RAPID BIOMAX THERMOPHILIC COMPOSTING EFFECTS ON QUALITY, NUTRIENT RELEASE AND FERTILISER VALUE OF CHICKEN LITTER COMPOSTS

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ABSTRACT

Large accumulation of organic waste produced from intensive animal production systems pose challenges for disposal and direct land application of these materials adversely affect the environment. Composting aids to reduce waste volume and produce a stable product, rich in nutrients, that is valuable for soil fertility improvement. The Biomax system has been developed as a system to rapidly compost organic wastes at 70-80 ^{o}C in a 24 h period. The stability, quality and fertiliser value of the compost needs to be understood. Considering the high temperatures involved it would be essential to establish whether or not the addition of the enzyme is entirely necessary in the process. The objectives of this study was to determine effects of the Biomax composting time and enzyme addition on chicken litter compost stability, quality, nutrient release, in soils, and dry matter yield and nutrient uptake of spinach. Biomax composts were produced from mixtures of chicken litter and other organic wastes with (W) and without (N) the BM1 enzyme. Compost samples were collected after 1, 6, 12, 18 and 24 h of composting, and analysed for pH, EC, total C, N, and P, exchangeable bases, trace elements, fulvic and humic acids and *Escherichia coli* and *Salmonella* spp. An incubation study was carried out with final composts (after 24 h of composting) applied to soil at 0, 1, 2 and 3 % (w/w) and destructive sampling was done after 0, 7, 14, 28, 42 and 56 days of incubation. The samples were analysed for pH, mineral N, available P, and bases. A glasshouse experiment was also conducted using the final compost produced with the BM1 enzyme. The compost was applied as the nitrogen source to 3 kg soil at 0, 2.5, 5, 10 and 20 t/ha and spinach *(Spinacia oleracea)* grown for eight weeks.

The pH of compost with the BM1 enzyme decreased with composting time while the one without the enzyme increased between 1 and 12 h. Total C, electrical conductivity (EC), carbon to nitrogen ratio (C:N) and humification ratio (HR) were not affected by composting time for both composts. Total N increased up to 18 h of composting and became constant afterwards for both composts. Pathogenic organisms *E. coli* and *Salmonella* species were not detectable in all composts irrespective of composting time. In the incubation study NH_4-N levels initially were similar statistically for all rates of both composts, except for soil treated with 1% of compost with the enzyme, which had lower NH_4-N than that amended with 3% of the compost without the enzyme. Levels of soil NO_3-N showed rapid increase in all treatments including control between 14 and 28 days of incubation and remained constant thereafter. The amount of available P was higher in soil treated with 3 % of both composts. There was no differences in spinach tissue nitrogen concentrations among the different application rates of Biomax compost. Spinach dry matter yield and N uptake improved with addition of compost. The findings of this study implied that the Biomax system is not effective in stabilising chicken litter into compost but it effectively sterilizes the organic waste materials and that the resultant composts rapidly release nutrients at sufficient rates to improve dry matter yield and nutrient uptake of spinach.

Keywords: Biomax compost, mineralisation , nutrient composition, plant nutrient uptake, spinach (*Spinacia oleracea*).

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CHAPTER 1

GENERAL INTRODUCTION

Global increases in population, and the associated need for more food including milk, eggs and meat, have resulted in production of large quantities of organic wastes in intensive animal production systems, designed to meet such increase in demand (Adeoye *et al.*, 2014). For many decades landfill has been used as one of the technique of disposal of organic wastes in many developing countries, including South Africa (Taiwo, 2011; Strategy National Organic Waste Composting, 2013). However, organic waste degradation at landfills could result in leachates that contain high levels of nutrients, heavy metals and toxins; a major environmental concern. The pollutants may leach into groundwater resources thereby posing health risk to animals and humans (Gao *et al.*, 2010a; Taiwo, 2011). While waste disposal has proved to be a major challenge in these systems, there is need for greater quantities and variety of source of plant nutrients in order to meet the increased requirements for feed (for these production systems), and food and fibre for humans (Atiyeh *et al.*, 2000).

Organic wastes from animal production systems contain large quantities of nutrients and could contribute as organic fertilisers. For example, on average fresh poultry manure contains about 3-5% nitrogen, 1.5-3.5 % phosphorus and 1.5-3.0 % potassium and considerable amount of micro-nutrients (Amanullah *et al.*, 2010). However, land application of fresh wastes for extended periods may pollute air, soil and water, and pose human health risks. Direct application of the wastes, as fertiliser, is limited by their instability, which leads to odours and losses, through gaseous emissions, of nitrogen (NH_3), carbon (CO_2; CH_4) and sulphur (H_2S) (Atiyeh *et al.*, 2000; Khalil *et al.*, 2005; Petersen et *al.*, 2007). The ammonia volatized from manure pollutes the air by the peculiar odour (Tanksley & Martin, 2003). The fresh wastes could also contain large populations of pathogenic organisms like *Escherichia* *coli* (E. coli) and *Salmonella* spp (Kenyangi & Blok, 2013), which pose risks to human health, including haemorrhagic diarrhoea from infection with *E. coli* (Sobsey *et al.,* 2001; Atwill *et al.,* 2012). Therefore, pre-treatment can be a viable strategy for management of these organic wastes (Castillo *et al.,* 2005). Composting has been widely accepted as a viable pre-treatment of organic wastes.

Composting is a biological process that is facilitated by microorganisms to break down organic waste materials into a more stable and useable product (Bernal *et al.,* 2009). Thermophilic composting has been widely used for stabilisation and sterilisation of organic wastes (Godley *et al.,* 2004), and to kill weed seeds, through promotion of microbial activity and the high (thermophilic) temperatures. While this strategy has been popular, the process takes a long-time (at least two months) and could result in nitrogen losses due to ammonification (Sweeten & Auvermann, 2008). In order to reduce these nitrogen losses, vermicomposting has been promoted and is currently being utilised but the disadvantages of this technique include losses of nutrients through leaching, although leachate can be collected. Furthermore the method takes a long time and does not eliminate pathogens unless a pre-thermophilic step is included (Misra *et al.,* 2003; Mupondi *et al.,* 2010). Development of rapid thermophilic composting could shorten the process and sterilise the products with minimal nutrient losses.

A Singapore-based company, Biomax, has developed a Rapid Thermophilic Digestion System that produces compost-like product within 24 h; greatly shorter than the over two months of normal thermophilic composting. The system operates at 70-80 ^{o}C with the aid of an enzyme, BM1 (identity not disclosed by the manufacturer), and the dependence of this system on high temperature appears to suggest that it is a shortened thermophilic composting process. The short composting time could minimise ammonia losses while high temperatures helps to get rid of pathogenic organisms. The Biomax composting technology is being introduced in South Africa on an experimental scale. It is essential to test its effectiveness in terms of stabilisation and sterilisation of organic wastes produced in intensive animal production systems in South Africa.

While testing its effectiveness, it could be important to determine whether the time could be shortened further, while achieving similar results, considering the electricity costs for maintaining 70-80 ^{o}C for 24 h. The need to use the BM1 enzyme in each “composting” cycle suggests that the user of the technology depends heavily on the supplier for the enzyme in the long term, with major cost implications. Considering the high temperatures involved it would be essential to establish whether or not the addition of the enzyme is entirely necessary in the process. The value of the compost produced will depend on its release of the nutrients.

While chicken manure compost is known as a good source of macro and micro nutrients (Qureshi *et al.*, 2014), it is essential to investigate nutrient release patterns and effectiveness, as fertiliser, of the Biomax composts. The general objective of this study was to evaluate the effects of the Biomax composting system on quality, nutrient release and fertiliser value of chicken litter composts.

The specific objectives were:

(a) To determine the effect of process time of Biomax composting on compost stability, nutrient composition and pathogen composition.
(b) To determine the effect of enzyme addition on compost quality parameters.
(c) To determine the nutrient release patterns of the Biomax composts in soils.

(d) To determine the effects of the Biomax compost on spinach plant growth and biomass yield.

To achieve the stated objectives the following hypotheses were tested:

(a) The Biomax composting time will increase the stability, nutrient composition and reduce pathogen composition within the 24 h cycle.

(b) The addition of the BM1 enzyme improves compost quality parameters.

(c) Application of Biomax compost with enzyme will improve release of nutrients compared to compost without enzyme.

(d) Biomax compost addition will increase spinach growth, dry matter and nutrient uptake.

CHAPTER 2

THERMOPHILIC COMPOSTING OF ORGANIC WASTES FROM INTENSIVE ANIMAL PRODUCTION SYSTEMS AND FERTILISER VALUE OF COMPOSTS: A REVIEW

2.1 Introduction

Animal manure are natural organic materials that are ubiquitous in large quantities where intensive animal production is practised. Disposal of these materials is a major problem worldwide because of the negative effect on the environment (Petersen *et al.,* 2007). On the other hand these materials are known as rich source of nutrients required by crops (Whitemore, 2007). As a result, for many decades they have been used and applied directly to soil as agriculture amendment, with benefits that they improve soil physiochemical properties and supplying nutrients for plant growth (Westermen & Bicudo, 2005). However limitation of using raw animal manure as fertilizer amendment is that they encompass large amounts of pathogen organisms especially chicken litter manure which can pose a risk to environment and human health (Chen & Jiang, 2014). Hence Gao *et al.,* (2010a), postulated the pre-treatment of raw organic manure prior to its application to soil through composting technique which is actually recommended to breakdown the life cycle of pathogens.

2.2 Organic waste production and management in intensive animal production systems

Intensive animal production is the prevalent practise adopted worldwide nowadays due to an ever increasing human population that demands animal products such as meat, eggs and milk (Laguë & Eng, 2000; Adeoye *et al.,* 2014). In attempting to meet the demands, large amounts of animal waste are produced (Bolan *et al.,* 2010). The build-up of these organic waste materials pose a disposal risk and safety to the environment unless there are viable strategies to be used which are environment friendly and cost-effective (Hooda *et al,* 2000; Westerman & Bicudo, 2005; Adeoye *et al.,* 2014). Furthermore organic wastes produced from animal production varies in terms of handling and nutrient content (Chastain *et al.,* 2001). According to Lorimor (2000), Moreki & Keaikitse (2013) and Yardimci (2013), there are three forms of organic wastes produced from animal production systems which are solid, semi-solid and liquid form depending on handling. The solid form is the raw manure without addition of water while semi-solid is the raw manure that is has with bedding material or litter added whereas liquid form is the raw manure flashed with water from the animal houses. Dairy and piggery organic wastes are the ones that have been produced in all forms compared to poultry manure which can only be produced in solid and semi solid forms (Yardimci, 2013).

These organic waste materials is that they contain essential nutrients that are required for plant growth which are nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), manganese (Mn), copper (Cu), zinc (Zn), boron (B), iron (Fe), and molybdenum (Mo) and some heavy metals (Van Horn *et al*., 1994; Chastain *et al.,* 2001; Bolan *et al.,* 2010; Faridullah *et al.,* 2014). These nutrients originate from the feed, medication, supplements and water consumed by animals (Chastain *et al.,* 2001). Combs *et al.,* (2001) determined the nutrient elements found in solid organic manure of dairy, swine and poultry on a dry mass basis (Table 2.1).

All the studies that have been conducted to date conclude that animal organic wastes can be used for several purposes including use as fertilizer and soil amendment, energy recovery (heat, liquid, electricity), production of chemicals (volatile organic acids, ammonium products, alcohol) and animal feed (Salminen & Rintala, 2002; Davalos *et al.,* 2002; Westermen & Bicudo, 2005; Bolan *et al.,* 2010). However animal manure has been used frequently as fertilizer and soil amendment on agricultural lands due to its high nutritional value (Kelleher *et al.,* 2002; Westermen & Bicudo, 2005). On the other hand, there are a number of challenges associated with the utilization of raw organic manure on agricultural lands and management which adversely affect the environment, animals and humans (Martinez & Burton, 2003; Westerman & Bicudo, 2005).

Table 2.1: Total nutrient concentrations and trace elements determined in poultry, dairy and swine solid manure on dry weight basis (Combs *at el.*, 2001)

Nutrients	Poultry	Dairy	Swine
N (%)	4.03	2.27	1.85
P (%)	1.70	0.56	1.60
K (%)	2.11	0.62	1.87
Ca (%)	5.49	1.62	2.03
Mg (%)	0.53	0.68	0.51
S (%)	0.51	0.27	0.41
Al (%)	0.18	0.13	1.48
Fe (%)	0.17	0.12	1.12
Na (%)	0.53	0.07	0.52
Zn (mg/kg)	328	90	608
B (mg/kg)	53	25	30
Mn (mg/kg)	419	163	844
Cu (mg/kg)	437	27	381
Se (mg/kg)	1.47	0.58	1.81
As (mg/kg)	20.7	0.29	7.7
Co (mg/kg)	1.7	0.80	4.7
Cr (mg/kg)	9.0	2.83	26.6

According to Van Horn *et al.* (1994); Hooda *et al.* (2000); Oenema *et al.* (2007), poor management and over-application of raw organic animal wastes on agricultural lands produces odours gases to the atmosphere that can cause respiratory diseases in animals and humans. The losses of nutrient through sub-surface drainage to groundwater thereby affecting water quality. Furthermore land application of organic manure as fertilizer releases greenhouse gases such as carbon dioxide, methane and nitrous oxides, which are implicated in ozone depletion and global warming (Davalos *et al.*, 2002; Bolan *et al.*, 2010). Martinez & Burton (2003), summarized the forms of mineral N (including gases), phosphate and methane from manures produced by intensive animal production systems and their effects on the environment (Table 2.2).

Table 2.2: List of current concerns with the use of livestock manure and impacts of their degree on the environment (Martinez & Burton, 2003).

Environmental Concern/issue	Environmental and other impacts	Scale of agricultural contribution	Scale of impact
Nitrate (NO_3^-)	Water quality Eutrophication Health Economic loss to farmers cost of removal	Major source	Local: on-farm surface waters. Regional: surface waters; catchment; aquifers National/international: maritime waters
Nitrite (NO_2^-)	Water quality fish stocks and health	Major source	Local: on-farm surface waters. Regional: surface waters and wells.
Ammonia (NH_3)	"Acid rain" acidification of soils eutrophication of natural systems Direct toxicity	Major source (>85%)	Local: on-farm deposition. Regional: deposition on natural ecosystems National/international : cross boundary transfer of NH_3 and deposition
Nitrous oxide (N_2O)	Greenhouse gas global warming Ozone interactions	Substantial (likely to increase in importance as other sources decrease)	Global
Nitric oxide (NO)	Tropospheric ozone precursor	"minor"?	Global
Phosphorus (P)	Water quality eutrophication Health toxins from algal bloom Economic cost of removal	Substantial – increasing as industrial point sources decrease	Local: on-farm surface waters Regional: surface waters, catchments National/international: maritime waters (cross boundary transfer)
Methane (CH_4)	Greenhouse gas Global warming	Substantial	Global

Furthermore Irshad *et al.* (2013), postulated that land application of raw animal manure may lead to the immobilization of plant nutrients and results in phytotoxicity due to inadequate decomposition of organic matter. A considerable body of literature exists regarding viable management strategies to stabilize livestock manure, before their use and disposal to minimize their negative impact on the environment. For example, vermicompost, anaerobic digestion, and composting strategies could be used (Westerman & Bicudo, 2005; Irshad *et al.*, 2013; Yardimci, 2013; Nayak & Kalamdhad, 2014). Among the management techniques, composting has become the most preferred method due to its economic and environmental benefits (Bernal *et al.,* 2009). Furthermore the method aids in reducing bulk volumes of animal manure thereby eliminating pathogens, parasites and weeds seeds during the process leading to a finer stable and matured end product called compost (Tam & Tiquia, 1999; Bernal *et al.,* 2009; Irshad *et al.,* 2013; Martinez-Blanco *et al*., 2013).

A number of studies have shown that the use of compost as organic fertilizer on agriculture lands may improve soil health and enhance nutrient availability for plant growth (Martinez-Blanco *et al*., 2013). Cooperband (2002) & Martinez-Blanco *et al*. (2013), reported that the benefits associated with the addition of compost include soil structure improvement, promotion of soil water retention capacity, an increase in soil fertility and cation exchange capacity and enhanced soil microbial activity.

2.3 Thermophilic composting

Composting is defined as a natural process where the decomposition of organic waste materials is carried out under controlled aerobic conditions by various microorganisms into a valuable end product free of pathogens and weeds called compost (Tuomela *et al*., 2000; Cooperband, 2002; Bernal *et al*., 2009; Hubbe *et al*., 2010; Chen *et al*., 2011). Sweeten & Auvermann (2008), reported that during composting process microorganism’s breakdown organic materials and generate heat, carbon dioxide and water (Figure 2.1).

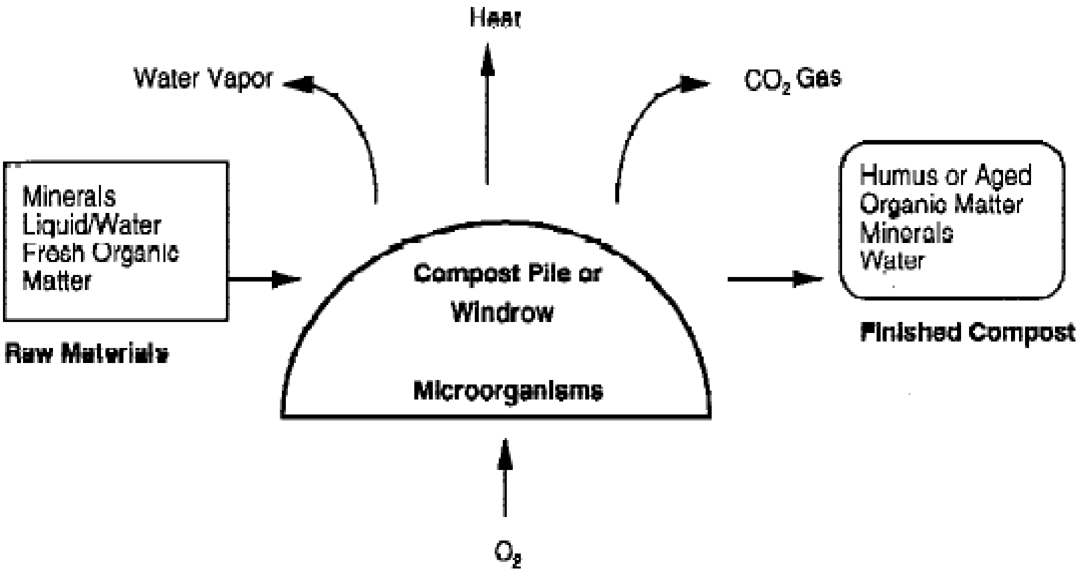

Figure 2.1: Aerobic thermophilic composting process (Pace *et al.*, 1995).

The effectiveness of the process depends upon various parameters present within the composting system (Liang *et al.*, 2003). Bernal *et al* (2009) categorised factors that significantly affect composting performance into two groups: those that depend on formulation of composting mixture such as C/N ratio, pH, particle size and porosity; and those dependent on composting process management which includes O_2 amounts, water content and temperature.

