PHOSPHORUS RELEASE CHARACTERISTICS AND QUANTIFICATION OF MICROBIAL POPULATION AT DIFFERENT STAGES OF PHOSPHO-COMPOST PRODUCTION

By

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RESEARCH 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

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16 September 20162016

DECLARATION

I declare that this research project entitled ‘In-situ measurement of phosphorus release characteristics and quantification of microbial population dynamics at different stages of phospho-compost production’ is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other institution.

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ABSTRACT

This study aimed at assess phosphorus (P) solubility and bioavailability from non-reactive Phalaborwa ground phosphate rock (GPR) using thermophilic co-composting technology. Two types of organic wastes (Cattle, CM and poultry manure, PM) were used to produce different mix ratios (5:5, 7:3, 8:2 and 9:1) of phospho-composts. Control compost of both manures without GPR addition were included. Samples of each compost heap were taken at mesophilic, thermophilic, cooling and maturity stages and used for bioquality and chemical tests. Microbial counts, enzyme activity, molecular analysis, and the quatification of different P forms and fractions were carried out on all compost samples. Results showed that the concentration of P measured in the different phospho-composts differed significantly ($p < 0.05$). The 8:2 mix ratio gave quantitatively higher P concentration in both CM- and PM-based phospho-composts. Organic P form had the lowest concentration when compared to other P forms and fractions while water soluble-P had the highest concentration as compared to other P fractions. The content of actinomycetes showed correlated positively with EC, phosphatase β-glucosidase, fungi and bacteria but negatively correlated with organic P, Ca-P water P and pH. There was a positive and significant correlation between electrical conductivity, enzyme activity (phosphatase, dehydrogenase and β-glucosidase), fungi, actinomycete and P fractions (Bray P1, Ca-P and Pi value). Acid phosphatase activity correlated negatively with water extractable P, organic P and Ca-P contents but revealed a positively significant correlation with bacteria, fungi and actinomycete counts.

Generally higher microbial counts were measured in CM- than PM-based phospho-composts but the concentrations varied with each microbial species. Highest fungi (7.27 CFU g^{-1}) and actinomycete (6.83 CFU g^{-1}) counts were generally recorded in the control composts, which was quantitatively higher in CM- than PM-based phospho-composts. Quantitatively higher enzyme activities were measured across compost types and mix ratios during the cooling phase phospho-compost production; but were statistically comparable to measured values at maturity phase. Acid phosphatase and β-glucosidase enzymes were predominately higher at maturity phase in all cattle manure-based phospho-compost excluding the 5:5 mix ratio. In PM-based phospho-compost, both β-glucosidase and phosphatase were higher at

initial phase with PM5:5 , PM9:1 and PM10:0. Dehydrogenase activities were predominately higher at thermophilic and cooling phase from both PM- and CM-based phospho-compost. Results of molecular analysis revealed that *Bacillus sp*. and *Acholeplasma cavigenitalium* sp. were dorminant in PM-based phospho-composts while *Pseudomonas sp*. and *Acholeplasma pleciae* dorminated the CM-based phospho-composts. In conclusion, results of this study revealed that the type of manure used exerts great influence on the bioquality parameters and the amount of P released.

Key words: Phospho-compost, Compost quality, Enzyme activities, Nutrient cycling, Ground phosphate rock, Phosphorus forms and fractions

DEDICATION

I dedicate this research to my mother Mrs Tengisa Mphephu Mkansi, all my children Ndzalama, Ntivo and to all my sisters, particularly sister Florah, who was responsible for taking care of my children during the period of my study.

ACKNOWLEDGEMENTS

I exalt my Heavenly father “Jehovah Jire” for granting life, wisdom, understanding and opportunity to further my studies. It was not easy, but through your comfort, mercy and help I have manage. I would like to acknowledge the support and useful comments, remarks and commitment given by my research supervisor, Prof FR Kutu. Thank you for believing in me and for being my father while studying. I really appreciate everything you have done for me. My gratitude also goes to my co-supervisor Mr OHJ Rhode of Agricultural Research Council-Grain Crops Institute, ARC-GCI, Potchefstroom, for your guidance, support and particularly, the exposure and training on the knowledge of microbial and molecular parts of my research. I was initially clueless but through his support and his team members, I was sufficiently exposed and trained hands-on; and are confident in certain aspect of soil microbiologist. I also give thanks to Deidré, Tebogo, and Connie who gladly provided me with the training on the methodology for molecular analysis at the ARC-GCI Soil Microbiology laboratory. Furthermore, I wish to sincerely appreciate the support of Prof KK Ayisi from RVARC, University of Limpopo, who showed strong interest in my work and also offered to provide financial assistance to cover part of the cost for study at ARC-GCI in Potchefstroom. Finally, I acknowledge with thanks, the grantholder link bursary award received from the National Research Foundation (NRF) South Africa for my study through the phospho-composting Technology project (Grant ID number 76215).

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CHAPTER 1

INTRODUCTION

1.1 Background information

Phosphorus (P) is a common limiting nutrient for plant growth in most soils around the world (Sharma *et al.,* 2013*; Y*adav and Verma, 2012). It plays such important roles as energy storage and transfer as adenosine di- and tri-phosphate during photosynthesis and respiration (Khan *et al.,* 2010). It also helps in root development, flower initiation and for seed and fruit development in crop production (Prasad, 2009; Achal *et al.,* 2007). Unlike nitrogen (N) deficiency problems that can be addressed through a number of cheaper alternative strategies, the problem of low soil-P condition is addressed only through expensive inorganic fertiliser application.

Attempts to replace the costly mineral P fertilizers with cheaper and readily available alternative have prompted the development of different strategies including the direct utilisation of P-rich indigenous ground phosphate rock (GPR) as a P source for crop production (Goenadi *et al.*, 2000). Such GPR has been successfully used in several ways including partial acidulation with synthetic organic acids and natural organic acids (Akande *et al.,* 2005). Such success stories were, however, not achieved in soils with pH above 7 (Akande *et al.,* 2005). The situation is further exacerbated by the use of insoluble phosphate rock typical of Phalaborwa rock, which requires significant chemical processing to promote the release of its P content for plant use.

1.2. Problem statement

Available literature (Vance *et al*., 2003) revealed that over 40% of the world soils are reported to be P-deficient while the acid-weathered soils of tropical and subtropical regions of the world are particularly prone to P deficiency. In South Africa, widespread low available P content had similaly been reported on cultivated soils particularly on resource-poor farmers' fields (Mandirigana *et al*., 2005; Kutu, 2008) thereby leading to low crop yield. The problem of low soil P has been attributed to the presence of ferric and aluminum sesquioxides, low organic matter content and poor management practices (Sims *et al*., 2005; Kutu, 2012). Achieving high crop yileds on such fields require substantial external inorganic P fertiliser addition, which are most often very expensive and not readily available. Thus, the high price of

mineral P fertilisers have contributed to a significant decline in its use by smallholder farmers.

Furthermore, numerous studies have revealed that phosphate-rich organic manure (PROM), a value added product obtained either by direct mixing or co-composting of high grade fine size phosphate rock, could be a useful and effective alternate to costly inorganic P fertilisers (Sekhar, 2000). Regretably, the beneficial effects of application of such product has been reportedly to saline and weakly alkaline soils (Sekhar and Aery, 2001) with no clarity on the usefulness under acidic soil conditions. Similarly, there is ambiquity about the fate of nutrients formed during the composting process. Hence, information about the fate of P mineralisation and transformation from GPR during co-composting process remain scanty and unclear.

1.3. Motivation of the study

There is an urgent need to substitute the current costly inorganic P fertiliser used for crop production for a cheaper, locally and readily available alternative. This is crucial to addressing the current low-P fertiliser use by small-scale farmers in South Africa and promote increase food production. Smallholder and commercial farmers will have access to cheaper and affordable single product that will retain P on their crop land. Composting is a 'close loop' recycling process that can help produce a clean and beneficial product; and will also serve as a good way to reduce the pathogen load in the soil (Zaborske, 2013). A number of studies have reported changes in microbial community and functionality during composting of synthetic food wastes (Ishii *et al*., 2000; Dees and Ghiorse, 2001) and animal manures (Sasaki *et al*., 2009). Similarly, co-composted GPR and manure has been reported to perform better than commercially available chemical fertiliser due to the presence of phosphate solubilising microbes (PSM) that helps to improve the efficiency of the final product (Sekhar, 2000). Hence, the additional knowledge generated from this study will possibly help to stimulate interest in the development and commercialisation of a product that can be used to increase soil P availability and plant uptake for increase crop production and food security.

1.4 Purpose of the study

1.4.1. Aim

The aim of this study is to assess P solubility and bioavailability of non-reactive Phalaborwa ground phosphate rock using the windrow thermophilic co-composting technology.

1.4.2. Objectives

The study specific objectives include among others, to:

i. quantify the amount and forms of P release during mesophilic, thermophilic, cooling and compost maturity phases of phospho-compost production from two different organic wastes.

ii. identify and quantify the population of microbial groups including P solubilising micro-organisms from GPR during the different stages of phospho-compost production from the two organic wastes, and

iii. quantify the activity of selected enzymes present during the different stages of phospho-compost production from the two organic wastes.

1.5 Hypotheses

The study attempts to address the following hypotheses:

i. The amount of P released from different organic wastes including its various transformations during the different stages of phospho-compost production are comparable.

ii. The population of microbial species (diversity) responsible for P solubilisation from GPR obtainable at different stages of phospho-compost production from the two different animal wastes are similar and comparable.

iii. The concentration of enzyme activity in compost samples differs with the type of the organic waste materials used and hence, exerts influence on the quality of the resulting compost and the amount of nutrient release.

CHAPTER 2

LITERATURE REVIEW

2.1 Compost production and use on crop land

Compost is a product of controlled natural decomposition of organic materials, which involve physical, chemical and biological transformation of feedstock sanitise through active heat generation by aerobic microbial action and stabilised to the point that it is beneficial for plant growth (Chatterjee *et al.*, 2013). Compost involves a highly complex biological process controlled by many species of bacteria, fungi and actinomycetes that covert a low-value materials into higher value product (Turner *et al.,* 2005). Active composting is typically characterised by a high temperature phase that sanitises the product and allows a high rate of decomposition followed by a low temperature phase that allows the product to stabilise while still decomposing at a slower rate (Erickson *et al.*, 2009). Hence, four different stages are recognized during a composting process that are controlied by changes in temperature. These stages are the mesophilic with moderate temperatures possibly rising to 45°C, thermophilic stage with high temperatures peaking at 80°C, stabilization or cooling with cool to ambient temperature, and matured/cured stages with no temperature changes (Chatterjee *et al.*, 2013). The final product should not contain virulent pathogens or viable seeds and it should be stable and suitable for use as a soil amendment (Stone *et al.,* 2001).

Compost has been used at the household or small farm level for recycling of organic matter and nutrients for thousands of years (Tognetti *et al.,* 2011). In recent years, more large-scale composting facilities are being established to manage and recycle organic wastes from urban areas in a more sustainable manner. Composts made from green wastes usually contain large amounts of N and P (Raj and Antil, 2011; Amlinger *et al.,* 2003). Composting therefore plays an important role in nutrient cycling (Chatterjee *et al.,* 2013); and are presently used as nutrient sources in farmlands, public and private gardens, parks, highway embankments, and landscaping (Turner *et al.,* 2005; Erickson *et al.,* 2009).

2.2 Importance of co-composting of ground phosphate rock

Mixing GPR with organic materials and composting them, technically described as co-composting, is another way of enhancing P solubility and the subsequent increase in P availability to plants from non-reactive phosphate rock (Afzal and Bano, 2008). Co-composting entails the addition of chemicals and/or non-biodegradable wastes (e.g. non-reactive GPR, biosolids etc.) with the usual composting materials such as yard waste, manure and sewage sludge to increase the reaction rates and the composition of the compost (Sanchez-Arias *et al*., 2008). The process has been reported to stimulate the growth of numerous types of bacteria and fungi that produce large amounts of organic acids and humic substances (Pathma and Sakthivel, 2012.). Phospho-composting has also been reported to offer additional environmental advantage of safe disposal of organic wastes (Dinis, 2010; Hellal *et al.*, 2013).

2.3 Microbial composting techniques and the role of P solubilising microbes

The main types of microbiologically facilitated composting techniques include windrow composting, static pile composting with forced aeration through the pile, and vermicomposting (Myburgh *et al.,* 2012). Windrow involves placing a mixture of organic wastes into long, narrow piles on a composting pad that are frequently turned using specialized agricultural implements (Ahmad *et al.,* 2007). Windrow composting can be completed in 12-20 weeks while it height ranges from 1 m for dense materials such as manures to 3.5 m for less dense materials such as leaves, and its width may vary from 1.5-6 m depending on the type of equipment used (Ahmad *et al.,* 2007). Turning usually exchanges the materials from the outside of the windrow with those from the interior; and also increases oxygen within the piles thereby leading to an aerobic process (Brodie *et al.,* 2000). The benefits is that all materials receive equal exposure to air at the surface and high temperatures inside the windrow thereby providing a uniform treatment process (Ahmad *et al.,* 2007).

Aerated static pile systems with forced air throughout the pile and does not require turning once the pile has been formed, thus allowing for larger piles to be produced (Ahmad *et al.,* 2007). The process often becomes anaerobic and often takes longer to complete and water still needs to be added to ensure optimal microbial activity

(Brodie *et al.,* 2000). It also increases the aerobic microorganisms activity, and it is described as an extremely fast process for producing a stable final product (Gao *et al.,* 2010). The pile may and may not be covered, a factor that has important implications for the circulation of air within the pile. Vermicompost on the other hand, is a mesophilic process that employs earthworms to stabilize organic residues (Anastasi *et al.,* 2005). Although vermiculture does not inherently include a thermophilic phase but material can be pre-composted through aerated static piles and windrow to remove substances toxic to earthworms, inactivate plant seeds and remove human and plant pathogens (Ahmad *et al.,* 2007). The quantity of soluble P, K and Mg appears to be increased, and worm-processed animal wastes have been shown to be suitable as plant growing media in vermicomposting technique (Ahmad *et al.,* 2007).

Phosphate solubilising micro-organisms are a group of beneficial microbes that are capable of converting organic and inorganic P from insoluble compounds into soluble P forms (Chen *et al*., 2006). The main function of PSM is the production of organic acids and acid phosphatases that play a major role in the mineralisation of organic P in the soil (Whitelaw, 2000). Micro-organisms contribute directly and indirectly to the soil health through their various beneficial or detrimental activities (Khan *et al.*,2007). Phosphate solubilising fungi and bacteria are described as effective micro-organisms in the mineralisation process (Gyaneshwar *et al.,* 2002). Most micro-organisms particularly *Pseudomonas, Bacillus, Aspergillus* and *Penicillium* are effective in releasing P from both organic and inorganic soil pools through solubilisation or mineralisation (Kang *et al.,* 2002). The most effective organisms in the dissolution of non-reactive phosphate rock are *Aspergillus niger* and *Penicillium bilaji* (Fenice *et al.,* 2000; Khan and Khan 2002) as well as *Pseudomonas cepacia* (Reyes *et al.,* 2002). *Aspergillus niger* has been reported to produce organic acids including citric, oxalic and gluconic acids that increase phosphate rock dissolution (Zaidi *et al.,* 2009). Solubilisation of inorganic P form by heterotrophic micro-organisms that dissolve phosphate minerals has also been reported through chelation thereby releasing P into the solution (He *et al*., 2002). Phosphorus solubilising activity is determined by the ability of microbes to release metabolites such as organic acids through which their hydroxyl and carboxyl group chelate the cation bound to phosphatic rock and finally gets converted to soluble P form (Trolove *et al.,* 2003).

2.4 Factors affecting the population of P solubilising microbes in composts

The structure and diversity of P solubilizing microbes in compost is influenced primarily by physical factors such as moisture and temperature, biotic factors including predation and competition, and chemical characteristics of the compost that include acidity, dissolved nutrients and salinity content (Silwana and Lucas, 2002, Balser *et al.,* 2001). The moisture content of the substrate is a key factor in determining the synthesis and secretion of desired products via involvement during composting processes of enzymatic action, biomass growth, and nutrient and gas transport (Bellon-Maurel, 2003). A number of researchers have reported that microbes require moisture to grow through which they obtain most of their nutrients from the environment (Boulter *et al.,* 2000, Silwana and Lucas, 2002, Partanen *et al.,* 2010). Low moisture content can caurse some microorganisms not to sustain their growth due to lack of water thus, higher moisture canl inhibit the transfer of oxygen to the substrate particles and the transfer of metabolic products such as carbon dioxide away from the substrate particle and could also foster contamination of the solid matrix (Monteil-Gonzalez *et al.,* 2004).

Temperature has also been described as a critical factor in determining the rate of decomposition that takes place during a co-composting process (Rebollido *et al.,* 2007). Temperature less than 20^{o}C slows decomposition process while above 59^{o}C kills some micro-organisms and reduces the diversity of micro-organisms (Raghavarao *et al.,* 2003). Lack of oxygen during co-composting can lead to microbial population reduction while oxygen can be provided by mixing or turning of the compost (Chatterjee *et al.,* 2013). Oxygen is necessary for bacterial respiration that drives the composting process (Boulter-Bitzer *et al.,* 2006). Together with the moisture content, an optimal aeration rate must be maintained to optimize microbial growth and activity (Raghavarao *et al.,* 2003). When the aeration rate is too low, mass transfer processes become limited and consequently either slows down the growth of microorganisms (Raghavarao *et al.,* 2003). On the other hand, if the rate of aeration is too high, the compost pile will become dry as a result of rapid evaporation of water from the pile (Gervais and Molin, 2003). This dehydration can lead to lower metabolic activity and dramatic changes in the microbial population and release of nutrients (Gervais and Molin, 2003). Changes in pH during composting

process provide another indication of the activity of microorganisms within the system (Bellon-Maurel, 2003). These changes are associated with the hydrolysis of proteins in the substrate with the production of ammonia and organic acids at different stages in the process (Bellon-Maurel, 2003).

2.5 Reactivity of GPR and P release

Naturally occurring phosphate rock differs widely in mineralogy and chemistry (Gholizadeh *et al.*, 2009). The reactivity or solubility of phosphate rocks is determined by the ability of the phosphate rock to release P contained therein for plant uptake (Zaidi and Khan, 2006). Chemical and mineralogical features are the major factors in determining the reactivity of a given phosphate rock (Chien *et al.,* 2010, Rania, 2011). The dissolution of phosphate rock can be improved in soils with low pH values. However, many studies have shown that acid soil and acid generating processes, including inorganic and organic acids, can lead to dissolution of phosphate at low soil pH (Villegas and Fortin, 2002, Fankem *et al*., 2006). Higher content of organic materials in a manure-ground phosphate rock mixture, promotes increased dissolution of the ground phosphate rock while reducing the calcium activity in the soil increases the dissolution of the phosphate rock (Hellal *et al.,* 2013). The reactivity of GPR is also increased by the activities of PSM particularly, *Aspergillus niger*, *Penicillium bilaji* and *Pseudomonas cepacia* ((Chien *et al.,* 2010, Rania, 2011). Fungi are the most effective in producing acid to dissolve phosphate rock than bacteria (Rania, 2011).. Certain plant species are also reported to exhibit mechanisms that are localised in the rhizosphere, which allow for the efficient use of P through phosphate rock dissolution (Zaidi and Khan, 2006).

2.6 Phosphorus transformation during composting and co- composting processes

Phosphorus transformation occurs in two different chemical forms that include inorganic and organic P. These forms differ in their behaviour and fate in compost (Hansen *et al*., 2004). Organic P forms are the most important source of available P for plant following mineralisation (Yadav and Verma, 2012) while inorganic P compound are unavailable to plant (Beech, 2001). The complex of organic P form represented by esters of orthophosphoric acid have been identified primarily as inositol phosphates, phospholipids and nucleic acids (Yadav and Verma, 2012).

