OPTIMIZATION OF THE VERMIDEGRADATION OF COW DUNG –WASTE PAPER MIXTURES

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DECLARATION

I, **Frank Oshioname Unuofin** declare that the thesis hereby submitted for the degree of Doctor of Philosophy at the University of Fort Hare is entirely my own independent work and has not previously been submitted at another University.

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GENERAL ABSTRACT

Vermicomposting is an eco-friendly waste management strategy. Its successful performance necessitate that key functioning parameters like earthworm stocking density, nutrient enrichment be established for each target waste/waste mixture. One main target waste mixture in South Africa, and in the University of Fort Hare in particular is waste paper mixed with cow dung and rock phosphate (RP) for phosphorus (P) enrichment. This study was carried out to address the following specific objectives, to determine (i) the effect of *Eisenia fetida* stocking density on the bioconversion of cow dung waste paper mixtures enriched with rock phosphate, (ii) an optimum application rate of low grade South African Rock Phosphate and time required for efficient vermicomposting of cow dung-waste paper mixtures, and (iii) to determine if the phosphorus in RP is responsible for improved biodegradation during the vermicomposting of cow dung-waste paper mixtures.

Results of this study revealed that bioconversion of cow dung waste paper mixtures enriched with RP was highly dependent on *E. fetida* stocking density and time. The stocking density of 12.5 g-worms kg^{-1} feedstock of the mixtures resulted in highest earthworm growth rate and humification of the waste mixture as reflected by a C: N ratio of < 12, polymerization index (PI) or humic acid/fulvic acid ratio of > 1.9, and a humification index of >13 for the cow dung waste paper mixtures. A germination test carried out also revealed that the resultant vermicompost had no inhibitory effect on the germination of tomato, carrot, and radish. Extractable P increased with stocking density up to 22.5 g-worm kg^{-1} feedstock, suggesting that for maximum P release from RP enriched wastes, a high stocking density should be considered.

Informed by an earlier study which demonstrated that RP improved vermidegration, a follow up study was done to determine the optimum amount of rock phosphate necessary for efficient vermidegradation of cow dung waste paper mixtures while ensuring a phosphorus rich vermicompost. The results showed that addition of RP at rates $\leq$ 1% P as RP efficiently enhanced the bioconversion of cow dung waste paper mixtures as reflected by low C: N ratio, high polymerization index (PI), HI and HR used as maturity indicators for matured compost. Final vermicompost products obtained at minimum amounts of RP application rates resulted highly humified vermicompost with finer morphological structure, with no inhibitory effect on the germination of tomato, carrot, and radish similar to the ones obtained at higher RP rates. The findings suggest that 1%P as RP application rate is optimum for efficient vermidegradation of cow dung waste paper mixtures.

Since P or Ca happen to be the most prevalent elements in most rock phosphate used for compost enrichment, a study was carried out to determine if P or Ca in RP is predominantly responsible for the improved biodegradation of cow-dung waste paper mixture observed during vermicomposting. Phosphorus sources in form of triple superphosphate (TSP), phosphoric acid (PHA) and Ca in form of calcium chloride ($CaCl_2$) salt were compared with rock phosphate.

The results from the study indicated that TSP, a water soluble P source, resulted in greater and faster degradation of the waste mixtures than RP while the Ca source had the least effect. With TSP incorporation the compost maturity C: N ratio of 12 was reached within 28 days while RP, PHA and $CaCl_2$ needed 42, 56 and more than 56 days, respectively. The results indicated that P was largely responsible for the enhanced bioconversion of the waste mixtures. This appeared linked to the effect of P to stimulate microbial growth as reflected by higher microbial biomass carbon levels where water soluble P sources were applied. The C: N ratios of the final vermicomposts at day 56 were 10, 11.5, 13, 14, and 23 for TSP, RP, PHA, Control (No P added) and $CaCl_2$ treatments, respectively. Although TSP gave superior superior performance, RP may still be the preferred additive in the vermicomposting of cow dung waste paper mixtures as it is cheaper and produces mature compost in a shorter period of 8 weeks.

Generally, the results of this study have shown that the vemidegradation of cow dung waste paper mixtures can be optimized through adoption of an *E. fetida* stocking density of 12.5g-worm kg^{-1} and an RP incorporation rate of 1% P as RP. However, higher rates of RPincorporation may be adopted where final vermicomposts with higher P fertilizer value are desired. Phosphorus appears to be the RP constituent responsible for its ability to enhance the vermidegradation of cow dung waste paper mixtures. Future studies should explore the effectiveness of other P-bearing minerals for their effectiveness in enhancing vemidegradation.

PREFACE

The abstract of this thesis shows the summary of the detailed work which is explained in the following emerging six chapters. Chapter 1 encloses the broad introduction that ascertains the justification for the study. Chapter 2 is the literature review which establishes the need for the study, the causes of the problem, and possible solutions to the problem and the potential of the studied solution in resolving the identified problem. Chapter 3 reports on the findings of a study that sought to establish an optimum (*Eisenia fetida*) earthworm stocking density for cow dung paper waste mixtures that resulted in vermicomposts of good nutritional quality and whose use will not jeopardize human health. This chapter has been published in the Journal of Waste management and the phblished article is appended as Appendix 1. Chapter 4 reports on the optimization rates of rock phosphate (RP) incorporation to cow dung waste paper mixtures for optimum vermi-degradation and improved P content of the resultant vermicomposts. Chapter 5 reports on the result of a study that sought to shed light on the RP constituent that could be responsible for its effect to improve the biodegradation of cow dung waste paper mixtures. Chapter 6 is a synthesis of all experimental findings in relation to the objectives of the study. It ends with general discussions, conclusions and recommendations for future studies.