2.3.1 C/N ratio

The carbon to nitrogen ratio of organic materials is the most considered parameter when formulating composting mixture since microorganisms utilize carbon as the source of energy and ingest nitrogen for protein synthesis during degradation of organic materials (Bernal *et al.*, 2009; Hubbe *et al.*, 2010). The proportion of C/N required by microbe's averages about 30 parts carbon and 1 part nitrogen (Bernal *et al.*, 2009; Chen *et al.*, 2011). According to Tuomela *et al*. (2000), C/N values vary depending on the substrate and suggested an optimum value of 25-40. Haung *et al.* (2004), mentioned that C/N at 25-30 is considered the optimum ratio for composting. Cooperband (2002), mentioned that as a general rule if C/N is lower than 20:1, the microbes will use all the N for their own metabolic needs therefore proposed C/N ratio range from 25:1 to 35:1 as ideal for starting. As noted by Bernal *et al.* (2009); Gao *et al*. (2010b) & Chen *et al*. (2011), if the C/N ratio is too high, it means there is too little nitrogen therefore decomposition process slows. On the other side too low C/N ratio means there is excess of nitrogen that can be possibly lost through volatilization and leaching from the composted mixture.

2.3.2 Organic material pH

Several groups of researchers have studied the effect of pH during composting of various organic materials (Sundberg *et al.,* 2004; Bernal *et al.,* 2009; Hubbe *et al.,* 2010). As noted by Chen *et al.* (2011), pH governs the growth and activity of microorganisms and fate of nitrogen during composting. Whereas Bernal *et al.* (2009) suggested pH ranging between 5.5 and 8.0 to be optimum for microorganisms, Chen *et al.* (2011) suggested that the optimum pH values were around 6.0 to 7.5 and 5.0 to 8.0 for bacteria and fungi, respectively. In general terms, the pH decreases below 6.0 during the initial stages of composting due the release of organic acids and thereafter increases to 9.0 in the thermophilic stage of composting as a result of ammonia production (Sundberg *et al.,* 2004; Sweeten & Auvermann, 2008; Hubbe *et al.,* 2010;). Tuomela *et al.* (2000), reviewed related literature and found that the end product of composting always has a neutral pH value.

2.3.3 Particle size and porosity

The particle size of organic materials matters when formulating the composting mixture since they form surface area for functioning of the microorganisms and adequate pores for aeration (Bernal *et al*., 2009; Hubbe *et al.*, 2010). As reported by Hubbe *et al*. (2010), the reduction of the organic materials particle size helps in accelerating the decomposition process. Therefore, larger particle sizes of organic materials reduces the surface area thereby reduces the rate of decomposition (Bernal *et al.,* 2009). Conversely the smaller the particle sizes of the composted materials, the greater the rate of the decomposition (Pace *et al.,* 1995; Bernal *et al.,* 2009). Hubbe *et al.* (2010) reported that the recommended particle sizes should be between 2.5 and 7.2 cm. Porosity relates to the empty pores in the composting mass and Bernal *et al.* (2009) proposed that air filled pores of composting pile should be in a range of 35-50 %.

2.3.4 Oxygen concentrations

Thermophilic composting is the process that is facilitated by microorganisms that are aerobic and require oxygen to survive (Pace *et al.,* 1995; Liang *et al.,* 2003; Hubbe *et al.,* 2010). Chen *et al.* (2011) stated that microbes utilize oxygen during composting as they oxidize carbon for energy to produce CO_2. The oxygen is supplied by introducing air into the composting mass through air forced system or by turning the pile mechanically (Pace *et al.,* 1995; Sweeten & Auvermann, 2008). Inadequate supply of oxygen will turn the composting process into anaerobic condition thereby slowing decomposition of organic matter and generating odours (Pace *et al.,* 1995; Hubbe *et al.,* 2010; Chen *et al.,* 2011). According to Bernal *et al.* (2009) optimum oxygen concentration required for accomplishing good composting ranges between 15 and 20 %.

2.3.5 Moisture Content

The moisture content of the composting mass is necessary since it supports the metabolism processes of microbes (Pace *et al.,* 1995; Liang *et al.,* 2003). The water content of organic materials combined when formulating the compost pile varies (Bernal *et al.,* 2009; Chen *et al.,* 2011). Therefore, to accomplish the best compost mixture, moisture levels should be optimized (Hubbe *et al.,* 2010). Pace *et al.* (1995), stated that the optimum moisture levels should range between 40 to 65 % for a composting mixture whereas Liang *et al* (2003), Bernal *et al.* (2009) and Chen *et al.* (2011), later suggested a 50 to 60 % moisture content . Low moisture levels (i.e. below 40 % of the compost mass) affects microbial activity and slows down the decomposition of organic matter (Pace *et al.,* 1995; Liang *et al.,* 2003; Sweeten & Auvermann, 2008). If the moisture content in the compost mixture is too high (i.e. above 60 %), the water will displace the air in the pore spaces of the composting materials thereby creating anaerobic conditions (Liang *et al.,* 2003; Sweeten & Auvermann, 2008; Bernal *et al.,* 2009; Chen *et al,* 2011). Sweeten & Auvermann (2008), stated that excessive moisture lowers the temperature thereby prolonging compost stability and maturity.

2.3.6 Temperature

Temperature is considered as one of the important parameter that affects the composting process (Liang *et al.,* 2003). During the process, microbes breakdown organic materials by generating energy in the form of heat (Cooperband, 2002; Bernal *et al.,* 2009). The accumulation of this heat possibly increases the temperature of the composted mixture (Bernal *et al.,* 2009). The increase in temperature during the composting process goes through a number of specific phases (Tuomela *et al.,* 2000; Cooperband, 2002; Liang *et al.,* 2003). The initial phase is mesophilic, this stage normally starts off at ambient temperature (10-40 ^{0}C) where microbes consumes the easily degradable compounds thereafter it increases rapidly (Tuomela *et al.,* 2000; Chen *et al,* 2011; Bernal *et al.,* 2009). As the temperature increases thermophilic microorganisms colonize the mixture at temperatures above 40 ^{0}C (Chen *et al.,* 2011) and within 24 to 72 hours the temperature in the compost mixture will increase to 54 to 65 ^{0}C and maintained there for several days or weeks depending of the type of feedstock (Cooperband, 2002; Chen *et al,* 2011). These thermophilic temperatures are suitable to kill pathogen organisms, weed seeds and fly larves (Pace *et al.,* 1995; Cooperband 2002; Bernal *et al.,* 2009). Bernal *et al.* (2009), reported that optimum temperature should range between 40-65 ^{0}C for the composting process while Liang *et al.*

(2003), reported optimum temperature range of 52-60 ^{0}C for composting. Furthermore Liang *et al.* (2003), mentioned that if the temperature reaches or exceeds about 82 ^{0}C the microbial activity will be adversely inhibited while Chen *et al.* (2011), stated that temperatures above 71 ^{0}C inhibit microbial activity. After the thermophilic phase, microorganisms are left with less easily degradable materials to decompose therefore microbial activity and temperature decrease and the compost mixture is recolonized by mesophilic microorganisms (Bernal *et al.,* 2009; Chen *et al,* 2011). This phase is called curing, and is important for the stability and maturity of the compost (Pace *et al.,* 1995; Cooperband, 2002; Sweeten & Auvermann, 2008).

2.3.7 Microorganisms and Inoculation

Thermophilic composting is the process that is entirely facilitated by microorganisms (Bernal *et al.*, 2009). During the process, various types of microorganisms rise and fall in succession with the change in temperature (Chen *et al.*, 2011). As mentioned by Bernal *et al*. (2009), bacterial organisms are usually predominant at early stage of composting while fungi exists throughout the process but are inhibited at moisture content below 35 % and temperature above 60 ^{0}C. In addition, at the curing stage actinomycetes and fungi are the most predominant organisms degrading the remaining resistant polymers. Del Carmen *et al.* (2006), suggested that certain microbial inoculation can be used to enhance the composting process, although microbesare present naturally in the organic wastes. Furthermore Ltibke (2000) and Del Carmen *et al.* (2006), stated that microbial inoculant products are known to function best in breaking down complex particles into small particles with hi-speed during composting and enhancing characteristics of the end product. In a study conducted by Zhou *et al.* (2015), inoculation enhanced temperature of the pile and degradation of lignocelluloses when they added three different microbial inoculum during co-composting process of dairy manure and rice straw.

2.3.8 Time

According to Hubbe *et al.* (2010), time can be used as an independent factor to determine when the compost is ready. The length of the time required for composting depends on the parameters listed above (C/N, moisture, temperature, oxygen amounts, particle size and porosity and pH), feedstock, composting method and management (Pace *et al.,* 1995; Cooperband, 2002). Sweeten & Auvermann (2008), reported that the minimum composting time when using turned windrow method is 1 month followed by at least 2 months of the curing phase after which the compost is ready for use. Goyal *et al.* (2005), composted different organic wastes for period a of 90 days using windrow mechanical aeration method. In addition Tognetti *et al.* (2007), composted municipal organic wastes for about 130 days using the static pile method, whereas Raj & Antil (2011), composted agro-industrial wastes over a period of 150 days using windrow mechanical aeration method. Therefore it is on the basis of the length of thermophilic composting that new technological approaches are desirable to shorten the time of composting.

2.4 Compost quality

For compost to be utilized as amendment in agricultural soils, an assessment of stability or maturity of the material is desirable (Bernal *et al.,* 2009; Hubbe *et al.,* 2010; Chowdhury *et al.,* 2013). Stability is often defined as stable amounts of organic matter whereas maturity refers to compost material with free phytotoxic compounds and pathogens organisms (Cooperband *et al.,* 2003; Chowdhury *et al.,* 2013). Nonetheless Bernal *et al.* (2009) and Chowdhury *et al.* (2013), mentioned that the processes of stability and maturity go hand-inhand normally since phytotoxic compounds are created by microorganisms in unstable compost. A number of authors have suggested a suite of parameters including threshold values to be used to assess stability and maturity of compost (Cooperband *et al.*, 2003; Bernal *et al.*, 2009; Kristine & McCartney, 2010; Chowdhury *et al.*, 2013). Carbon and nitrogen ratio in solid phase, cation exchange capacity (CEC), pH, electrical conductivity (EC), mineral nitrogen, organic matter humification and temperature have been the most used chemical parameters (Cooperband *et al.*, 2003; Gao *et al.*, 2010b; Kristine & McCartney, 2010; Raj & Antil, 2011; Chowdhury *et al.*, 2013). In general, the C:N ratio decreases as the composting process progresses due to losses of carbon in the form of carbon dioxide with ultimate compost stabilization (Kristine & McCartney, 2010; Raj & Antil, 2011). Different C:N ratio threshold values have been proposed in literature, for example Bernai *et al*. (1998), proposed that C:N ratio of compost of less than 12 may be the indication that it is suitable to be added to soil whereas Goyal *et al*. (2005), stated C:N ratio ranges between 15-20 as the indication of a matured compost. In addition Raj & Antil (2011), mentioned that a threshold value of C:N ratio below 20 is a good indication of a matured compost but C:N ratio less than 15 is more preferable. The cation exchange capacity tends to increase during composting as the organic materials are decomposed by microorganisms producing carboxyl and phenolic groups (Chowdhury *et al.*, 2013). Furthermore CEC has been frequently used as one of the maturity indices (Kuo *et al.*, 2004; Bernal *et al.*, 2009; Chaudhry *et al.*, 2013). The CEC threshold (optimum) value of 67 meq/100 g has been used to evaluate degree of compost maturity produced from city refuse (Bernal *et al.* 1998; Chowdhury *et al.*, 2013). However Bernal *et al.* (2009), argued that the value cannot be considered for composts produced from animal manure, since the limit (67 meq/100 g) can be reached at preliminary stages of the compost preparation. The pH of the composting end product has been considered as one of the parameters that indicates stability and maturity (Bernal *et al*., 1998; Cooperband *et al.*, 2003; Bernal *et al.*, 2009). Chowdhury (2013) and Kristine & McCartney (2010), postulated that compost pH that is between neutral ranges may be a good indication of maturity therefore suitable for agricultural application. Electrical conductivity (EC) is the amount of soluble salts in the compost product (Gao *et al.,* 2010b; Kristine & McCartney, 2010). It has been used to evaluate compost stability and maturity (Cooperband *et al.,* 2003; Bernal *et al.,* 2009). According to Gao *et al*. (2010b), the suggested EC stable limit value is 3000 µs cm^{-1} whereas Chowdhury. (2013), mentioned an optimum EC value of 4000 µs/cm. Inorganic forms of nitrogen (NH_4-N; NO_3-N) have been used as criteria to evaluate the compost stability and quality (Sanchez-Menedero *et al.,* 2001; Bernal *et al.,* 2009; Kristine & McCartney, 2010). As noted by Sanchez-Menedero *et al.* (2001) and Bernal *et al.* (2009), compost with a high content of NH_4-N above 400 mg/ kg shows immaturity and according to Bernal *et al.,* 1998; Gao *et al.* (2010b) and Kristine & McCartney (2010), the limit value of 400 mg/ kg suggests that the compost is stable and matured. Furthermore among the maturity indices ammonium and nitrate ratio has been considered as one of the parameters (Cooperband *et al.,* 2003; Kuo *et al.,* 2004). A limit value of 0.16 % was established by Sanchez-Menedero *et al.* (2001); Bernal *et al.* (2009) and Kristine & McCartney (2010), for NH_4:NO_3 ratio as the indication of a stable and matured compost of different kinds. The maturity of compost can be assessed by the degree of OM humification (Bernal *et al.,* 2009). During composting amounts of humic acids (HA), humification index (HI) and ratio of humic to fulvic acids increase as a result of decrease in fulvic acids (FA), showing humification of organic matter (Benito *et al.,* 2003; Kristine & McCartney 2010; Raj & Antil, 2011). Bernal *et al.* (1998) and Raj & Antil (2011), reported that compost with an HA:FA ratio above 1.9 value which has been proposed for the city refuse and sludge compost is the indication of maturity. According to Bernal *et al*. (2009), amounts of FA $\leq$ 12.5 g/kg and humification ratio (HR) $\geq$ 6.0 were suggested as maturity indices for a manure compost produced from different organic wastes. Raj & Antil (2011), mentioned that HI increased with composting time and proposed HI> 30 % as the maturity index for compost prepared from different farms and agro-industrial wastes. However, a number of authors argue that humification parameters cannot be reliably used as indication of compost maturity (Benito *et al.,* 2003; Bernal *et al*. 2009; Kristine & McCartney 2010), due to the fact that the initial organic materials used when formulating composting affects final values of humification parameters used for maturity index (Bernal *et al.,* 2009; Kristine & McCartney, 2010). As noted by Cooperband *et al.* (2003), temperature is one of the parameters that have been used to assess the stability and maturity of compost. Bernal *et al.* (2009), stated that temperature above 55 ^{0}C is suitable for killing pathogenic organisms and according to Raj & Antil (2011), temperature indicates a good degree of compost stability during thermophilic composting when approaching the ambient level.

2.5 Nutrient release from compost added to soil

Stabilized organic composts have been used as fertilizer in agricultural soil due to their ability to improve soil health (Arslan *et al.,* 2008; Canali *et al.,* 2011).The benefits of adding composts to soil includes the improvement of soil aggregate, structure and fertility, increasing microorganisms population, enhancing water holding capacity and cation exchange capacity of the soil (Arslan *et al.*, 2008; Angelova *et al.*, 2013). However nutrient release from organic fertilizer varies when added to soil due to properties of the original source and characteristics of the soil (Canali *et al.,* 2011). A number of authors have studied the dynamics of nutrient release of various composts under laboratory incubation experiment (Gagnon & Simard, 1999; Heenkende & Parama, 2010; Barral *et al.,* 2011). Preush *et al.* (2002), determined the availability of N and P in composted and uncomposted poultry litter mixed with sandy loam soil through incubation for about 120 days and observed low concentrations of NH_4-N (1 to 2 mg/kg) throughout the study. This was due to a stable compost product that was more resistant to nutrient release. Moreover there was no significant effects on the soil NO_3-N amounts for the duration of the incubation period. Burgos *et al.* (2006), observed high amounts of NH_4-N (38.2 mg/kg) at week 0 when the municipal solid waste compost was blended with sandy soil for an incubation period of 40 weeks. The NH_4-N content decreased for 6 weeks after which it remained constant while the NO_3-N amounts increased until the end of incubation, this was due to the nitrification process. Furthermore Ebid *et al.* (2007), studied nitrogen mineralization in soil amended with composted tea leaves, coffee wastes and kitchen garbage and incubated for a period of 63 days. They observed the highest NH_4-N concentrations during the 21 days of incubation due to ammonification, thereafter the NH_4-N content decreased gradually after 42 days in all composted mixtures as a result of N immobilization. On the other hand, the composted kitchen garbage had the highest amounts of NO_3-N during the first 7 days while the other composted mixtures increased in similar trend as time lapsed, the increase in NO_3-N corresponded with decrease in NH_4-N, thereby indicating a rapid conversion of NH_4-N to NO_3-N by nitrifying bacteria. In a study conducted by Ebid *et al.* (2007), P release patterns were not identical in composted tea leaves, coffee waste and kitchen garbage during a incubation. The wide variations was explained by immobilization of P by soil microbes and compost materials. Leconte *et al.* (2011) studied available P when sandy soil was blended with poultry manures compost separated at different particle size and incubated for a period of 16 weeks. They observed that poultry compost with 1 to 5 and 5 to 10 mm fractions released high amounts of mineral P (76-185 mg/kg) at week 0 and (86-340 mg/kg) at week 16 due to the mineralization of P during the incubation period. Barral *et al.* (2011) observed adsorption of P when the municipal solid waste compost was added to schist and granite soils and incubated for a period of 90 days. This was attributed to fixation and immobilization of P by soil microorganisms. Furthermore, Ebid *et al*. (2007) concluded that the release patterns of K, Mg and Ca were not the same during the incubation study of soil mixed with organic composts. The wide inconsistency in nutrient release of exchangeable bases was related to the immobilization of K, Mg and Ca by compost materials. In contrast, Barral *et al.* (2011) observed an increase in amounts of soil exchangeable bases (K, Ca & Mg) when municipal solid waste compost was blended with schist and granite soil 90-day incubation study. The release of K, Ca and Mg were approximately 150, 800 and 30 mg/kg, respectively, and this was the results of compost addition which had high concentrations of these elements. Shivay *et al.* (2010) studied nutrient release of micro-elements (Cu, Zn and Mn) when rock mineral flour and city wastes compost were added to quartz sand under incubation period of 140 days. The results showed the release of Cu and Mn during the first 21 days of incubation due to compost application effect afterwards remain constant until the end of the period which was the indication of adsorption and redox reaction condition in the medium. On the contrary, soil Zn amounts were negative throughout the incubation which was an indication of the adsorption of Zn from the city waste compost.