Inositol P represents a series of phosphate esters that range from monophosphates up to hexaphosphates (Turner and Leytem, 2004). Phytic acid, $(CH)_6(H_2PO_4)_6$, has six orthophosphate groups attached to each carbon atom in the benzene ring. Phytin, a Ca-Mg salt of phytic acid, is the most abundant of the known organophosphorous compounds. Phospholipids P-containing fatty compounds are insoluble in water but are readily utilized and synthesized by microorganisms (Beech, 2001). Some of the most common phospholipids, which constitute 1-5% of total organic P in soils, are derivatives of glycerol whose rate of release from organic sources in soil is rapid (Deubel *et al.,* 2000).

Nucleic acids occur in all living cells and are produced during the decomposition of residues by soil microorganisms (Henri *et al.,* 2008). Two distinct forms of nucleic acids, ribonucleic acid (RNA) and deoxyribonuclic acid (DNA), are release into the soil in greater quantities than inositol phosphates, and are more readily broken down (Henri *et al.,* 2008). Most inorganic P compounds fall under one of the two groups, in which calcium is the most dominant controlling cation (Ca-P) and those in which iron (Fe-P) and aluminium (Al-P) are the controlling cations that are found in more weathered soils, especially in their lower horizons (Reyes *et al.,* 2007). The simpler compounds of Ca are readily available for plant growth (Aseri *et al.,* 2009) while hydroxy phosphates such as strengite [$FePO_4.2H_2O$] and variscite [$AlPO_4.2H_2O$] present in extremely small quantities are too insoluble and do not contribute much to plant nutrition (Tarafdar *et al.,* 2003)

2.7 Dynamics of enzyme activity during composting and its role in compost quality

The process of composting is driven by the microbial activity of organisms that lead to the degradation of the substrate (Schloss *et al.,* 2003). The shift in environmental conditions in compost piles from mesophilic to thermophilic also represents a shift in the microbial community structure (Ishii *et al.,* 2000). The main organisms responsible for composting are bacteria and fungi, while all other microbes play minor roles in the decomposition process (Trautmann and Olynciw, 2002). Bacteria dominate most compost piles throughout all the phases of composting. Mesophilic bacteria predominate during the first and last stages of composting while thermophilic bacteria dominate during the active phase where the pathogenic prokaryotes are killed (Schloss *et al.,* 2005, Ishii *et al.,* 2000). Bacteria within the

compost pile include actinomycetes, which are filamentous bacteria responsible for degrading complex compounds like cellulose, lignin, chitin and proteins (Trautmann and Olynciw, 2002). Also notable is the Bacillus genera that dominate during the thermophilic phase (Schloss *et al.,* 2005).

The consideration of enzyme activity throughout the composting process gives valuable knowledge related to the dynamics of important nutritional elements like C, N or P; and contributes to a better understanding of the transformations that take place during composting (Wong and Fang, 2000). Dehydrogenase differs from other enzymes as is not related to specific element cycles, but to overall microbial activity because of its role in the respiratory chain (Castaldi *et al.,* 2008). The dehydrogenase activity is considered to be the general index of biological activity due to its role on the oxidative phosphorelation process (Delgado *et al*., 2004). According to Tiquia *et al.*, (2002), the highest dehydrogenase activity levels is related to a higher microbial activity while its lower values is detected at phases such as the maturation stage where physico-chemical processes gain prominence at the expense of microbial ones (Benitez *et al*., 2005).

Other enzymes such as β-glucosidase and phosphatase are responsible for certain cycles which occurs during the composting process. The β-glucosidase is responsible for the hydrolysis of various β-glucosides such as cellobiose, with glucose as the final product (Yu *et al*., 2007). The level of this activity is determined by the presence of readily metabolizable substrates; hence, composting phases characterised by a higher availability of such compounds should be associated to greater β-glucosidase activity (Schloss *et al*., 2005). Yu *et al.* (2007) reported that maximum levels of β-glucosidase activity were observed at the initial stage and during the maturation stage. Phosphatase catalyses the hydrolysis of organic P compounds to different inorganic forms that plants can metabolize (Benitez *et al*., 2005). This enzymatic activity plays critical roles in P cycles and it is considered a general microbial indicator, although some phosphatases are synthesized not only by microorganisms but also by plants (Makoi and Ndakidemi, 2008).

2.8 Assessing microbial diversity during composting: inpact on compost quality

Definig the diversity and structure of microbial communities of compost through their constituent population has been of considerable interest in addressing such basic ecological concern of establishing the similarity between microbial communities in mature compost and those from the different feedstocks used during different composting methods (Tiquia and Michel, 2002). The composition of microbial communities during composting is influenced by many factors including temperature, pH, water content, C/N, etc (Balser *et al.,* 2001). The major microbiological components of compost are bacteria and fungi and actinomycetes (Karnchanawong and Nissaikla, 2014). These organisms, needed for composting, are found in compost feedstock and required to maintain an active microbial population during composting (Tsai *et al.,* 2007; Ke *et al.,* 2010).

Earlier studies revealed that the major bacterial groups at the beginning of the composting process are mesophilic organic acid-producing bacteria such as Lactobacillus and Acetobacter specie (Partanen *et al.,* 2010). During the thermophilic stage, gram-positive bacteria such as *Bacillus spp.* and *Actinobacteria*, become dominant (Sundberg, 2004). Evidences abound in literature that the most efficient composting process is achieved by mixed communities of bacteria and fungi (Pandey *et al.,* 2000). Microbes are major drivers of the composting process, and as such, characterization and Identification of microorganisms in compost is important to better understand degradation mechanisms. Microbial community structures change during a composting process due to change in temperature and chemical conditions leading to increased broader range of microorganisms (Chatterjee *et al.,* 2013). Available report also suggests that microbial population in compost depend upon feedstock and composting methods (Dees and Ghiorse, 2001). Maturity and stability of compost can also be assessed by determining the changes in chemical structure of the compost over time (Tang *et al.,* 2006) Compost maturity is usually associated with the readiness of the material for its intended use (agriculture, horticulture, landfilling). Compost stability is an important characteristic; and under certain conditions, immature, poorly stabilised composts can cause problems (Mondini *et al.,* 2004). Stability is normally achieved by the end of the actively managed composting phase; and the period required to achieve this will depend

upon the types of feedstock materials, and management of the composting process (Tang *et al.,* 2006). The end product should not contain pathogens or viable seeds, and it should be stable and suitable for use as a soil amendment. The number of bacteria in soil is influenced primarily by the amount and quality of food available. Other factors include physical factors (moisture and temperature), biotic factors (predation and competition), chemical characteristics of the soil such as acidity, dissolved nutrients and salinity contents (Tuner *et al.*, 2007).

2.9 Impact of compost application on soil P availability

Phosphorus exist in soil either in the organic and inorganic form (Yadav and Vermer, 2012). Organic P undergoes mineralisation and immobilisation with the aid of soil bacteria and growing plants (Magette *et al.*, 2007). Compost application improves soil quality by enhancing aeration, water status and aggregate stability that stimulate plant growth and increases the availability of nutritive elements (Amlinge *et al.*, 2007). Although the effect of compost application on P availability has been reported as not very clear and often inconsistent (Gichangi and Mnkeni, 2009; Hanč *et al*., 2008), the types of organic waste composted and the composting process usually affect compost P availability to plant (Magette *et al.,* 2007). Results of study by Courtney and Mullen (2008) suggest that organic P sources are more effective for plant absorption than inorganic P. Contrarily, compost application has been reported to bring about increased P-availability in P fixing soil (Guppy *et al*., 2005, Gichangi, 2009) while the presence fo humic substances in composts leads to improve P bioavailability in acid soil (Hua *et al.*, 2008). The addition of high quantities of soluble P have been reported to lead to soil saturation while part of the added P remains in the available form and can be easily leached (Kleinman *et al*., 2000).

2.10 Impact of P fractions and transformation on soil P availability

Phosphorus transformation is governed by two major factors that include biotic and abiotic factors (Frossard *et al*., 2000). Azzez and Averbeke (2010) revealed that abiotic factors control nutrients transfer between non-living pools and soluble form and this is done through dissolution and adsorption process. Biotic factors include microbial activity that influence the change from organic into inorganic P and *vice versa*, through the processes of mineralisation and immobilisation (Azzez and

Averbeke, 2010). Fang (2000) indicated that iron hydroxides and organic carbon probably play important roles in controlling the geochemistry of inorganic and organic P, respectively in the Tanshui estuarine system. Similarly, Yadav and Verma (2012) indicated that P is adsorb from the soil in the form of soluble orthophosphate ions ($H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}) whose availability to plants is generally in the order of $H_2PO_4^- > HPO_4^{3-} > PO_4^{3-}$.

The type of orthophosphate ion present in soil solution is dependent on soil reaction (pH), biological processes and interaction processes with soil solid phases that include (i) the concentrations of metal cations such as Ca, Fe and Al; and (iii) the concentrations of competing inorganic especially bicarbonate and possibly sulphate, and organic ligands such as carboxylic anions (Hinsinger, 2001). The occurrence of primary orthophosphate ($H_2PO_4^-$ ion) favoured by low pH while higher pH favours HPO_4^{-2} ions (Reddy 2006). According to Whitelaw (2000), increase in soil pH from acidic to alkaline condition results first in the formation of HPO_4^{2-} ion and subsequently to PO_4^{3-}. Application of large amount of P fertiliser enters into the immobile pools through precipitation reaction with highly reactive Al^{3+} and Fe^{3+} in acidic soil and Ca^{2+} in calcareous or normal soils (Gyaneshwar *et al*., 2002; Hao *et al*., 2002). Organic P found in manure is approximately up to 30% of total P content. The different P fractions usually differ in their mobility, bioavailability and their chemical behaviour (Jalali and Ranjbar, 2010). Larger fraction of P available to crops was reportedly maintained due to higher rate of organic than inorganic P cyclying through the addition of manure (Reddy *et al.,* 2000). However, Jalali and Ranjbar (2010) reported that Ca-P, Fe-P, Al-P and organic P fractions are relatively active depending on the chemical and physical properties of soil.

2.11 Impact of compost application on soil fertility management

Compost application on soil has been reported to increase the activities of organic C, N and P decomposing enzymes, the microbial biomass, N content and the hyphal length (Amlinger *et al*., 2003). Vaddella *et al*. (2011) reported that compost had clearly greater effects than fresh farmyard manure and straw plus chemical fertilisers for the enrichment of organic C and N in the particle size fractions. Bernal *et al.* (2009) and Chalhoub *et al.,* (2013) reported that the N availability in composts depends on the stability of their organic matter, therefore on compost maturity and

also on their physico-chemical characteristics. Immature compost usually means high $NH4^+$-N concentration which can be easily lost through NH_3 volatilization following land application (Doydora *et al.,* 2011). During composting, $NH4^+$-N can either be mineralized following transformation from organic to inorganic forms or immobilized into microbial cells. According to Cooperband *et al*. (2003), the C/N ratio is an indicator of compost maturity and quality that is often commonly used to predict N availability. Composts with high C/N ratio (>15) often limit N availability due to immobilisation of N in the soil (Amlinger *et al.*, 2003; Gutser *et al.*, 2005). The dynamics of N in soil after compost incorporation can also be affected by environmental conditions (e.g., soil type, climate) and management practices such as the rate and frequency of compost application (Amlinger *et al.,* 2003).

2.12 Microbiological Identification methodologies

Different kinds of identification methods have been developed to satisfy the need for microbial diversity information in compost and/or soil samples. The choice of method depends on the level of detail desired, the sample size, and the time available for sample processing (Myburgh *et al.,* 2012). Microbiological identification methods involves culture-based protocols that may be laborious and biochemical methods (Tang *et al.,* 2004). Most molecular biology techniques employ Polymerase Chain Reaction (PCR) as an essential step in the process of genetic fingerprinting (Tang *et al.,* 2004). According to Spiegelman *et al.* (2005), PCR is defined as the exponential amplification of a DNA fragments and the product can be divided based on nucleic acid content to produce a pattern of bands on a gel that will give an indication of the diversity of the microbial community (Alfreider *et al.,* 2002). The main fingerprinting methods that lead to characterization of microbial diversity include Amplified ribosomal DNA restriction analysis (ARDRA), denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), functional PCR and direct cloning and sequencing (Spiegelman *et al.,* 2005).

However, PCR has been reported to have certain limitations that may hinder the accuracy of the results such as highly sensitive to environmental conditions and small amounts of contamination can lead to quantitatively different results (Alfreider *et al.,* 2002). Polymerases used to elongate the DNA strands may incorrectly insert or delete nucleotides thereby creating incorrect sequences that may go undetected

because the polymerases would be unable to correctly check the strands (Spiegelman *et al.,* 2005). PCR introduces many chimeric sequences, which occur when a foreign DNA strand is annealed to a prematurely terminated amplicon and is copied to completion in subsequent PCR cycles (Spiegelman *et al.,* 2005). Moreover, PCRs of longer products have been reported to be less efficient and less accurate and has been described as a troubling issue in intricate microbial communities because of the differences in size and copy number of the various organisms (Spiegelman *et al.,* 2005). Additionally, DGGE is used to separate rDNA in a polyacrylamide gel containing a linear gradient of DNA denaturing compounds, based on differences in G-C content of the sequences (Alfreider *et al.,* 2002). As the DNA moves along the gel, the increased concentration gradient forces it to become single stranded, but it does not become completely denatured because of the presence of a GC clamp that is incorporated into one of the primers for the PCR amplification (Alfreider *et al,* 2002).

Compost research completed using DGGE has produce microbial diversity data and identification when coupled with 16S rDNA sequencing (Pedro *et al.,* 2001, Ishii *et al.,* 2000). A major advantage of the DGGE method is that bands can be cut out from the gel and then sequenced to obtain phylogenetic information (Alfreider et al., 2002). It is also relatively easy to determine the changes in the microbial community through the absence or presence of bands on the gel (Pedro *et al.,* 2001). The problems associated with DGGE include difficult calibrations to ensure optimal separation conditions and DNA fragment lengths are limited to 500bp (Alfreider *et al,* 2002). In addition, large amounts of DNA – as much as 500 ng are needed for good resolution with DGGE. PCR biases can lead to incorrect conclusions about the components of a given sample by either under or over- representing certain groups (Spiegelman, 2005). Similar to DGGE is TGGE (Temperature Gradient Gel Electrophoresis) which separates rDNA in the exact manner as DGGE, but uses temperature at the denaturing gradient as opposed to chemical denaturants (Spiegelman, 2005). The advantages and limitations of TGGE are the same as that of DGGE (Alfreider *et al.,* 2002). Although, there has been no completed microbial community analyses on compost samples using TGGE, several have been done on soil samples (Alfreider *et al.,* 2002).

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Description of study site

This study comprised of three different phases namely, the preparation of phospho-composts, laboratory determination of P release characteristics and the identification and quantification of microbial diversity and activity in the compost samples taken at at mesophilic, thermophilic, cooling and maturity stages during the composting process. The preparation of phospho-composts was done at the University of Limpopo Experimental farm, Syferkuil (23°50’36.86”S and 29°40’54.99”E) using windrow pile composting process. Quantification of the amount of P released and the release characteristics from the phospho-composts was done through laboratory analyses, which was performed in the Soil Science laboratory at the University of Limpopo. All microbial assays and determination were carried out at the Soil Microbiology laboratory at Agricultural Research Council (ARC)-Grain Crops Institute, Potchefstroom.

3.2 Preparation of phospho-composts and samples collection

Poultry manure (PM), cattle manure (CM) and ground phosphate rock (GPR) were the materials needed for preparation of the phospho-compost. The collection of poultry and cattle manure was done from a nearby farm at Solomondale, which is approximately 20 km away from the University of Limpopo Campus.The GPR used was obtained from the Foskor mining company, Phalaborwa. Windrow piles of the different mix ratios were 5:5; 7:3; 8:2; 9:1 (w/w) were prepared on a concrete floor for the two different organic wastes (poultry and cattle manure) while a 10:0 mix ratio, representing the control without GPR addition, was included. Thus, a total of ten phospho-composts were produced through aerobic-thermophilic co-composting process. Turning of the composts was done at 2-weekly interval to provide proper aeration. The moisture content of the composts were maintained below the water-holding capacity during the composting period so as not to slow down microbial activities. Composting was continued for a period of fourteen weeks when compost temperature has attained possible maximum levels and drops; and finally reaches full maturity or compost curing.

Temperature readings from the different phospho-composts were taken and recorded (four replicates per heap) prior to compost turning using Hanna instrument (HI 9043) K type thermocouple thermometer. Three replicate phospho-compost samples were randomly taken per heap during the mesophilic, thermophilic, stabilisation or cooling and compost maturity stages; and bulked to obtain a representative sample. Compost samples for moisture and P release determination were stored in the refrigerator while those used for microbial assays, and the DNA extraction and analyses for microbial identification were stored at between -20^{o}C to 4^{o}C until ready for use.

3.3 Chemical characterisation of the phospho-compost samples

3.3.1 Procedures for P determination in the compost samples

The quantification of P released in each compost sample was done by measuring various P forms namely, water extractable, Bray P, organic and inorganic P fractions (calcium extractable- and Iron bound-P) in the compost samples. The various P determinations were performed using standard procedures as described below.

3.3.1.1 Extraction and determination of available Bray P1

Bray P1 in the phospho-compost samples was obtained by the methods describe by Bray and Kurtz (1945). Each representative phospho-compost samples was sub-sampled, air-dried, grounded and passed through a 2 mm sieve.The Bray 1 solution used was prepared by mixing 30 ml of NH_4F (37 g of NH_4F dissolved in one litre distilled water) solution with 50 ml of HCl in 1000 ml volumetric flask then made up to the mark with distilled water. About 6.67 g phospho-compost sample was subsequently weighed and transferred into 100 ml extraction bottles. Fifty millilitres of Bray 1 solution was then added and the mixture was hand shaken for 1 minute. The resulting suspension was filtered through Whatman no. 42 filter paper and the extract used for P determination in three replicates.

The Bray 1 P extraction process was followed by the preparation of Reagents A and B for colour development. Reagent A was prepared by 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 2.5 N sulphuric acid (148 ml concentrated sulphuric acid to 1 L) in a 2 L volumetric flask and distilled water was added to make the mark. 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 were 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. The colour development in standard P solutions and 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; and thoroughly mixed together. These were left to stand for 30 minutes so as to allow for the full development of the molybdenum blue colour and the absorbance subsequently read on T60 UV-visible spectrophotometer at a wavelength of 882 nm.

3.3.1.2 Procedure for the extraction and determination of water extractable P

Water extractable P was determined by adapting the procedure described by Pierzynski (2000) in which 2 g of phospho-compost grounded to pass through 2 mm mesh sieve and dried in an oven at 60^{o}C for 48 hours was transferred into 50 mℓ centrifuge tubes and shaken for 60 minutes with distilled water. The solution was then centrifuged at 6000rpm for 15 minutes. After filtering the solution with Whatman number 42 filter paper; and the pH was subsequently adjusted to 2.0 using HCl. The concentration of P in the extract was determined by measuring its absorbance using UV spectrophotometer at 882 nm wavelength.

3.3.1.3 Procedure for extraction and determination of organic P

Organic P in the phospho-compost samples was obtained following the modified method described by Wang *et al.* (2013). About 1g finely ground phospho-compost sample was weighed and burnt in muffle furnace at temperature of 500^{o}C for 4 hours. The samples was allowed to cool for an hour and transferred to 100 ml beaker and 30 mℓ of 0.5M H_2SO_4 added. Each sample was stirred and left for 24 hours and then filtered through Whatman filter paper no. 42 into a clean labelled 100

mℓ volumetric flask. The filtrate was filled to the mark using distilled water. The filtrate was later used for P determination.

3.3.1.4 Procedure for extraction and determining calcium-extractable-P

Calcium extractable phosphorus (Ca-P) was performed using the methods adapted by Sharpley, (2000) in which 1 g of grounded phospho-compost was weighed and transferred into a 40 mℓ centrifuge tube. About 25 mℓ of 0.01 M$CaCl_2$ was added andshaken for an hour on a reciprocating shaker. The content was centrifuged at 4000 rpm for 10 minutes, andsolution filtered through Whatman No. 42 filter paper.Determination of water or dilute salt extractable P content in extract was performed colorimetrically using UV spectrophotometer at 882 nm.