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LIST OF ABBREVIATONS AND ACRONYMS

^{13}C-NMR	^{13}C nuclear magnetic resonance spectroscopy
ANOVAR	Repeated measure analysis of variance
C: N	Carbon to nitrogen ratio
$CaCl_2$	Calcium chloride
CEC	Cation exchange capacity
C_{EX}	Extractable carbon
C_{FA}	Fluvic acid carbon
C_{HA}	Humic acid carbon
CRD	Completely randomized design
CV	Coefficient of variation
EC	Electrical conductivity
E_C	Organic carbon extracted from fumigated – unfumigated soil
FA	Fluvic acid
FTIR	Fourier Transmission infra red
GI	Germination index
HA	Humic acid
HI	Humification index
HR	Humification ratio
K_{EC}	Proportion of microbial carbon extracted from compost
LSD	Least significant difference
MBC	Microbial biomass carbon
MC	Moisture content
NH_4: NO_3	Ammonium –N to Nitrite-N ratio
PHA	Phosphoric acid

P_i	Inorganic phosphorus
P_o	Organic phosphorus
PR	Phosphate rock
PI	Polymerization index
P_t	Total Phosphorus
RRE	Relative root elongation
RSG	Relative seed germination
SEM	Scanning electron microscopy
SOUR	Specific oxygen uptake rate
TGA	Thermogravimetric Analysis
TSP	Triple superphosphate

CHAPTER ONE

1 GENERAL INTRODUCTION

Solid waste management is a major environmental challenge in the world due to increasing population, urbanisation, intensive agriculture and industrialization. Huge amounts of wastes, for example, are generated from intensive livestock farming in form of animal excreta, while different institutions also produce a lot of solid wastes such as waste papers, waste food, grass clippings and cardboards. However, out of 1.7 – 1.9 billion metric tons of Municipal Solid Waste (MSW) quantity estimated being generated worldwide (Chalmin and Gaillochet, 2009), South Africa produce over 42 million cubic metres every year, with Gauteng Province contributing 42%, Western Cape province 20%, Mpumalanga Province 12%, KwaZulu Natal province 8%, Eastern Cape Province 5%, Free state Province 4%, Northern Cape province 4%, Limpopo Province 4% and Northwest province 2 % respectively (Muzenda *et al.*, 2012). Out of the 42 million tonnes solid waste that South Africa generated, waste papers form the huge part.

The production and consumption of waste paper in South Africa has increased by 2.1 million tonnes to 2.7 million tonnes per year from 2009 to 2012 (PRASA, 2013). At the University of Fort Hare, waste paper generation increased from 2.4 tonnes per month in 2007 to 3.2 tonnes per month in 2010 because of increase in student intake (Mupondi, 2010). This upward trend is estimated to have increased to 5.6 tonnes per month in 2013 for the same reason according to Richard Scott (Xerox manager, UFH Branch, a personal conversation, 15-05-2013). In most places, the waste paper is either incinerated; land fills or disposed of in dump sites along with other wastes. This often causes air pollution and ground water pollution.

Composting has been found to be a viable approach for managing organic waste (Mbuligwe et *al*., 2002). There are two main forms of composting, namely, thermophilic composting and vermicomposting. Thermophilic composting is the most common form of composting in which organic wastes undergo a microbiologically mediated process facilitating the mineralisation and incomplete humification of the organic matter, leading to a heterogeneous final product, free of phytotoxicity and pathogens and with certain humic properties (Bernal *et al*., 2009).

During the first stage of the process, the simple organic carbon compounds are simply mineralised and metabolised by the microorganisms, generating CO_2, NH_3, H_2O, organic acids and heat. The build-up of this heat increases the temperature up to ($\geq 75^0C$), in a predominantly aerobic environment (Vallini *et al*., 2002; Hubbe *et al*., 2010; Mupondi, 2010). The benefits of composting, thus, involve reduction of volume of the wastes, the destruction of weed seeds, and pathogenic microorganisms. However, thermophilic composting takes a long time, requires a high frequency of material turning, is associated with loss of nutrients during the long composting process and inhibition of decomposition (Ndegwa and Thompson, 2001, Bernal *et al*., 2009).

Vermicomposting, on the other hand, involves the bio-oxidation and stabilization of organic material by the combined activity of earthworms and microorganisms. The microorganisms biochemically breakdown the organic matter, whereas earthworms serve as modifiers of the process, as they aerate, condition and fragment the substrate, thereby drastically alter the microbial activity and consequently minimizing frequent turning of material and loss of nutrients. By their mixing action, earthworms modify the physical and chemical status of the organic matter by gradually reducing the ratio of C: N thereby increasing the surface area exposed to microorganism for favourable activity and further decomposition (Tognetti *et al.*, 2005, Mupondi, 2010). It has been reported that the action of earthworms during vermicomposting is physical/mechanical and biochemical (Edwards and Bohlen, 1996). This mean that substrate aeration, mixing, which is manually done by frequent turning, is achieved by earthworm mixing action, while biochemical process is effected by microbial decomposition of the substrate in the intestines of the earthworms.

