhioub, K. and P. Morel. 2013. Evaluation of Compost Maturity, Hydrophysical and Physicochemical Properties: Indicators for Use as a Component of Growing Media. Compost Science and Utilization 19:226-234.

Ryckeboer, J., Mergaert, J., Vaes, K., Klammer, S., De Clercq, D., Coosemans, J., Insam, H., and J. Swings. 2003. A survey of bacteria and fungi occurring during composting and self-heating processes. Annals Microbiology 53: 349-410.

Salazar, F.J., Chadwick, D., Pain, B.F., Hatch, D., and E. Owen. 2005. Nitrogen budgets for three cropping systems fertilised with cattle manure. Bioresource Technology. 96: 235-245.

Sánchez-Monedero, M.A., Roig, A., Cegarra, J., and M.P. Bernal. 2001. Relationships between water-soluble carbohydrate and phenol fractions and the humification indices of different organic wastes during composting. Bioresource Technology 78: 301-308.

Schinner, F., and W. Von Mersi. 1990. Xylanase-, CM-cellulase- and invertase activity in soil: an improved method. Soil Biology and Biochemistry 22: 511-515.

Sherman, R. 2005. Large-scale organic materials composting. North Carolina Cooperative Extension Services. Available online at: http://www.bae.ncsu.edu/bae/programs/extension/pblicat/vermcompost/ag593.pdf.

Smith, D.C., and J.C. Hughes. 2002. Changes in chemical properties and temperature during the degradation of organic wastes subjected to simple composting protocols suitable for small-scale farming, and quality of the mature compost. South African Journal of Plant and Soil Science 19:53-60.

Sundberg, C. Improving Compost Process Efficiency by Controlling Aeration, Temperature and pH. Doctoral Thesis submitted to the Faculty of Natural Resources and Agriculture, Swedish University of Agricultural Sciences, Sweden, 10 -17pp.

USDA. 2002. National Engineering Handbook, NRCS, U.S. Department of Agriculture, Washington, D.C., Part 637.

Tabatabai MA, Dick WA. 2002. Enzymes in soil: research and developments in measuring activities. In: Burns RG and Dick RP, (eds.), Enzymes in the Environment. Marcel Dekker, New York, pp. 567–596.

Tiquia, S.M. 2002. Evolution of extracellular enzyme activities during manure composting. Journal of Applied Microbiology 92: 764-775.

Tiquia, S., Wan, J., and N. Tam. 2002. Dynamic of yard trimmings composting as determined by dehydrogenase activity, ATP content, arginine, ammonification and nitrification potential. Process Biochemistry 37: 1057-1065.

Wang, P., Changa, C.M., Watson, M.E., Dick, W.A., Chen, Y., and H.A.J. Hoitink. 2004. Maturity indices for composted dairy and pig manures. Soil Biology and Biochemistry 36: 767-776.

Wickramatilake, A., Kouno, K., and T. Nagaoka. 2010. Compost amendment enhances the biological properties of Andosols and improves phosphorus utilization from added rock phosphate. Soil Science and Plant Nutrition 56 (4): 607-616.

Yang, X. B., Ying, G. G., Peng, P. A., Wang, L., Zhao, J. L., Zhang, L. J., Yuan, P., and H. P. He. 2008. Influence of biochars on plant uptake and dissipation of two

pesticides in an agricultural soil. Journal of Agricultural Food Chemistry 58: 7915 - 7921.

Zmora-Nahum, S., Markovitch, O., Tarchitzky, J., and Y. Chen. 2005. Dissolved organic carbon (DOC) as a parameter of compost maturity. Soil Biology and Biochemistry 37: 2109-2116.

APPENDICES

Appendix 1: Analysis of Variance Table for BrayP1mg/kg

Source	SS	MS	P
Reps	0.60	0.300	
SampleID	1025.76	205.153	0.0000
Sdate	1730.78	346.155	0.0000
SampleID*Sdate	124.76	4.990	0.0000
Error	88.81	1.269	

Appendix 2: Analysis of Variance Table for LogACT

Source	SS	MS	P
Reps	0.0101	0.00503	
SampleID	5.5862	1.11724	0.0000
Sdate	47.1230	9.42460	0.0000
SampleID*Sdate	14.1776	0.56710	0.0000
Error	0.9487	0.01355	

Appendix 3: Analysis of Variance Table for LogMEA

Source	SS	MS	P
Reps	0.0094	0.00469	
SampleID	0.7423	0.14847	0.0000
Sdate	21.3927	4.27854	0.0000
SampleID*Sdate	6.3582	0.25433	0.0000
Error	0.9270	0.01324	

Appendix 4: Analysis of Variance Table for LogNA

Source	**SS**	**MS**	**P**
Reps	0.0193	0.00964	
SampleID	0.9830	0.19659	0.0000
Sdate	10.8550	2.17099	0.0000
SampleID*Sdate	3.9053	0.15621	0.0000
Error	0.2835	0.00405	

Appendix 5: Analysis of Variance Table for NH_4N

Source	**SS**	**MS**	**P**
Reps	982.9	491.473	
SampleID	850.2	170.035	0.8627
Sdate	4465.0	892.995	0.0920
SampleID*Sdate	8551.3	342.053	0.7767
Error	31548.1	450.686	