Calculations:

$$WSP\ or\ CaSP\ (mg\ P/kg) = (\text{P in extract, mg/L}) \times \frac{vol.extract\ (L)}{mass\ of\ compost\ (kg)} \times Dilution\ factor$$

where: WSP = water soluble P and CaSP = Calcium extractable P

3.3.1.5 Iron oxide impregnated P determination

The determination of iron oxide impregnated-P (Pi) concentration in each compost sample was done by adapting the procedure described by Chardon (2000) using Whiteman filter paper No. 40.

3.3.1.5.1 Preparation of reagents

Acidified $FeCl_3$ solution was prepared by dissolving 100 g $FeCl_3$ in 110 mℓ concentrated HCl in 1 L volumetric flask and diluted to mark using distilled water. Another 5% NH_4OH was prepared by diluting 50 mℓ of NH_4OH to 1000ml volumetric flask and filled to the mark with distilled water. The first extraction solution (0.01 M $CaCl_2$) was produced by dissolving 14.7 g $CaCl_2{\cdot}2H_2O$ with distilled water and made up to 1 L. The second extraction solution (2.5 M H_2SO_4) was done by diluting 140 mℓ of concentrated H_2SO_4 to 750 mℓ using distilled water; and made up to 1 L mark after cooling. The reagent (0.1 M H_2SO_4) was made by diluting 40 mℓ of 2.5 M H_2SO_4 to 1 L with distilled water.

3.3.1.5.2 Preparation of filter papers

The filter papers to be used were first immersed in an acidified $FeCl_3$ solution for five minutes; andlater allowed to dry for an hour. Immediately after drying, the filter papers were quickly pulled through 2.7 M NH_4OH solution (prepared immediately after drying) using plastic tweezers leading to the formation of amorphous iron-oxides on the filter paper. Each filter paper was immediately washed with distilled water to remove any loose iron-oxides. Thereafter,the filter papers were allowedto completely dry,and filter paper was thenfolded and entered into prepared nets made of polyethylene and tied with fishing line as shown below..

Figure 1. Iron oxide impregnated-P filter papers inside polyethylene net

3.3.1.5.3 Extraction procedure and determination of Iron oxide impregnated P content

One gramme of each grounded phospho-compost together with 1 bag containing filter paper strip was shaken for 16 hours with 40 mℓ of 0.01 M $CaCl_2$. The filter paper was properly rinsed with distilled to remove all phospho-compost immediately after shaking. Then the filter paper was shaken for another 60 minutes with 40 mℓ of 0.1 M H_2SO_4. The solution was then measured with UV spectrophotometer at a wavelength of 882 nm.

3.4 Determination of pH and EC of phospho-compost samples

All Phospho-compost samples pH was determined using modiified method of Hanlon (2015). Procedure for pH and EC were similar. About 10 g finely grounded phospho-compost sample was weighed in a 100 mℓ beaker in which 25 mℓ of de-ionised water was poured and stirred for 5 seconds. The samples were allowed to stand for 50 minutes then stired. After that the samples were again allowed to stand for 10 minutes. The pH value was measured using lasec crison GLP 21 pH meter while EC was similarly measured using lasec Jenway 4510 conductivity meter. Both the pH and EC meters were calibrated an hour before the measurement.

3.5 Microbial population enumeration

Population count of bacteria, actinomycetes and fungi in the various compost samples was done through serial dilution as described by Benson (2002). Different microbial growth media designed to be selective for heterotrophic microbes, actinomycetes and filamentous fungi were used in 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) marketed by Biolab, Midrand, and South Africa. Actinomycete was isolated and enumerated on actinomycete isolation agar formulated by Sigma-Aldrich, South Africa. Filamentous fungal count on malt extract agar, MEA, (Biolab (Merck), South Africa) was used and supplemented with 30 ppm chloramphenicol and 50 ppm streptomycin prepared according to manufacturer's recommendations. These various media were sterilized at 121^{o}C for 15 minutes and made into pour plates each consisting 90mm diameter petri dish containing the isolation medium. A dilution series that ranged from 10^{-1} to 10^{-5} was prepared in triplicate using 1 g of soil in 9ml of saline solution and a 100 µL 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 bacteria count, and 7 days for actinomycetes and fungi counts. The various counts,\ expressed as coliform unit per gram (CFU g^{-1}), were transformed into logarithm prior to statistical analysis.

3.6 Enzyme activity determination

Enzyme activities were determined colorimetrically using enzyme-specific procedures. Once the colour was developed, a microplate spectrophotometer reader was used in determining the absorbance at the specified wavelength. Compost samples were passed through 2 mm sieve and kept at 4°C. Enzyme assays were performed 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, 1994; Schomburg and Schomburg, 2009). All data generated were subsequently corrected for moisture content during the data analysis.

β-glucosidase was determined by adapting the procedure described by Rainey and Oren, (2006). 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 1 hour with calcium chloride and tris(hydroxymethyl) aminomethane (THAM) before filtering through a Whatman no. 2v filter paper. The absorbance of released *p*-nitrophenol (ρNP) was tested with a microplate reader at 405 nm immediately after yellow colour development.

The dehydrogenase assay was done according to Bisswanger (2011). 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 after 30 minutes. Controls were performed in all cases by adding the substrate after the reaction was stopped, and before filtration of the soil suspension.

The procedure used for acid phosphatase assay was adapted from Gerday (2007) 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 after yellow colour development with a microplate

reader at 405 nm. All Enzyme activies were expressed in units of nmol h^{-1} g^{-1} and calculated by the following equations:

3.7 Microbial community analysis for identification of PSM in compost samples

Identification of PSM present in each compost sample for microbial community analysis was done using molecular technique of denaturing gradient gel electrophoresis (DGGE) described by Pedro *et al*., (2001). The DGGE procedure is a useful uncultured method that can separate DNA fragments by amplification using polymerase chain reaction (PCR) based on the differences in base-pair sequences and visualisinge the bacterial community as a band fingerprint (Yamamoto, 2009 and Cleary, *et al.*, 2012). DNA was isolated from all composted samples and subjected to PCR for 16S rRNA amplification. Amplicons were then used in DGGE to obtain a dominant bacterial and fungi profile. Bands excised from DGGE gels were re-amplified and cleaned. The cleaned bands were then sequenced. Sequence data was edited and BLASTn searches used for identification. The details of the procedures used are as described below.

3.7.1 Total genomic DNA isolation

Direct DNA extractions from compost samples were done using the Machery-Nagel Nucleospin Soil kit (Macherey Nagel, Germany). Two hundred and fifty milligrams of sample material were used in accordance to the manufacturer's instructions. The Macherey-Nagel Nucleospin Soil kit has a choice of two lysis buffers and an additional enhancer that can be used in combination with these buffers. All possible lysis conditions were tested during the optimisation process with lysis buffer SL2 with added enhancer SX delivering the best results. Subsequently, a NanoDrop™ 1000 Spectrophotometer (Thermo Fischer Scientific, USA) was used to determine the DNA concentration and quality (i.e. A260/A280 and A260/A230).

3.7.2 16S rRNA gene PCR amplification

A standard conventional polymerase chain reaction (PCR) using universal bacterial primers (Table 1) was used for 16S rRNA gene amplification. Genomic DNA was amplified using primer set 338fGC/518r in a TC-Plus thermal cycler (Techne, UK). The reaction was carried out in 50 µL volumes and contained 25 µL double strength

MyTaq PCR mastermix (Bioline, UK), 100pmol of each primer, 1 µL isolated genomic DNA and 22 µL PCR-grade water (Bioline, UK). PCR cycling conditions for the amplification consisted of an initial denaturation at 95°C for 300 s followed by 35 cycles at 63°C for 45 s, 72°C for 60 s and a final extension step at 72°C for 600 s.

3.7.3 Agarose gel electrophoresis of PCR products

Recovery of PCR amplicons were determined by horizontal electrophoresis using 5 µL of PCR product mixed with 5 µL of 6X Orange loading dye (Thermo Fischer Scientific, USA). A 1.5% (w/v) agarose gel was prepared in 1X TAE buffer (Bio-Rad, UK). The agarose gel solution was heated in a microwave until fully dissolved, cooled and 10 µL of 10 ml/mg Ethidium bromide (EtBr) was added and mixed to enable visualisation under UV light. Gels were electrophoresed for 45 minutes at 80V in a BioRad Wide MiniSub Cell (BioRad, UK). Images were captured using a Gel Doc XR+ molecular imager (BioRad, UK) with Image Lab (version 3.0) software.

3.7.4 PCR-Denaturing gel electrophoresis

The 16S PCR amplicons were subjected to DGGE as described by Muyzer *et al.* (1993) to study bacterial communities. An 8% (v/v) acrylamide gels (Sigma-Aldrich, USA) were prepared with 40 to 60% (where 100% denaturant is equivalent to 7 M urea and 40% (v/v) formamide) linear denaturing gradients. Equal volumes of PCR products were loaded on these gels and subjected to electrophoresis in 0.5X TAE buffer (20 mM Tris, 10mM actate, 0.5 mM Na_2EDTA, pH 7.8) at 60ºC and 70 V for 500 min using the Dcode^{TM} Universal Mutation system (BioRad Laboratories, Ltd, South Africa). The DGGE banding patterns described as products were visualised by GelRed (Biotium, USA) staining and UV translumination. The DGGE bands were excised using a sterile scalpel, suspended in 50 µL PCR grade water (Bioline, UK) and stored overnight at 4°C. Samples were frozen causing the acrylamide gels to contact and release the inherent DNA.

3.7.5 PCR amplification of excised DNA

The freshly released DNA were then re-amplified using the same primers but without the GC-clamp. The same cycling conditions as described for 16S rDNA PCR

amplification were used. PCR success was again evaluated using agarose gel electrophoresis as earlier discussed in section 3.6.3 above.

3.7.6 Sequencing

16S rRNA sequencing of the re-amplified excised DNA was performed at the Central Analytical Facility of Stellenbosch University, South Africa. Chromatograms were viewed in Chromas Lite and BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST) to determine the identity of the amplified sequencesSequences for the V1/V3 region of 16S rDNA were obtained using an ABI genetic sequencer (Lifetechnologies, USA). The reverse sequences were aligned with Bioedit software and identified by comparing sequencing results with known sequences using the BLAST program (http://www.ncbi.nlm.nih.gov./BLAST

Table 1. List of primer sequences used for 16S rRNA amplification

Primer Set	**Sequence**	**Expected Product Size**	**Reference**
338f*	ACTCCTACGGGAGGCAGCAG		Muyzer *et al*., 1993
518r	ATTACCGCGGCTGCTGG	200 bp	Lane *et al.*, 1991

*GC clamp added to the 5'end of primers (338f), 5'CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G 3'.

3.8 Data analysis

The P, microbial population counts and enzyme activities data obtained from the different phospho-composts samples were first, transformed to logarithm base 10 to reduce it to two decimal places and thereafter subjected to analysis of variance (ANOVA) using Statistix 10.0 software. This was followed by the use of LSD test ($\alpha \leq 0.05$) to carry out mean comparison. Pearson correlation analysis was performed to determine the degree of relationship between all measured variables. Regression

analysis was also performed to determine the response of P release from the various phospho-composts to the measured enzyme activities and microbial population concentration.

3.9 Procedure for evaluating seed germination percentage on different phospho-compost samples

In an attempt to assess the degree of maturity of the various phospho-composts, percent seed germination test was performed following the procedure described by Wei *et al.,* (2000), Selim *et al.,* (2012) and Pivato *et al.*, (2014). About 10 g of each phospho-compost sample was filtered using 50 ml distilled water. Small white haricot beans seeds were surface sterilised by immersing in 75% (m/v) ethanol for three minutes followed by transferring in 0.001 $HgCl_2$ solution (which was prepared by adding 0.27 g of $HgCl_2$ in 1000 mℓ volumetric flask and filled to the mark with distilled water) for two minutes. The seed was then thouroughly washed with distilled water. Ten milliliters of each phospho-compost extract was poured into a filter paper inside the petri dish, then 10 seed were placed in each petri dish with filter paper. The petri dish were sealed with tape to minimize water loss while allowing air penetration. The control was perfomed by pouring 10 ml of distilled water with 10 seed in a petri dish containing no. 42 Whatman filter paper. All samples were done in triplicate. And then were incubated in the dark for three days at 25^{o}C room temperature. The seed germination percentage was calculated as follows:

$$Seed\ germination\ (\%) = \frac{No\ seeds\ germinated\ in\ compost\ extract}{No\ seeds\ of\ germinated\ in\ control\ (water)} \times 100$$

CHAPTER 4

RESULTS

4.1 Temperature readings during the co-composting process

Figures 2 and 3 below showed temperature trends of the various phospho-composts across composting period. The highest measurement of 46.25^{o}C was obtained with PM10:0 mix ratio from PM-based phospho-compost during the thermophilic phase while the lowest temperature of -0.88^{o}C was measured from PM-based phospho-compost with PM10:0 mix ratio during the initial phase of composting. Similarly, the highest temperature of 45.40^{o}C was measured at 8 weeks during composting from CM10:0 mix ratio from CM-based phospho-compost during the initial phase while the lowest measurements of 4.78^{o}C was measured at first week of sampling date with CM8:2. Temperature ranges from 4.78 to 45.40^{o}C, and -0.88 to 46.25^{o}C for CM and PM-based phospho-compost, respectively (Figure 2 and 3). PM5:5 gave the lower temperature relative to the other PM-mixed ratios across the different composting phases. Temperature increases with the decrease in GPR addition. Table 2 below showed that the measn measured temperature in the different phospho-compost mixed ratios over the entire co-composting period differed significantly ($p < 0.05$); being generally higher in the control than GPR mixed composts.

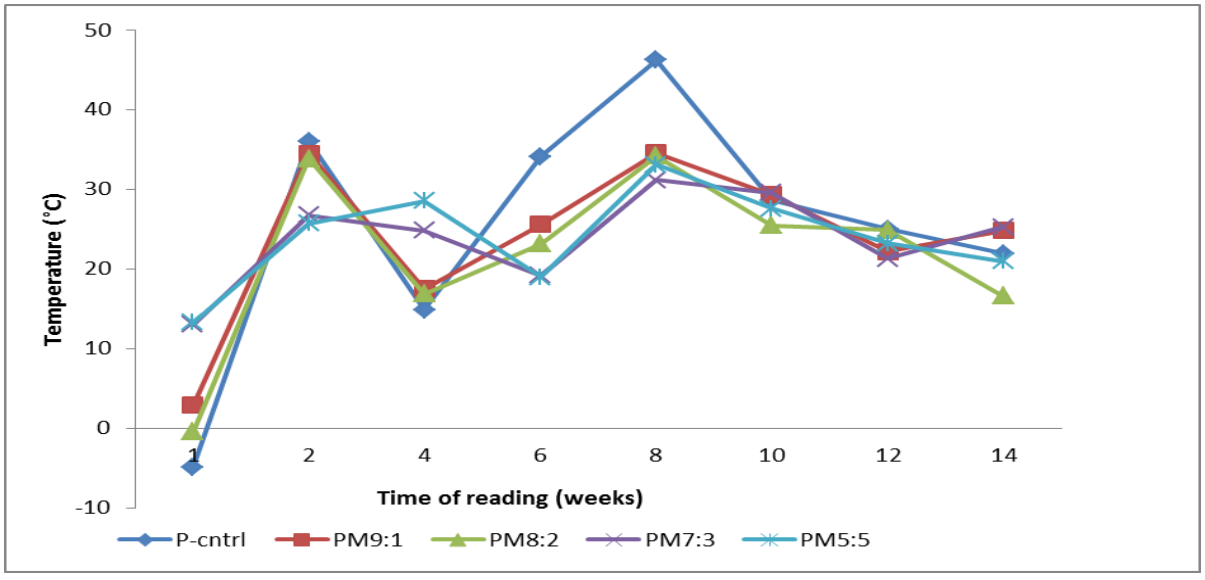

Figure 2: Changes in temperature during the composting period in the poultry manure-based phospho-compost mix ratios (PM = Poultry manure) (P-cntrl = poultry manure control)

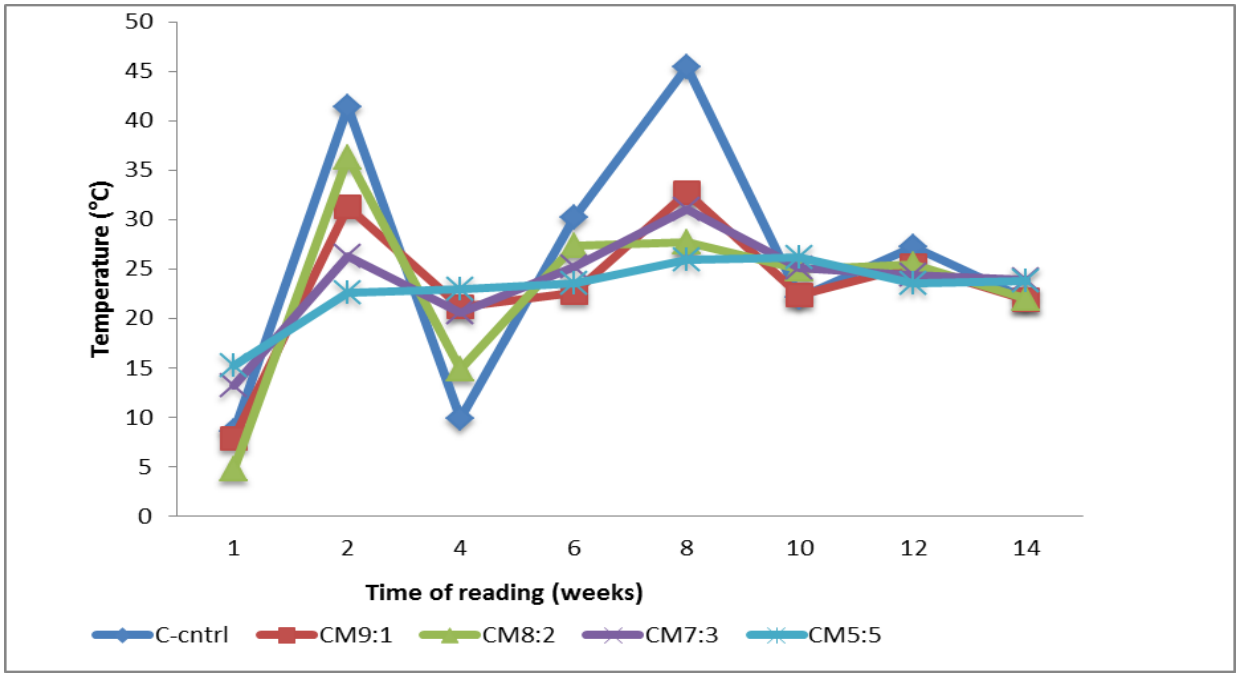

Figure 3: Changes in temperature during the composting period in the cattle manure-based phospho-compost mix ratios (C-contrl =cattle manure control) (CM=cattle manure)

Table 2. Mean maximum temperature measured in the different phospho-compost mixed ratios.

Phospho-compost mixed ratios	Mean temperature (^{o}C)
CM5:5	22.97c
CM7:3	23.87b
CM8:2	22.93c
CM9:1	23.15bc
CM10:0	25.85a
PM5:5	23.91b
PM7:3	23.87b
PM8:2	21.79d
PM9:1	23.96b
PM10:0	25.23a

Different letters in the same column indicates significant difference at $p<0.05$; PM = poultry manure while CM = cattle manure

4.2 Phospho-compost quality assessment parameters at maturity

4.2.1 Chemical characterisation of manures and phospho-composts

Table 3 shows the results of chemical analyses of the raw organic manures used for production of different phospho-compost as well as the final phospho-composts produced. The total N ranged from 0.32 to 1.05% in the CM-based phospho-composts but varied between 0.41 to 1.46% in the PM-based phospho-composts suggesting a generally higher N content in the PM--based phospho-composts. The content of total N decrease with increase in GPR addition. The total P level ranged from 0.12 to 3.31% in CM-based phospho-composts and varied between 1.34 to 3.61% in PM-based phospho-composts. The content of total P extracted increased with increase in GPR additional with higher P content in poultry than cattle phospho-composts. Higher level of Cu and Mn was present in the PM- than CM-based phospho-composts whereas Zn content in both PM and CM used was increasing with a decrease in GPR addition. The concentration of these two micro-elements Cu and Mn increased with an increase in GPR addition. The highest EC values was obtained with CM-based phospho-compost when compared with PM-based phospho-composts. The value of EC varied from 12.83 mS cm^{-1} to 20.54 mS cm^{-1} from CM-based phospho-composts and from 8.49 mS cm^{-1} to 19.28 mS cm^{-1} in PM-based phospho-composts. The values increased with increase in GPR addition in both PM- and CM-based phospho-composts.