Vermicomposting is being used as a solid waste management technology for agricultural waste (Garg *et al*., 2006, Suthar, S., 2007., Aalok *et al*., 2008), city garbage (Indrajaat *et al*., 2011, Garg *et al*., 2011, Ansari, 2011), kitchen waste (Garg *et al*., 2011, Ansari, 2011, Alok ., 2010), paper waste (Mupondi., 2010, Alok ., 2010, Lim *et al*., 2012, Manyuchi *et al*., 2013b, Manyuchi, 2013a) and biosolid (Garg *et al*., 2006, Suthar, S., 2007). So far, work on earthworm stocking density has focused mostly on its influence on growth and maturation of the earthworms than on the vermicomposting of the substrates (Dominguez and Edwards. 1997). Generally, earthworms grew amd matured fast in low than high stocking densities (Ndegwa *et al*., 2000). This suggested that, the amount of earthworms inoculated into waste during vermicomposting process could determine the degree of decomposition and dissolution of different substrate. It was therefore, needful to establish an ideal earthworm stocking density for varying compositions of the different waste mixtures in order to optimize the process.

Ndegwa *et al*. (2000) reported that 1.6 kg-worm m^{-2} was the ideal optimum stocking density for the bioconversion of biosolid and paper mulch as substrate. Yadav *et al*. (2011) reported a stocking density rate of 3 kg-worms m^{-2} as optimum for the bio-conversion of human faeces and observed maximum total biomass, growth and reproduction at high stocking rates. Neuhauser *et al*., (1980) reported an ideal *E. fetida* stocking density of 0.8 kg-worms m^{-2} for horse manure and 2.9 kg-worms m^{-2} for activated sludge. They observed that using regression analysis, the earthworm production increased with population density while growth declined.

Singh and Kaur (2013) reported an optimum stocking density of 12.5 g-worms kg^{-1} feed of *E. fetida* for chemical sludge and spent carbon obtained from industrial soft drinks. Dominguez and Edwards (1997) reported that *Eisenia andrei* showed faster worm growth but less sexual maturation at 0.25 kg-worms m^{-2} population density. Thus the ideal earthworm stocking density for waste biodegradation seem to vary with substrate type. Mupondi *et al*. (2010) used an earthworm stocking density of 1.6 kg-worm m^{-2} at a feeding rate of 0.75 kg feed kg worm^{-1} day^{-1} in their study on the bioconversion of diary manure and waste paper mixtures. This rate, which was found to be ideal for bio solids by Ndegwa *et al.* (2000), may however, not be the ideal stocking density for cow-dung-waste paper mixtures due to differences in the quality/ composition of the two substrates. Therefore, it is imperative to determine the optimum earthworm stocking density necessary for efficient vermicomposting of cow-dung-waste paper mixtures using *Eisenia fetida*.

Composts are poor sources of plant nutrients in comparison to inorganic fertilizers (Mupondi, 2010; Yan *et al*., 2012; Matiullah and Muhammad, 2012). They are especially low in phosphorus (P) which compromises their acceptance as alternatives to the more expensive inorganic fertilizers. Phospho-composting has been used to improve the quality of composts through enrichment with P-bearing minerals like phosphate rock and other additives (Kaur and Singh, 2012). Mupondi (2010) investigated the co-vermicomposting of waste print paper with dairy manure. The author found that dairy manure co-vermicomposted well with waste paper and that inclusion of RP not only increased the P content of the resultant vermicompost but it also accelerated the bioconversion of the cow dung-waste paper mixtures. The rates of RP incorporation investigated ranged from 2 to 8% P as RP. The extent of humification of the cow dung waste-paper mixture increased with increasing rate of RP application but the increases were not statistically significant at rates above 2% P (Mupondi, 2010), suggesting that lower rates of RP incorporation could achieve the same result. This indicated a need to investigate the effectiveness of lower rate of RP incorporation on the vermicomposting of cow dung-waste paper mixtures.

Generally, the study by Mupondi (2010) indicated the potential of managing waste paper through vermicomposting with dairy manure, and also the potential of enhancing the process using RP which agreed with the work of (Biswas and Narayanasamy, 2006; Mishra, 1992). These studies did not, however, establish why RP incorporation was able to accelerate the bioconversion of cow-dung-waste paper mixtures.

Most rock phosphates used for nutrient enrichment during vermicomposting of various wastes contain calcium and phosphorus as the predominant elements in terms of composition. The RP used by Mupondi *et al* (2010) was obtained from Phalaborwa in Limpopo Province, South Africa and was of the following chemical properties: P_2O_5- 40.3%; CaO-54.6%, MgO-0.26%; cadmium-2.2 mg kg^{-1}, chromium -18.05 mg kg^{-1}, copper -5.85 mg kg^{-1}, lead -6.05 mg kg^{-1} and zinc -13.22 mg kg^{-1}. It was, therefore, hypothesized that since Ca and P were the dominant nutrient elements in RP they could be responsible for the observed improved bioconversion of cow dung waste paper mixtures. Therefore, the main objectives of this study were to find ways of optimizing the vermi-degradation of cow-dung-waste paper mixtures through establishing the optimum earthworm stocking density and optimum RP application rate; and to determine whether phosphorus and calcium played key roles in enhancing the bioconversion of cow-dung-waste paper mixtures.

STUDY OBJECTIVES AND HYPOTHESES

1.1 The specific objectives of this study were to:

a). Evaluate the effect of *Eisenia fetida* stocking density on the bioconversion of cow-dung waste-paper mixtures enriched with rock phosphate (RP) and identify an ideal stocking density.

b). Determine the rate of Phalaborwa RP (low grade South African Rock Phosphate) incorporation and time required for efficient vermicomposting of cow dung-waste paper mixtures.

c). Determine if the phosphorus and calcium in RP could be responsible for improved biodegradation during the vermicomposting of cow dung-waste paper mixtures.