2.6 Effect of compost application as organic fertilizer on crop growth and nutrient uptake

The use of organic compost as fertilizer has been adopted and promoted by agronomists as the remedy to enhance poor soil fertility and crop production (Indriyati, 2014). The effect of various organic fertilizers on growth and yield of different crops have been studied by several authors (Ibrahim *et al.,* 2008; Asgharipour & Rafiei, 2011; Indriyati, 2014; Korai *et al.,* 2014; Iqbal *et al.,* 2014) under greenhouse experiment. Ibrahim *et al.,* (2008), observed a significant increase in oven dry matter yield of wheat crop with a minimum value of 19.57 g/pot when various levels of compost were added whereas Asgharipour & Rafiei (2011), reported dry weight yield value (19 g/pot) of basil crop when commercial city refuse compost was used over control. In a study conducted by Indriyati (2014), the komatsuna crop showed higher values of dry weight yield (51.27, 91.13 and 105 g/pot) when it was planted in soils containing residues of chicken manure composts with different content of N. On other hand, Korai *et al.* (2014), observed that maize crops gave higher dry matter yields that coincided with an increase in N, P and K plant uptake when sugarcane press-mud biocompost was applied due to sufficient mineralisation of nutrients by microorganisms into soil solution for plant uptake. Similarly Shu (2005), observed a significant increase in nutrient uptake of N, P and K in rice plant when pea-rice hull compost was used compared to the cattle dung-tea compost and chemical fertilizer under greenhouse experiment. As noted by Leytem *et al*. (2011), most published studies have shown less focus on the effect of compost manure on the uptake of Ca, Mg and trace elements such as Fe, Mn and Zn. In a study conducted by Malik *et al.* (2013), wheat crop showed an increase in nutrient uptake of plant tissue when different organic amendments were used including biogenic waste compost compared to control soil. Furthermore, Leytem *et al*. (2011) observed a decrease in Ca, Mg and Mn plant tissue concentrations of silage corn when composted dairy manure was applied as amendment, whereas Zn amounts showed an increase with increasing compost rate. On the contrary in a study conducted by Iqbal *et al* (2014), the addition of P enriched compost on soil and maize crop systems showed a higher increase on plant tissue and soil residual amounts of micronutrients (Cu, Zn, Fe and Mn) with an increase in compost rate. Korai *et al.* (2014) observed a significant increase in soil residual organic matter, available P and N compared with the control after the addition of biocompost. In a study conducted by Duong *et al.* (2012), soil organic matter, electrical conductivity, available P and N showed significant increases after application of six compost types with different texture. Both Duong *et al.* (2011) and Korai *et al.* (2014), observed no effects of compost on soil pH. Moreover, Demir & Gulser (2015) observed low values of exchangeable Ca compared to the control soil after application of rice husk compost whereas exchangeable Mg concentrations increased with increasing compost rate over the control.

2.7 Potential of Biomax technology for composting poultry in South Africa

In South Africa, poultry production includes meat and eggs produce but broilers production have been fast growing over the past five decades because it is an affordable source of meat protein than all the other animal meats according the Department of Agriculture Fisheries & Forestry (Department of Agriculture Foresrty and Fisheries, 2012). Department of Agriculture Foresrty and Fisheries (2013) reported that in 2012 broiler meat production in South Africa was the largest segment at 17.6 % among other animal products that contributed 48.2%. The broiler meat production is practised throughout the provinces of South Africa, with KwaZulu-Natal being the fourth largest producer (Figure 2.2).

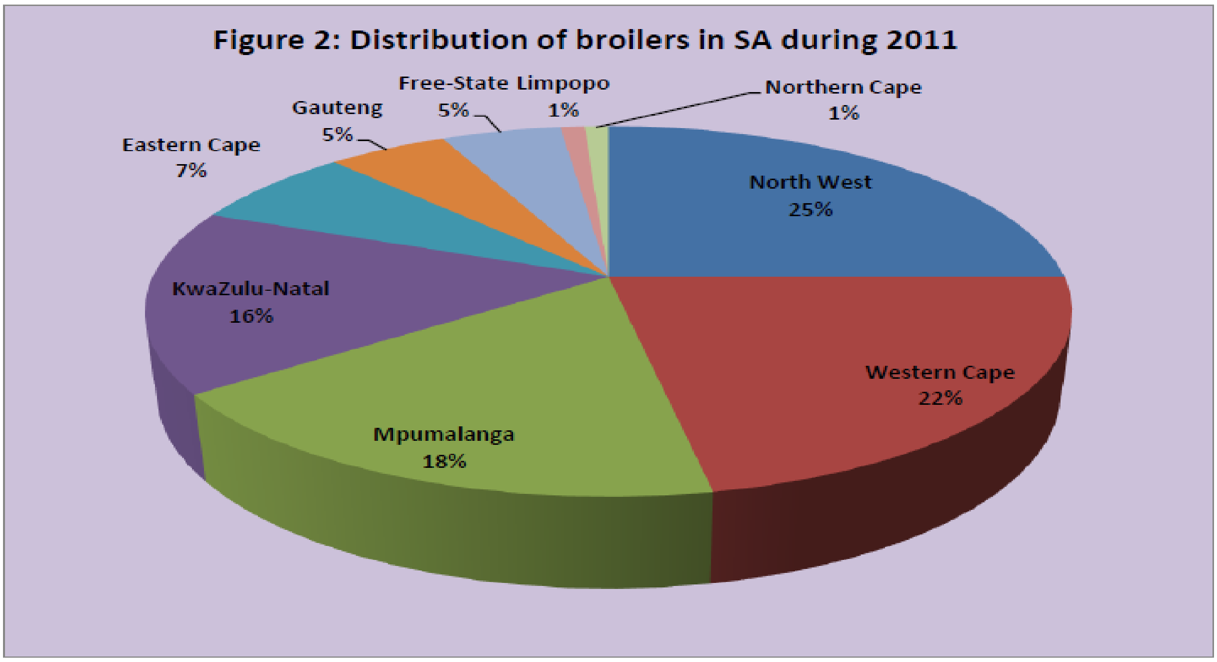

Figure 2.2 Statistics of broiler meat production in South Africa 2011 (Department of Agriculture Foresrty and Fisheries, 2012).

Malapo (2009), highlighted that the production of large organic waste material is one of the major challenges that poultry industries are facing in South Africa, and these material are known to have a negative impact on the environment. Therefore, recommended composting as disposal strategy. However since thermophilic composting process takes three or more months, Biomax Technologies company, in Singapore saw the need to shorten the process by designing a new technological system called Rapid Thermophilic Digestion technology (RTD) (Tong, n.d). The system functions with the BM1 enzyme, which is recommended to speed up the degradation process within a 24 h period. During the process, RTD mixes organic materials under conducive aerobic condition at temperature levels maintained between 70 to 80 ^{0}C inside the digester. After 24 hours the raw organic materials are considered fully decomposed into compost, which can be used as organic fertilizer (Tong, n.d). RGS Smith Drumnadrochit Farm, situated in KwaZulu-Natal, South Africa, purchased the system for the composting of their poultry litter. However the dynamics in composition of the chemical parameters and pathogens during RTD composting process are not clearly understood. Furthermore the nutrient release patterns and fertilizer value of the composts needs to be established.

2.8 Conclusion

The disposal of organic wastes coming from the intensive animal production system is a major challenge worldwide. Thermophilic composting has been used as the traditional technique to reduce and convert organic animal manure wastes into a usable product called compost. However the challenge about thermophilic composting process is the length of time it takes to produce compost. The Biomax technology has a potential to produce compost from organic wastes within a 24 h period, the changes in the chemical composition, stability and composition of pathogens of the product during the composting period, are not clearly understood. The nutrient release patterns and fertilizer value of the compost needs to be established.

CHAPTER 3

STABILISING AND STERILIZING EFFECTS OF BIOMAX COMPOSITING ON CHICKEN LITTER AND OTHER ORGANIC WASTES

3.1 Introduction

About 20 million tons of the waste generated in South Africa per annum is organic (Kasner, 2012). The organic wastes typically originate mainly from animal and plant sources and are therefore biodegradable (Strategy National Organic Waste Composting, 2013). Disposal and management of these organic wastes is the main problem worldwide. Traditionally organic materials have been directly applied on land as organic fertiliser since they contain nutrients that plants need. However usage of these raw organic materials is limited due to gaseous emission, odour smell, pathogen population and loss of nutrients and flies attraction (Bernal *et al.,* 2009; Amanullah *et al.,* 2010).

Major organic wastes produced in South Africa, with potential for use in agriculture include sewage sludge and organic chicken litter waste. Griessel (1979), stated that rate of chicken litter manure production in South Africa per annum was about 373 200 tons with possibility that it will increase to about 800 000 tons by the year 2000. Therefore there is a need to process these organic materials before they can be applied on land to prevent nutrient loss and reduce waste. Composting has been found as one of the best technique that can be utilized to reduce the volume of organic waste and convert it into more useable product rich in nutrients (Bernal *et al*., 2009; Gao *et al.,* 2010a; Amanullah *et al.,* 2010; Hubbe *et al.,* 2010). However the rapid accumulation of chicken litter manure suggests that the slow thermophilic composting may not be fast enough to process these organic wastes and the

Biomax composting technology that as showed to produce compost within 24 h has a good potential.

The RGS Smith Drumnadrochit Farm in the Midlands, KwaZulu-Natal, is involved in intensive poultry (chicken) production, including layers and broilers. These systems produce large amounts of chicken litter, and other organic wastes. The Biomax composting technology is being tested for its effectiveness for stabilising and sterilising the organic wastes to produce compost that could be used an organic fertilizer, in South Africa. Since the BM1 enzyme and electricity are among the major costs of the process, it is essential to test effects of composting time with or without the enzyme on the quality of the compost. The objectives of this study were to determine the effect of (a) Biomax composting time on chemical properties and composition of pathogens in chicken litter composts and (b) addition of the BM1 enzyme on compost quality parameters. The hypotheses were:

(a) The Biomax composting time will increase the stability, nutrient composition and reduce pathogen composition within the 24 h cycle.
(b) The addition of the BM1 enzyme will improve compost quality parameters.

3.2 Materials and Methods

3.2.1 Biomax composting

The chicken manure composts were produced using the Biomax Rapid Thermophilic Digestion technology at RGS Smith Drumnadrochit Farm in the Midlands, KwaZulu-Natal. A variety of organic materials were mixed and composted according to farmer practice. The organic materials consisted of chicken litter (42 bins), egg shells plus grass (4 bins), feed mill sweepings (2 bins), woodchips (8 bins), papers plus grass (1 bag) and reworks (2 wheelbarrows). Reworks were residual composts from the previous composting batches. The bins and bags were “50 kg” size. All the materials were integrated into a 4000 L Biomax Rapid Thermophilic Digester. Thereafter the machine was started to mix the inputs together.

About 1 kg of BM1 enzyme was split added during the process of composting at different temperatures of 62 and 73.2 ^{o}C to accelerate decomposition as the machine was continuously mixing. The temperature was maintained at 70-80 ^{o}C for 24 h. During the process, “compost” samples were taken at 1, 6, 12, 18 and 24 h of composting for characterisation of nutrients composition and stability of compost. Another run of the procedure was carried out without enzyme, in order to determine effect of enzyme on the composting process. All the composts samples were dried to constant weight in an oven at 70^{o}C, ground and sieved through a 2 mm sieve before analysis.

3.2.2 Compost pH and electrical conductivity

For compost pH and electrical conductivity (EC) determination, the samples were stirred in distilled water at a 1:10 (compost: water) ratio for 5 seconds and allowed to stand for 50 minutes before stirring again and allowing to stand for ten minutes. The pH was determined using PHM 210 standard meter. The same procedure was also followed to determine compost pH in 1.0 M KCl. The same water supernatant solutions used for pH analysis were used for determination of EC using CDM 210 conductivity meter as described by Rayment & Lyons (2011).

3.2.3 Total carbon and total and mineral nitrogen

The Leco TruMac CNS/NS Carbon/Nitrogen/Sulfur Determinator was used for determination of total C and N (Leco Corporation, 2012). The procedure is based on dry combustion of air dried compost samples (0.2 g) in crucibles and subjected to 1450 ^{o}C furnace temperature for about 6 minutes per sample. Mineral nitrogen in the compost (NH_4-N and NO_3-N) were extracted with 2M KCl (Kalra & Maynard, 1991). Air dry compost (2.5 g) was placed into a

100 ml centrifuge tubes and 25 ml of 2M KCl was added, shaken for 30 minutes, and filtered through Whatman no 5 filter paper before analysis for nitrate-N with Gallery Discrete Autoanalyser (Scientific Thermo Fisher, 2014), and for ammonium-N using the UV/VIS spectrophotometer following the salicylate-nitroprusside colorimetric method, after development of a blue colour (Anderson & Ingram, 1993).

3.2.4 Total and available phosphorus

Total P was extracted by microwave digestion system (CEM Corporation, 2014). For this purpose, compost samples were further sieved (<0.5 mm), for homogeneity, and 0.5 g of each compost was weighed into digestion vessels. Five ml of both perchloric and nitric acids were added and allowed to predigest by standing for 15 minutes before sealing the vessels. The samples were digested at 200^{o}C for 30 minutes, allowed to cool before filtration through Whatman no 5 filter paper. The filtrates were then diluted with deionised water in 25 ml volumetric flasks and analysed with Gallery.

Available P was extracted with Ambic-2 procedure (The Non-Affiliated Soil Analysis Work Committee, 1990). Compost samples (2.5 g) were weighed and placed into extracting bottles to which 25 ml of Ambic-2 solution (0.25 mol dm^{-3} NH_4HCO_3 + 0.01 mol dm^{-3} Na_2 EDTA + 0.01 mol dm^{-3} NH_4F + Superfloc) was added. The suspension was shaken on a reciprocating horizontal shaker at 400 revolution per minute (rpm) for 10 minutes, and then filtered through a Whatman no 5 filter paper (The Non-Affiliated Soil Analysis Work Committee, 1990) and the filtrate analysed for P with the Gallery Discrete Autoanalyser.

3.2.5 Humic and fulvic acids

Humic acids were extracted and analysed as described by Mupondi *et al.* (2010). Compost samples (2.5 g) were weighed into centrifuged tubes and 50 ml of 0.1 M NaOH added, shaken for 4 h at 180 oscillations per minute and centrifuged for 15 minutes at 4000 rpm. The supernatant was divided into two fractions; one was stored for analyses of total extractable fraction (EX) while the other was adjusted to pH 2 by adding concentrated H_2SO_4 and allowed to coagulate for 24 h at 4 ^{o}C (Sanchez-Monedero *et al.,* 1996). The precipitates comprised of humic acids fraction (HA), while fulvic acids (FA) remained in solution (Mupondi *et al.,* 2010). The dissolved fulvic acids were separated by centrifugation (as above) and stored for analysis. Total C in the extractable fractions (EX) and FA were determined using the modified Walkley-Black method (Sanchez-Monedero *et al.,* 1996). A 5 ml aliquot of extract was transferred into 250 Erlenmeyer flask and 10 ml of 0.167 M $K_2Cr_2O_7$ added, and the flasks swirled to mix the solution uniformly. Concentrated sulphuric acid (20 ml) was added rapidly, the flasks swirled again gently till the sample and reagents were thoroughly mixed, and then the contents were swirled more vigorously for one minute. The flasks were allowed to cool under the fume hood for 30 min, and 150 ml of de-ionized water and 10 ml concentrated ortho-phosphoric acid were added. O-phenanthroline hydrate indicator (1 ml) was added and excess dichromate was titrated against 0.5 M ferrous ammonium sulphate ($(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$) solution.

The carbon concentration of humic acid fractions (CHA) were calculated by subtracting fulvic acids fraction C (CFA) from total extractable fraction C (CEX). Humification ratio (HR) was estimated using the following equation:

1) $HR = (C_{EX}/C) \times 100$

The humification index (HI) was estimated using the following formula:

2) $HI = (C_{HA}/C) \times 100$

3.2.6 Exchangeable bases and extractable micro-elements

Exchangeable Ca, Mg, Na and K, in the composts were extracted using ammonium acetate (NH_4OAc) at pH 7. Compost samples (5.0 g) were placed in centrifuge tubes and 50 ml of 1M NH_4OAc solution added, and shaken on a reciprocating horizontal shaker at 180 oscillations per minute for 30 minutes. The suspension was filtered through Whatman no 5 filter paper and the filtrate analysed using the atomic absorption spectrophotometer (AAS). The CEC was estimated from the sum of bases (The Non-Affiliated Soil Analysis Work Committee, 1990).

Available Cu, Zn, Co, Fe and Mn were extracted by 1 % EDTA (di-sodium salt). Five grams of compost was placed in centrifuge tubes and 50 ml of 1 % EDTA was added and shaken for 1 h, before filtration through Whatman no 5 filter paper (The Non-Affiliated Soil Analysis Work Committee, 1990) and analysed with Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES 720 Varian).

3.2.7 Escherichia coli and Salmonella species

Fresh subsamples of the composts were analysed for *E. coli* and *Salmonella* species by KZN Agriculture, Environmental Affairs and Rural Development’s Allerton Provincial Veterinary Laboratory. For *Escherichia coli* determination, serial dilutions of samples were prepared with buffered peptone water (BPW) and 1 ml of the diluted samples were pipetted into a petri dish. Thereafter, cooled molten Rapid *E coli* 2 Agar was added to each plate and swirled clockwise and anti-clockwise to allow thorough dispersion and mixing of the sample with the media. Afterwards the media were allowed to solidify at room temperature for approximately 20 minutes then incubated (inverted) for 21 h. Plates with 15 to 150 of colonies were selected for counting of E *coli* colonies (Official Method 966.24).

For *Salmonella* spp, compost sample (8 g) was added to 80 ml of buffered peptone water (BPW) and the suspension was incubated at 37 °C for 18 h. Thereafter 0.1 ml of the culture was transferred to a tube containing 10 ml of preheated (41.5 °C) Rappaport-vassiliadis Salmonella (RVS) broth. The inoculated RVS broth was incubated at 41.5 °C for 24 h. After incubation the culture obtained from RVS broth was inoculated by looping the surface of one 90 mm Xylose lysine deoxycholate (XLD) agar, so that well-isolated colonies could be obtained. The petri-dishes were inverted and placed in the incubator set at 37°C. After 24 h of incubation the plates were examined for the presence of typical colonies of *Salmonella* (Official Method 17.1ISO 6576:2003).

3.2.8 Statistical Analysis

GenStat 14th edition (VSN International, 2011) software was used for statistical analysis in this study. Two way analysis of variance (ANOVA) was used to determine the effect of sampling time and addition of enzyme during composting process on all measured parameters.

3.3 Results

3.3.1 pH

The trends of pH, in both H_2O and KCl, for compost with enzyme decreased with increase in composting time between 1 and 12 h, while it increased without enzyme (Figure 3.1).The compost with the enzyme had higher pH than without, up to 6 h of composting period whereas without enzyme had higher pH than with enzyme between 12 and 18 h. The pH of the compost with enzyme (W) remained constant between 12 and 24 of composting period while that without enzyme (N) decreased between 18 and 24 h.There was not difference between the composts, in terms of pH at 24 h.