The results of the chemical composition of the phospho-composts showed that GPR addition has a great influence on all micro and micro-elements contents in the cured composts. The Ca content of the phospho-composts was relatively high in PM and it ranged from 4.6 to 51.0% in CM-based and from 28.5 to 81.7%. in PM based-phospho-compost. The Na content was the same (0.08%) in all CM-based phospho-compost except that it was 0.09% at the control and varied between 0.08-0.09% in bothPM-based phospho-compost. The Mg content of the phospho-composts ranged from 0.85 to 1.21% in the CM- and from 1.62 to 3.15 PM-based phospho-composts while the K content ranged from 1.71 to 2.48% in the CM- and from 3.24 to 5.67 in the PM- based phospho-composts suggesting higher content of Mg and K in PM-based phospho compost. The value of Fe varied between 1338 and 1515 mg kg^{-1} in CM- and from 1347 to 1521 mg kg^{-1}in PM-based phospho compost. The contents of

pH, EC, OC, P Ca, Mg, Na, Zn and Cu measured from GPR ammendant composts were higher than in the control and raw materials for both CM- and PM- based phospho-compost. However, the contents of N, Fe, Mn and K in both CM and PM-based phospho-composts were low compared to unamended control.

4.2.2 Percent seed germination test in mature compost

The percent seed germination test differed significantly ($p < 0.05$) across the different phospho-compost mixed ratios and manure types used (Figure 4). Generally, the CM-based phospho-composts gave higher percent germination when compared to PM-based phospho-composts. Percent seed germination ranged from 31.7 to 85.0% and from 35.0 to 80.8% in CM- and PM-based phospho-composts, respectively. The highest percent germination was observed in the cured or matured phospho-composts; being highest in 8:2 mix ratio for CM (85.0%) and PM-based (80.8%). The percent germination obtained from 8:2 mix ratio for both PM and CM-based phospho-composts differed significantly from all other composts. A significant ($P<0.001$) phospho-compost types × sampling dates interaction effect on percent seed germination was observed (Figure 5). The highest percent germination for both CM- and PM-based phospho-composts was obtained at compost maturity stage while the lowest percentage was obtained in compost samples obtained at the initial stage.

Table 3: Chemical composition and moisture content of raw organic manure used for different phospho-composts

Compost types	pH (H_2O)	EC* mS cm^{-1}	% total OC	% total N	% total P	% K	% Ca	% Mg	% Na	Zn	Cu	Fe	Mn	Moisture (%)
										(mg kg^{-1})				
CM-raw	7.28	12.83	4.28	0.60	0.12	1.71	4.6	0.85	0.08	839	8	1338	152	4.4
CM5:5	8.42	20.54	7.34	0.54	3.31	2.34	51.0	1.17	0.08	780	20	1470	148	1.0
CM7:3	8.36	20.44	6.60	0.32	2.28	2.28	46.1	1.14	0.08	810	16	1472	154	3.2
CM8:2	8.43	19.42	5.60	0.50	1.90	2.42	48.9	1.21	0.08	822	16	1441	164	3.6
CM9:1	8.39	20.31	6.20	0.60	1.29	2.28	27.3	1.14	0.08	844	14	1515	182	6.1
CM10:0	7.32	16.65	4.47	1.05	0.53	2.00	8.1	1.00	0.09	860	10	1456	187	4.9
PM-raw	7.67	8.49	5.14	1.3	1.35	4.70	28.5	2.35	0.09	871	41	1347	556	7.2
PM5:5	9.20	14.88	7.86	0.48	3.61	3.24	81.7	1.62	0.08	751	30	1423	272	0.8
PM7:3	8.93	16.28	7.15	0.41	2.63	4.03	78.5	2.01	0.08	779	43	1446	346	2.8
PM8:2	8.42	19.28	6.02	0.59	1.70	5.02	70.8	2.51	0.09	860	44	1471	492	5.6
PM9:1	9.58	16.16	6.61	1.46	2.68	5.67	57.6	2.84	0.09	888	49	1521	578	6.0
PM10:0	7.20	12.56	5.25	1.1	1.34	5.44	57.2	3.15	0.09	918	54	1505	648	9.0

CM = cattle manure; PM = poultry manure

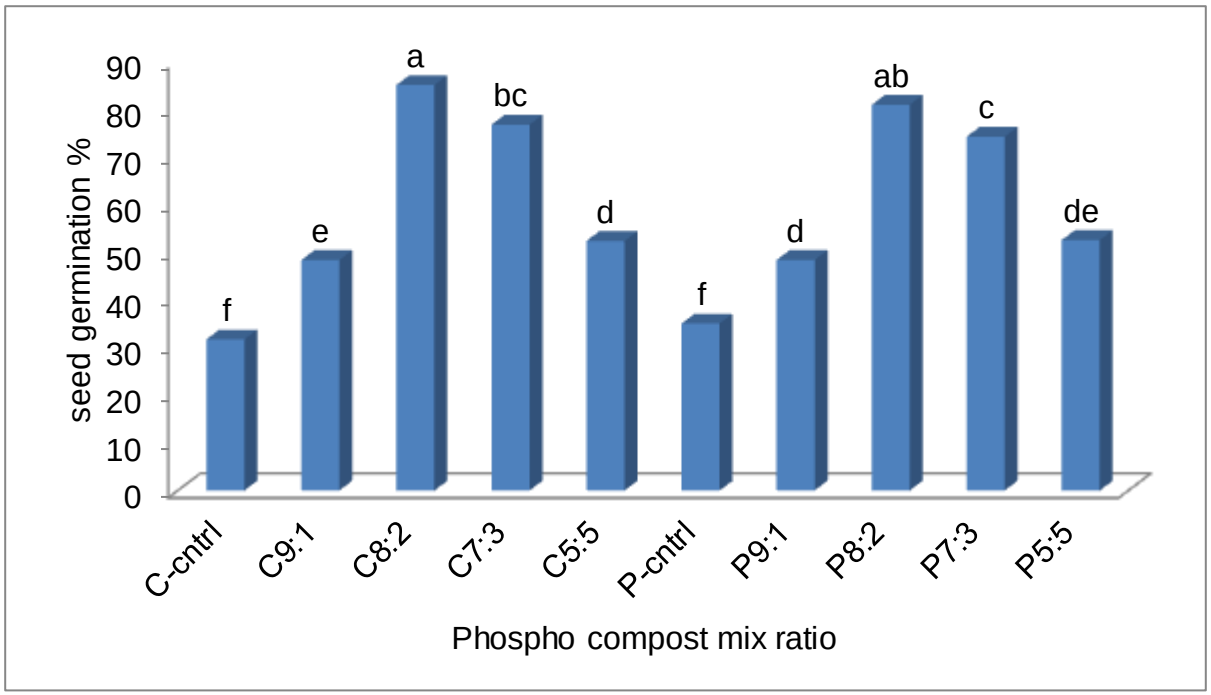

Figure 4: Mean percent seed germination percentage in the different phospho-compost mixed ratios across the different sampling dates (Bars with different letter indicate significant difference at $p<0.05$; P = poultry manure, C= cattle manure, Cntrl = control)

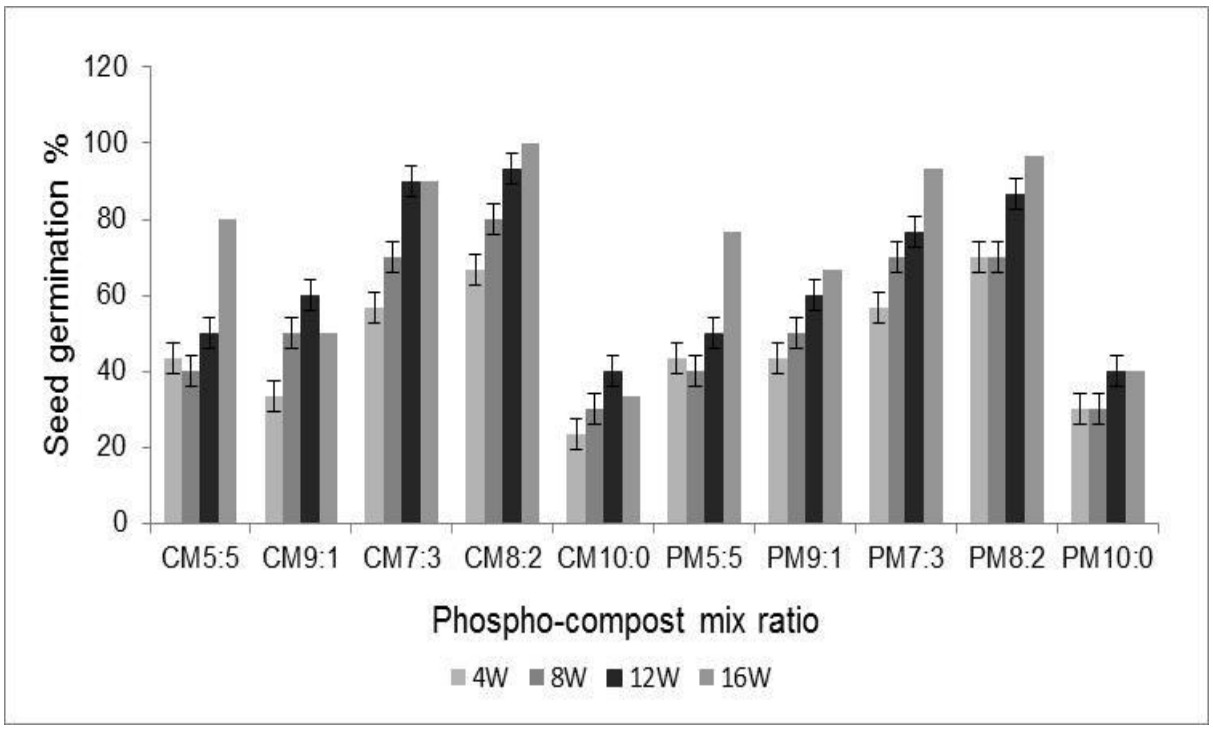

Figure 5: Phospho-compost mix ratio × compost sampling time interaction effect on percent seed germination (CM = cattle manure, PM= poultry manure, bar = standard error of different phospho-compost mix ratios)

4.3 Treatments and interaction effects on microbial count and enzyme activities

Variation in phospho-compost types, compost sampling dates and their interaction effects exerted highly significant ($p < 0.001$) effects on the measured microbial counts and enzyme activities (Table 5).

4.3.1 Bacterial counts

Mean bacterial count measured ranged from 2.05 to 2.90 CFU g^{-1} and from 1.17 to 2.50 CFU g^{-1} in CM- and PM-based phospho-composts, respectively. The 5:5 mix ratio from both CM and PM-based phospho-compost had the highest bacterial counts; being generally higher in the CM-based phospho-compost (Table 5). The highest count was obtained in all CM-based phospho-composts at compost maturity (curing stage) while the 5:5 mix ratio similarly gave the highest count 4.69 (CFU g^{-1}) in the PM-based phospho-composts during the maturity stage (Figure 6). The CM10:0 had the lowest count obtained during thermophilic phase. However, there was no bacteria count detection at mesophilic phase for 5:5 and 10:0 mix ratios of PM-based phospho-composts; thermophilic phase for 10:0 mix ratio of CM-based, 9:1, 8:2 and 10:0 mix ratios of PM-based phospho-composts, cooling phase for 7:3 mix ratio of PM-based compost, and at matured phase for 8:2 mix ratio of PM-based phospho-compost (Figure 6).

4.3.2 Fungal counts

Mean fungal counts measured varied between 6.36 and 7.27 CFU g^{-1} in CM-based phospho-compost and between 4.96 and 6.11 CFU g^{-1} in the PM-based phospho-compost (Table 5). The highest mean count of 7.27 CFU g^{-1} and 6.11 CFU g^{-1}, respectively in CM- and PM-based phospho-composts was both from the 5:5 mix ratio. The lowest fungal count of 5.97 CFU g^{-1} was measured during the thermophilic phase from PM8:2 while the measured fungal counts in the various phospho-composts increased with increase in GPR addition and *vice versa* (Figure 7).

4.3.3 Actinomycete counts

Mean actinomycete counts in CM-based phospho-composts were higher than those of PM-based phospho-composts; and ranged from 5.94 to 6.83 CFUg^{-1} and from

4.41 to 4.99 CFU g^{-1} in CM-based and PM-based phospho-composts, respectively (Table 5). The highest mean count of 6.83 CFU g^{-1} was measured in 10:0 mix ratio of CM-based phospho-composts while the least count of 4.41 CFU g^{-1} was measured in the 10:0 mix ratio of PM-based phospho-composts. The 8:2 mix ratio of PM-based phospho-composts gave the higher mean count of 7.71 CFU g^{-1} obtained at the cured phase (Figure 8). In contrast, the highest count of 7.89 CFU g^{-1} measured in the CM-based phospho-composts was obtained in the 10:0 mix ratio at maturity phase. The actinomycete count in the CM-based phospho-composts was generally higher than those of PM-based phospho-composts except in PM8:2 during the curing phase (Figure 8). The measured counts of actinomycetes generally increased with the increase in GPR addition.

4.3.4 β-glucosidase activity

Mean content of β-glucosidase activity measured ranged from 138.506 to 212.905 g kg^{-1} hr^{-1} in the CM-based phospho-compost samples but varied between 32.659 and 78.483 g kg^{-1} hr^{-1} in the PM-based phospho-composts (Table 5). In the CM-based phospho-composts, the highest mean content of 212.905 g kg^{-1} hr^{-1} obtained from 10:0 mix ratio at maturity phase while the least mean content of 32.659 g kg^{-1} hr^{-1} was with 5:5 mix ratio at the mesophilic stage (Figure 9). Similarly, highest (153.522 g kg^{-1} hr^{-1} at the compost cooling phase) and least (16.832 g kg^{-1} h^{-1} at the mesophilic phase) mean contents obtained in PM-based phospho-composts were from 8:2 and 7:3 mixed ratios, respectively. The mean content of β-glucosidase actIvities measured was generally higher in CM-based than the PM-based phospho-composts.

Table 4: P-Values for ANOVA for microbial counts and enzyme activities in the different phospho-composts across different sampling dates

Sources of variation	Df	Bacteria	Fungi	Actinomycete	Phosphatase	β-glucosidase	Dehydrogenase
Sampling dates (SD)	3	***	***	***	***	***	***
Compost types (CT)	9	***	***	***	***	***	***
C x SD interaction	27	***	***	***	***	***	***

df implies degree of freedom; *, **, *** implies significant at 5%, 1% & 0.1%, respectively; ns implies not significant at $P \leq 0.05$; CV implies coefficient of variation

Table 5: Influence of sampling dates and phospho-compost types on microbial counts and enzyme activity

	Bacteria (CFU g^{-1})	Fungi (CFU g^{-1})	Actinomycete (CFU g^{-1})	Phosphatase (mg kg^{-1} hr^{-1})	Dehydrogenase (µg INF g^{-1} $2hr^{-1}$)	β-glucosidase (g kg^{-1} hr^{-1})
Sampling dates						
4W	2.25b	6.43a	5.12d	2561.4ab	425.75c	16.633b
8W	1.44c	5.97c	5.39c	2362.8b	592.80b	107.590ab
12W	2.21b	6.43b	6.00a	2736.1a	618.45a	124.584a
16W	2.73a	5.99c	578b	2621.3ab	617.60a	119.356ab
SEM	0.0709	0.0733	0.0549	209.80	6.5835	9.55
Compost types						
CM5:5	2.90a	6.56c	6.44c	3045.8d	558.50c	138.506d
CM7:3	2.75b	6.61c	6.64b	3399.4cd	566.88abc	154.784cd
CM8:2	2.59cd	6.36d	5,94 d	4083.6b	561.37bc	185.951b
CM9:1	2.67bc	6.80b	6,36 c	3765.5bc	576.13ab	171.454bc
CM10:0	2.05e	7.27a	6.83a	4675.8a	516.50d	212.905a
PM5:5	2.50d	6.11e	4.77f	717.3f	558.13c	32.659f
PM7:3	1.55g	5.29h	4.52g	1505.4e	572.25abc	68.544e
PM8:2	1.56g	5.70f	4.99e	1504.1e	582.13a	68.498e
PM9:1	1.17h	5.51g	4.83f	1723.8e	567.88abc	78.483e
PM10:0	1.81f	4.96i	4.41h	1287.6e	576.75ab	58.624e
SEM	0.112	0.116	0.087	331.7	10.4	15.1

Different letters in the same column indicates significant difference at $p<0.05$; SEM implies standard error of mean;PM = poultry manure while CM = cattle manure

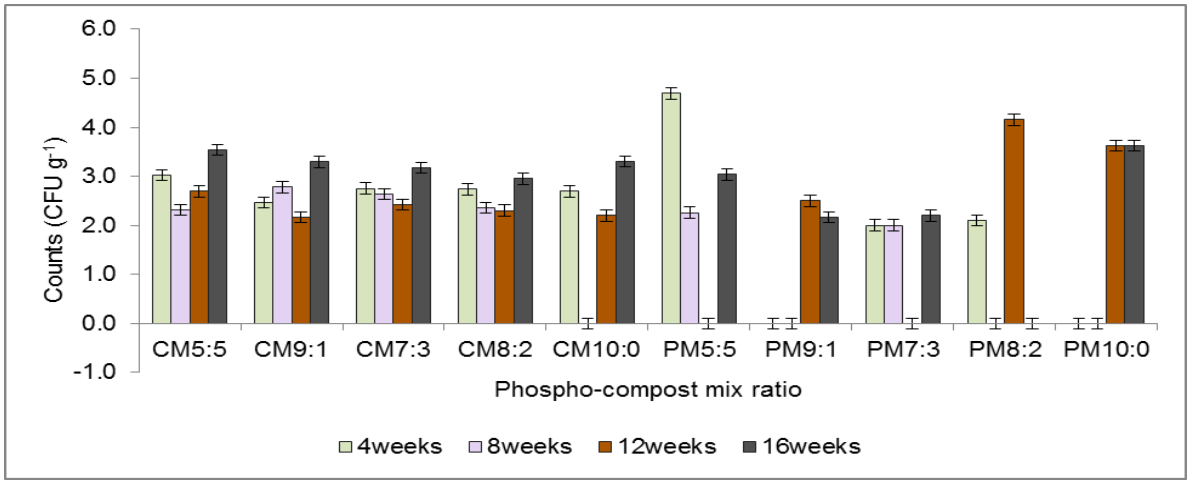

Figure 6: Phospho-compost x sampling date interaction effects on bacteria count (bar = standard error of different phospho-compost mix ratios)

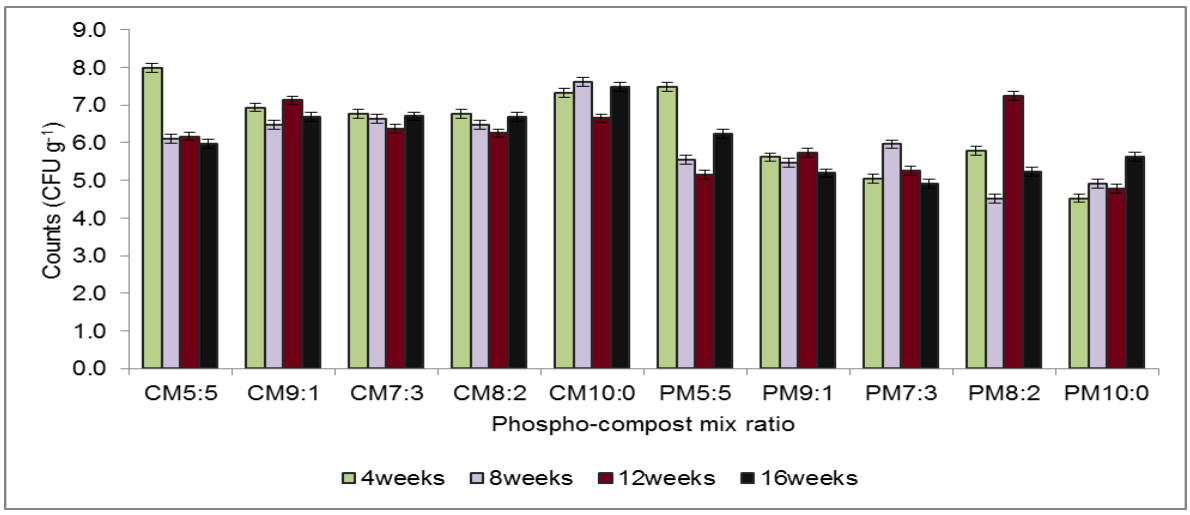

Figure 7: Phospho-compost x sampling date interaction effects on fungi count (bar = standard error of different phospho-compost mix ratios)