Appendix 6: Analysis of Variance Table for NO_3N

Source	**SS**	**MS**	**P**
Reps	95.1	47.537	
SampleID	1141.0	228.197	0.0000
Sdate	719.9	143.985	0.0004
SampleID*Sdate	13095.1	523.805	0.0000
Error	1944.0	27.772	

Appendix 7: Analysis of Variance Table for urease

Source	**SS**	**MS**	**P**
Reps	15.13	15.125	
Sdate	741.25	148.249	0.0000
SampleID	447.16	89.432	0.0015
SampleID*Sdate	1670.22	66.809	0.0002
Error	630.83	18.024	

Appendix 8: Analysis of Variance Table for dehydrogenase

Source	**SS**	**MS**	**P**
Reps	79.8	79.80	
Sdate	32777.5	6555.50	0.0000
SampleID	4251.6	850.34	0.0000
SampleID*Sdate	10231.6	409.26	0.0000
Error	49180.2	52.56	

Appendix 9:Analysis of Variance Table for glucosidase

Source	**SS**	**MS**	**P**
Reps	59518.3	59518.3	
Sdate	4.405E+08	8.810E+07	0.0000
SampleID	1.581E+07	3162072	0.0000
SampleID*Sdate	8.696E+07	3478474	0.0000
Error	1630014	46571.8	

Appendix 10: Analysis of Variance Table for phosphatase

Source	**SS**	**MS**	**P**
Reps	6645.12	6645.12	
Sdate	7.784E+07	1.556E+07	0.0000
SampleID	2549212	509842	0.0000
SampleID*Sdate	1.784+07	713620	0.0000
Error	1377689	39362.5	

Appendix 11: The abstract (as approved) for the paper presented during January 2015 Combined Congress

QUANTIFICATION OF MICROBIAL POPULATION DYNAMICS AND ENZYME ACTIVITY IN SOIL AMENDED WITH COMPOSTS OF VARYING DEGREE OF MATURITY

S Shikwambana[1], FR Kutu[2] and OF Madiba[1]

[1]University of Limpopo, School of Agricultural and Environmental Science, P/Bag X1106,Sovenga 0727, South Africa

[2]Department of Crop Science, Faculty of Agriculture, Science and Technology, North West University (Mafikeng Campus), P/Bag X2046, Mmabatho 2735, South Africa

E-mail: 200630087sydney@gmail.com

INTRODUCTION

The activity and functional diversity of microorganisms contribute to the stability and productivity of agro-ecosystems. Soil microorganisms and enzyme activities have been suggested as potential indicators of soil quality due to their crucial role in soil fertility management. Hence, management practices that can enhance microbial diversity and soil enzyme activities are essential for improving soil health and soil fertility status. The aim of the study was to assess the effects of compost maturity on bio-quality indicators of compost amended soil.

MATERIALS AND METHODS

Cattle manure-rich compost was prepared through thermophilic windrow composting using cattle manure and wood chips mixed at a proportion of 4:1 (w/w) to achieve a C:N ratio of 30:1. This compost was sampled at regular interval of 30 days until 150 days. Compost samples of varying degrees of maturity were air-dried, pulverised and mixed with 1.2 kg surface soil at an equivalent rate of 100 kg P ha^{-1}. Each compost amended soil was transferred into well labelled plastic pot for incubation. Sampling of incubated amended soils was performed at 7 days interval until 42 days; and used for microbial count and enzyme activity assessments. Data generated were analysed as factorial experiment using SYSTAT package. Treatment and interaction effects were evaluated using Fisher protected least significant difference at probability level of 5%.

RESULTS AND DISCUSSION

The chemical composition of the different composts including the C/N and C/P ratios are fairly similar and comparable. Variation in compost maturity date, incubation time and their interaction exerted significant effects on the measured microbial counts and enzyme activities. The highest count of actinomycetes (6.18 CFU g^{-1}), bacterial (6.73 CFU g^{-1}) and fungi (3.06 CFU g^{-1}) were obtained during the 42-day incubation period. Similarly, the highest concentration of ß-glucosidase (3076 mg kg^{-1} hr^{-1}), phosphatase (1480 mg kg^{-1} hr^{-1}), dehydrogenase (120.07 µg INF g^{-1} $2hr^{-1}$) and urease (26.15 mg kg^{-1} $2hr^{-1}$) were obtained during the 42-days incubation period. The concentrations of bio-quality parameters measured in the compost-amended soils were highest in compost sampled at 90 days, except for phosphatase, indicating the peak of the thermophilic process. Bacteria and fungi counts were highest at 14 days of the 42-day after incubation but highest at 21 days for actinomycete. Significantly higher ß-glucosidase, phosphatase and urease activities were obtained at 42 days after incubation while quantitatively higher dehydrogenase activity was obtained at 7 days after incubation.

CONCLUSION

The bio-quality parameters of composts are influenced by compost maturity and incubation time. The highest enzyme activity and microbial population counts were reached at 60 and 90 days, respectively during composting. The use of mature compost with desirable level of bio-quality indicators is crucial for fertility management and improved soil health.

Keywords: Compost maturity, enzyme activities, microbial count, soil health, soil fertility