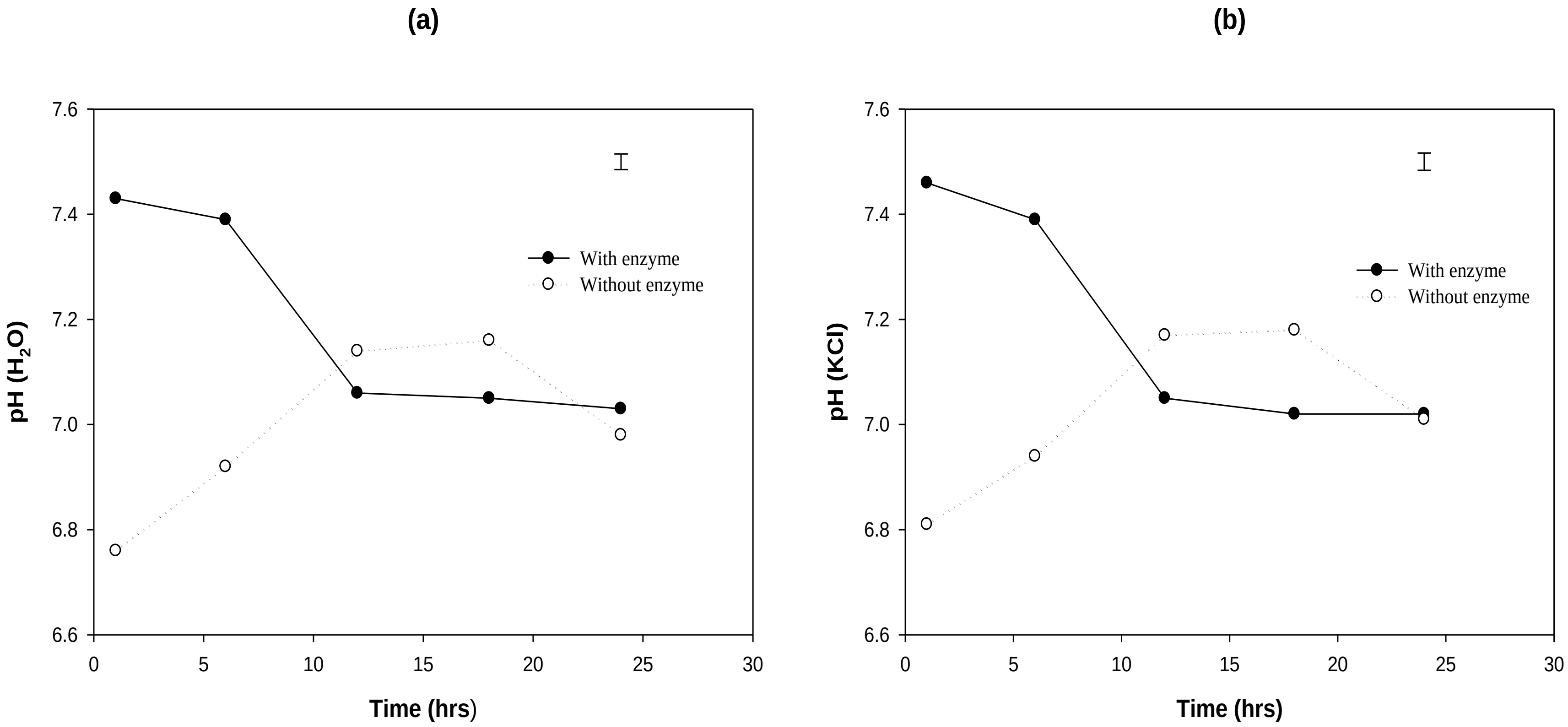

Figure 3. 1. pH values of Biomax composts over composting time: (a) pH in water and (b) pH in KCl at p≤0.05. . Error bar indicate least significant difference (LSD) at $p < 0.05$

3.3.2 Total C and total N and Electrical conductivity

Total C contents did not significantly changed throughout composting process for both composts, while total N increased up to 18 h of composting after which it became constant (Table 3.1). The C:N of the compost with enzyme remained constant throughout the composting period, the one without the enzyme increased in the first 6 h and decline between 12 and 18 h of composting. The final composts had similar C:N ratios irrespective of whether the enzyme was added or not (Table 3.1). Electrical conductivity values of the composts, with or without enzyme, were slightly affected by composting time.The compost with enzyme had lower levels of EC throughtout composting time than without enzyme. The EC of compost with enzyme remained constant between 18 and 24 h while it increased for the compost without the enzyme (Table 3.1).

Table 3.1 Selected chemical properties of Biomax composts sampled at different hours during composting process.

Sample	Time (h)	EC (dS/m)	Total N (%)	Total C (%)	C:N
With enzyme	1	0.11a	2.9a	30.0a	10.2a
	6	0.10b	2.8b	29.5a	10.4a
	12	0.11a	2.9a	29.9a	10.3a
	18	0.10b	3.0c	30.9b	10.3a
	24	0.10b	3.0c	30.7ab	10.3a
Without enzyme	1	0.12c	2.9a	29.5a	10.1ba
	6	0.12c	2.8b	29.9a	10.7c
	12	0.12c	2.9a	30.1ab	10.5ca
	18	0.12c	3.0c	29.8a	10.1ba
	24	0.13d	3.0c	30.3ab	10.1ba
LSD ($P \leq 0.05$)		0.01	0.04	0.84	0.28

W = Biomax compost with enzyme; N = Biomax compost without enzyme; C:N = carbon-to-nitrogen ratio

3.3.3 Mineral nitrogen

Concentrations of NH_4-N generally decreased with composting time for both composts. Compost without (N) enzyme had higher NH_4-N release, only after 1 h than with enzyme (W). Conversely concentrations of NH_4-N were high between 6 and 24 h of composting with enzyme over without enzyme (Figure 3.2a). In the final composts, NH_4-N was greater in the compost with the enzyme than without. Concentrations of NO_3-N increased between 6 and 18h with enzyme and between 12 and 18 h without the enzyme and there was not change between 18 and 24 h of composting. The compost produced without enzyme had lower nitrate concentrations than that with the enzyme from 12 h of composting and beyond (Figure 3.2 b).

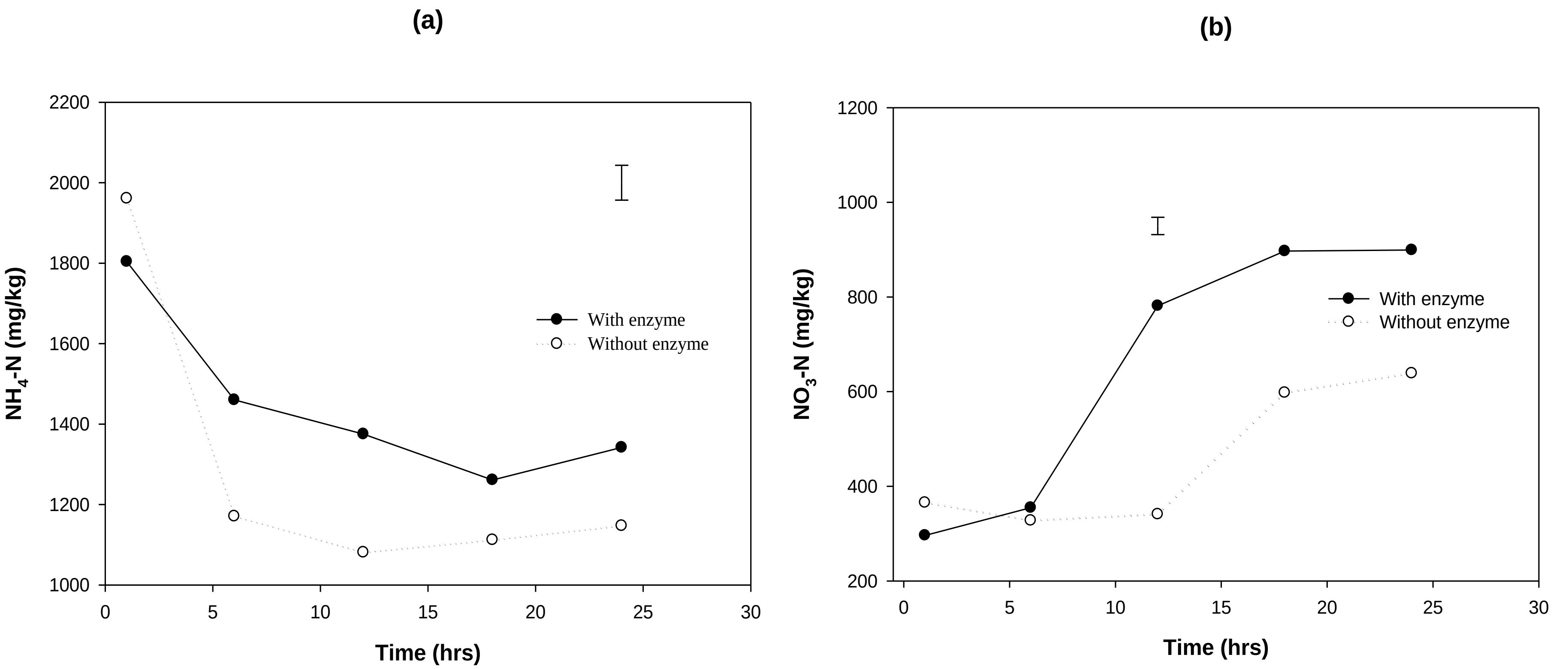

Figure 3.2. Available concentrations of mineral nitrogen of Biomax composts at different sampling time during process of Rapid Biomax composting. (a) NH_4-N and (b) NO_3-N. Error bar indicate least significant difference (LSD) at $p < 0.05$.

3.3.4 Mineral N (NH_4-N + NO_3-N)

Concentrations of mineral N decreased in the first 6 h of composting, with greater decline without the enzyme, and afterwards increased between 12 and 24 h (Figure 3.3). The compost with the enzyme had higher mineral N than without starting from 6 h of composting process.

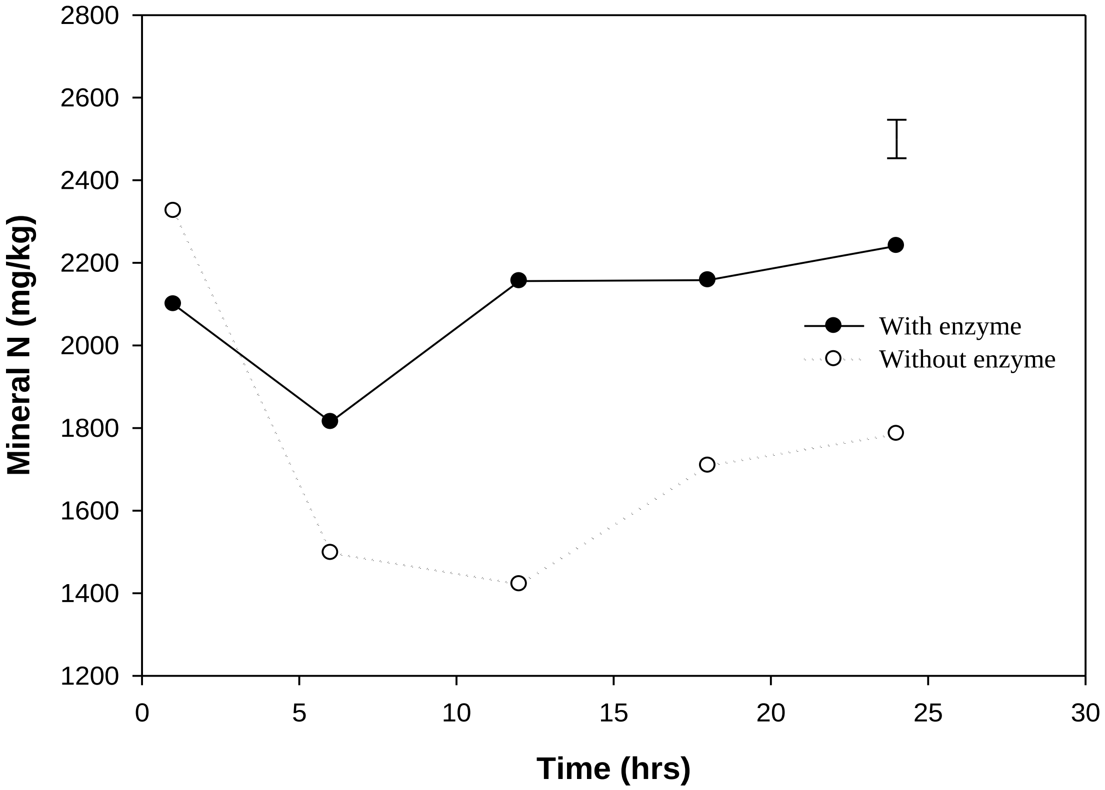

Figure 3.3. Mineral N concentrations of Biomax composts throughout Biomax thermophilic composting process.

3.3.5 Total P and available P

Total P concentrations increased up to 6 h with the enzyme and between 6 and 12 h of composting without the enzyme, and thereafter it remained constant (Figure 3.4a). In the final composts, the compost without the enzyme had greater total P than the one with it (Figure 3.4a). Soluble P was higher during the first hour of composting for both composts. Afterwards available P declined drastically between 6 and 24 h of the composting period for both composts with or without the enzyme (Figure 3.4 b).

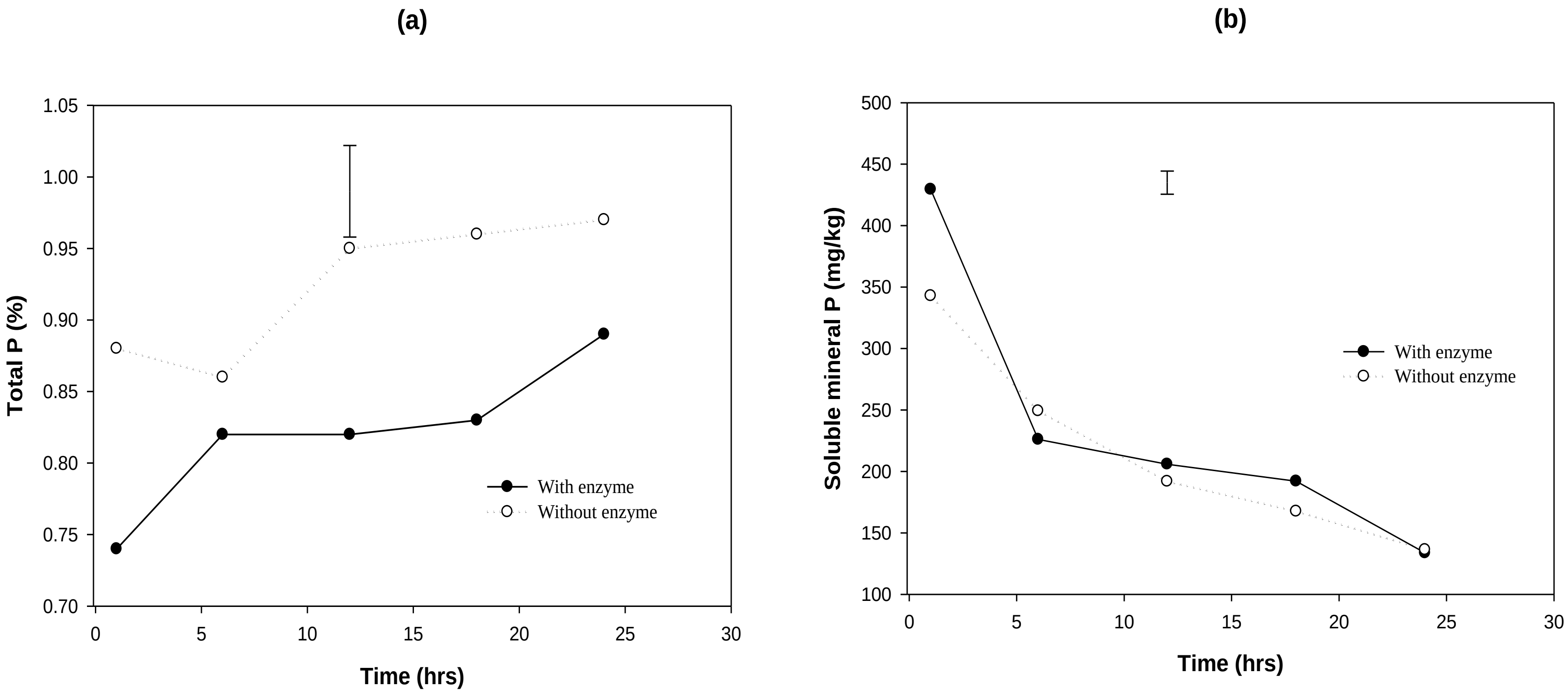

Figure 3. 4. Change in concentrations of total P of Biomax composts at different sampling hours (a); Available mineral P concentrations of Biomax composts over the period time during composting (b). Error bar indicate LSD at 0.05 level of significance.

3.3.6 Humic and fulvic acids

Fulvic acid (CFA) concentration declined with composting time for composts with the enzyme, while the compost without the enzyme showed an increasing pattern. In contrast, humic acids (CHA) concentration and humification index (HI) in the compost with the enzyme increased with time while no clear pattern was shown for the compost without enzyme (Table 3.2). The final composts with the enzyme had higher concentrations of humic acids and HI, and lower CFA, than without. The humification ratio was not affected by addition of the enzyme or by composting time. were higher after 18 and 24 h than the intial levels, with no change without the enzyme

Table 3.2 Selected maturity parameters of Biomax composts.

Samples	Time (hrs)	CFA (%)	CHA	CHA/CFA	HI (%)	HR
	1	0.40a	0.26a	0.64a	0.86a	2.21
	6	0.36b	0.42a	1.17ab	1.43a	2.65
With enzyme	12	0.33c	0.45a	1.35b	1.50a	2.61
	18	0.31d	0.52ba	1.70b	1.69ba	2.69
	24	0.27e	0.61ba	2.24cb	1.99ba	2.88
	1	0.30d	0.26a	0.88ab	1.68ba	2.69
	6	0.36b	0.37a	1.03ab	1.23a	2.43
Without enzyme	12	0.32cd	0.34a	1.08ab	1.15a	2.21
	18	0.35b	0.47ba	1.35b	1.58a	2.74
	24	0.39a	0.33a	0.85ab	1.08a	2.36
LSD ($P \leq 0.05$)		0.01	0.21	0.59	0.81	0.82

CFA = fulvic acid carbon; CHA = humic acid carbon; *HI* = humification index; *HR* = humification ratio.

3.3.7 Exchangeable bases, CEC and extractable micro-nutrients

There were no clear trends in concentration of Mg, K, Na and CEC with composting time but Ca concentrations decreased between 1 and 12 h then increased between 12 and 18 h for compost with enzyme, while without the enzyme there was no clear trend. Composts with the enzyme had higher CEC and concentrations of exchangeable Ca, Mg and Na and lower concentration of K than those without the enzyme (Table 3.3).

Concentrations of Zn, Cu and Mn decreased, with time of composting with the enzyme while they increased for composting without the enzyme (Table 3.4). There was no clear trend in concentrations of extractable Fe of composts with composting time with or without the enzyme.

Table 3.3 Concentrations of exchangeable bases of Biomax composts over sampling time.

Sample	Time	Ca	Mg	Na	K	CEC
	(h)		(cmol/kg)			(cmol/kg)
With enzyme	1	48.4a	24.6a	38.4a	85.6a	197.0a
	6	44.3b	28.0b	37.6a	87.9a	197.0a
	12	31.3c	24.9a	31.0c	76.2b	163.4b
	18	42.4d	33.1c	39.2a	92.3c	207.0c
	24	38.8e	34.3c	37.3ab	91.7c	202.0d
Without enzyme	1	35.5f	21.2d	22.9d	93.9c	173.5e
	6	28.9g	15.3e	19.9e	97.2d	161.3b
	12	32.4c	16.7e	23.3d	96.1d	168.4f
	18	31.4c	20.7d	23.2d	94.0cd	169.4f
	24	29.2g	25.4a	23.7d	94.0cd	172.3e
LSD ($P \leq 0.05$)		1.57	1.52	1.63	2.62	4.57

Table 3.4 Micro- nutrients concentrations of Biomax composts during composting process

Compost	Time	Zn	Cu	Mn	Fe
	(hrs)		(mg/kg)		
With enzyme	1	133.8	5.1	167.0	186.0a
	6	127.0	5.0	160.3	142.8b
	12	125.3	4.6	163.7	119.1b
	18	125.4	4.4	162.6	177.1a
	24	120.3	3.7	143.5	171.2a
Without enzyme	1	99.9	4.4	161.0	124.1b
	6	105.0	5.8	155.9	199.9a
	12	109.0	5.6	152.5	189.5a
	18	113.9	6.0	152.0	147.4ba
	24	128.6	6.2	201.7	165.0ab
LSD ($P \leq 0.05$)		4.3	0.4	7.1	29.6

3.3.8 Pathogens

E. coli and *Salmonella* species were not detectable in all the composts irrespective of composting time or whether or not the enzyme was added (Appendix 1).