1.2 Hypothesis of the Study:

a). There is an ideal *Eisenia fetida* stocking density for the bioconversion of cow dung- waste paper mixtures fortified with rock phosphate (RP).

b). The bioconversion of cow dung-waste paper mixtures can be achieved with rates of RP application supplying less than 2% P.

c). Phosphate Rock improves the vermicomposting of cow dung-waste paper mixtures through the phosphorus or calcium it supplies during the process.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Introduction

Disposal of solid organic waste generated from all three streams of waste (municipal waste, commercial and industrial waste and construction or demolition waste) are one of the main challenges facing both developed and developing countries due to population explosion, growing industrialization and rapid urbanisation (Mufeed *et al*., 2008, Rajeev *et al*., 2011). These wastes are disposed into dumpsites, landfills and /or incinerated, causing ground water contamination, air pollution and production of methane gas. In view of this, a sustainable eco-friendly waste management strategy is advocated.

Vermicomposting and composting are two of the best known ways for the biological stabilization of organic solid wastes; however, vermicomposting is proved to be the best sustainable biotechnological process of managing and converting the ever increasing amounts of biodegradable solid organic waste into organic fertilizer in an eco-friendly way through the joint actions of microorganisms and earthworms (Lazanco *et al*., 2008, Nandita and Arun, 2012). This literature review focused on the different biodegradable wastes production, comparison between thermophilic composting and vermicomposting, vermicomposting of different wastes and the need for optimizing vermidegradation of organic waste for nutrient enrichment during vermicomposting or composting.

2.2 Biodegradable waste Generation

Human activities have led to the generation of unavoidable by-product called waste. Economy bloom and rising standard of living in most countries of the world have increased the quantity and complexity of waste generated. In addition, industrialisation and the provision of expanded health-care facilities have added significant quantities of industrial hazardous waste and biomedical waste into the waste stream with likely severe environmental and human health consequences (Muzenda *et al*., 2013).

There are two distinct classes of waste: general waste (municipal waste) and hazardous waste (some industrial and health-care waste). Municipal waste can be generally categorized into: Bio-degradable waste (paper, food and kitchen waste, green waste), recyclable material (paper, glass, bottles, cans, metals, certain plastics, fabrics), inert waste (construction and demolition waste, dirt, rocks and debris), electrical and electronic waste, composite wastes (clothing, tetra packs, waste plastics), hazardous waste, toxic waste (pesticides, herbicides, fungicides) and medical wastes (Muzenda *et al*., 2013). Biodegradable wastes are generated from kitchens, fast food joints, domentaries, shopping mulls, agricultural farms and institutions in form of food waste, kitchen waste, paper waste, leaves and grass clipping and agricultural waste (animal and crop) respectively.

2.3 Thermophilic composting

Thermophilic composting is a biochemical process which involves accelerated degradation of organic matter by microorganisms under controlled conditions (Singh *et al*., 2010; Bernal *et al*., 1998). These waste substances go through thermophilic phase ($\geq 75^0$C) which sanitize and eliminate pathogens to produce a stabilized final product that is free of phytotoxic compounds and contains humic properties (Lazanco *et al*., 2008). Thermo-composting is the most economical and sustainable alternative for onsite organic waste management because its operation is easy and can be conducted in contained space. Although, thermo-composting has been adopted as the basic method for on-site waste decomposition, it has disadvantages such as long duration of the process, frequent aeration required, loss of nutrients (e.g. gassing off of nitrogen) and a heterogeneous end product (Nair *et al*., 2006). So, the need to find a quicker and cost-effective alternative technique is required. In view of this, vermicomposting has been reported to be a feasible, cost effective and quick method for solid wastes management.

2.4 Factors influencing Composting Process

The composting process is influenced by temperature, carbon to nitrogen ratio, pH, air and moisture content. Moisture is essential for microbial action during composting process, which increases the rate of decomposition of the waste material. The level of moisture content must be high enough to ensure adequate biological stabilization rates, however, not too high to block air pores that could limit the rate of oxygen transport and thus inhibit the rate of biological activity (Haug, 1993). Most of the composting processes operate within moisture content of 40-60% (Haug, 1993).

Temperature in the composting process determines the type of microorganisms present at both the mesophilic and thermophilic phase, the rate of metabolic activities and organic waste decomposition (Haug, 1993). Air is essential for the activities of the aerobic microorganisms, and its movement aid the removal of water from wet substrates through drying, heat and carbon dioxide from decomposing organic matter which control the temperature in the process (Haug, 1993).

Carbon to nitrogen ratio (C: N) is important in the composting process because cellular synthesis is initiated with nitrogen and carbon which form the largest portion of the organic molecules in the cell (Haug, 1993). In this process, oxidation of carbon produces energy which is metabolized to synthesize cellular constituents. Nitrogen on the other hand, is a component of protoplasm, protein and amino acids. Hence, the growth and multiplication of any organism depends largely on the presence of nitrogen. During the active phase of composting, microbes use up 15 to 30 parts of carbon for each part of nitrogen. Therefore, for favourable composting, a C: N ratio of less than or equal to 30 should be maintained. Higher C: N ratio slows down the composting process while very low C: N ratio causes loss of nitrogen in form of ammonia (Inbar *et al.*, 1993).