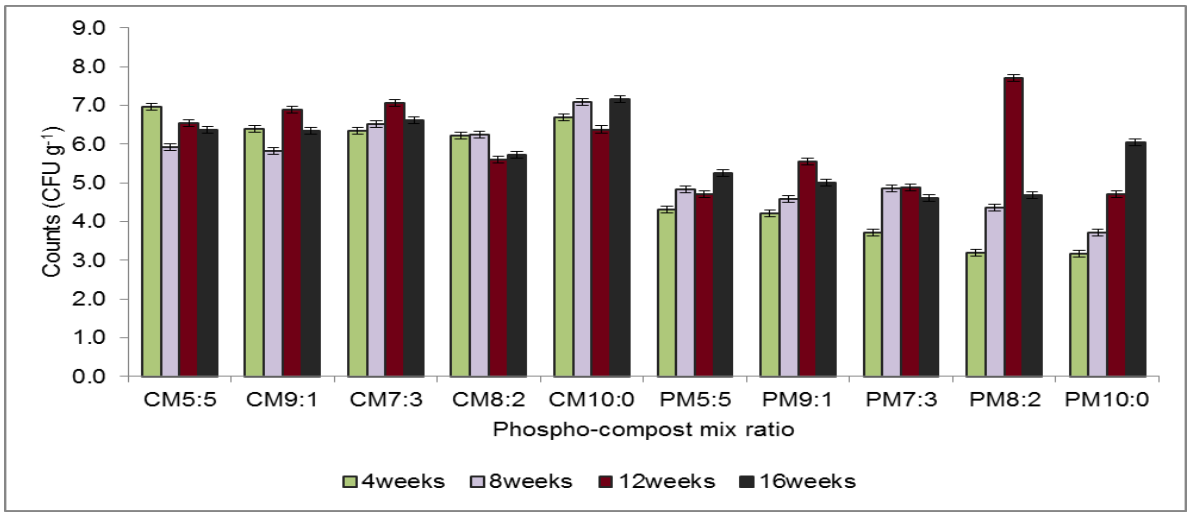

Figure 8: Phospho-compost x sampling date interaction effects on actinomycete count (bar = standard error of different phospho-compost mix ratios)

4.3.5 Dehydrogenase activity

The content of dehydrogenase activity ranged from 516.50 to 558.50 µg INF g^{-1} $2h^{-1}$ in the CM-based phospho-compost and from 558.13 to 582.13 µg INF g^{-1} $2h^{-1}$ in the PM-based phospho-compost (Table 5). The highest content was from PM-based phospho-compost obtained in the 8:2 mix ratio during the thermophilic phase, which did not differ significantly from the measured content in 10:0 mix ratio. The measured mean contents of dehydrogenase activity in CM-based and PM-based phospho-composts at the different composting phases were however statistically comparable (Figure 10).

4.3.6 Acid phosphatase activity

The content of acid phosphatase activity ranged from 3045.8 to 4675.8 mg kg^{-1} h^{-1} in the CM-based phospho-composts but varied between 717.3 and 1723.8 mg kg^{-1} h^{-1} in the PM-based phospho-composts (Table 5). Highest mean content of 4675.8 mg kg^{-1} h^{-1} measured in 10:0 mix ratio from CM-based phospho-compost was obtained at maturity phase while the least content of 717.3 mg kg^{-1} h^{-1} was obtained in 5:5 mix ratio from PM-based phospho-compost at cooling phase A significant ($p < 0.01$) phospho-composts × sampling dates interaction effect on phosphatase activities was obtained with generally higher contents in CM-based phospho-composts (Figure 11). In PM-based phopho-composts, the 8:2 mix ratio gave the highest content of 3371.3 mg kg^{-1} h^{-1} during the cooling phase (12W) while the 10:0 mix ratio gave the highest mean content of 4877.7 mg kg^{-1} h^{-1} in the CM-based phospho-composts.

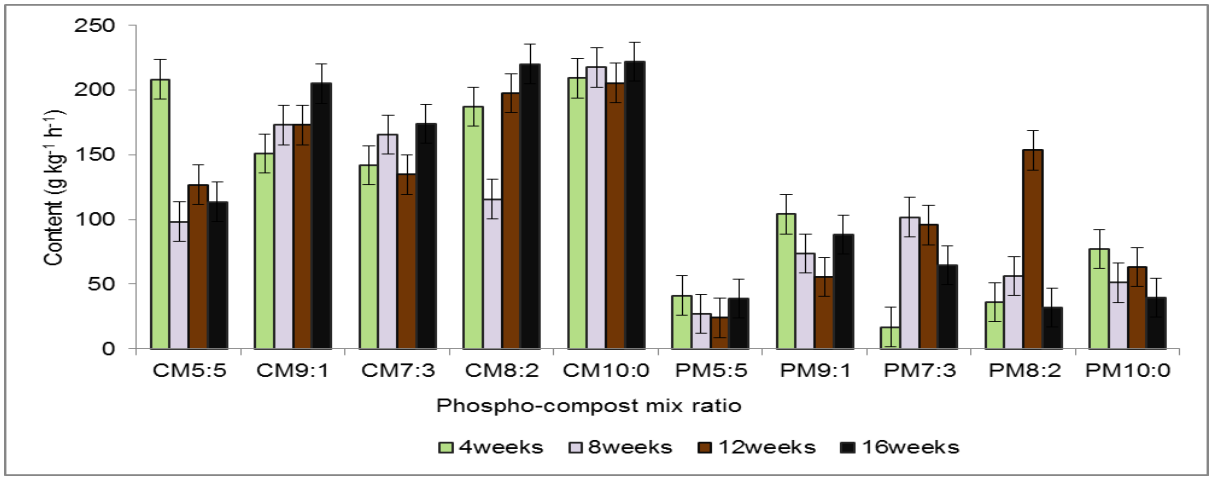

Figure 9: Phospho-compost x sampling date interaction effect on β-glucosidase activity (bar = standard error of different phospho-compost mix ratios)

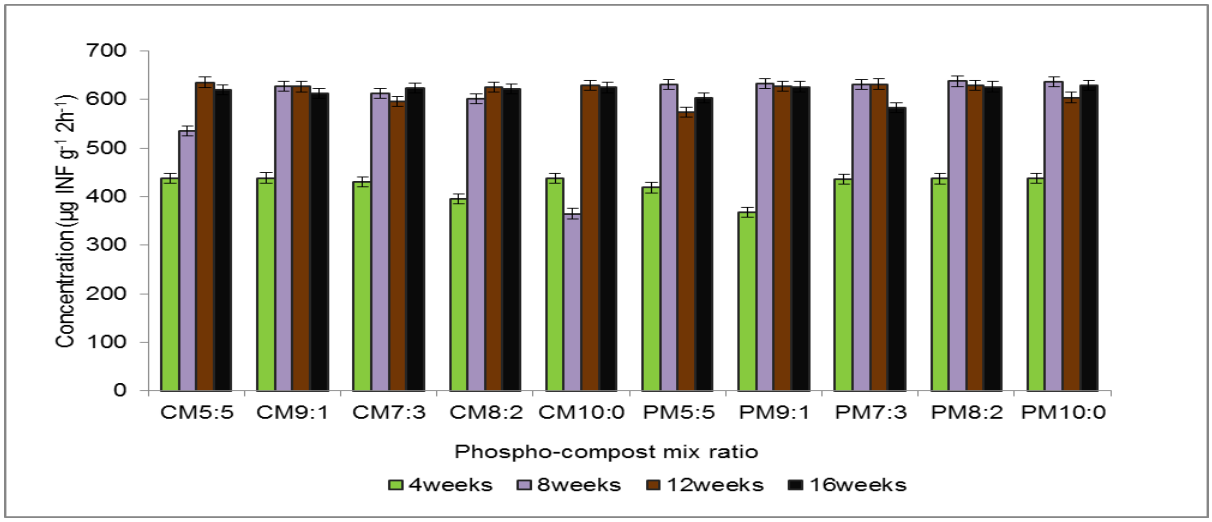

Figure 10: Phospho-compost x sampling date interaction effect on dehydrogenase activity (bar = standard error of different phospho-compost mix ratios)

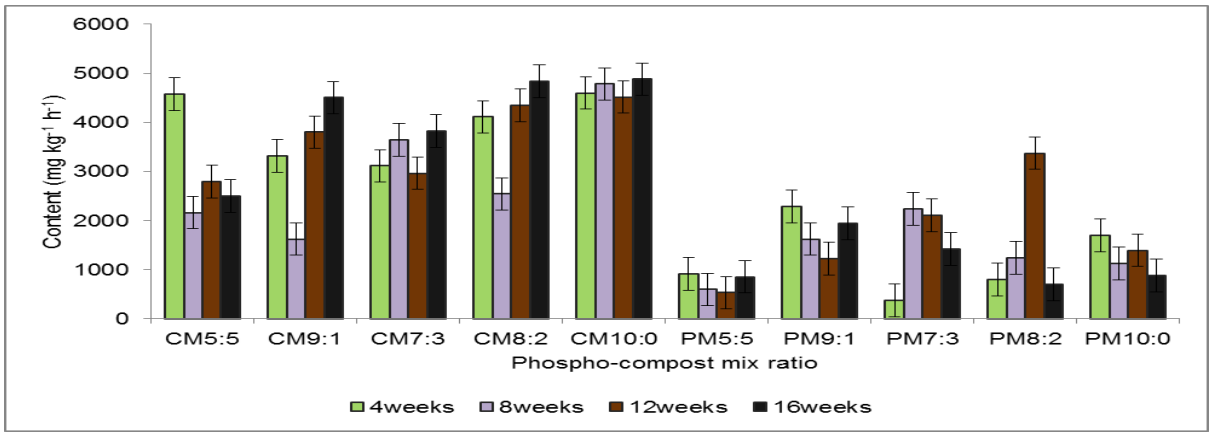

Figure 11: Phospho-compost x sampling date interaction effect on phosphatase activity (bar = standard error of different phospho-compost mix ratios)

4.4 Treatments and interaction effects on pH, EC and P forms and fractions

The p-values from analysis of variance for the measured P concentration of each P forms and fractions measured in the various phospho-compost samples are as represented in Table 6 below. A significant ($p < 0.001$) treatments and phospho-compost types × sampling dates interaction effects on measured values of all P forms and fractions as well as electrical conductivity were obtained. The mean measured P concentrations from the various phospho-composts increased along the order of organic P< Bray1 P< Calcium P< water extractable P< Pi-value (Table 7). The 8:2 mix ratio from PM-based phospho-composts gave equally highest amount of ca-P and Fe-P while the 10:0 mix ratio from CM- based phospho-compost gave the least P concentrations for all forms and fractions. Generally, the PM-based phospho-composts gave the higher P concentrations than the CM-based phospho-composts for all forms and fractions.

The mean content of P released from CM-based phospho-composts across the different phases varied between 1.50 mg kg^{-1} and 20.89 mg kg^{-1}, 30.45 mg kg^{-1} and 144.56 mg kg^{-1}, 18.5 mg kg^{-1} and 704.5 mg kg^{-1}, 675.6 mg kg^{-1} and 1193.6 mg kg^{-1} and between 1427.9 mg kg^{-1} and 3712.8 mg kg^{-1}, respectively for organic P, Bray P1, Ca-P, water extractable P and Pi values. Similarly, the mean content of P measured from PM-based phospho-composts across the different phases ranged from 5.31 mg kg^{-1} to 32.41 mg kg^{-1}, 41.20 mg kg^{-1} to 149.02 mg kg^{-1}, 102.9 mg kg^{-1} to 1585.6 mg kg^{-1}, 102.9 mg kg^{-1} to 1585.6 mg kg^{-1}, and 2389.2 mg kg^{-1} to 6659.7 mg kg^{-1}, respectively for organic P, Bray P1, Ca-P, water extractable P and Pi-values. The 5:5 mix ratio of CM-based phospho-composts gave quantitatively highest (37 mg kg^{-1}) organic P content at the mesophilic phase which is significantly same as the measured contents from the 5:5 and 8:2 mix ratios of PM-based phospho-composts but both obtained at the thermophilic phase (Figure 12).

The 8:2 mix ratio of CM-based phospho-composts gave the highest (241 mg kg^{-1}) Bray P1 content obtained at compost maturity while the highest (228 mg kg^{-1}) Bray P1 content was obtained from the 7:3 mix ratio of PM-based phospho-compost during the thermophilic phase (Figure 13). The iron-oxide impregnated P (Pi) content of 4770 mg kg^{-1} measured from 8:2 mix ratio of CM-based phospho-composts at compost maturity phase as well as 11982 mg kg^{-1} similarly obtained from the 8:2 mix

ratio of PM-based phospho-compost at the cooling phase represented the highest concentration measured (Figure 14). Calcium P content of 1202 mg kg^{-1} measured in CM-based phospho-composts and 2403 mg kg^{-1} in PM-based phospho-compost both from the 8:2 mix ratio and at the maturity phase represented the highest concentration (Figure 15). The water extractable P content of 1677 mg kg^{-1} measured in 8:2 mix ratio from CM-based phospho-composts during the maturity stage and 3023 mg kg^{-1} in 9:1 mix ratio from PM-based phospho-compost during mature phase represented the highest concentration (Figure 16). The content of P in the different phospho-compost samples increased with increase in GPR addition. The concentration of organic P represented the least fraction when compared to other P fractions while Pi value constituted the highest fraction in all the phospho-composts produced.

Table 6: ANOVA significance levels of compost types, compost sampling dates and their interaction effects on compost pH, electrical conductivity and P forms and fractions in the different phospho-composts across different sampling dates

Treatments	Df	pH	EC	Organic P	Bray P1	Water soluble P	Inorganic P	Ca-P
Compost types (C)	9	**	***	***	***	****	***	***
Sampling dates (SD)	3	ns	***	***	***	***	***	***
C×SD interaction	27	ns	***	***	***	***	***	***

df implies degree of freedom; *, **, *** implies significant at 5%, 1% & 0.1%, respectively; ns implies not significant at P $\leq$ 0.05; CV implies coefficient of variation

Table 7: Influence of sampling dates and compost types on the release of P using different fractions

Treatments	Organic P (mg/kg)	Bray 1P (mg/kg)	Pi value (mg/kg)	Water P(mg/kg)	Ca-P(mg/kg)
Sampling date (SD)					
4W	20.41a	7.58e	393.0e	989.1e	108.14e
8W	14.67c	109.64c	2619.3d	1544.0d	250.54d
12W	10.37d	154.45a	4206.4c	1742.9c	710.95c
16W	16.39b	151.70b	4432.2	1869.7b	826.10b
SEM	0.67	1.21	154.66	15.48	24.89
Compost types (CT)					
CM5:5	14.32d	96.89e	2049.7g	850.3h	191.8h
CM7:3	19.08c	140.47c	2667.4e	1070.3f	508.8e
CM8:2	20.89c	144.56b	3712.8c	1193.6e	704.2d
CM9:1	7.64e	96.86e	2487.2ef	933.0g	318.5g
CM10:0	1.50g	30.45h	1427,9h	675.6i	18.5j
PM5:5	24.00b	118.65d	2389.2f	2301.4c	449.2f
PM7:3	22.93b	149.02a	4144.9b	2414.3b	792.4c
PM8:2	32.41a	148.33a	6659.7a	2502.2	1585.6a
PM9:1	16.00d	74.40f	4049b	2529.3a	943.7b
PM10:0	5.31f	41.20g	3033.6d	1830.4d	102.9i
SEM	1.05	1.92	244.54	24.48	39.35

Different letters in the same column indicates significant difference at $p<0.05$; SEM implies standard error of mean; PM= poultry manure while CM= cattle manure; sampling date is in weeks

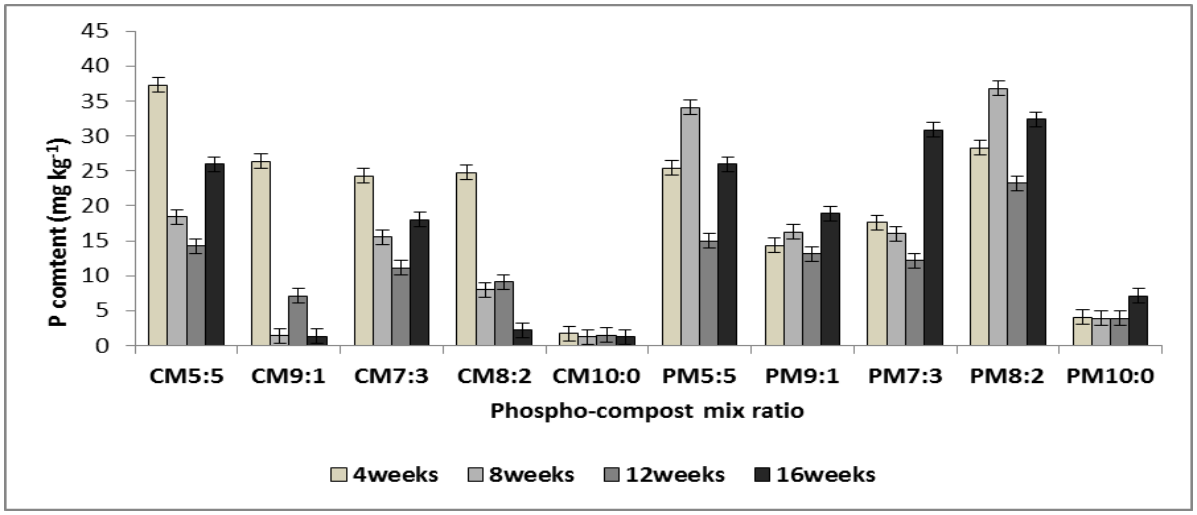

Figure 12: Phospho-compost x sampling date interaction effect on organic P content (mg/kg) (bar = standard error of different phospho-compost mix ratios)

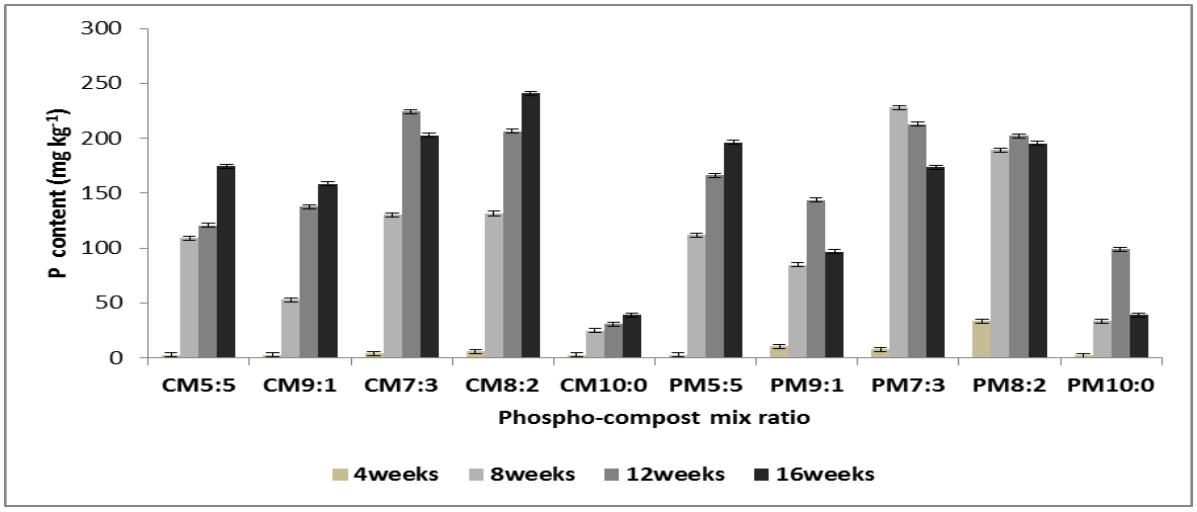

Figure 13: Phospho-compost x sampling date interaction effect on Bray P1 content (mg/kg) (bar = standard error of different phospho-compost mix ratios)