Table 3.5 Pathogenic organism's population of Biomax composts sampled over time during composting.

Samples	Time	E. Coli count	Salmonella species identified
	(h)	(cfu/g)	
With enzyme	1	0	Not detected
	6	0	Not detected
	12	0	Not detected
	18	0	Not detected
	24	0	Not detected
Without enzyme	1	0	Not detected
	6	0	Not detected
	12	0	Not detected
	18	0	Not detected
	24	0	Not detected

3.4 Discussion

The decrease in pH values for the compost with the enzyme within the first 12 h could be explained by production of nitrate and organic acids (Gao *et al.*, 2010a). This was supported by the results of NO_3-N, which increased with composting time. The nitrification process produces H^+ ions resulting in decline in pH (Gao *et al.,* 2010a; Shen *et al.,* 2011). On the other-hand, the increase in pH within 18 h of composting without the enzyme, which coincided with rapid decline in NH_4-N and no change in NO_3-N suggested that the pH increase was a result of formation of NH_3 gas, which eventually got lost. This was supported by the results of mineral N (sum of NH_4-N and NO_3-N), which was higher from 6-24 h of composting for the compost with the enzyme, than without the enzyme. These results were contrary to those of Gao *et al.* (2010a), who showed that pH and NH_4-N increased in the first 10 days and thereafter the levels of these parameters decreased and NO_3-N increased, during normal thermophilic composting of chicken litter. The difference could be explained by the limited time (24 h) of Biomax composting, which did not allow for the mineralisation processes to take place. Furthermore pH values during traditional thermophilic composting stabilised at 7.0, which was similar case to this study. However, the concentrations of NH_4-N and NO_3-N in Biomax composts were higher and this could be attributed to minimal losses in the closed system whereas, during normal thermophilic composting, nitrogen is lost through volatilization process when pH is above 7.0 (Tam & Tiquia, 1999). While the difference in pH suggested possible greater ammonia losses in the compost without the enzyme, the results of total N showed no significant difference between the composts, possibly because of the lower sensitivity of total N measurements. In a closed system, insignificant N losses could occur. The C/N ratio of end product of composting is one of the paramount parameters used to measure maturity of the compost. The losses in OM reduces the dry weight mass and decreases C/N ratio (Bernal *et al.,* 2009). After 24 h of composting the C/N ratio for both composts were lower than 20 suggested by Nayak & Kalamdhad (2014) for matured compost. While such C/N suggested that the composts could have been mature, the initial composting mixture had low initial C/N ratios, because the chicken litter and bulking agents were not mixed according the C/N ratio requirements for composting. The initial C/N required for composting is between 25 and 30 and as such the farmer practice of mixing wastes for Biomax composting resulted in a lower than ideal C/N ratio. Such a low C/N could encourage ammonia losses during composting (Ogunwade *et al.,* 2008; Wang *et al.,* 2013). However, the results of total N and total C suggest that minimal losses of these elements occurred during the composting period, possibly because a closed system was used (Sanchez-Monedero *et al.,* 2001). The EC values, which ranged between 0.10 to 0.13 dS/m, which were lower than 4 dS/m throughout the composting period, for both composts, indicated that the salt concentration was within the acceptable range, with limited salinity potential (Nayak &

Kalamdhad, 2014). The increase of amount of total P for both composts could be explained by biomass reduction which resulted in an increase of P concentration of the existing minerals (Nayak & Kalamdhad 2014).. These results were similar to those of Chaudhry et *al.* (2013) which showed that total P increased during conventional thermophilic composting of chicken litter. However total P concentrations were lower compared to those of normal thermophilic composting. The decrease in inorganic P could be attributed to the fact that soluble phosphorus decreases with humification, so phosphorous solubility during the decomposition was immobilized by microorganisms (Shyamala & Belagali, 2012). Considering the relatively high pH values in the composts, P reduction could have been a result of precipitation of calcium phosphates (Eneji *et al.*, 2003). This is supported by the results of exchangeable Ca, which showed a general decline for compost with enzyme between 1 and 12 h and without between 21 and 24 h during composting. The high amounts of Ca, Mg and Na of compost with enzyme can be attributed to greater decomposition of organic matter and mineralisation of these elements (Hubbe *et al.,* 2010). These results were contrary to those of Jr Orrico (2010) which showed that concentrations of exchangeable Ca, Mg, Na and K increased during normal thermophilic composting of chicken litter but these concentrations were lower compared to those of Biomax composts.

Additionally this difference may be due to addition of inoculum BM1 enzyme that greatly speed up the digestion of chicken manure. CEC is one of the parameter that is used to determine stability and maturity of compost. The relatively higher CEC values of the compost with the enzyme could be a result of organic matter humification process (Bernal *et al.,* 1998; Gao *et al.,* 2010b).This was supported by the higher humic acid, CHA/CFA ratio and HI, in the compost with enzyme than the other, especially at the end of composting processes (Bernal *et al.,* 1998). The decrease in amounts of fulvic acid and the increase humic acids, in the compost with the enzyme suggest that the enzyme encouraged humification and facilitated maturity of the compost. This was supported by the results of CHA/CFA ratio (Bernal *et al.,* 1998) and HI, which indicated that humification of organic matter occurred (Raj & Antil, 2011) when the enzyme was added. In generally the CHA/CFA ≥1.6 is considered as indication of a mature compost (Ko *et al.,* 2008). In this study the CHA/CFA ratio was > 1.6 for compost with the enzyme, after 18 and 24 h of composting. According to a review by Bernal *et al.* (2009), the HR ≥ 7.0, HI ≥ 3.5 and CHA/CFA (polymerisation index) ≥ 1.0, are the agronomically established limits for a stable and matured compost. These results were similar to those of Bernal *et al.* (1998) which showed that CHA/CFA, HI and HR increased with time during normal thermophilic composting of chicken litter. The concentrations of these parameters were higher than those of Biomax composts and the difference could be explained by humification of organic matter and the pronounced decrease in CFA (Bernal *et al.,* 1998).

Although the final pH of both composts (pH 7.0) suggested maturity of the composts, the values of HR and HI which were ≤7.0 and ≤3.5 respectively after composting indicating that the composts were immature and unstable (Bernal *et al.,* 2009).

The decrease concentrations of extractable Zn, Cu and Mn of compost with time in the enzyme treatment coincided with increase in humification, while lower humification in the compost without the enzyme could have resulted in increase in extractable Zn, Cu and Mn concentrations with time at the end of composting. Humic substances (CFA and CHA acids) bind metal cations and reduce their mobility (Pettit, 2004). The lack of *E. coli* and *Salmonella* spp pathogenic organisms in both composts, even after only 1 h of composting, could be due to the high composting temperatures of 70-80^{o}C. These results were contrary to those of Gao *et al.* (2010a), who showed that pathogenic organisms were eliminated in 3 days during conventional thermophilic composting of chicken litter. The difference could be explained by the higher temperatures used in the Biomax system than the above 55^{o}C in normal thermophillic composting.The temperatures above 55°C are suitable to kill pathogenic organisms during composting (Bernal *et al.*, 2009). This finding suggested that the use of the Biomax system for composting organic wastes is effective in sterilising the waste from pathogenic organisms within the first few hours of the process.

3.5 Conclusion

Although the chemical composition of Biomax composts with BM1 enzyme differs from those without the enzyme, the technology does not stabilise chicken litter and other organic wastes into a compost irrespective of composting time. The technology was effective in sterilising the organic wastes, from *E. coli* and *Salmonella* spp. Longer composting periods, and mixing of organic materilas based on recommended C/N ratio, may need to be tested for better compost stability and nutrient release of the composts.

CHAPTER 4

NUTRIENT RELEASE POTENTIAL OF BIOMAX COMPOSTS

4.1 Introduction

The use of inorganic fertilizers as the source of essential nutrients has been predominate for over 50 years now (Shu, 2005) because they contain nutrients (e.g., $NH4^+$, NO_3^-, HPO_4^- and K^+) in the readily available forms (Murugan & Swarnam, 2013). However continuous application of inorganic fertilisers may amount of organic matter and acidify soils, leading to unfavourable effects on microbial activity and fertility status of the soil (Adediran *et al.*, 2005; Shu, 2005). Organic materials have been used to replenish the fertility of soils.

Use of organic fertiliser supplies nutrients and improves chemical, physical and biological properties of the soil thus enhancing soil fertility (Adediran *et al.*, 2003; Abbasi, 2007). Chicken manure has been the most desirable organic material because of its high nitrogen (3-5 %), phosphorus (1.5-3.5 %) and potassium (1.5-3.0 %) content (Amanullah *et al.*, 2010). Composting is used to convert the unstable organic materials into a black end product called humus (Anwar *et al.*, 2015). In addition to normal thermophilic and vermi-composting, the Biomax Rapid Thermophilic Digester is being tested for the quality of the composts produced. The value of an organic fertiliser as a source of nutrient depends on its nutrient release patterns in the soil.

Composted organic material are slow release fertilisers, which release nutrients like N and P through mineralisation by microbial activity while K is mainly in readily available form (Manitoba, 2013). Nutrient release from organic materials is affected by the properties of the original source and the properties of the soil to which the material is applied (Murugan & Swarnam, 2013). In soil, nutrient release from organic matter into soil solution is governed by biochemical (mineralisation and immobilization) and physiochemical (adsorption, desorption, dissolution and precipitation) processes. These processes are functions of soil temperature, soil aeration, soil water, soil organic matter, cation exchange capacity (CEC), and soil pH (Comerford, 2005; Manitoba, 2013). According to Murugan & Swarnam (2013), application of poultry manure to acid soil at a rate of 120 kg N/ ha significantly increased N mineralisation (NH_4^+- NO_3^{2-}) during the first week of incubation relative to the control. In addition Heenkende & Paraman (2010), observed higher release of soil NH_4^+, ranging from 45 to 58 mg/kg, after 7 to 21 days of incubation with seri-compost, prepared with silk worm pupae waste and farm yard manure, and applied at a rate of 100 kg N/ ha . Higher release of NO_3^- ranging from 16.8 to 112 mg/kg, was observed between 0 to 21 days of incubation for the same application rate.

The results in Chapter 3 showed that the Biomax Rapid digestion process stabilises pH, and increased mineral N and humification parameters. It is essential to understand the nutrient release patterns of Biomax compost in soils as these will have implications on nutrient availability and fertiliser value of the composts. The objective of this study was to determine nutrient release patterns of Biomax compost in soil. The specific objective was to determine the effects of Biomax compost type (with and without enzyme) and application rates on mineral N and P and exchangeable bases release in the soil during incubation. The hypothesis was that application of Biomax compost with enzyme will better release nutrients compared to compost without enzyme.

4.2 Materials and methods

4.2.1 Soil

The soil used in this study was collected from the 0 - 20 cm depth of a Glenrosa soil form at Ukulinga Research Farm of the University of KwaZulu-Natal in Pietermaritzburg, using an auger. The average annual rainfall is 695 mm and most comes in summer. The mean temperatures are 27^{o}C and 20.5^{o}C in summer and winter respectively (Mdlambuzi, 2014). The samples were mixed thoroughly to make composite sample. The soil sample was air-dried for seven days, crushed and sieved (< 2 mm) and stored in a dry place before analysis. The samples were analysed for pH, EC, bases, extractable P, micro-nutrients, mineral N, soil organic carbon, particle size and field capacity.

pH

Soil pH was measured in 1: 2.5 soil: solution ratio in both H_2O and KCl. Ten grams of air-dried soil was placed in a beaker and 25 ml of water (or KCl solution) was added. The contents were stirred with a glass rod for 5 seconds and allowed to stand for 50 minutes before stirring again and allowing to stand for ten minutes. The pH was determined using standard meter PHM 210 (Okalebo *et al.,* 2000).

Exchangeable bases

Exchangeable Ca, Mg, Na and K were extracted using 1 M ammonium acetate (pH 7) and the concentrations determined using the atomic absorption spectrophotometer (AAS) (The Non-Affiliated Soil Analysis Work Committee, 1990). Five grams of soil was placed in an extracting bottle and 50 ml NH_4OAc solution was added to the soil. The suspension was shaken on a reciprocating shaker at 180 oscillations per minute for 60 minutes, before filtration through Whatman no 42 filter paper and the filtrate was analysed for Ca, Mg, K and Na.

Available P

Available P was extracted with the Ambic-2 method (The Non-Affiliated Soil Analysis Work Committee, 1990). Soil (2.5 g) was placed in an extracting bottle and 50 ml of Ambic-2 solution was added. The content was shaken for 10 minutes. The extract was filtered through a Whatman no 5 filter paper and the filtrate was collected and analysed with Gallery Discrete Autoanalyser.

Micro-nutrients

Copper, Zn, Fe and Mn were extracted with 1 % ethylenediaminetetraacetic acid (EDTA). The soil (5 g) was placed in centrifuge tube, 50 ml of 1 % EDTA added, and the soil suspension shaken for 1 hour, before filtration through Whatman no. 542 filter paper (The Non-Affiliated Soil Analysis Work Committee, 1990). The filtrates were then analysed for Cu, Zn, Fe and Mn with ICP.

Total carbon and total and mineral nitrogen

Total C and N were analysed using the Leco TruMac CNS/NS autoanalyser (Leco Corporation 2012). Air dry soil samples (0.2 g) were placed in crucibles and subject to furnace temperature (1450 ^{o}C) for 6 minutes per sample as detailed in the instrument manual.

For extraction of mineral N, 5 g of soil was placed in a centrifuge tube and 50 ml of 2M KCl added to soil before shaking at 180 rpm for 30 min. The suspension was filtered through

Whatman no. 5 filter paper before analysis of NH_4-N and NO_3-N (Kalra & Maynard, 1991). Ammonium-N was determined using a UV/VIS spectrophotometer after development of a blue colour using salicylate-nitroprusside (Anderson & Ingram, 1993) while nitrate-N was analysed with Gallery.

Soil organic carbon

Soil organic carbon was determined using the Walkley-Black method (The Non-Affiliated Soil Analysis Work Committee, 1990). Air dried soil samples (1g) were transferred into a 500 ml separately in Erlenmeyer flask and 10 ml of 0.167 M $K_2Cr_2O_7$ solution was added. The flasks were swirled before 20 ml of concentrated sulphuric acid was added rapidly. The flasks were swirled again to thoroughly mix the content, and allowed to cool for 30 min before 150 ml of de-ionized water and 10 ml concentrated ortho-phosphoric acid were added. The excess dichromate was back-titrated against 0.5 M ferrous ammonium sulphate solution.

Particle size distribution

Soil samples (50 g) were weighed into 400 mL beakers and saturated with distilled water before addition of 10 mL of 10% sodium hexametaphosphate. The suspensions were allowed to stand for 10 minutes and transferred into a dispersing cup thereafter 300 mL of water was added and mixed thoroughly for 2 minutes using high-speed electric mixer. The sediments were transferred into a 1 litre graduated cylinder and made up to the mark by water. The plunger was inserted into the cylinder to further mix the sediments thoroughly and hydrometer was inserted into the suspensions soon after the sedimentation was complete for about 10 s before taking the first reading (R_1) then removed and wiped. The cylinders were allowed to stand undisturbed for 6 h thereafter a hydrometer was reinserted for about 10 s before taking the next reading (R_2) exactly after 6 hours. Soil particle size (clay, sand and silt) were calculated and texture triangle used for determination of soil texture (Gee & Or, 2002).

Field Capacity

Field capacity was determined using a pressure plate apparatus (Gebregiorgis & Savange, 2007). Soil cores were placed on ceramic plate and saturated with water for 24 h, before being placed in a pressure chamber at matric potential of -1/ 3 bar until the excess water was drained. Gravimetric moisture content was determined after oven-drying at 105^oC for 24 h.

4.2.2 Compost

Biomax composts produced after 24h of composting with and without enzyme were used in this incubation study. The characteristics of the composts are as detailed in Chapter 3. A summary of the characteristics is given in Table 4.1.

Table 4.1. Chemical properties of Biomax composts

Parameters	Biomax compost with enzyme	Biomax compost without enzyme
pH $_{H2O}$	7.03	6.98
pH $_{KCl}$	7.02	7.01
EC (dS/cm)	0.10	0.13
Total N (%)	2.98	2.99
Total C (%)	30.72	30.33
Ca (cmol/kg)	38.72	29.30
Mg (cmol/kg)	34.33	25.44
Na (cmol/kg)	37.30	23.69
K (cmol/kg)	91.66	93.96
Available P (mg/kg)	133.74	136.3
Total P (%)	0.89	0.97
NO_3-N (mg/kg)	899.2	638.8
NH_4-N (mg/kg)	1342	1147
C:N	10.30	10.14
HI	1.99	1.08
HR	2.88	2.36

W = Biomax compost with enzyme*;* *N* = Biomax compost without enzyme

4.2.3 Incubation study

The incubation study was conducted in a constant temperature room at 25 °C for 56 days. The experiment was laid out in a completely randomized design with three replicate for each treatment. The treatments were the two Biomax composts, presented in Table 4.1, applied to the soil at 0, 1, 2 and 3 % (w/w). Air dried soil (equivalent to 100 g oven-dry soil) was placed in 450 ml plastic vials before addition of the treatments. The vials had 8 holes perforated right around the rim to allow gaseous exchange without rapid drying. The amended soils were then moistened to field capacity (24 %) with distilled water and incubated. Thereafter, moisture correction was done weekly after determination of weight loss. There were enough replicates to allow for destructive sampling after 0, 14, 28, 42 and 56 days of incubation. At each sampling, the samples were stored in fridge at 4°C before analysis of pH, NH_4-N and NO_3-N, available P, and exchangeable bases. The exchangeable bases were only analysed for samples collected after 0, 28 and 56 days of incubation. The methods of analysis of all the parameters were as described under soil characterisation. Soil pH was measured in 1:2.5 soils: water ratio. Extractable P was determined calorimetrically following Ambic – 2 extraction and determined using Gallery Discrete Autoanalyser. Mineral nitrogen (NH_4^+-N and NO_3-N) were extracted with 2 M KCl solution (Kalra & Maynard, 1991) and NO_3-N was determined using Gallery Discrete Autoanalyser and NH_4-N by calorimetric method (Anderson & Ingram, 1993). Exchangeable bases were extracted by ammonium acetate at pH 7 and analysed with the AAS (The Non-Affiliated Soil Analysis Work Committee, 1990).

4.2.4 Statistical analysis

Statistical analysis was done with GenStat 14th edition software (VSN International, 2011). A two-way analysis of variance (ANOVA) was used to determine effects of compost type (with or without the BM1 enzyme) and compost application rates on concentrations of mineral nitrogen, phosphorus and bases for the different incubation times.

4.3 Results

4.3.1 Soil characteristics

The soil used was loamy with 22% clay, pH 5.9, 1.8% organic C and 18 mg P kg^{-1}, among other parameters (Table 4.2). The soil also contained 950 mg $kg^{-1}$of NH_4-N and 9.3 mg kg^{-1} of NO_3-N.

Table 4. 2. Characterized chemical and physical properties of soil.