Microbial activity during composting is limited either by high or low pH. Hence, optimum pH range for most bacterial activities is between 6.0 and 7.5, whereas fungi thrive well within pH ranges of 5.5-8.0 (Daiz *et al.*, 1993). As a result of the release of carbon dioxide and ammonia during organic matter decomposition, both high and low pH values are buffered back towards neutrality as composting progresses (Haug, 1993). Hence, the adjustment of the pH of the starting substrate is not required since carbon dioxide and ammonia are the end products of all organic and protein decomposition.

2.5 Vermicomposting

Vermicomposting is a simple technological process of composting which involves the use of certain species of earthworms for bio-oxidative conversion and stabilization of biodegradable solid waste to give a homogenous product (Venkatesh and Eevera, 2008, Loh *et al.*, 2005). Earthworm's role during vermicomposting is both physical/mechanical and biochemical. The physical process involves substrate aeration and mixing, as well as actual grinding of materials in the gut of the earthworm whereas, microbial decomposition of the substrate in the intestines of the earthworms is done biochemically, which result important plant nutrient such as N, P, K and Ca that are converted to more soluble plant form. (Frederickson *et al*., 2007) reported accelerated organic matter stabilization during vermicomposting of organic waste, whereas (Arancon *et al.,* 2005) reported more chelating and phytohormonal elements having high content of microbial matter and stable humic substances.

Different species of earthworm have been used for vermicomposting of various wastes and over 1800 species of earthworms worldwide have been estimated (Edward and Lofty, 1972). However, the most commonly used is the *Eisenia fetida*, commonly called the “compost worm”, “red worm” and “red wiggler”. Microorganisms that grow and live on the waste materials such as fungi, protozoa, algae, bacteria and actinomycetes during vermicomposting serve as a means of nourishment to the earthworms in that order of importance. The vermicomposting method needs to be maintained at temperatures of 15 to 25^{o}C and a moisture content of 60 to 80% suitable for earhworm and microbes survival. In addition, earthworms are much active and biodegrade organic solid wastes at a comparatively narrower level of 15 to 25 cm beneath the surface of compost bed.

Though vermicomposting is a common practice in most Asian countries and the Western world, there are few reports of vermicomposting research in Africa and even less in South Africa. Vermicomposting research in South Africa has mainly been on a laboratory scale and only focused on how climatic factors such as moisture and temperature affect the life cycles of different species of earthworm (Reinecke and Venter, 1987). There is little or no information on the effectiveness of vermicomposting on the bioconversion of locally generated wastes such as livestock manures, paper and green wastes.

2.6 Fundamental conditions for vermicomposting

To achieve a successful vermicomposting process, certain essential conditions must be met. These conditions include:

(i) Bedding

Bedding is any material that provides the worms with a relatively stable habitat. This habitat must be able to absorb and retain water fairly for the worm to thrive. Various bedding materials such as: paper from municipal stream, newspaper, peat moss, corn silage, sawdust, cardboard, paper mill sludge horse manure have been used during vermicomposting (Latifah *et al.,* 2009; Fatehi and Shayegan, 2010).

(ii) Temperature

Responses of earthworms to temperature changes are fairly complex. The potential of several species of earthworms to grow and produce cocoon is temperature dependent (Dominguez, 2004). Similarly, the optimal temperature limit for earthworm survival varies between species (Mupondi, 2010). *Esienia fetida* thrives well at optimal temperature of 25^{o}C but can tolerate temperatures between 0 and 35^{o}C (Dominguez, 2004). Generally, temperatures below 10^{o}C result in reduced feeding activity; below 4^{o}C cocoon production and development of young earthworms ceases (Edwards and Bohlen, 1996). But in extreme temperature conditions, there is hibernation and migration of earthworms to deeper layers of the vermiboxes for protection. Earthworms can acclimatize to temperature in autumn and survive the winter, but cannot survive for long periods in freezing conditions (Dominguez, 2004).

(iii) Moisture

The body weight of earthworms is made up of 70-90% water; therefore, earthworms can only survive when dehydration is prevented (Edwards and Lofty, 1972). Moist environment is essential for the survival and growth of earthworm as they need moist skin for their respiration metabolism. Edwards, 1988 reported an optimum moisture content range for most species used in vermicomposting systems to be between 50-90%. *Eisenia fetida* can thrive in moisture ranges between 50 - 90% (Dominguez, 2004) but in animal wastes it grows rapidly in moisture ranges between 80 and 90%. However, Reinnecke and Venter (1987) reported optimum moisture content of above 70% in cow manure for *E.fetida* whereas, *E.andrei* propagated in pig manure grew and matured best between 65 and 90% moisture content, with 85% as the optimum (Dominguez and Edwards, 1997).

The moisture content appropriate for clitellate cocoon-producing *Eisenia fetida* in separated cow manure ranges from 50% to 80% for adults, but juvenile earthworms prefer a moisture range of 65% to 70% since clitellum development occurred in earthworms at moisture contents at that range, but are delayed at 55-60% moisture range. Low moisture condition retards growth and sexual development. The development of clitella by earthworms of same age can vary under different moisture conditions at different times Reinnecke and Venter (1987).

(iv) Aeration

Earthworms obtain oxygen by diffusion through the body wall and also exspire carbon dioxide by diffusion since they lack specialized respiratory organs. Earthworms have high sensitivity to anaerobic conditions, thus, low oxygen concentration of around 55 to 65% result in depressed respiration rates (Edwards and Bohlen, 1996). Feeding activities are reduced under these suboptimal conditions. Migration of individual *E. fetida* and other species have been reported in large numbers from a water-saturated substrate where oxygen conditions had been depleted or in which carbon dioxide or hydrogen sulfide had accumulated. Nevertheless, they can live for long periods in aerated water, such as in trickling filters in wastewater treatment plants. Oxygen may be depleted if earthworm beds are kept too wet or if too much feed is introduced.