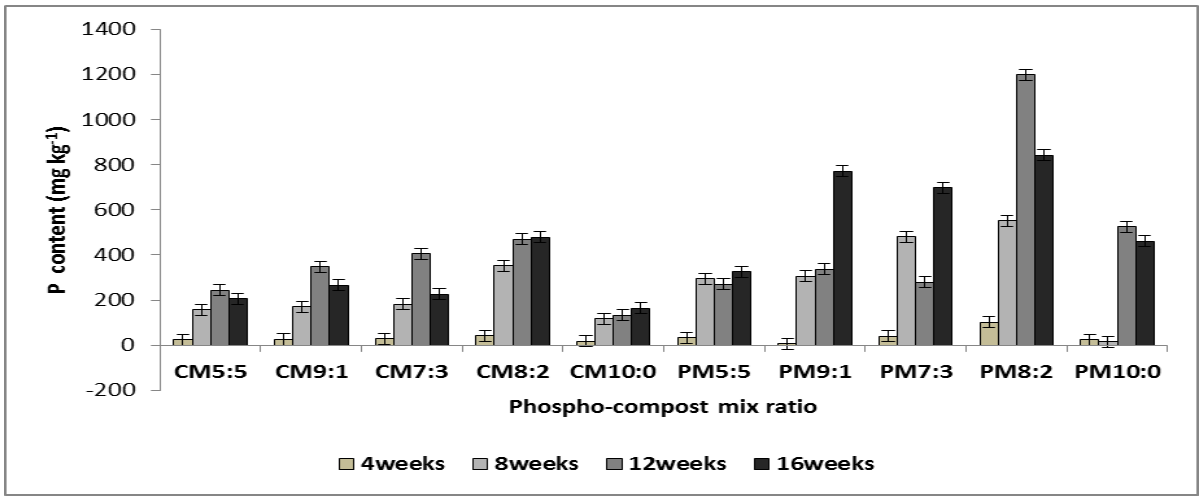

Figure 14: Phospho-compost x sampling date interaction effect on Fe-oxide impregnated P (Pi) content (mg/kg) (bar = standard error of different phospho-compost mix ratios)

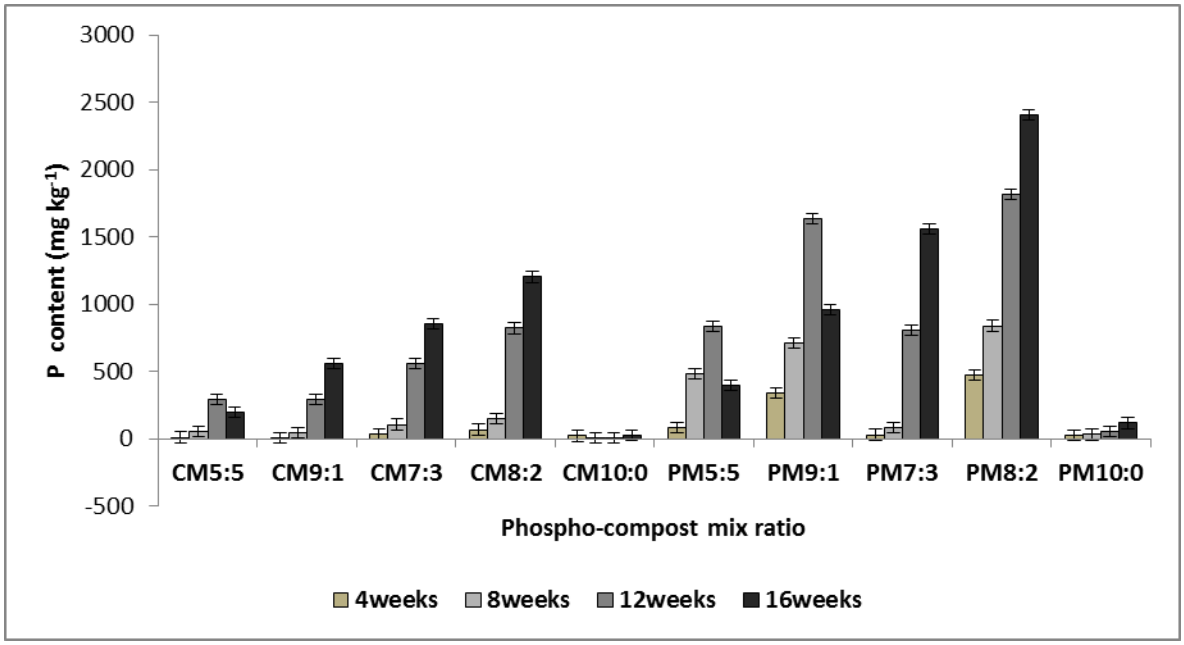

Figure 15: Phospho-compost x sampling date interaction effect on Ca-P content (mg/kg) (bar = standard error of different phospho-compost mix ratios)

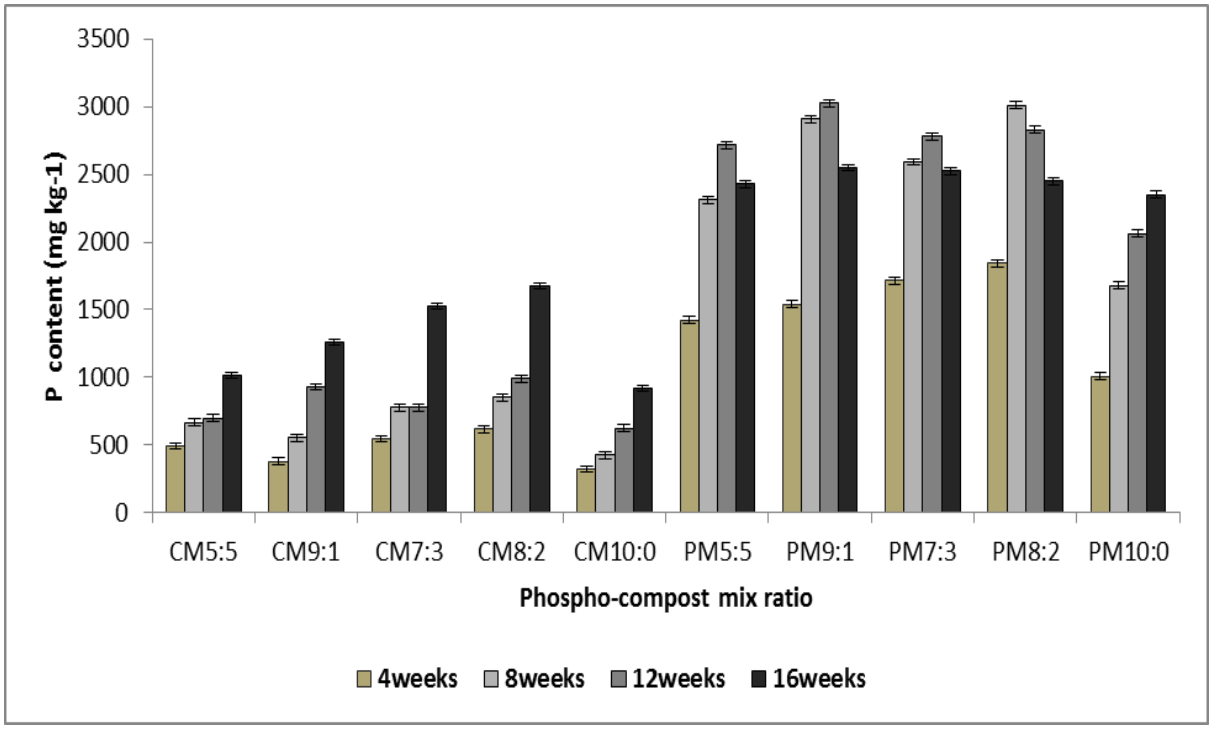

Figure 16: Phospho-compost x sampling date interaction effect on water extractable P content (mg/kg) (bar = standard error of different phospho-compost mix ratios)

4.5 Microbial functional diversity in phospho-compost samples

4.5.1 DNA isolation

Genomic DNA was successfully extracted from the forty compost samples. Figure 17 below shows a typical example of an ethidium stained 1% (w/v) agarose gel, which is indicative of the quality and quantity of DNA isolated from the compost samples.

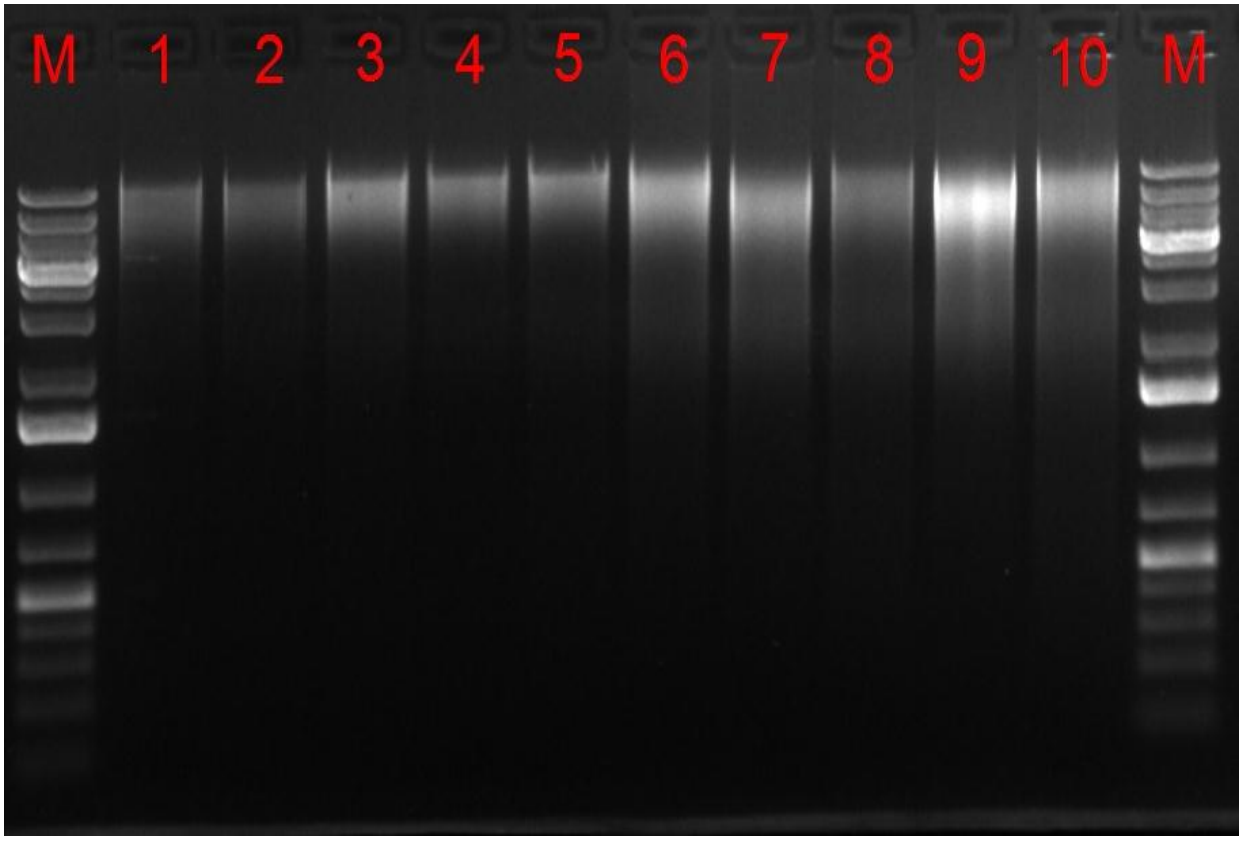

Figure: 17: Photo of 1% (w/v) agarose gel showing successful DNA extraction (A 100bp DNA ladder (Thermo Scientific, US) served as molecular weight marker (MW), lane M. Lanes 1-10 = soil DNA from samples).

4.5.2 Amplification of 16S rDNA

Figure 18 below shows a typical PCR amplicons of the genomic DNA isolated from the phospho-composts after amplification. Spectrophotometric analysis of the isolated DNA indicated that the DNA was of good quality; and the DNA yield ranged from 11.9 to 313.7 ng uL^{-1} with average 260/280 nm values for both cattle manure-based and poultry manure-based compost representing an average ratio of 1.67 indicating good purity (Appendix A).

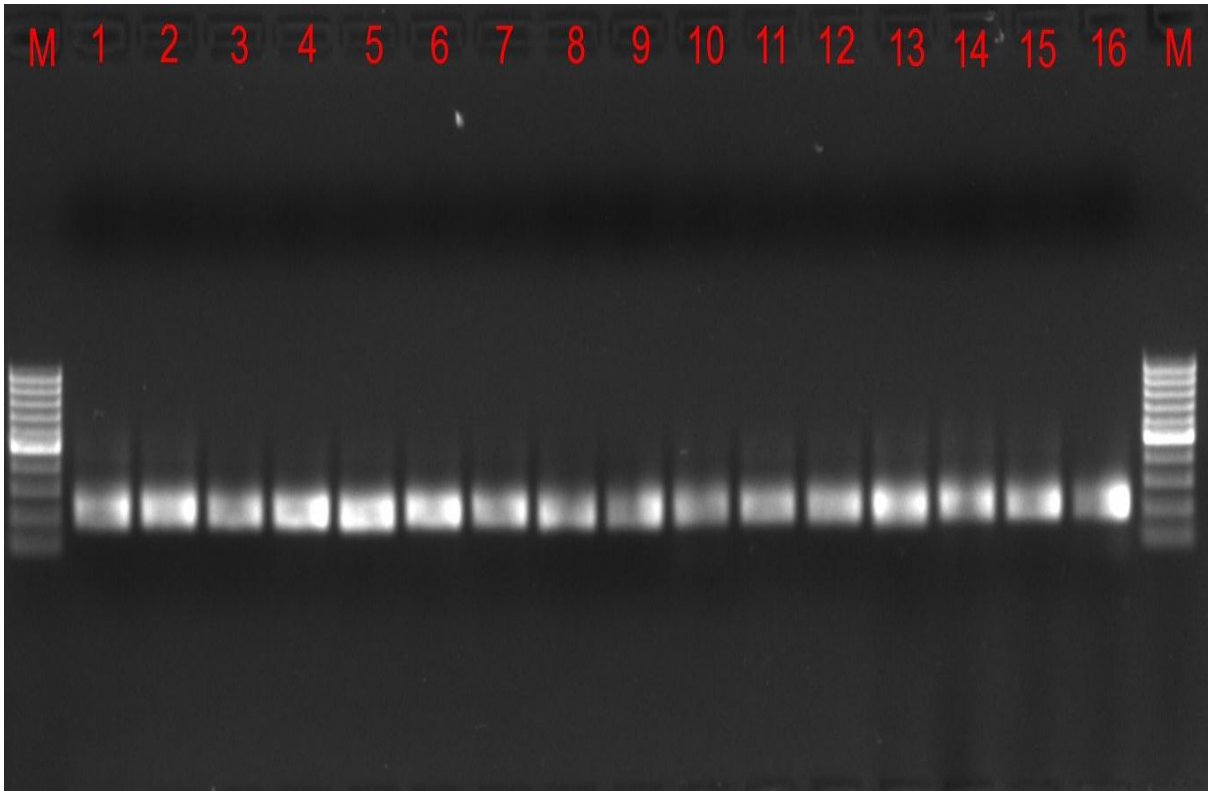

Figure 18: Photo of 1% (w/v) agarose gel showing successful DNA amplication (A 100bp DNA ladder (Thermo Scientific, US) served as molecular weight marker (MW), lane M. 1-16 = PCR amplicons from soil DNA from samples).

4.5.3 Denaturing Gradient Gel Electrophoresis

4.5.3.1 16S rDNA profiling

Various samples over the composting process were used for DGGE analysis. Figures 19 and 20 below provide the prokaryotic 16S rDNA gels. the DGGE banding profiles differed amongst treatments.

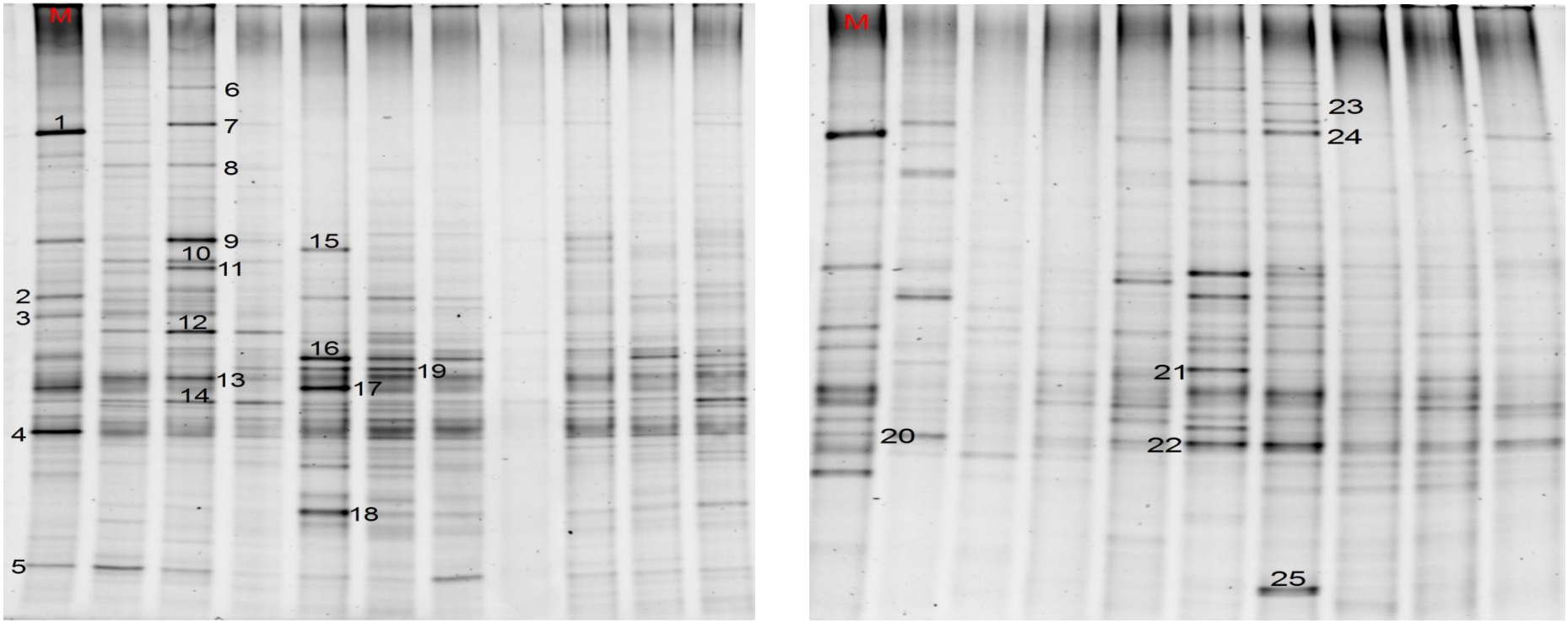

Fig 19 : DGG profiles of bacterial diversity community isolates from cattle manure-based composted samples (Left hand side - Cattle manure-based phospho-composts: Lanes 1-11 correspond to samples 1-11 in Table A.3 in Appendix 3.C-control sample 1 served as marker (M), lane 1. Sequences 1-5 were taken from lane 1, 6-14 from lane 3, 15-18 from lane 5, 19 from lane 7. Right hand side - Cattle manure based-phopspho-composts: Lanes 1-10 correspond with samples 1-10 in Table A.3 (Appendix 3).C-control sample 1 served as marker (M), lane 1.Sequences 20 were taken from lane 2, 21 from lane 6, 25 from lane 6, 22-23 from lane 7).