Parameters	Value
pH $_{H20}$	5.9
pH $_{KCl}$	5.9
EC (dS/m)	0.63
Exchangeable Ca (cmolc/kg)	5.17
Exchangeable Mg (cmolc/kg)	1.18
Exchangeable Na (cmolc/kg)	0.46
Exchangeable K (cmolc/kg)	0.36
CEC (cmolc/kg)	7.17
Available P (mg/kg)	18.0
NO_3-N (mg/kg)	9.29
NH_4-N (mg/kg)	9500
OC (%)	1. 8
Extractable Zn (mg/kg)	11.5
Extractable Cu (mg/kg)	5.88
Extractable Mn (mg/kg)	403.2
Extractable Fe (mg/kg)	290.6
Total N (%)	0.24
Total C (%)	2.1
Clay (%)	22
Sand (%)	46
Silt (%)	32

4.3.2 pH

Soil pH in both H_2O and KCl increased with increase in application rates for both composts (Figure 4.1). Compost produced with enzyme resulted in lower pH than that without the enzyme throughout incubation period (Figure 4.1). For all the application rates, for both composts, soil pH decreased gradually in the first 28 days and then remained constant up to the end of the incubation (56 days), except for the control, which increased between 28 and 56 days of incubation.

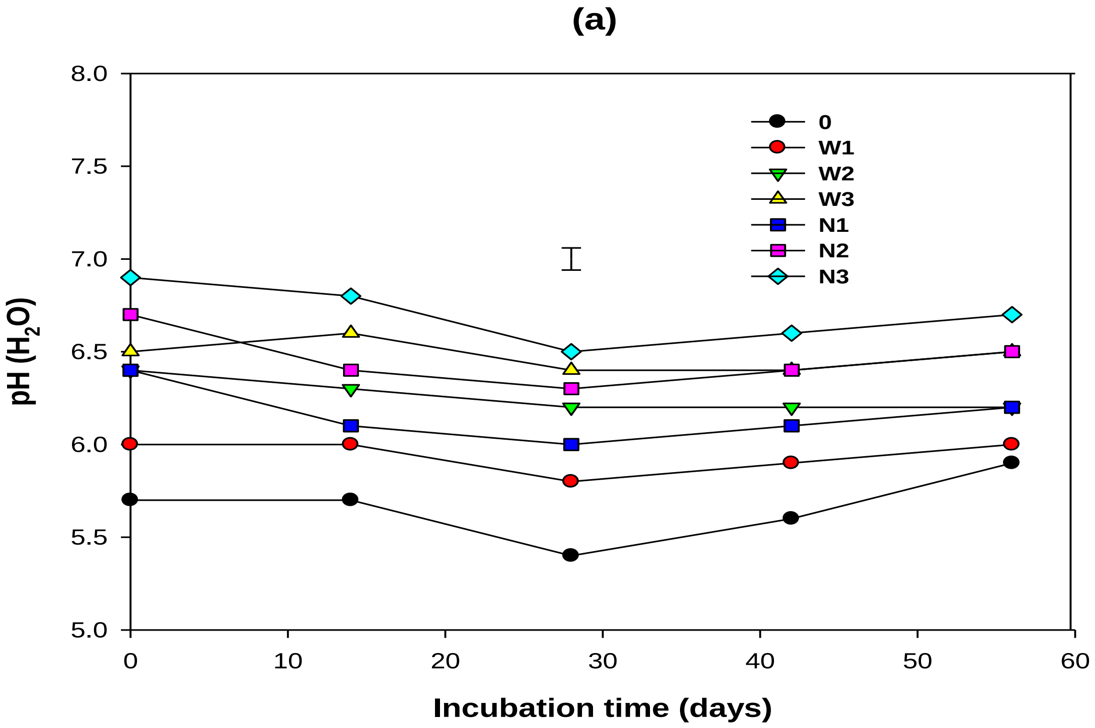

Figure 4. 1. Variations in soil pH during incubation of different application rates of Biomax composts with (W) and without (N) enzyme. Error bars indicate LSD at p ≤0.05. The 0 = control; W1, 2 and 3 (w/w) indicates application rates of Biomax compost with enzyme; N 1, 2 and 3 (w/w) Biomax compost application rates without enzyme.

4.3.3 Mineral nitrogen (NH_4-N and NO_3-N)

Initial levels of NH_4-N were statistically similar for all the rates of both composts, except that the soil treated with 1% of compost produced with the enzyme had lower NH_4-N than that amended with 3% of compost without the enzyme. Whereas the NH_4-N of all other rates of the two composts remained constant up to 28 days of incubation, that of the 3% compost with the enzyme increased. Statistically there was no change in NH_4-N at all rates of the compost without the enzyme while that with the enzyme declined betweeen days 28 and 42 days after which they remained constant. Compost without enzyme had higher concentrations of NH_4-N at all rates than the one with enzyme.

Soil NO_3-N were initially low at all rates of both composts, and were similar to the control. There was a rapid increase in NO_3-N in all treatments (including the control) between 14 and 28 days of incubation, and a decline at 42 days after which the levels remained constant (Figure 4.2). From 28 to 56 days of incubation, the NO_3-N levels increased with rate of application of both composts, with no differences between the compost type of the same rate. The control had the lowest NO3-N levels throughout the incubation period.

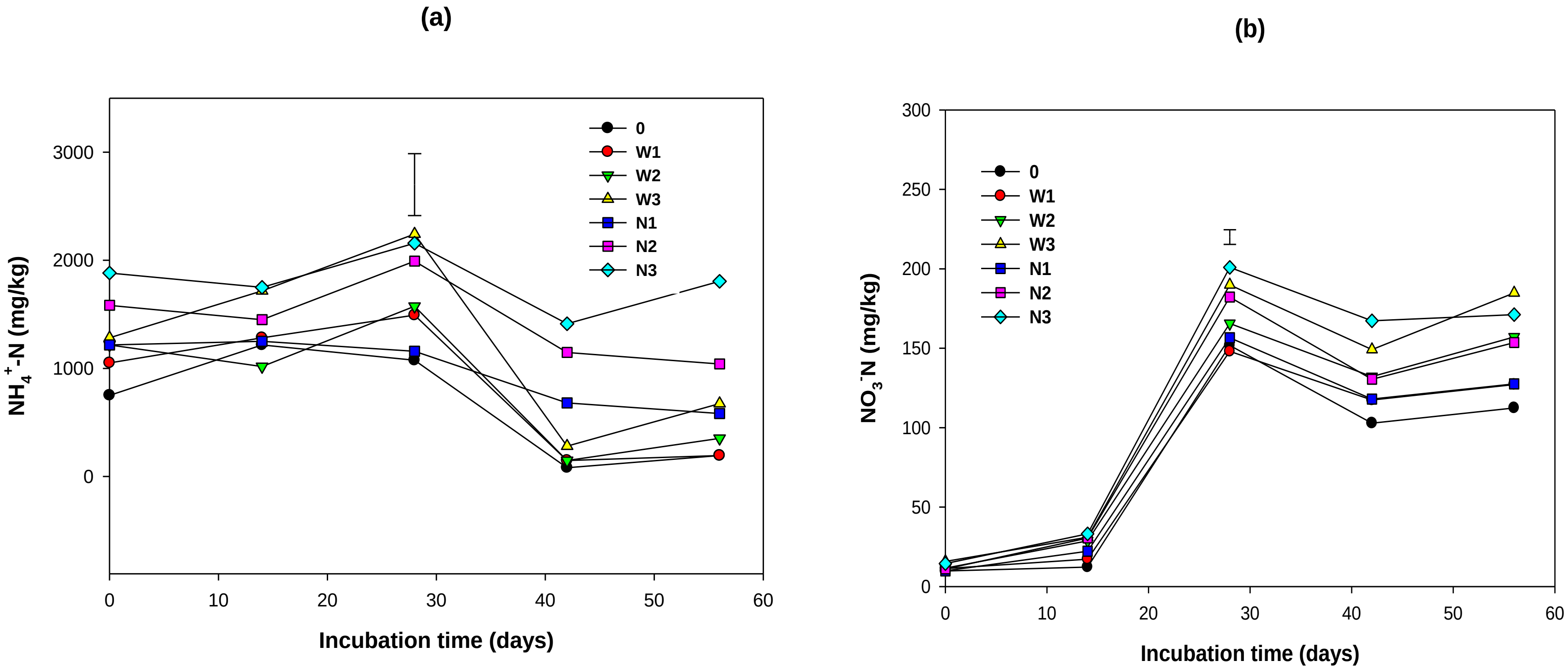

Figure 4.2 . Changes in of NH_4^+ and NO_3^- (mg/kg) concentration during incubation of soil with different application rates. 0 = control; W1,2 and 3 (w/w) indicates application rates of Biomax compost with enzyme; N 1, 2 and 3 (w/w) Biomax compost application rates without enzyme.

4.3.4 Mineral P

Available P remained constant throughout the incubation period for all treatments except the 3% rate, which declined in the first 14 days and then increased up to 28 days beyond which it remained constant for both composts (Figure 4.3). At all sampling times, available P was higher at higher application rates, and there were no differences between the composts at each the same application rate.

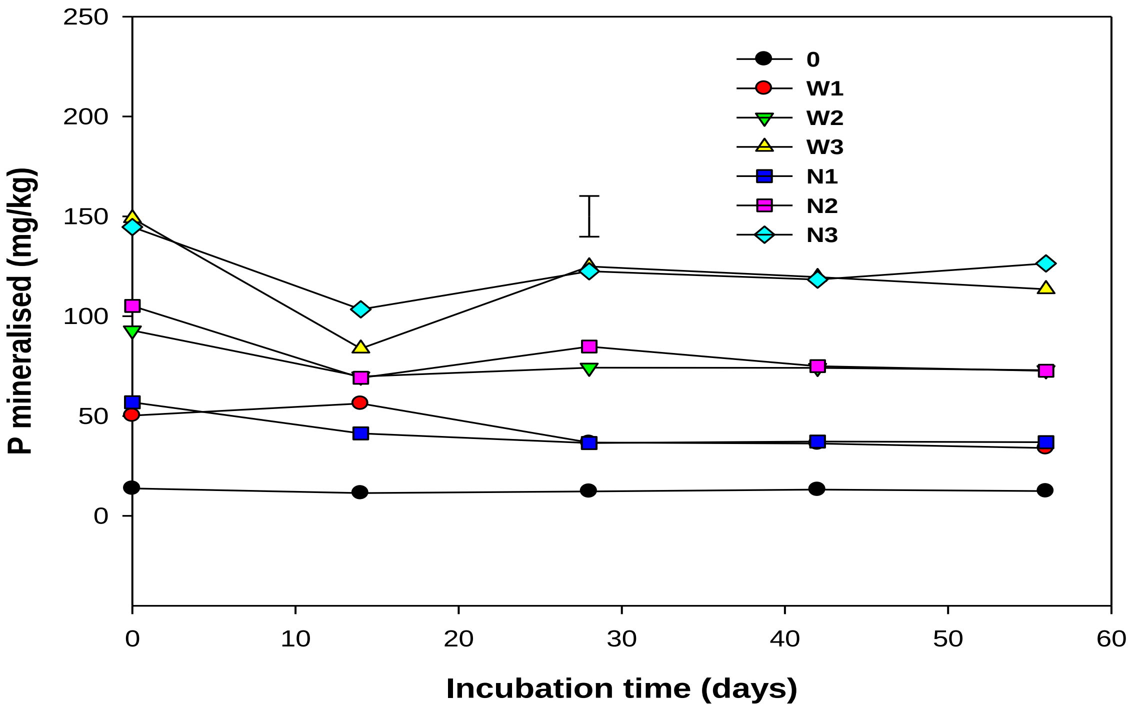

Figure 4.3. Variation of available P concentrations during incubation of soil with different application rates of the two composts. The error bar represents the least significant difference at $p<0.05$ level. 0 = control; W1, 2 and 3 (w/w) indicates application rates of Biomax compost with enzyme; N 1, 2 and 3 (w/w) Biomax compost application rates without enzyme.

4.3.5 Ca and K concentrations

Exchangeable Ca concentrations increased with increase in application rates, but decrease with incubation time for both composts, with higher exchangeable Ca in soils treated with compost produced without the enzyme than with the enzyme (Figure 4.4a). The concentration of Ca decreased with incubation time for all compost rates between 0 to 56 days (Figure 4.4a). Concentrations of soil K increased with increase in application rates for both composts (Figure 4.4b). Soil K concentrations generally declined in the first 28 days and then increased between 28 and 56 days of incubation except for 1 % and 2% rates of compost produce without the enzyme in which K decreased and increased, respectively, with increase in incubation time. Soil amended with compost without enzyme showed higher concentrations of K than with the enzyme at 2 % and 3 % compost rates.

4.3.6 Mg and Na

Soil exchangeable Mg (Figure 4.5a) and Na (Figure 4.5b) concentrations increased with increase in application rate for both composts. Except for the control and the 1% compost produced with the enzyme, all other treatments resulted in increase in exchangeable Mg and Na with incubation time, particularly between 28 and 56 days. For all application rates, composts without the enzyme resulted in greater available Mg and Na than with the enzyme throughout the incubation time. The control had the lowest Mg and Na at all sampling times.

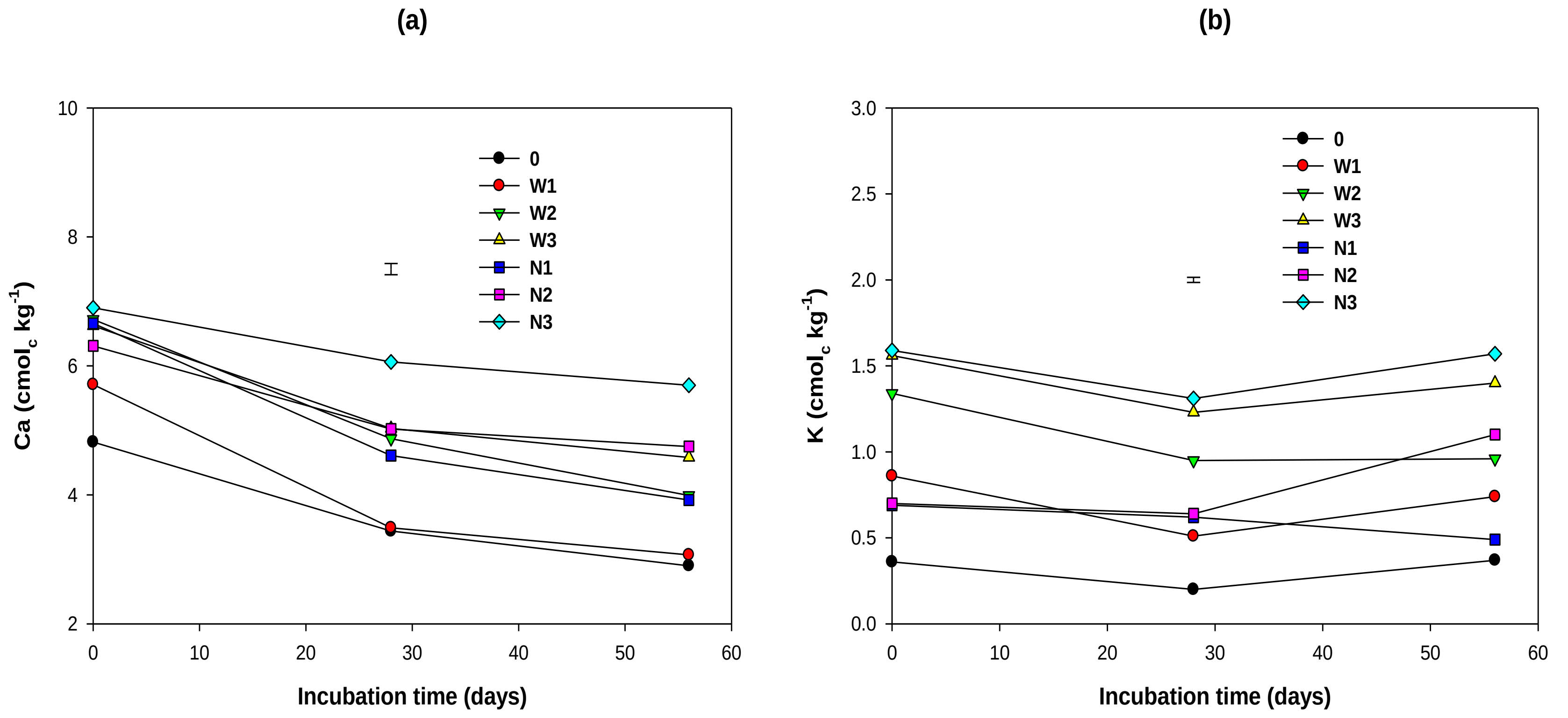

Figure 4. 4 Variation of Ca and K concentrations (cmol$_c$ kg^{-1}) during incubation of soil with different application rates. The error bar represents the least significant difference at $p<0.05$ level. 0 = control; W 1, 2 and 3 (w/w) indicates application rates of Biomax compost with enzyme; N 1, 2 and 3 (w/w) Biomax compost application rates without enzyme.

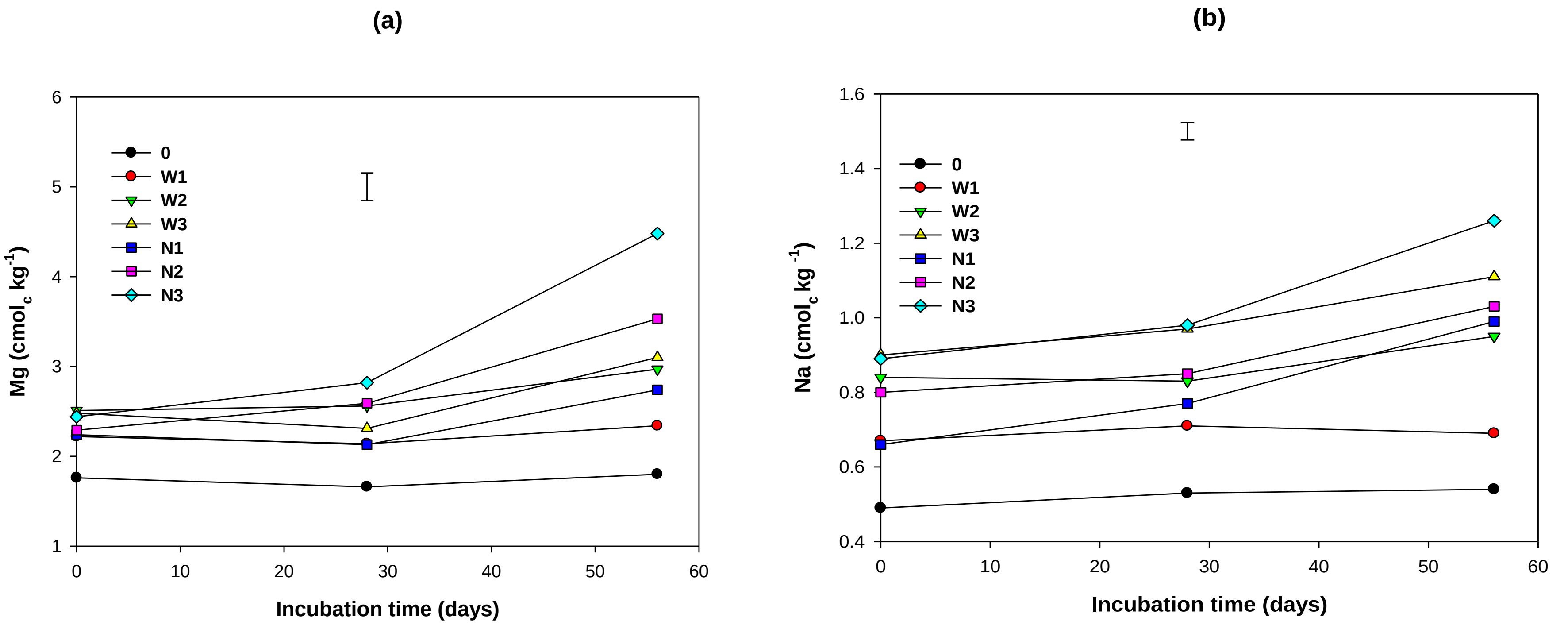

Figure 4. 5 Variation of Mg and Na concentrations (cmol$_c$ kg^{-1}) during incubation of soil with different application rates. The error bar represents the least significant difference at $p<0.05$ level. 0 = control; W 1, 2 and 3 (w/w) indicates application rates of Biomax compost with enzyme; N 1, 2 and 3(w/w) indicates application rates of Biomax compost without enzyme.