(v) pH

Generally, epigeic earthworms are acid tolerant and tend to move towards the more acid material, when given a choice in a pH gradient with a pH preference 5.0. Nevertheless, they avoid acid materials of pH less than 4.5 because prolonged contact with such acidity could have lethal effects (Dominguez, 2004). An optimal pH range of greater than 5 and less than 9 was reported to be ideal for *Eisenia fetida* survival (Edwards, 1995, Singh *et al*., 2005).

(vi) C: N ratio

Organic carbon and inorganic nitrogen have been reported to play a vital role during cell division, growth and metabolism in all living organisms including earthworms (Ndegwa and Thompson, 2000). According to (Aira *et al*., 2006), substrates that have low C: N ratio generates matured clitelate earthworm population with higher mean individual weight compared to high C: N ratio whereas, substrates with higher C: N ratio produced more of hatchlings and juveniles. To achieve quick growth and maturation of earthworms, vermidegradation and stabilization of organic wastes during vermicomposting, a suitable C: N ratio for different materials is required. (Ashish *et al*., 2013) reported that higher loss in total organic carbon, chemical oxygen demand, and soluble biochemical oxygen demand, total nitrogen and phosphorus and stabilised compost are achieved at a C: N ratio of 30 during vermicomposting of sewage sludge with cattle manure. According to Kendie, (2009), substrates that have low C: N ratio increases available phosphorus, potassium, organic carbon and earthworm's growth and production.

(Vii) Worm stocking density

Population density has been one of the key factors determining the growth and reproduction of waste composting earthworms (*E. fetida, E. andrei*). Neuhauser *et al*. (1980) reported a decline in growth and reproduction of *E. fetida* when studying the effects of population density on growth and reproduction with respect to increase in earthworm density and using linear regression, the potential stocking density of *E. fetida* was estimated to be 0.8 kg-worms m^{-2} for bioconversion of horse manure and 2.9 kg-worms m^{-2} on activated sludge.

Similarly, Dominguez and Edwards (1997) in a related study reported that individual worms developed faster at lowest population density, whereas, total biomass production was maximum at the highest population density. They observed that sexual maturation of worms was faster at higher densities than at lower stocking densities with *Eisenia Andrei.* Yadav *et al*. (2011) reported a stocking density rate of 0.5 and 3 kg-worms m^{-2} for the bio-conversion of human faeces and observed maximum total biomass, growth and reproduction at high stocking rates. Suthar (2012) reported that low stocking density favour maximum individual biomass production, while cocoons production rate was better with high density. Mupondi, (2010) observed maximum total biomass, growth and reproduction using a stocking density of 1.6 kg-worm m^{-2} at a feeding rate of 0.75 kg feed kg $worm^{-1}day^{-1}$ for bioconversion of cow dung-waste paper mixtures . Therefore, to achieve a rapid turnover of waste materials during vermicomposting, high stocking rate is preferred.

2.7 Differences between vermicomposting and composting

In contrast with compost formed from the same substrates, vermicompost is richer in content of available nutrients after passing through the worm gut (Zhenjun, 2004). In addition, vermicompost after the same period of maturity become more effective for soil fertility improvement than compost. A comparison between composting and vermicomposting is presented in Table 2.1 (Somani, 2008).

Table 2.1 Comparison of composting and vermicomposting with some advantages of process, product and application

Parameter	Composting	Vermicomposting
Heating requirement	Remained and controlled	Avoiding excess heat
By-product	Non	Worm biomass
Period of decomposition	Long (≥ 6 Months)	Less
Decomposition degree of OSW material	Incomplete in large and heterogeneous particles	Complete in small and homogeneous particles
Smell of product	Still bad odour	Less bad odour
Available salt content in product	Increased	Greater increase
pH level of product	Less reduced, pH (>7)	Reduced near to neutral
C/N ratio of product	Decreased	Greate reduction
Available N-P-K in product	Increased	Greater increase
Humification and stabilisation of product	Increased	Greater increase
Pathogenic microbes in product	Reduced	Greater reduction
Texture and structure of soil with product	Improved	Greater improvement
Yield of plant crops with product amendment	Increased	Greater increase

Adapted from Frederickson *et al*., (2007); Lazcano *et al*., (2008) and Somani, (2008)

2.8 Vermicomposting of different organic solid waste

Vermicomposting as a technology has been successfully harnessed worldwide to manage different organic solid waste from domestic, commercial, institutional, industrial and agricultural activities of the society. In general, wastes produced from these streams consist of paper and cartons, food scraps, glass, cans, clothes, animal manure, human faeces, vegetable waste, fruits, grass clippings, leaves and demolition debris. During vermicomposting of these wastes, earthworms consume, and assimilate organic material through symbiotic microorganisms in their gut, changing it into finer, humidified, microbiologically active vermicast. The resultant vermicompost is stable and homogeneous with good aesthetics value; reduced levels of contaminants; and is a precious, profitable and better plant growth medium (Gupta and Garg, 2008).