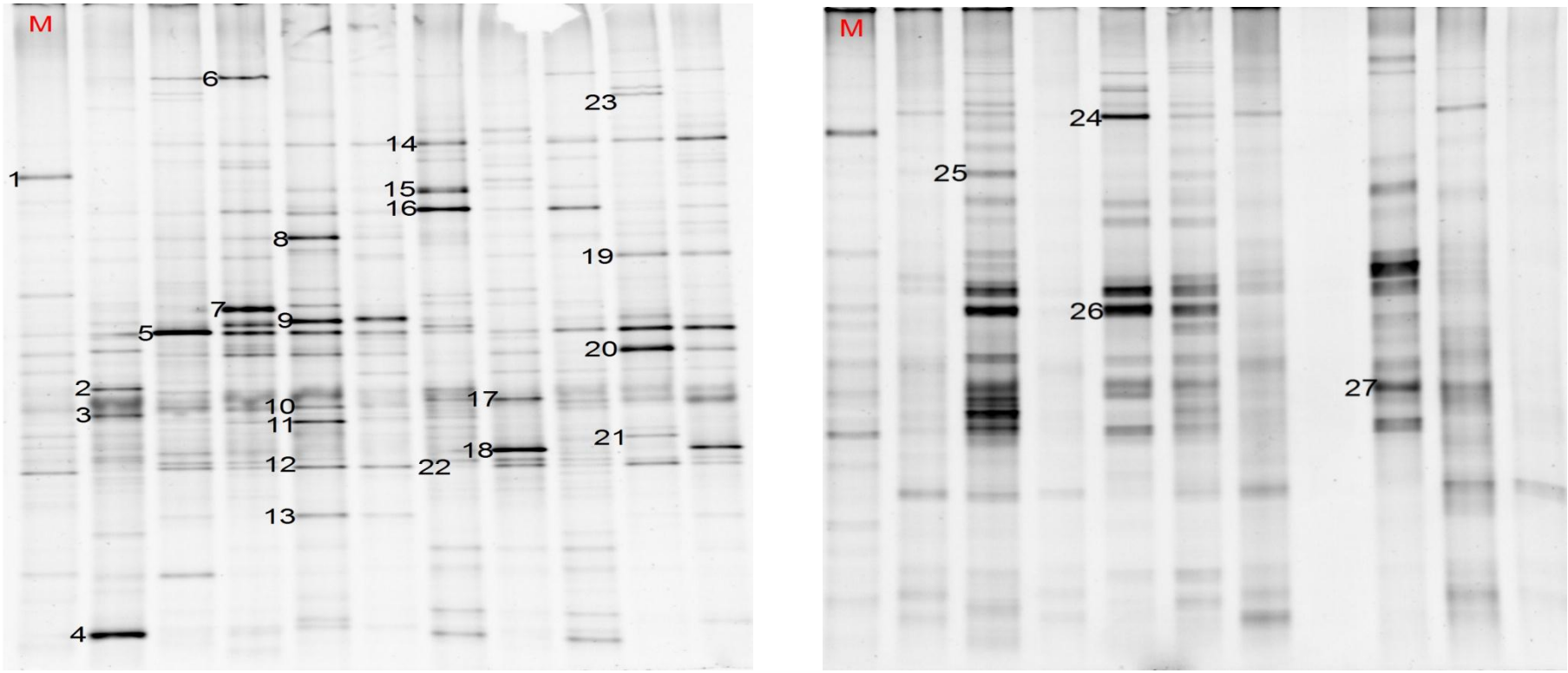

Fig 20 DGG profiles of bacterial diversity between communities isolates from poultry manure-based composted samples (Left hand side - poultry manure-based-phospho-composts: Lanes 1-11 correspond to samples 1-11 in Table A.4 of Appendix 3. C-control sample 1 served as marker (M), lane 1.Sequences 1 were taken from lane 1, 2-4 from lane 2, 6-7 from lane 4, 8-13 from lane 5 and 14-16 from lane 7, 17-18 from lane 8, 19-21, 23 from lane 9 and 22 from lane 7.Right hand side - poultry manure based-phopspho-composts: Lanes 1-11 correspond with samples 1-11 in Table A.3 (Appendix 2).C-control sample 1 served as marker (M), lane 1.Sequences 25 were taken from lane 3, 24, 26from lane 5, 6-10 from lane 3, 27 from lane 9).

4.5.3.2 Sequencing results

It is important to stress that not all bands on the gels were sequenced due to financial limitation but bands from the marker (M) were selected and used as reference. However, the sequencing results as displayed in Table 8 revealed that *Bacillus* and *Acholeplasma* spp. were the most common isolates detected from the poutltry manure-based compost samples. For cattle manure-based compost samples the most common isolates identified were *Acholeplasma* and *Pseudomonas* spp. as well as members in the phylum Bacteroides (Yamamoto *et al.,* 2009). These organisms are usually found in soil and also at different stages in the composting process. Based on the current DGGE and sequencing technique utilised, there were several unidentified microbial species that abound in the compost samples at the different sampling stages. Among the list of identified bacteria, the *Bacillus* sp. and *Acholeplasma cavigenitalium* were commonly found in the various mix ratios of the poultry manure-based phospho-composts while *Pseudomonas* sp. and *Acholeplasma pleciae* were obtained in the cattle manure-based phospho-composts. The quantification of the abundance of the identified microbial species in the phospho-composts was however not achieved based on our DGGE technique.

Table 8: Microbial functional diversity in phospho-compost samples taken at different stages of compost production

Compost Types	Sampling stages			
	4W	8W	12W	16W
CM-control	Uncultured *Flavobacteriaceae bacterium* clone 6347, Uncultured Bacteroidetes *bacterium* clone Upland_75_2525, *Pseudomonas sp*. BAS203i, Uncultured *bacterium* clone	Uncultured *bacterium* clone JFR0701_jaa45e08, Uncultured Bacteroidetes *bacterium*, Uncultured *bacterium* clone JFR0701_jaa45e08, Uncultured *bacterium* clone GD2:G9RA0RH03HF80V, *Acholeplasma pleciae* strain NBRC 100476	Uncultured Bacteriodetes*bacterium* clone Upland_75_2525, Uncultured *bacterium* clone FO109.33, Uncultured *bacterium* clone NT-4-68, Uncultured *Pseudomonas sp*. Clone DVASW J273, Uncultured *bacterium* clone JFR0701_jaa45e08	Uncultured Bacteriodetes*bacterium*, *Acholeplasma pleciae* strain NBRC 100476, Uncultured *Flavobacteriaceae bacterium* clone 6347
CM9:1	Uncultured Bacteroidetes *bacterium* clone Upland_75_2525, Uncultured *bacterium* clone FO109.33, Uncultured bacterium clone NT-4-68, Uncultured *Pseudomonas sp*. Clone DVASW J273, Uncultured *bacterium* clone JFR0701_jaa45e08	Uncultured Bacteroidetes *bacterium* clone Upland_75_2525, Uncultured Bacteroidetes*bacterium*, Uncultured *bacterium* clone GD2:G9RA0RH03HF80V, *Acholeplasmapleciae* strain NBRC 100476, Uncultured *Flavobacteriaceae bacterium* clone 6347	Uncultured Bacteriodetes *bacterium* clone Upland_75_2525, Uncultured *bacterium* clone FO109.33, *Pseudomonas sp*. BAS203i, Uncultured *Pseudomonas sp*. Clone DVASW J273, Uncultured *bacterium* clone JFR0701_jaa45e08	Uncultured *bacterium* clone GD2:G9RA0RH03HF80V
CM8:2	Uncultured Bacteriodetes *bacterium* clone Upland_75_2525, Uncultured *bacterium* clone FO109.33, *Pseudomonas sp*. BAS203i, Uncultured *Pseudomonas sp*. Clone DVASW J273, Uncultured *bacterium* clone JFR0701_jaa45e08	Uncultured *bacterium* clone GD2:G9RA0RH03HF80V	Uncultured *bacterium* clone NT-4-68, Uncultured *bacterium* clone JFR0701_jaa45e08	Uncultured Bacteriodetes*bacterium* clone Upland_75_2525, Uncultured *bacterium* clone NT-4-68, *Pseudomonas sp*. BAS203i, Uncultured *Flavobacteriaceae bacterium* clone C146300287
CM7:3	Uncultured *bacterium* clone NT-4-68, Uncultured *bacterium* clone JFR0701_jaa45e08	Uncultured Bacteriodetes*bacterium* clone Upland_75_2525, Uncultured *bacterium* clone NT-4-68, *Pseudomonas sp*. BAS203i, Uncultured *Flavobacteriaceae*	Uncultured *bacterium* clone JFR0701_jaa45e08	Uncultured Bacteriodetes *bacterium* clone Upland_75_2525

		bacterium clone C146300287		
CM5:5	Uncultured *bacterium* clone JFR0701_jaa45e08, Uncultured *Flavobacteriaceae bacterium* clone C146300287, Uncultured Bacteriodetes *bacterium*, Uncultured *bacterium* clone	Uncultured Bacteroidetes*bacterium* clone Upland_75_2525	Uncultured *bacterium* clone JFR0701_jaa45e08, Uncultured Bacteriodetes *bacterium*, Uncultured *bacterium* clone JFR0701_jaa45e08, Uncultured *bacterium* clone GD2:G9RA0RH03HF80V, *Acholeplasma pleciae* strain NBRC 100476	Uncultured Bacteriodetes*bacterium* clone Upland_75_2525, Uncultured *bacterium* clone NT-4-68
PM-control	Uncultured *bacterium* isolate DGGE gel band 288, Uncultured *bacterium* clone Hmb2-30	Uncultured *bacterium* isolate DGGE gel band 288, Uncultured *bacterium* clone MBIOS-29, *Acholeplasma cavigenitalium* strain GP3, Uncultured *bacterium* clone N1_1_2042, Uncultured *bacterium* clone YC12	Uncultured *bacterium* isolate DGGE gel band 288, Uncultured *bacterium* clone Hmb2-30	Uncultured *bacterium* isolate DGGE gel band 288, Uncultured *bacterium* clone MBIOS-29, *Acholeplasma cavigenitalium* strain GP3, Uncultured *bacterium* clone N1_1_2042, Uncultured bacterium clone YC12
PM9:1	Uncultured *bacterium* clone Hmb2-30, Uncultured *bacterium* clone GDIC2IK01DPSBB, Uncultured *bacterium* clone EBP1709, *Bacillus sp*. S3-R6TC-BA1 clone 1, Uncultured *bacterium* clone MBIOS-29, Uncultured *bacterium* clone YC12, *Acholeplasmacavigenitalium* strain GP3	*Bacillus sp*. S3-R6TC-BA1 clone 1, *Acholeplasma cavigenitalium* strain GP3, Uncultured *bacterium* clone N1_1_2042, Uncultured *bacterium* clone YC12, Uncultured *bacterium* clone YC12	Uncultured *bacterium* clone Hmb2-30, Uncultured *bacterium* clone GDIC2IK01DPSBB, Uncultured *bacterium* clone EBP1709, *Bacillus sp*. S3-R6TC-BA1 clone 1, Uncultured *bacterium* clone MBIOS-29, Uncultured *bacterium* clone YC12, *Acholeplasmacavigenitalium* strain GP3	*Bacillus sp*. S3-R6TC-BA1 clone 1, *Acholeplasma cavigenitalium* strain GP3, Uncultured *bacterium* clone N1_1_2042, Uncultured *bacterium* clone YC12, Uncultured *bacterium* clone YC12
PM8:2	Uncultured *bacterium* clone Hmb2-30, Uncultured *bacterium* clone GDIC2IK01DPSB, *Bacillus sp*. S3-R6TC-BA1 clone 1B, Uncultured *bacterium* clone 3g07, Uncultured *bacterium* clone EBP1709, Uncultured *bacterium* clone MBIOS-29	Uncultured *bacterium* clone Hmb2-30, Uncultured *bacterium* clone GDIC2IK01DPSBB, *Acholeplasmacavigenitalium* strain GP3, Uncultured *bacterium* clone YC12	Uncultured *bacterium* clone Hmb2-30, Uncultured *bacterium* clone GDIC2IK01DPSBB, Uncultured *bacterium* clone 3g07, Uncultured *bacterium* clone EBP1709, *Bacillus sp*. S3-R6TC-BA1 clone 1, Uncultured *bacterium* clone MBIOS-29	Uncultured *bacterium* clone Hmb2-30, Uncultured *bacterium* clone GDIC2IK01DPSBB, *Acholeplasma cavigenitalium* strain GP3, Uncultured *bacterium* clone YC12
PM7:3	Uncultured *bacterium* clone Hmb2-30,	Uncultured *bacterium* clone Hmb2-30,	Uncultured *bacterium* clone Hmb2-30,	Uncultured *bacterium* clone Hmb2-30,

	Uncultured *bacterium* clone EBP1709, Uncultured *bacterium* clone 01d11, *Bacillus sp*. S3-R6TC-BA1 clone 1, Uncultured *bacterium* clone RL178_aan65b07, Uncultured *bacterium* clone MBIOS-29, Uncultured *bacterium* clone BS09	Uncultured *bacterium* clone 01d11, *Bacillus sp*. S3-R6TC-BA1 clone 1, Uncultured *bacterium* clone LB_16	Uncultured *bacterium* clone GDIC2IK01DPSBB, Uncultured *bacterium* clone 3g07, Uncultured *bacterium* clone EBP1709, Uncultured *bacterium* clone 01d11, *Bacillus sp*. S3-R6TC-BA1 clone 1, Uncultured *bacterium* clone RL178_aan65b07, Uncultured *bacterium* clone MBIOS-29	Uncultured *bacterium* clone 01d11, *Bacillus sp*. S3-R6TC-BA1 clone 1, Uncultured *bacterium* clone LB_16
PM5:5	Uncultured *bacterium* clone Hmb2-30, *Bacillus sp*. S3-R6TC-BA1 clone 1, Uncultured *bacterium* clone RL178_aan65b07, Uncultured *bacterium* clone MBIOS-29, Uncultured *bacterium* clone BS09, *Acholeplasmacavigenitalium* strain GP3	Uncultured *bacterium* clone Hmb2-30, Uncultured *bacterium* clone GDIC2IK01DPSBB, *Bacillus sp*. S3-R6TC-BA1 clone 1, *Acholeplasma cavigenitalium* strain GP3, Uncultured *bacterium* clone N1_1_2042, Uncultured *bacterium* clone YC12	Uncultured *bacterium* clone Hmb2-30, *Bacillus sp*. S3-R6TC-BA1 clone 1, Uncultured *bacterium* clone RL178_aan65b07, Uncultured *bacterium* clone MBIOS-29, Uncultured *bacterium* clone BS09, *Acholeplasma cavigenitalium* strain GP3	Uncultured *bacterium* clone Hmb2-30, Uncultured *bacterium* clone GDIC2IK01DPSBB, *Bacillus sp*. S3-R6TC-BA1 clone 1, *Acholeplasma cavigenitalium* strain GP3, Uncultured *bacterium* clone N1_1_2042, Uncultured *bacterium* clone YC12

4.6 Correlation matrix and regression analysis

Statistical analyses contained in Tables 10 below indicated that pH of phospho-compost samples was negative but significantly (P<0.05) correlated with EC (-0.2878***), phosphatase (-0.2874***), β-glucosidase (-0.2874***), fungi (-0.2717**) and actinomycetes (-0.2919***) and positively correlated with water-P (0.3668***). Actinomycetes correlated positively with EC (0.5045***), phosphatase(0.7289***), β-glucosidase(0.7289), fungi (0.7653***) and bacteria(0.4853***) but negatively with organic P(-0.1875), water P (-0.4877***) and pH(-0.2919***). There was a positive and significant correlation between EC, enzyme activity (phosphatase (0.3726***), dehydrogenase (0.3726***) and β-glucosidase (0.3726***)), fungi(0.2191**), actinomycete(0.5045) and P fractions (Bray P1 (0.4182***), Ca-P(0.2443**) and Pi value(0.2531**)). Acid phosphatase activity correlated negatively with water extractable P(-0.6467***), organic P(-0.4021***) and Ca-P (-2143**) contents but revealed a positively significant correlation with bacteria(0.2678**), fungi (0.6944***) and actinomycete (0.7289***) counts. The bacteria counts in the different phospho-compost samples collected at the various compost production phases was positively and signicantly (p<0.05) correlated with fungi (0.5154****)and actinomycete (0.4853***) numbers but negatively correlated with water extractable P (-0.2493**) content. A positive and significant correlation was obtained between β-glucosidase activity and bacteria (2678**), fungi (6944***) and actinomycete (0.7289***) counts while a negative correlation was found between β-glucosidase activity and Ca-P(0.2143**), water P (-0.6467***) and organic P (-0.4021***) fractions. There was a positive correlation between dehydrogenase activity, water extractable P (0.4412***), Pi value(0.5704***), Bray P1 (0.6966***)and Ca-P (0.3979***); while dehydrogenase activity had a negative correlation with fungi (-0.2791**)count.

Table 9: Polynomial regression analysis for Bray P1 of the different phospho-composts

Predictor variables	Coefficient	Standard error	T-statistic	P-value
Phosphatase	-52.3664	21.0819	-2.48	0.0145
Dehydrogenase	0.62110	0.11646	5.33	0.0000
Bglucosidase	1.15005	0.46298	2.48	0.0145
Bacteria	4.57435	8.05533	0.57	0.5713
Fungi	-86.8081	14.3896	-6.03	0.0000
Actinomycete	63.0941	12.2449	5.15	0.0000
Pi value	0.02271	3.728E-03	6.08	0.0000
Ca-P	0.10295	0.02155	4.78	0.0000
Water P	-0.02592	8.084E-03	-3.21	0.0018

Table 10: Pearson correlation matrix between pH, EC, enzyme activities, microbial population counts and phosphorus forms and fractions in the composts

	pH	EC	Phospha	Dehydrog	β-glucosid	Bacteria	Fungi	Actinom	Bray P1	Org. P	Ca-P	Water P	P_i-value
pH	1												
EC	-0,2878***	1											
Phospha	-0.2874***	0.3726***	1										
Dehydrog	0.0548	0.3726***	-0.0822	1									
β-glucosid	-0.2874***	0.3726***	-	-0.0822	1								
Bacteria	-0.0378	0.1548	0.2678**	0.0815	0.2678**	1							
Fungi	-0.2717**	0.2191**	0.6944***	-0.2791**	0.6944***	0.5154****	1						
Actinonom	-0.2919***	0.5045***	0.7289***	0.1192	0.7289***	0.4853***	0.7653***	1					
Brya P1	0.0122	0.4182****	-0.0547	0.6966***	-0.0547	0.0037	-0.2278**	0.1117	1				
Organic P	0.0090	0.0730	-0.4021***	-0.1417	-0.4021***	0.0231	-0.0912	-0.1875**	0.0920	1			
Ca-P	-0.0432	0.2443**	-0.2143**	0.3979***	-0.2143**	-0.1512	-0.2903***	-0.0827	0.6170	0.2981***	1		
Water P	0.3668***	-0.1226	-0.6467***	0.4412***	-0.6467***	-0.2493**	-0.6128***	-0.4877***	0.4747***	0.3002***	0.6324***	1	
P_i-value	0.0276	0.2531**	-0.1580	0.5704***	-0.1580	0.0823	-2483**	0.084	0.6594***	0.1733*	0.7542***	0.6077***	1

Phospha= phosphatase, dehydrog=dehydrogenase, -glucosid=-glucosidase, actinom=actinomycete, org-P=organic P, Ca-P= calcium P

CHAPTER 5

DISCUSSIONS

In general, the mixed ratios of the different phospho-composts had a greater influence on the microbial population counts (bacteria, fungi and actinomycete), enzymes activities (β-glucosidase, phosphatase and dehydrogenase), P release characteristics, percent seed germination and measured temperature changes observed during phospho-compost production. In this study, temperature was used as an important parameter to monitor composting process. The temperature measured in the different phospho-composts reached thermophilic phase during the first 5 weeks of composting then decreased to ambient temperature at compost maturity suggesting that the organic matter in the compost was stabilized and the composts cured. This is in agreement with earlier study reported by Lemunier *et al.* (2005). Dui-an *et al.* (2013) also studied changes in P fractions and N forms during composting of raw pig manure with rice straw and found that the temperature of the compost reached thermophilic phase in 1 week, which was maintained for nearly 2 weeks, and then decreased to ambient temperature on day 49.