4.4 Discussion

The increase in pH of the soil with increasing compost rate, was a reflection that the addition of Biomax composts, which had high pH values, moderated the soil pH. These results were similar to those of Leconte *et al.* (2011) which showed that the addition of thermophilic composted poultry manure in different fractions increased the pH to close to nuetral. The decrease in soil pH during the first 28 days coincides with an increase in nitrate-N concentration (Dou *et al.,* 1996). The low pH values of the soil treated with compost produced with the enzyme may be due to the production of humic and organic acids during the decompostion of organic matter by microorganisms (Yu *et al*., 2013). The gradual decline in the NH_4-N mineralisation for both composts between 28 to 42 days of incubation can be attributed to N immobilization (Amanullah *et al.,* 2007) and this was confirmed by the decrease in NO_3-N concentration between 28 and 42 days. These results were similar to those of Amanullah *et al.* (2007), which showed that NH_4-N mineralisation declined with varying concentrations whereas the soil NO_3-N increased after the addition of thermophilic composted chicken litter followed by 105 days of incubation. Moreover N mineralisation concentrations of Biomax composts were higher compared to those of thermophilic composted chicken litter during incubation (Amanullah *et al.* 2007). This difference could be explained by higher concentrations of mineral N in the composts.

The higher NH_4-N concentration (1147 mg/ kg) in the compost without the enzyme could be explained by the instability of the initial compost, with lower mineral N which then mineralised during the incubation in the soil, than the one with the enzyme. As noted by Sanchez-Menedero *et al.* (2001) and Bernal *et al.* (2009), compost with NH_4-N content above 400 mg/ kg showed immaturity .The rapid increase of soil NO_3-N concentrations between 14 to 28 days of incubation for both composts at all rates was an indication of the nitrification process. These results were similar to those of Heenkende & Parama (2010), which showed an increase in NO_3-N within 21 days of incubation after the application of thermophilic composted chicken litter.

The results of pH confirms the occurrence of nitrification at pH > 4.5. Xiao *et al.* (2014) reported that the a pH of less than 4.5 affects growth and activity of nitrifying microorganisms. An increase in the soil available P concentrations between 14 and 28 days, for both composts at 3 % application rate, could be attributed to mineralisation of P from compost decompostion by microorganisms (Abbasi *et al.*, 2015). The available P and pH showed similar trends after 28 day of incubation for both composts. The pH of 6 and 7.5 in this study was ideal for P availability because pH values below 6 or above 7.5 directly affect P availability (Fuentes *et al.*, 2006).

These results were contrary to those reported by Yu *et al.* (2013), which showed that concentrations of available P increased during the first 30 days of incubation and therafter decreased following the application of the thermophilic chicken litter compost. This difference could be explained by the combination of mineralisation and adsorption of P by soil clay particles. The general decrease in extractable soil Ca concentrations for both composts with incubation time may be explained by the adsorption of Ca by soil particles and compost materials. Since Ca is a cation ion, it is adsorbed in the soil to the surface clay and organic matter which are negatively charged (Brady & Weil, 1999).

Furthermore, the prominent decrease of soil Ca at all compost rates was the reflection of adsorption of Ca by compost material (Brady & Weil, 1999). These results were contrary to those obtained by Ch'ng *et al.* (2014), which showed that concentrations of excheangeable Ca increased with the application rate of composted chicken litter during the incubation period of 90 days. This could be attributed to relatively high concentration of Ca from the compost. The increase in the soil K concentrations between 28 and 56 days of incubation was a reflection of high concentrations of K from the composts and the release of K during the decomposition of organic matter. The increase in the soil exchangeable Mg and Na for both composts between 28 and 56 days could be attributed to the release of these elements during the decomposition of composts (Hargreaves *et al.*, 2008). Although the compost containing no enzyme had higher total Ca, Mg and Na, the compost with the enzyme had lower exchangeable bases (Ca, Mg and Na) concentrations, which could be a result of immobilization of the bases by compost with enzyme.

4.5 Conclusion

Biomax composts without the enzyme resulted in greater available N, P and bases than with the enzyme. Higher rates of the composts resulted in greater available nutrients in the soil.The effect of nutrient release on the fertilizer value of the composts needs to be established under glasshouse and field experiments.

CHAPTER 5

BIOMAX COMPOST AS NITROGEN FERTILIZER FOR SPINACH *(SPINACIA OLERACEA).*

5.1 Introduction

Composted chicken manure has been used successfully for a long time as remedy in improving soil degradation and decline in productivity that is caused by conventional tillage and lack of soil conservation practices (Hernandez *et al.*, 2014). Utilization of organic compost as remedy is based on its ability to improve physical, chemical and biological properties of soil thereby having positive effect on crop production (Deportes *et al.*, 1995; Rigane & Medhioub, 2011). The agronomic practice of utilization of organic composts does not only represent low-cost disposal method but it also recycles essential nutrients for plant growth. Use of compost can only show negative impact on plant-soil system when is excessively applied than required, as results runoff and leaching process of nutrients will be prevalent phenomenon.

Furthermore beneficial effects of compost on crop production and soil properties are related to the amount of organic composts applied (Wong *et al.*, 1999). Therefore it is essential to investigate the effects of application rates on soil-plant system as to determine benefits of using composts as fertilizer compared to conventional inorganic fertiliser. Kara *et al*. (2006), observed increase in NH_4 mineralisation during the first week of incubation, thereafter NH_4^+ was nitrified into NO_3^- that accumulated as the experiment was progressing when poultry compost was added to a silt loam soil under an incubation study of 84 days. The Biomax composts from chicken litter contain significant concentration of nutrients. Result in Chapter 4 showed that NH_4-N and NO_3-N was released rapidly within 28 days of incubation in soil, which suggested that the Biomax composts could have potential as a nitrogen fertiliser. The specific objective for this study was to determine effect of different application rates of Biomax compost as nitrogen source on yield and nutrient uptake of spinach *(Spinacia oleracea).* The hypothesis was that application of Biomax compost will increase spinach growth, dry matter and nutrient uptake.

5.2 Materials and Methods

The study was conducted as a pot experiment under glasshouse conditions at the University of KwaZulu-Natal (UKZN), Pietermaritzburg campus (29°36′S 30° 23′E). The temperatures ranged from 19 - 28°C. The soil used in the incubation study was also used for this study, and its characteristics are detailed in Chapter 4. The compost with enzyme used had pH 7.45, 2.98 % N, 30.72 % C, 133.74 mg P kg^{-1}, 33000 mg K /kg and C: N ratio of 10.30 among other parameters (Table 4.2).

5.2.1 Pot experiment

The experiment was laid out in a randomized complete block design, with three replicates. The air dried and sieved soil (<2 mm) was treated with 0, 2.5, 5, 10 and 20 t/ha of Biomax compost produced with enzyme to vary the N rates, with the 10 and 20 t/ha supplying 3 and 6 times the recommended N rate (111.63 kg N/ha), based on total N. Phosphorus and K supplied by the composts were topped up with sodium dihydrogen phosphate (NaH_2PO_4) and potassium chloride (KCl), respectively, to achieve recommended rates. The recommended application rates (kg/ha) were 111.63 N, 246.9 P and 306.02 K. The rates translated to 0.076 g N, 0.167 g P and 0.21 K per pot with 2.2 kg soil.

Six 8-week old spinach seedlings *(Spinacia oleracea)* were transplanted to each pot and thinned to two plants per pot after two weeks. The plants were irrigated with tap water when required, to ensure that water was not limiting, and were allowed to grow for eight weeks and harvested by cutting with a scissors at 1 cm above the soil surface. The harvested plants were rinsed with water and oven dried at 65°C for 48 h to determine dry weight. The tissue samples were ground to <1 mm and stored for analysis of total C, N, P, Ca, Mg, Na and K. The soil from the pots were air dried for a week and sieved (<2mm) before analysis of pH, EC, mineral nitrogen, available P, exchangeable bases and trace elements.

5.2.2 Plant tissue analysis

Total C and N in plant tissue were analysed using Leco Trumac Autoanalyser as described in Chapter 3. For analysis of tissue P, bases and micronutrients, the plant tissue was digested using a microwave digester (CEM Corporation, 2014). The ground tissue samples (0.18 g) were digested with a mixture of 7 ml of nitric (HNO_3) acid and 1 ml of hydrogen peroxide (H_2O_2) following the installed procedure for plant materials. After complete digestion, the samples were allowed to cool and the solution was diluted to 50 ml using deionised water and analysed for total P, Ca, Mg, Na and K using the ICP (Vummiti, 2015).

5.2.3 Analysis of soils

Soil pH, EC and mineral nitrogen were determined following procedures described in Chapters 4. The soils (2.5 g) were placed in centrifuges tubes and 25 ml of Mehlich 3 extraction solution (0.2 N CH_3CHOOH+0.25 N NH_4NO_3+0.015 N NH_4F+0.013 N HNO_3+0.001 M EDTA) was added to each tube and placed on reciprocating shaker for 5 minutes at 200 oscillation per minute. The samples were centrifuged at 8 300 rpm for 4 min and filtered through 1.2 g of activated carbon (charcoal granular) placed on Whatman No. 2 filter paper to decolorize the filtrate. The filtrates were analysed for P, Ca, Mg and K on ICP (Wolf & Beegle, 2009).

5.2.4 Statistical analysis

Statistical analysis were performed using 14th edition of GenStat software (VSN International, 2011). One-way analysis of variance (ANOVA) was conducted to determine effect of application rates of Biomax compost as source of nitrogen on the measured parameters.

5.3 Results

5.3.1 Spinach dry matter yield, plant tissue nitrogen and carbon content

Spinach dry matter yield increased as the Biomax compost application rate increased (Table 5.1). Dry matter yield increase was proportional to the application rate of compost, with 1.6 and 2.1 g/pot for the 10 and 20 t/ha, respectively, while the mineral fertiliser treatment had 1.73 g/pot. There were no differences in spinach tissue nitrogen (N) concentrations among the different application rates of Biomax compost, except that the highest N rate had greater tissue N concentration than the control (Table 5.1). However, N uptake of spinach followed similar trend as dry matter. The mineral fertiliser treatment had N uptake of 0.030 gN/pot. All treatments had similar tissue C except the positive control (fertiliser), which had greater concentration (Table 5.1). Although there were no significant differences in tissue C/N ratio among treatments, the values appreared to decrease with increase in compost application rate.

Table 5.1. Dry matter, N uptake, Total content of N, C & C:N ratio as the results of Biomax compost application rates 0 (control), 2.5, 5, 10, 20 t/ha and the recommended nitrogen fertilizer application rate on Ukulinga soil. The LSD means least significant difference.

Treatment	Dry matter	Total N	Total C	C:N	N-uptake
	(g)	(%)			(g N/pot)
0 t compost/ha	0.9130a	1.44a	36.3a	25a	0.013a
2.5 t compost/ha	1.1670b	1.72a	35.2a	21a	0.020b
5 t compost/ha	1.4330c	1.66a	36.2a	22a	0.024b
10 t compost/ha	1.6000c	1.76a	37.1ab	21a	0.028cd
20 t compost/ha	2.1000e	1.85ab	36.0a	20ab	0.039d
Mineral N	1.7330cd	1.72a	37.9b	22a	0.030c
LSD	0.2470	0.325	1.60	4.6	0.0064

5.3.2 Other nutrients in plant tissue

The highest tissue P concentration was in the 20 t/ha treatment which was not significantly different from the 10 and 5 t/ha rates (Table 5.2) The positive control (mineral fertiliser) had the least tissue P followed by the negative control, with the 2.5 t/ha having a higher value than negative and positive control. The 20 t/ha and negative control treatments had higher Ca than most of the other treatments. Tissue Mg concentration was higher in the 20 t/ha than all other treatments.The 20 t/ha treatment had greater tissue K concentration than the other compost treatments, which had greater values than the mineral N and control treatments. In addition uptake of these elements generally increased with increase in compost application rates except Ca tissue that increase only after 20 t/ha (Table 5.3). The highest uptake of these nutrients were observed in the 20 t/ha treatment. Mineral treatment did not differ that much in Ca, Mg and K amounts with most compost tretments.

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Table 5.2. Spinach tissue nutrient concentration at increasing Biomax compost application rates.

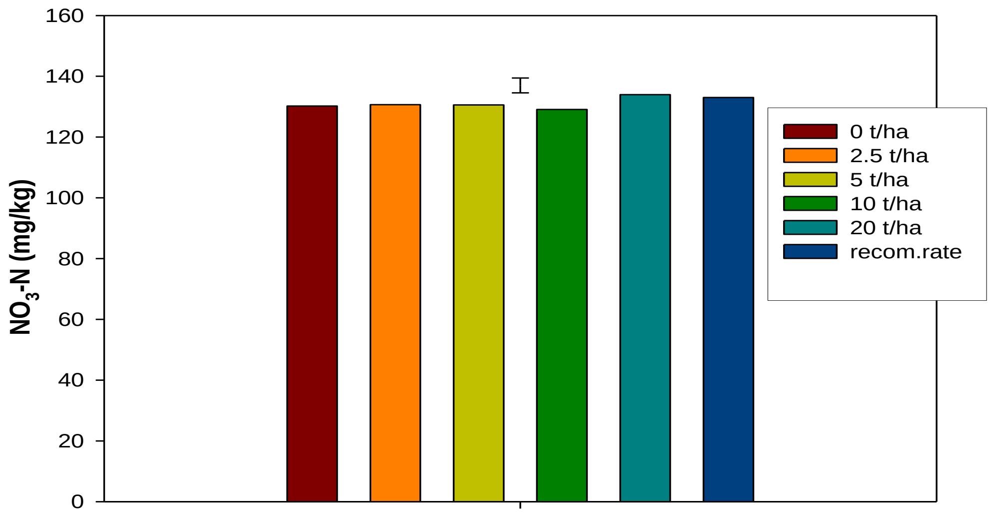
Treatment	P	Ca	Mg	K
		(%)		
0 t compost/ha	0.35a	0.16ab	0.12a	0.48a
2.5 t compost/ha	0.64b	0.13a	0.12a	0.65b
5 t compost/ha	0.72c	0.14a	0.11a	0.63b
10 t compost/ha	0.74c	0.13a	0.10a	0.56c
20 t compost/ha	0.76c	0.18b	0.16b	0.71db
Mineral N	0.29d	0.11a	0.12a	0.52ac
LSD	0.05	0.04	0.04	0.07

Table 5.3. Uptake of P, Ca, Mg and K as the results of Biomax compost application rates 0 (control), 2.5, 5, 10, 20 t/ha and the recommended nitrogen fertilizer application rate on Ukulinga soil. The LSD means least significant difference.

Treatment	P-uptake	Ca-uptake	Mg-uptake	K-uptake
	(g P/pot)	(g Ca/pot)	(g Mg/pot)	(g K/pot)
0 t compost/ha	0.003	0.002	0.001	0.004
2.5 t compost/ha	0.008	0.002	0.001	0.008
5 t compost/ha	0.010	0.002	0.002	0.009
10 t compost/ha	0.012	0.002	0.002	0.009
20 t compost/ha	0.016	0.004	0.004	0.015
Mineral N	0.005	0.002	0.002	0.009
LSD	0.002	0.001	0.001	0.002

5.3.3 Residual soil mineral nitrogen

At harvest there were no significant differences in concentration of NO_3^- in the soil among the treatments. However, NH_4^+ increased with increase in compost rate. The 5 t/ha compost treatment had lower NH_4^+ concentration than the mineral N fertiliser treatment which had lower levels than the 10t/ha and 20 t/ha compost treatments (Figure 5.1).



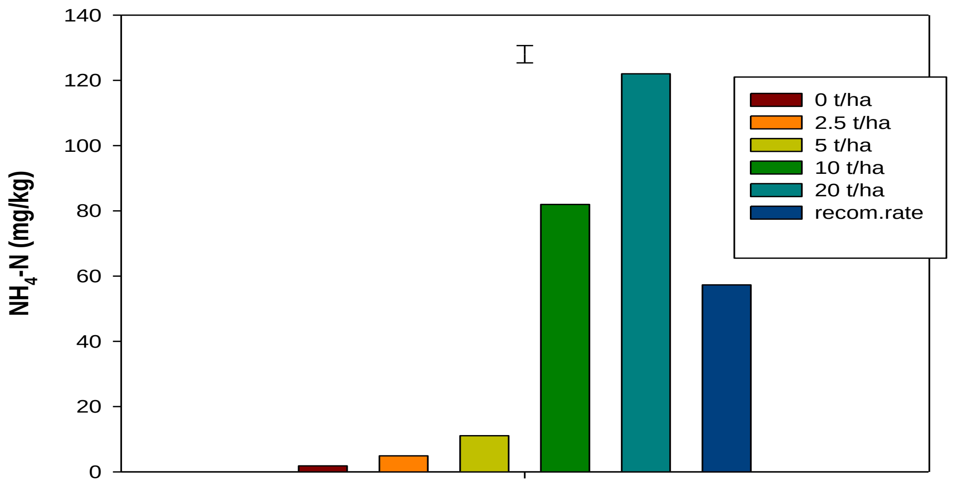


Figure 5.1. Concentrations of mineral nitrogen in soil after harvest of spinach fertilized with Biomax compost at relative application rates. The error bars denotes LSD at $p<0.05$.

5.3.4 Residual soil chemical composition

Soil pH remained constant with increase in application rates in all treatments (Table 5.4). Soil EC increased with increase in compost application rate. The mineral N treatment had lower EC than all manure treatments, but was higher than in the negative control. The 5 and 10 t/ha compost treatments had the least available soil P, which was similar to the negative control (Table 5.4). The 20 t/ha compost treatment had the highest soil P which was not statistically significant compared to the 2.5 t/ha and the mineral N treatments. The mineral N fertiliser treatment had the lowest exchangeable Ca, followed by the negative control, and the compost treatments had greater levels, with the 20 t/ha having the greatest Ca (Table 5.4). The 20 t/ha compost treatment had highest levels of Mg, while positive control had lower. On the other hand, the negative control and 2.5, 5 and 10 t/ha compost rates had similar concentrations of Mg. The least exchangeable soil K was in the 5 and 10 t/ha manure treatments, followed by 2.5 and 20 t/ha, which had lower levels than the negative control and the mineral N fertiliser treatment (Table 5.4).

Table 5.4. Residual soil characteristics after harvest of spinach from the different Biomax compost treatments.