Essential plant nutrients in the material are released and transformed into the forms that are more soluble and available than parent substrate during the process (Ndegwa and Thompson, 2001). Various studies have revealed the potential of using epigeic earthworm species in the bioconversion of a wide range of organic solid waste such as animal excreta (Suthar, 2008; Khwairakpam and Bhargava, 2009), sewage sludge (Kaushik and Garg, 2004; Loh *et al*., 2005), human faeces (Yadav *et al*., 2010), crop residual and agricultural wastes (Reddy *et al*., 2005; Shalabi, 2006), raw organic wastes Gajalakshmi *et al*., 2004), waste paper in smal1 and large-scale projects (Mupondi, 2010; Muddasir and Agrawal, 2013).

Vegetables and fruit wastes from processing industries are good sources of organic material and plant nutrients, therefore, vermicomposting can probably be used to convert fruit and vegetable peels to organic manure (Avunish *et al*., 2010). Garg and Gupta (2011) reported that the vermicomposting of cow dung spiked with pre-consumer processing vegetable to contained high nutrient levels without heavy metals. Vasanthy *et al*. (2012) reported that vermicomposting of market waste (vegetable waste) and garden waste were successfully converted to valuable manure within 24 days with higher plant essential nutrients. According to Azizi *et al*. (2012) sawdust based spent mushroom compost, an organic waste and biomass residue media for pea sprout cultivation was reused for earthworm propagation and thus reported higher proliferation of the earthworm in a shorter period of 50 days compared with previous work on vermicomposting of saw dust-based spent mushroom compost that lasted for 70 days.

Various studies on paper waste composting or vermicomposting have only explored the possibility of using paper mill sludge as feed or bulking agent for epigeic earthworm species with the addition of nitrogen rich additives for augmentation (Butt, 1993; Ceccanti and Masciandaro, 1999). Gajelakshmi *et al*. (2001) studied the effect of different earthworms species on the biodegradation of paper waste mixed with cow dung at 6:1(w/w) and reported that *Eudrilus eugeniae* and *Lampito mauritii* performed better than others with highest weight biomass. Mupondi, (2010) studied the optimum pre-composting period and nutrient improvement by adding rock phosphate and thus reported better value added compost. Muddasir and Agrawal, (2013) worked on the management of paper waste using *Eudrilus eugeniae* to examine its vermicomposting potential and reported finding well humified final compost.

The stocking density of earthworms (that is the biomass per unit surface area) and feeding rate are known to affect the biomass growth and reproduction, quality and quantity of vermicompost product. Several studies are reported on this aspect with different feeds. For example, Nadaffi *et al.* (2006) reported a stocking density of 1.36 kg –worm m^{-2} for highest bioconversion of waste activated sludge into earthworm biomass and volatile solid reduction. Singh and Kaur, (2013) reported an optimum stocking density of 12.5 g-worms kg^{-1}-feedstock which translated to be (0.05 kg- worms m^{-2}) and a feeding rate of 0.75 kg-feed kg-worms^{-1} day^{-1} for biodegradation of chemical sludge and spent carbon obtained from industrial soft drinks. Ndegwa *et al*. (2000) reported a stocking density of 1.6 kg-worms m^{-2} and a feeding rate of 1.25 kg-feed kg-worm^{-1}day^{-1} for highest bioconversion of the substrate into earthworm biomass, and the best vermicompost was obtained at the same stocking density and a feeding rate of 0.75 kg-feed kg-worms^{-1} day^{-1} when paper mulch was used as the substrate. However, the specificity of optimum stocking density for bioconversion of waste paper into humified compost is not known. Dominguez and Edwards, (1997) studied the effect of stocking rate on growth and maturation of *E. andrei* in pig manure and concluded that, whereas individual worms grew more and faster at the lowest population density, the total biomass production was maximum at the higher population density. At higher ratesof *E. andrei*, they noted that the worms sexually matured faster than in the lower stocking rates. Monroy *et al*. (2006) showed that stocking densities have a strong effect on mature weight of earthworm and cocoon production. However, there is little or no information on stocking density for the bioconversion of cow-dung-waste paper mixtures.

2.9 Benefits of optimizing the vermidegradation of organic waste

Organic wastes differ in their chemical composition as well as in the degree of their decomposition. Wastes are classified into bio-degradable and non bio-biodegradable. Biodegradable waste includes kitchen waste, animal waste, crop residues and general agricultural waste whereas, non biodegradable wastes includes inert waste (construction and demolition waste and debris), electrical and electronic waste, hazardous waste, toxic waste (pesticides, herbicides, fungicides) and medical wastes (Muzenda *et al*., 2013).

Optimization of stocking density, application rate of additives for nutrient enrichment, bulking materials, feeding rate and operating condition creates an ideal optimal level that is suitable for rapid decomposition of various target waste/waste to achieve a progressive vermicomposting process.

(a) Effect of optimizing stocking density on vermicomposting of organic waste

Optimization is a way of acertaining the minimum application rate or stocking density of earthworm into vermicomposting medium to achieve an ideal standard density suitable for producing vermicompost products from different kind of waste mixtures, since various stocking density and feeding rate always affect reproduction, quality and quantity of vermicompost product (Elvira *et al*., 1998). For example, Singh and Kaur, (2013) reported an optimum stocking density of 12.5 g-worms kg^{-1} –feedstock (0.05kg-worms M^{-2}) highest for biodegradation of chemical sludge and spent carbon obtained from industrial soft drinks. Ndegwa *et al*. (2000) reported a stocking density of 1.6 kg-worms m^{-2} and a feeding rate of 1.25 kg-feedstock kg-worm^{-1}day^{-1} for highest bioconversion of the substrate into earthworm biomass, and the best vermicompost was obtained at the same stocking density and a feeding rate of 0.75 kg-feedstock kg-worms^{-1} day^{-1} when paper mulch was used as the substrate. Giraddi, (2008) reported a stocking density of 0.11 kg-worms m^{-2} for highest bioconversion of agricultural waste into earthworms' biomass, population growth, cocoon production, with best vermicompost produced at the same rate. However, the specificity of optimum stocking density for bioconversion of waste paper into humified compost is not known.