The value of EC in the different phospho-composts increased at the beginning and thermophilic phase because of the mineralisation of organic matter and solubilisation of GRP with the release of organic acids, but decreased at the latter phase of co-composting due to the precipitation of mineral salts and the stabilisation of organic matter. This finding is in agreement with the work by Gómez-Brandón *et al.* (2008), who reported that EC increased after the active phase probably due to the release of soluble salts like ammonium and phosphate resulting from the decomposition of easily biodegradable organic substrate. The sequence of P forms and fractions of organic P<Bray1 P< Calcium P< water P< Pi-value obtained in phospho-compost for this study differs significantly from those reported in earlier studies measured in soil (Kulhanek *et al*., 2009; Wunscher *et al*., 2013). The low lowest level of organic P fraction obtained in this study is in agreement with earlier work by Eghball (2003), and Eghball *et al.* (2004), who studied the leaching of P fractions following manure and compost application and found that organic P was the smallest fraction of the total P in the applied manure and compost. However, the concentrations of all forms of P fractions increased due to the decomposition of organic matter and the mass

losses during composting. The results of this study showed that higher concetration of P fractions was higher at maturity phase and at phosphate amendment compost than as compared to unammended compost , this might be due to greater mobilization of P from phosphate rock. This aligned with report by Sibi (2011), who indicated that rock phosphate charged compost enhanced the content of total P in the final product when compared to untreated compost..

The 8:2 mix ratio gave the highest P concentration for all P fractions in both PM- and CM-based phopho-composts. This observation correspond with the previous study by Chauke *et al.* (2014) who reported that 8:2 mix ratio for both poultry manure and sewage sludge gave the highest residual soil P over all other phospho-compost treatments following soil amendment with phospho-composts. The higher measured P concentration in PM- than CM-based phospho-composts reported in this study may be attributed to the higher P content in poultry manure. This is in agreement with the earlier study reported by Dikinya and Mufwanzala (2010), who reported higher P and other nutrient contents in poultry manure than other animal manures such as pig and kraal manures. Similarly, Teppei *et al.* (2010) reported that the P concentration in broiler litter compost was greater than for cattle manure compost with most of the P in cattle manure compost being inorganic while those of broiler litter compost containing more of organic P. Increase in the amount of phosphorus in the compost with time was reported by Tripathi and Bhardwaj (2004), which is similar to this study.

Results of study on percent seed germination assessments suggest very high sensitivity to the maturity of the different phospho-compost mixed ratio. The highest percent germination was obtained in the 8:2 mix ratio with the maximum value obtained in the mature compost for both PM- and CM-based phospho-composts. Previous study by Gómez-Brandón *et al*. (2008) revealed that the germination index was 0% in the initial cattle manure, and reached a value of 24% during the active phase then, at maturation stage, a germination indices of 87% was recorded which was the highest to all after 270 days of maturation which is more related to this study. According to the study done by Komilis and Tziouvaras (2009), the cow manure derived composts gave higher percent germination when compared to poultry manure derived composts, which is related to this study. The authors also

revealed that immature compost had a negative impact on plant growth, which also agrees with the current study where germination percentage was higher at maturity phase. The lower germination percentage in poultry as compared to cattle might be due to lower CO_2 production and oxgen consumption in poultry manure than in cattle manure (Gómez-Brandón *et al.,* 2008). Results obtained from my current study suggest that the actual amounts of available P measured in these phospho-composts depended mostly on the types of manure used and the mixed ratios of GPR with manure. This is in agreement with the earlier work by Li *et al.* (2014) who reported that large variation in the P contents of bio-wastes depend on the source of waste and the treatments methods such as aerobic and anaerobic digestion, and the addition of inorganic materials typical of the GPR used in this study. According to study done by Hansen *et al*. (2004) total P measured and the forms of organic P in manure will depend on the nature of the animal manure and the type of storage. Phosphorus in manures can vary widely depending on animal physiology, species and age, composition of diets, duration of manure storage, moisture content and type of bedding material (Atia and Mallarino, 2002; McDowell and Stewart, 2005).

Bacteria counts was increasing with the increase in GPR additional and decreasing with the decrease in GPR addition in both cattle and poultry manure based phospho-compost. The higher abundance of population counts for bacteria, fungi and actinomycete in cattle manure-based than poultry manure-based phospho-compost might be due to favourable condition in CM-based phospho-compost than in PM-based phospho-compost. These microbial parameters were also positively correlated with each other. Awasthia *et al*. (2015) reported maximum increase in microbial population during the early stages of composting depending on initial substrate used and environmental conditions during composting. Chandna *et al.* (2014) also reported increased bacteria population during the mesophilic stage that gradually decreased from thermophilic through cooling to maturation phase. According to Albrecht *et al.* (2010) this might be due to changes in environmental factors such as temperature, pH or moisture that are specific to the stage of composting. Similar view was expressed by Kim *et al*. (2008) who attributed the fluctuation in microbial counts in compost to changes in temperature and moisture contents. Tiquia *et al*. (2002) also observed a drop in actinomycetes and fungi populations during composting process as the temperature began to peak; and then increased and

exceeded that of their initial numbers. Increase in fungi and actinomycete counts with an increase in GPR addition was particularly observed in CM-based phospho-composts.

Results from this study suggest that the various microbial species present in the phospho-composts were able to solubilise P from the Phalaborwa GPR to a varying degrees as indicated by the higher P released in the different phospho-compost mix ratios. The concentrations of enzyme activity were decreased to various extent with increase in GPR addition possibly due to the different mix ratios used. Kazemi *et al.* (2014) reported that the content of the different starting materials of a compost influence the overall level of enzyme activity in the final compost product. The high concentrations of dehydrogenase, β-glucosidase and phosphatase activity at the cooling phase of composting imply high microbial activity and high decomposition rate. Yu *et al*. (2007) reported that maximum levels of β-glucosidase activity were observed at the initial stage and during the maturation stage; thus suggesting agreement with my findings. The maximum concentration of dehydrogenase activity observed at thermophilic phase and during the cooling stage of composting in my study was similarly reported by Bernal *et al*. (2009).

Furthermore, Vargas-Garcia *et al*. (2010) stated that higher dehydrogenase activity values were related to the higher microbial activity and large account of mesophilic and thermophilic bacteria while lower dehydrogenase activity values was associated with the maturation phase. In the beginning, phosphodiesterase activities were high in both composts and also at maturity phase. Renée (2004), obtained a highest increase in soluble P content after five weeks of composting manure with phosphate rock. Soil pH and nutrients availability was also reported to be the main drivers of microbial community composition (Waldrop *et al.,* 2006). Lelei and Onwonga (2014), studied the response of microbial populations, soil available P and yield of Lupin (*Lupinusalbus L cv. Amiga*) to application of Minjingu phosphate rock in a greenhouse study. Their results showed an increase in soil available P from solubilised Minjingu phosphate rock and rhizo deposition by white lupin that caused higher fungal numbers at 56 day after sowing.

The significant phospho-compost types × sampling dates interaction effects on microbial counts and enzyme activities reported in this study is in agreement with

earlier results by Lucas *et al.* (2007) who revealed that small additions of N-containing organic compounds caused changes in the soil microbial community structures, which though did not necessarily have an impact on extracellular enzyme activity. This was further supported by the work of Stark *et al.* (2008) who reported that no direct relationships existed between microbial community structure, enzyme activities and N mineralisation. The *Bacillus* sp. and *Acholeplasma cavigenitalium* were commonly found in the various mix ratios of the poultry manure-based phospho-composts, while *Pseudomonas* sp. and *Acholeplasma pleciae* were obtained in the cattle manure-based phospho-composts. The higher microbial counts in CM-based phospho-composts may be attributed to the highly favourable condition such as oxygen consumption and CO_2 production as compared to PM-based phospho-composts which is potential toxic environment formed from a toxic elements. According to Bolan *et al.* (2004), poultry manure contains appreciable quantities of potentially toxic metals such as arsenic, copper and zinc, these elements can become toxic to plants, can adversely affect organisms.

The increased concentration of acid phosphatase activity reported in this study during the mesophilic phase, which also increased toward compost maturity contradicted earlier report by Ros *et al.* (2006) who found that alkaline phosphatase activity increased at the beginning and reached maximum value at compost maturity. According to Awasthia *et al*. (2015) and Goyal *et al*. (2005) the maximum activity of phosphatase at the maturation phase was due to the abundance of organic phosphorus compounds. The observed increase phosphatase activity after the mesophilic phase also contradicts earlier findings by Raut *et al.* (2008) who found that alkaline phosphatase activity decreased after thermophilic phase of composting. The result was however, similar to findings by Dui-an *et al*. (2013) who reported that alkaline phosphatase activity decreased at the beginning of composting, and then increased to a plateau value from 31 days. The results of this study further revealed that where P fractions (water extractable P, organic P and Ca-P contents) was higher, the concentration of phosphatase was lower. This is because acid phosphatase activity plays a key role in organic P mineralization and this enzyme is activated when P availability is low (Kim *et al.,* 2008).

CHAPTER 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The aim of this study was to assess P solubility and bioavailability of non-reactive Phalaborwa ground phosphate using the aerobic thermophilic co-composting technology. The content of P fractions and P availability in the different phospho-composts increased differently depending on the GPR mix ratios and composting phase during co-composting process. When comparison were made, higher P concentrations were mineralized from PM- than CM-based phospho-composts. However, during the mesophilic stage of the co-composting process, P concentration was found to be lower when compared to the last stage of co-composting which was higher in both CM- and PM-based phospho-composts. The 8:2 mix ratio gave higher P concentration when compared to other mix ratios in both PM- and CM-based phospho-composts. Seed germination percentage, which is a measure of maturity index, also differed significantly ($p<0.05$) across phospho-compost mix ratios and sampling date. Percent seed germination was higher in CM- than PM-based phospho-composts; and generally highest in fully cured composts. In the course of our investigation we noted that, activities of enzymes and available P indices were significantly higher during the maturation phase across all mix ratios, but higher in 8:2 than other ratios. Poultry manure based phospho-compost showed higher P fractions than cattle-based phospho-compost . Microbial population results showed that the population count of fungi, actinomycete and bacteria were not a limiting factor in phospho- compost production from all different mixed ratios. However this study also revealed that compost mixed ratio had an influence on availability of P forms and fractions. Although the amount and diversity of micro-organisms measured were higher in cattle manure-based composts when compared to poultry manure, higher P release was obtained from poultry manure-based phospho-compost, which was particularly higher in the 8:2 mix ratio. Based on the current DGGE and sequencing technique utilised, there were several unidentified microbial species that bounded in the compost samples at the different sampling stages. Among the list of identified bacteria, the Bacillus sp. and Acholeplasma cavigenitalium strain GP3 were commonly found in the PM-based phospho-composts while Pseudomonas sp. and Acholeplasma pleciae were dominant in the CM-based phospho-composts. The quantification of the abundance and identification

of microbial species in the phospho-composts was however not effectively achieved based on our DGGE technique. There were several other unidentified microbes found in the various phospho-compost samples collected at the different composting phases. However, these are preliminary findings on the use of the DNA molecular technique in providing understanding of microbial diversity and/or functionality in compost study particularly for nutrient mineralisation and transformation during composting process.

The results of this study indicate that aerobic thermophilic co-composting of animal manure such as cattle and poultry manures is beneficial for the solubilisation of non-reactive GPR typical of Phalaborwa phosphate rock. However, the PM-based phospho-composts at 8:2 mix ratio is preferable to CM-based phospho-composts due to the higher P concentration released. In terms of the use and management of the various phospho-compost by farmers, it is important to highlight that immature compost should not be applied to the land as it will negatively affect availability of P, germination and crop production as a whole. The GPR need to be at optimum ratio in order to find optimum P release.

Finally, future works need to consider other molecular techniques such as the use of next generation sequencing (NGS) or Quantitative Insight into Microbial Ecology (QIIME) technologies. Such will provide better identification and quantification of microbial species composition in each phospho-compost at the key composting stages of mesophilic, thermophilic, cooling and curing phases. Fungal composition was not dealt with at this stage, but will also need to be investigated in providing a clearer representation of interactions taking place in the co-composting process.

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List of appendices

Appendix 1: *Table A.1.Various treatments loaded on DGGE gel*

Lane	**Sample name**
1	C-10:0 sample 1
2	C-9:1 sample1
3	C-8:2 sample 1
4	C-7:3 sample 1
5	C-5:5 sample 1
6	C-10:0 sample 2
7	C-9:1 sample2
8	C-8:2 sample 2
9	C-7:3 sample 2
10	C-5:5 sample 2
11	C-10:0 sample 3
12	C-9:1 sample 3
13	C-8:2 sample 3
14	C-7:3 sample 3
15	C-5:5 sample 3
16	C-10:0 sample 4
17	C9:1 sample 4
18	C8:2 sample 4
19	C-7:3 sample 4
20	C-5:5 sample 4

Appendix 2: Table A .2.Various treatments loaded on DGGE gel

Lane	Sample name
1	C-control sample 1
2	P-10:0 sample 1
3	P-9:1 sample 1
4	P-8:2 sample 1
5	P-7:3 sample 1
6	P-5:5 sample 1
8	P-10:0 sample 2
9	P-9:1 sample 2
10	P-8:2 sample 2
11	P-7:3 sample 2
12	P-5:5 sample 2
14	C-10:0 sample 1
15	P-10:0 sample 3
16	P-9:1 sample 3
17	P-8:2 sample 3
18	P-7:3 sample 3
19	P-5:5 sample 3
20	P-10:0 sample 4
21	P-9:1 sample 4
22	P-8:2 sample 4
23	P-7:3 sample 4
24	P-5:5 sample 4

Appendix 3: Table A.3. Identities of 16S rDNA sequences present on DGGE gel

Nr of DGGE Bands	Identification of sequence names	Nr of lanes present
C1	Uncultured *Flavobacteriaceae bacterium* clone 6347	Lane 1
2	No significant similarity found	Lanes 1,5,6,7,10 and 11
3	No significant similarity found	Lane 1
4	No significant similarity found	Lanes 1,10 and 11
5	Uncultured Bacteroidetes *bacterium* clone Upland_75_2525	Lanes 1,2,3,7,9,10 and 11
6	Uncultured *bacterium* clone FO109.33	Lane 3 and 2
7	Uncultured *bacterium* clone NT-4-68	Lanes 2,4,9 and 11
8	No significant similarity found	Lanes 2 and 3
9	*Pseudomonas sp*. BAS203i	Lanes 1,3 and 9
10	Uncultured *Pseudomonas sp*. Clone DVASW J273	Lanes 2,3
11	No significant similarity found	Lanes 2,3 and 11
12	Uncultured *bacterium* clone JFR0701_jaa45e08	Lanes 2,3,4,5,6
13	No significant similarity found	Lanes 2,3
14	No significant similarity found	Lanes 1,2,3,4,5,6,7 and 8
15	Uncultured *Flavobacteriaceae bacterium* clone C146300287	Lanes 5 and 9
16	Uncultured Bacteroidetes *bacterium*	Lanes 5,6 and 7
17	Uncultured *bacterium* clone	Lane 1,5
18	No significant similarity found	Lane 5
19	No significant similarity found	Lanes 4,5 and 6

20	No significant similarity found	Lane 1
21	Uncultured *bacterium* clone JFR0701_jaa45e08	Lane 6
22	Uncultured *bacterium* clone GD2:G9RA0RH03HF80V	Lanes 6,7 and 8
23	No significant similarity found	Lane 7
24	*Acholeplasma pleciae* strain NBRC 100476	Lane 6 and 7
C25	Uncultured *Flavobacteriaceae bacterium* clone 6347	Lane 7

Appendix 4: Table A.4. Identities of 16S rDNA sequences present on DGGE gel

Nr of DGGE Bands	Identification of sequence names	Nr of lanes present
P1	Uncultured *Flavobacteriaceae bacterium* clone 6347	Lane 1
2	No significant similarity found	Lane 2
3	No significant similarity found	Lane 2
4	Uncultured *bacterium* isolate DGGE gel band 288	Lane 2 and 7
5	Uncultured *bacterium* clone Hmb2-30	Lane 2,3,4,5,6,9,10 and 11
6	Uncultured *bacterium* clone GDIC2IK01DPSBB	Lane 3,4,9 and 11
7	Uncultured *bacterium* clone 3g07	Lanes 4
8	Uncultured *bacterium* clone EBP1709	Lanes 3,4,5,
9	Uncultured *bacterium* clone 01d11	Lane 5 and 10
10	No significant similarity found	Lane 5
11	No significant similarity found	Lanes 5
12	*Bacillus sp*. S3-R6TC-BA1 clone 1	Lanes 3,4,5,6,8,10 and 11
13	Uncultured *bacterium* clone RL178_aan65b07	Lanes 5 and 6
14	Uncultured *bacterium* clone MBIOS-29	Lanes 3,4,5,6,7
15	No significant similarity found	Lanes 5 and 7
16	*Acholeplasma cavigenitalium* strain GP3	Lanes 7,8,9 and 11
17	Uncultured *bacterium* clone N1_1_2042	Lanes 7,8 and 11
18	Uncultured *bacterium* clone YC12	Lanes 8 and 11
19	No significant similarity found	Lanes 4,10 and 11
20	No significant similarity found	Lanes 10 and 11
21	No significant similarity found	Lanes 10
22	Uncultured *bacterium* clone YC12	Lanes 3,7,8,9
23	Uncultured *bacterium* clone LB_16	Lane 10

24	Uncultured *bacterium* clone BS09	Lanes 5 and 6
25	*Acholeplasma cavigenitalium* strain GP3	Lanes 3 and 6
26	No significant similarity found	Lanes 3, 5 and 6
P27	No significant similarity found	Lanes 5, 6,9 and 10

Appendix 5: Mean sum of square for microbial counts from different phospho-compost produced

Sources of variance	Degree of freedom	Actinomycete	Bacteria	Fungi
Treatments (Trts)	9	10.9627***	4.39194***	6.50334***
Sampling date (SD)	3	4.6353***	8.58536***	1.31901***
Trts*Sdate interaction	27	1.7475***	4.98858***	1.26276***

Appendix 6: Mean sum of square for the various enzyme activities

Source of variance	Degree of freedom	Phosphatase	B-glucosidase	Dehydrogenase
Sampling dates (SD)	3	487919ns	1.011E+09***	171865***
Treatments (Trts)	9	1.522E+07***	3.157E+10***	2717***
SD*Trts interaction	27	1079410***	2.238E+09***	4120***

Appendix 7: Mean sum of square for pH, EC and percent seed germination of phospho-compost samples

Source of variance	Degree of freedom	pH	EC	Seed germination
Sampling dates	4	9.581***	138.077***	152.41***
Treatments	9	3.197***	112.784***	160.94***
SD*Trts interaction	36	0.695	36.249***	5.70***

Appendix 8: Mean sum of square for the various P forms and fractions

Source of variance	Degree of freedom	Bray P1	Water soluble-P	Organic P	Ca-P	Pi value
Treatments (Trts)	9	29187***	8639621***	1351***	3307790***	3.291E+07***
Sampling date (SD)	4	106472***	4713863***	525***	3881199***	9.645E+07***
SD*Trts interaction	36	3755***	268635***	161***	329111***	6213417***

Appendix 9: Annova Table: Mean sum of square for temperature of phospho-compost samples

Source of variance	Degree of freedom	Week 2	Week4	Week 6	Week 8	Week10	Week 12	Week 14	Week 16
Temperature source	3	0.695	5.085	2.709	1.0343	9.304	4.9169	1.7483	1.8749
Treatments	9	179.44***	141.722***	120.307***	87.9278***	179.174***	27.8385***	12.1078***	24.5045***
Error	27	8.509	3.011	1.082	1.2787	7.473	1.1358	0.7812	0.9127