Treatment	pH		EC	P	Ca	Mg	K
(t/ha)	(H20)	(KCl)	(dS/m)	(mg/kg)	(cmol/kg)		
0	6	5	0.69	314a	12.12a	4.71a	3.75a
2.5	6	5	0.84	338ab	12.91b	4.59a	3.44b
5	6	5	0.84	300a	13.06b	4.47a	2.99c
10	6	5	0.90	302a	13.33b	4.60a	3.04c
20	6	5	1.28	401b	14.32c	4.80ab	3.55b
Mineral N	6	5	0.76	369ba	11.24d	3.82c	4.06d
LSD	0.18	0.09	0.02	65.56	0.74	0.28	0.19

5.4 Discussion

The use of the Biomax compost, as an organic N fertilizer showed significant increase in spinach yield compared to inorganic fertiliser, especially at higher rates. The increase in spinach yield with increase in application rates over negative and positive controls was the reflection of N (and other nutrients) released during compost decomposition (Arthur *et al.,* 2012). The similarity in the trend of N uptake and spinach drymatter yield suggested that the increase in yield was in response to N uptake. Furthermore the highest yield and nutrient plant uptake were recorded at 20 t/ha compost rate. These results were similar to those obtained by Indriyati (2014), which showed that incorporation of thermophilic chicken litter compost into soil resulted in increase of komatsuna dry matter and N-uptake over negative and positive control. The values of dry matter and N-uptake in this study (Biomax compost) were lower compared to those of Indriyati (2014). This difference could be explained by higher concentrations of plant readily available N after decomposition of compost with enzyme by mircoorganisms (Arthur *et al.,* 2012). Furthermore P, Ca, Mg and K plant uptake followed the same trends of spinach drymatter and N-uptake reflecting release of these nutrients during decomposition of Biomax compost by microorganisms (Leytem *et al*., 2011).

The increase in tissue P with increase in application rates, with the 20 t/ha being the highest, was the results of high levels of P from compost. The higher concentration of Ca than the control was the reflection of the soil Ca before planting, which was about 5.17 cmol/kg. In addition the high amounts of tissue Mg and K in 20 t/ha was due to high concentrations of Mg and K in compost. These results were similar to those of Preush *et al.* (2004), which showed that application of composted chicken litter increased plant uptake of P, Ca, Mg and K. The values of P, Mg, and K uptake in this study (Biomax compost) were higher than those of Preush *et al.* (2004). The difference was due to higher concentrations of P, Mg and K in the added thermophilic compost (Hirzel *et al*., 2007).

At harvest, the similar pH values in all treatments, including soils amended with Biomax compost, suggested that the composts did not affect soil pH. On the other hand, the increase in soil exchangeable Ca, Mg and K concentrations with increase in application rates, agreed with those of Warren *et al* (1993), when composted chicken litter was applied. However, the concentrations of these parameters were higher in this study compared to those of Warren *et al* (1993), which could be explained by from the addition of egg-shells during the production of Biomax compost (Mitchell, 2005).

Although there was an increase in soil EC with increase in rates, the values were still below 2 dS/m, which is an indication of a healthy soil (Arnold *et al.*, 2005). The trend was supported by increase in soil residual mineral N (NH_4^+- NO_3^-), which was the results of mineralisation of OM (Cavins *et al.*, 2000). Furthermore, higher NH_4^+ at higher compost rate at harvest, suggested that N mineralisation continued up to the end of the pot trials. These results were similar to those of Atiyeh *et al*. (2000), which showed that addition of thermophilic composted chicken litter increased soil mineral N and had high values of NH_4-N and NO_3-N compared to this study, this could be explained by higher N mineralisation of thermophilic chicken compost (Li *et al.,*2014). On the other hand Warren *et al.* (1993) showed that incorporation of chicken compost increased soil available P and had high figures of P compare to those of this study (Biomax compost), this could be attributed to higher release of P during mineralisation of compost (Fuentes *et al.*, 2006). The increase in soil P with increase in compost rates was the reflection of compost addition, which had significant amounts of P. On the contrary, the increase in soil P in of the control treatment compared with 5 t/ha and 10 t/ha compost rates could be a result of P uptake by the greater drymatter in response to higher N, compared to lower drymatter in the control.

5.5 Conclusion

Biomax compost, as N fertiliser increased dry matter and nutrient uptake of spinach. At least 10 t/ha of Biomax compost would be required to produce greater drymatter and uptake of N, P and bases than the recommended rate of mineral N fertiliser. Therefore positive effects of Biomax compost on drymatter and nutrient uptake of crops need to be tested under field conditions.

CHAPTER 6

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General Discussion

Land application of raw organic materials results in release of volatile compounds and loss of ammonia with odors and accumulation of pathogenic organisms in soil (Atiyeh *et al.,* 2000; Khalil *et al.,* 2005; Petersen et *al.,* 2007). Composting is used to stabilize the organic materials and sterilize it from pathogens (Godley *et al.,* 2004). The quality of the compost is determined by its chemical composition, nutrient release pattern and effects on crop growth. The Biomax Rapid thermophilic composting as been developed to produce composts at 70-80 ^{o}C within a 24 h period. The objective of this study was to determine effects of Biomax composting time and addition of the BM1 enzyme on compost quality and nutrient release and the fertilizer value of the composts.

Based on the low HI (<3.5)and HR (<7.0) indices (Bernal *et al.*, 2009), Biomax composting did not result in mature and stable compost, but eliminated *E. coli* and *Salmonella* spp and composting time did not have significant effects on total C, N and C/N ratio. The HI and HR for Biomax composts were lower compared to agronomically established limits for a stable and matured compost (Bernal *et al*., 2009) and the effect of high temperatures (70-80^{o}C) during composting could be responsible for eradicating pathogenic organisms. Work by Bernal *et al.* (2009) indicated that temperatures above 55 ^{o}C were suitable for eliminating pathogens. The non significant effect of composting on total C, N and C/N could have been the results of immaturity and instability of the composts coupled with minimal losses of N and C during composting in a closed system (Sanchez-Monedero *et al.,* 2001). Composting for at least 18 h resulted in increased mineral N and decline in mineral P. The increase in mineral N was a result of mineralisation of N from the chicken litter (Gao *et al.* 2010a). The decline in available P could have been the result of precipitation of by Ca, Cu, Zn, Mn, Fe phospahates (Tan, 2010). Mineral-N, HI and HR were increased, and available Zn, Cu and Mn were reduced by incorporation of the BM1 enzyme compared to without the enzyme, supporting the view that the enzyme is required to enhance mineralisation, while the reduction of micro nutrients in compost with enzyme was due to increase in humification (Pettit, 2004). Work by Tan (2010) showed that humic compounds were effective in binding mirconutrients during decomposition of OM. Increase in humification indicates advance in stability of the compost.

The similarity in total C, N and P concentrations and C/N in the composts could explain the similarity in trends of mineral N, particularly NO_3-N, and P in the incubation study. The higher NH_4-N during incubation of compost without the enzyme, than with the enzyme, could be explained by the lower HI and HR (lower stability) in the compost (Benal *et al.*, 2009), which released more NH_3. This finding was supported by the pH values which was higher in the compost without the enzyme, during incubation. The concentrations of NH_4-N and NO_3-N increased with rate of composts during incubation could be explained by the higher rates of total N added, which mineralised N and nitrified (Gao *et al.* 2010a). The increase in mineral N with compost rate could explain the results of N uptake in the pot trial, which resulted in increase in drymatter with increase in compost rate. The declined in NH_4-N while NO_3-N concentrations increased during incubation were in agreement with Preusch *et al.* (2002), who reported a similar trend in soil treated with 20 gN/kg composted chicken litter during 120 days of incubation period. Furthermore the decline in NH_4-N while NO_3-N concentrations increased during incubation were in agreement with Azeez & van Averbeke

(2010), who observed similar results in soil treated with 120 kg N/ha uncomposted chicken manure during an incubation period of 120 days.

The higher NO_3-N after 28 days incubation, than earlier days, suggested that spinach growing in such a medium would have access to high levels of available N between 28 and 56 days of the pot trial, resulting in greater uptake and biomass accumulation. These results were in agreement with those of Indriyati (2014), which showed that drymatter of komatsuna crop increased after incorpation of thermophilic composted chicken litter in the soil. Dikinya & Mufwanzala (2010) also observed similar trends, where drymatter of spinach increased with increase in rate of uncomposted chicken manure.

The increase in residual mineral N in the soil also reflected that N was mineralised from chicken manure (Dikinya & Mufwanzala, 2010), and some of the N was taken up by spinach resulting in increased drymatter. These findings were similar to those of Zai *et al.* (2008) and Dikinya & Mufwanzala (2010), which showed that addition of thermophlic chicken compost and uncomposted chicken litter, respectively, increased soil residual mineral N due to N mineralisation (Indriyati, 2014). Soil residual mineral-N amounts increased with compost rates but at lower levels compared to mineralised-N during incubation, possibly because some of the nitrogen was taken up by plants and assimilated by microorganisms during decomposition of compost (Manitoba, 2013). The Biomax compost applied at 10 t/ha appears to be the most appropriate for increased spinach drymatter and nutrient uptake and residual soil nutrients, whereas higher rates (20t/ha) could result in nitrate leaching losses from the soil and pollution of ground water (Syman & van der Waals, 2004).

In addition to N uptake, spinach drymatter yield could also be explained by uptake of P, Ca, Mg and K (Dikinya & Mufwanzala, 2010), which followed the same trend, and all of these macronutrients could have been released from the degradation of compost and mineralisation.

This view was supported by the results of the incubation study, which showed increases in available P, Ca, Mg and K, with increase in application rate. The trends of drymatter were similar to those of uptake of these macronutrients. These results were similar to those of Preush *et al.* (2004), which showed that uptake of Ca, Mg and K increased with rates of thermophilic composted chicken litter compared to the negative control. The increase in uptake of Ca, Mg and K by spinach with increasing rate compared to mineral-N inorganic fertilizer was due to greater release of these nutrients as indicated in incubation study. These nutrients were made available for plant uptake after decomposition of compost by mircoorganisms (Leytem *et al.*, 2011). Furthermore Ezeocha *et al.* (2014) reported that plant uptake of Ca and Mg increased with increasing rates of uncomposted chicken manure more than negative control due to greater release of excheangeable bases that were taken up by the plant (Dikinya & Mufwanzala, 2010). The higher levels of Ca concentration in the residual soil also support the view that the compost released these macronutrients, and the Ca could have originated from the egg-shells added during the production of the composts. The higher drymatter in the 5 and 10t/ha treatments, than the control could have resulted in greater uptake of P and K, leaving lower residual levels. The soil pH of 6.5, from the incubation study, suggested that availability of most nutrients, including phosphorous and exchangeable bases, were favoured.

Although the pot trial was done with the compost produced with the BM1 enzyme only, using the compost without the enzyme could have resulted in similar drymatter, N and P uptake based on the similarity of the mineralisation of these nutrients in the incubation study, and the composition in the initial composts. Residual soil N and P composition could also have been the same. Total N and P in the initial compost, and nitrate-N and mineral P in the incubation study, were similar in the two composts. Although mineral P concentration did not change with incubation time for both composts, Doydora *et al.* (2011) showed that soil amended with

200 kg P/ha uncomposted chicken litter increased P concentrations within 21 days of incubation, as a result of greater P mineralisation (Kumar *et al.*, 2015). Exchangeable Ca in the original compost and Ca release in the incubation study showed that the compost with the enzyme had higher levels, while levels of K were higher in the compost without the enzyme. Uptake and residual soil concentration of Ca could have been lower had the compost without the enzyme been used, while K uptake could have been higher.

In considering the larger quantities of chicken litter in South Africa, rapid composting strategies are required. However the conditions used for Biomax composting in 24 h with or without addition of BM1 enzyme, are inferior to normal thermophilic composting from a chemical composition perspective (Gao *et al.*, 2010a). This is because the composts are not stable and mature. Whereas Mupondi *et al*. (2010) showed that one week pre-composting to eliminate pathogens, the Biomax thermophilic composting eliminates pathogenic organisms within an hour of the process. Based on the instability and low composition of pathogens, these composts could just be behaving like uncomposted chicken litter that has been sterilised. Normally composting is done with material mixed to get a C/N of about 25 (Haung *et al.* 2004) and in this study the farmer practice was used and the C/N was 10:1. This anomaly could have contributed to the immaturity of the composts.

6.2 Conclusions

Biomax composting (within 24 h), with or without the BM1 enzyme, produces immature composts, which are free of pathogenic organisms. When added to soil, the Biomax compost without the enzyme releases more nutrients, than with the enzyme and most of the mineralisation occurs within the first 28 days. Addition of Biomax compost, as an organic fertilizer, increased spinach drymatter yield and uptake of N, P and bases. Compost rates ≥10 t/ha resulted in greater drymatter, nutrient uptake, and residual soil nutrients, than the inorganic fertiliser at the recommended rate.

It is recommended that the Rapid Biomax technology be adopted sterilise large amounts of organic wastes within a short space of time. Nutrient release dynamics from Biomax compost in different soils for more than 56 days need further investigation so as to fully understand the potential and limitations of using Biomax compost. Future studies should be conducted to fully understand the fertiliser value of Biomax composts with a variety of crops under field conditions.

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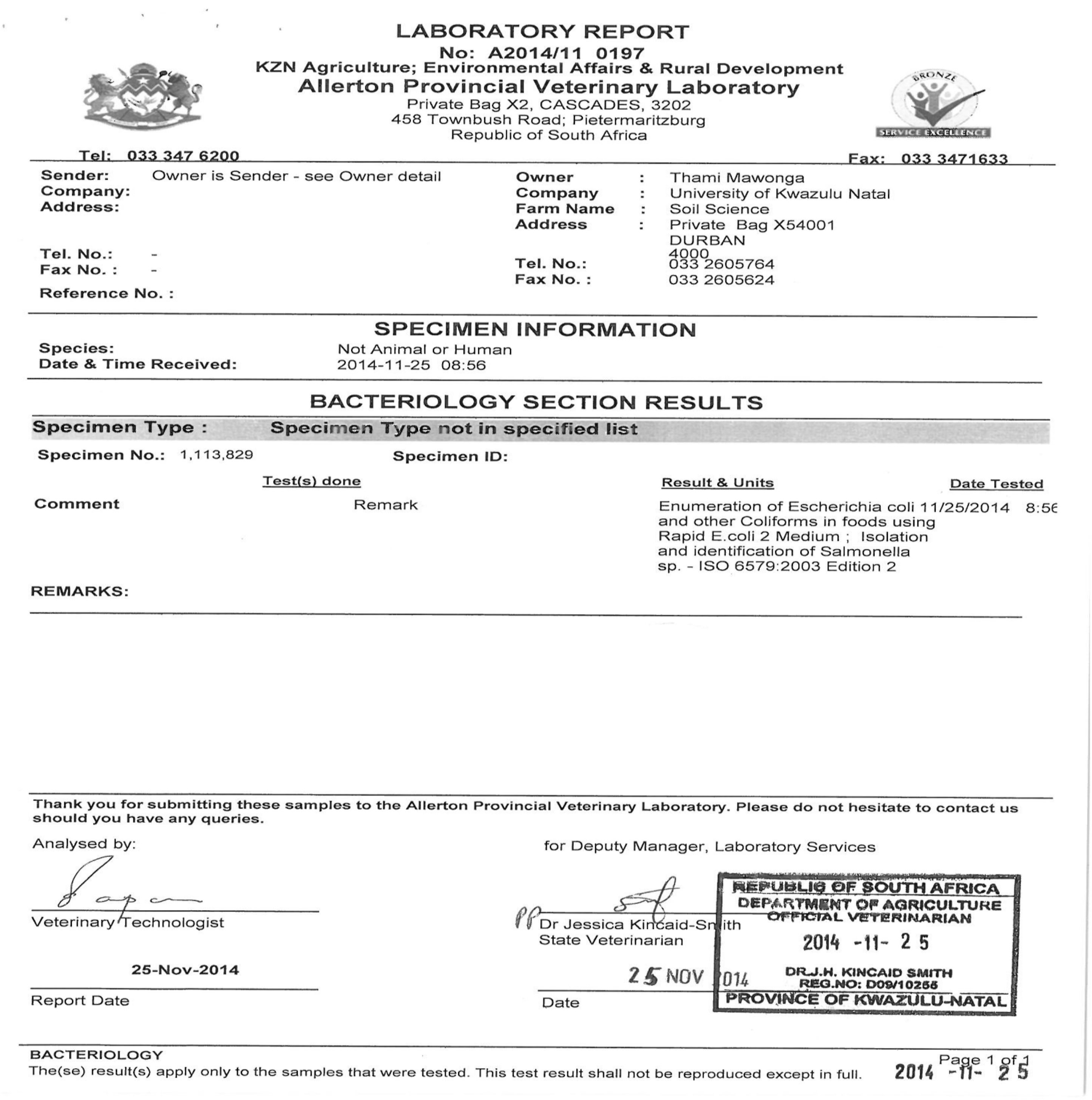
APPENDICES

Appendix 1: Pathogenic organism’s population of Biomax composts sampled over time during composting.



LABORATORY REPORT

No: A2014/11 0197

KZN Agriculture; Environmental Affairs & Rural Development

Allerton Provincial Veterinary Laboratory

Private Bag X2, CASCADES, 3202
458 Townbush Road; Pietermaritzburg
Republic of South Africa

BRONZE
SERVICE EXCELLENCE

Tel: 033 347 6200 **Fax: 033 3471633**

Sender: Owner is Sender - see Owner detail
Company:
Address:

Tel. No.: -
Fax No. : -

Reference No. :

Owner : Thami Mawonga
Company : University of Kwazulu Natal
Farm Name : Soil Science
Address : Private Bag X54001
DURBAN
4000
Tel. No.: 033 2605764
Fax No. : 033 2605624

SPECIMEN INFORMATION

Species: Not Animal or Human
Date & Time Received: 2014-11-25 08:56

BACTERIOLOGY SECTION RESULTS

Specimen Type : Specimen Type not in specified list

Specimen No.: 1,113,829 **Specimen ID:**

	Test(s) done	Result & Units	Date Tested
Comment	Remark	Enumeration of Escherichia coli and other Coliforms in foods using Rapid E.coli 2 Medium ; Isolation and identification of Salmonella sp. - ISO 6579:2003 Edition 2	11/25/2014 8:5

REMARKS:

Thank you for submitting these samples to the Allerton Provincial Veterinary Laboratory. Please do not hesitate to contact us should you have any queries.

Analysed by:

Veterinary Technologist

25-Nov-2014

Report Date

for Deputy Manager, Laboratory Services

PP Dr Jessica Kincaid-Smith
State Veterinarian

25 NOV 014

Date

REPUBLIC OF SOUTH AFRICA
DEPARTMENT OF AGRICULTURE
OFFICIAL VETERINARIAN
2014 -11- 25
DR.J.H. KINCAID SMITH
REG.NO: D09/10255
PROVINCE OF KWAZULU-NATAL

BACTERIOLOGY
The(se) result(s) apply only to the samples that were tested. This test result shall not be reproduced except in full. Page 1 of 1 2014 -11- 25

FINALE LABORATORIUM VERSLAG - FINAL LABORATORY REPORT

No: A2014/11_0197

RESULTS AND REPORT

		E. coli count	Salmonella species identified
LIMS Number	**Specimen ID**	cfu/g	-
1,113,817	Compost1	0	Not Detected
1,113,818	Compost2	0	Not Detected
1,113,819	Compost3	0	Not Detected
1,113,820	Compost4	0	Not Detected
1,113,821	Compost5	0	Not Detected
1,113,822	Compost6	0	Not Detected
1,113,823	Compost7	0	Not Detected
1,113,824	Compost8	0	Not Detected
1,113,825	Compost9	0	Not Detected
1,113,826	Compost10	0	Not Detected
1,113,827	Compost11	0	Not Detected
1,113,828	Compost12	0	Not Detected

Thank you for submitting these samples to Allerton Provincial Veterinary Laboratory. Please do not hesitate to contact us should you have any queries.

Analysed by:

pp Director Veterinary Services

2014 -11- 25