(b) **Effect of optimizing application rate of RP on vermicomposting of organic waste**

Enrichment of vermicompost with RP has been found to increase the nutrient contents, humification parameters, efficiency of decomposition and P fractions in the resultant vermicompost. Biswas and Narayanasamy, (2006) increased the total P content of straw compost from 0.37% in control to 2.20% by adding 4% P. Yan *et al*. (2012) increase the total P of rice straw vermicompost from 0.392% to 0.82% by adding 2% P as RP. Matiullah and Muhammad (2012) also increased the total P content in poultry litter to 1.02% by inoculating 4% P as RP. However, none of these studies explored the effect of added RP on the humification of the vermicompost. Mupondi (2010), however, investigated the effects of added RP ranging from 2 to 8% P to dairy manure waste paper mixtures on both the total P content and humification parameters. He showed that increasing rates of RP application resulted in increased available Pand total P content as well the humification parameters HI, HR and PI. The trend of the results was such that optimal rate of RP application was lower than the lowest rate of RP application of 2% P which he investigated. There was thus need to investigate the effectiveness of rates of RP application less than 2 % P on the bioconversion of cow dung waste paper mixtures.

2.10 Role of vermicompost in organic farming

The use of vermicompost for organic farming is reported to result in high food productivity with outstanding nutritional quality, whilst also improving the physical, chemical and biological properties of the soil (Gorakh and Keshav, 2011). Furthermore, the use of vermicompost in farming is reported to sequester huge amounts of atmospheric carbon, contributing to reduction of greenhouse gas emissions and thus mitigation of global warming (Rajeev *et al*., 2011). Besides, it is also reported that with the slow nutrient releasing pattern of vermicompost fertiliser compared with chemical fertilizer, significant amount of nutrient are conserved for plant use (Suhane, 2007). The release of humates or humic and fulvic acid from the long lasting carbon content in vermicompost have been reported to improve soil health and fertility in organic farming (Biswas and Narayanasamy, 2006).

2.11 Compost Enrichment

In bid to improve nutrient content of vermicompost which are usually poor in phosphorus in comparison with nitrogen due to the different chemical composition or nature of the feedstock. The combination of RP and phosphate-solubilising microorganisms has been used as an ameliorant of P deficiency in P deficient soils (Jader *et al.*, 2012; Iyer *et al.*, 2012; Saravana and Aruna, 2013; Biswas and Narayanasamy, 2006).

(1) Rock Phosphate

Rock phosphate (RP) is a naturally occurring mineral source of phosphate mined and processed into water soluble phosphate fertilizers. Ground RP is much less expensive than soluble phosphorus fertilizers, and is an amendment that is allowed by organic certification bodies (Zapata and Roy, 2004). Phosphate rocks generally are apatitic; containing varying percentages of P_2O_5 in calcium bound substance. In South Africa, RP is mined mainly in the Palaborwa area in the Limpopo Province (van Linden, 2004). The RP produced is of a low grade granitic type with low P and high heavy metal contents (van Linden, 2004). Biswas and Narayanasamy, (2006) reported that aerobic thermophilic compost prepared with addition of rock phosphate had more P than normal composts. They also reported significant increases in microbial biomass carbon as well as phosphatase activity in RP-enriched compost.

Matiullah and Muhammad, (2012) reported that the solubility enhancement of phosphorus from rock phosphate through composting with poultry litter could be harnessed. According to (Jeevan, 2007), compost prepared with rock phosphate increased the total phosphorus content from 0.2% to a maximum of 0.89% under both aerobic and anaerobic conditions.

In another study, Hellal *et al*. (2012) reported that RP solubility and sulfate ammonia increased during phospho-composting of organic waste. Jolanta *et al*. (2013) reported that the solubility of ground phosphate rock was increased, resulting from co-composting with organic substances, causing bioavailability of phosphorus. Vermicomposting has been reported also to increase the available P and nutrient contents of organic waste enriched with rock phosphate.

Yan et al. (2012) reported a 2.3 fold increase in available P, total P and humic substances by applying 2%P as RP into rice straw vermicompost. Jolanta *et al*. (2013) reported that a ratio of four parts of organic waste materials to one part of rock phosphate (dry-weight basis) was effective for their decomposition while, Mupondi (2010) reported increased available P from 434 mg kg^{-1} to 1312 mg kg^{-1}, HR from 1.33% to 20.5%, HI from 6.4% to 23.3% by investigating rates of RP incorporation ranging from 2 to 8% P as RP. The degree of humification of the resultant vermicompost increased with increasing rate of RP application but the magnitudes of increases were not statistically significant at rates above 2% P which indicated that adding RP at lower rates would give the same result. Therefore, enrichment of cow-dung-waste paper mixtures with lower RP application rate should be explored.

(2) Phosphate solubilising microorganisms

Phosphate solubilising microorganisms (PSM) through their various activities during organic matter decomposition are able to improve phosphorus nutrition and stimulate plant growth. Rock phosphate solubilization occurs as a result of the secretion of organic acids by microorganisms that reduces the pH or by complexing cations that are bound to phosphorus and resulting in the release of the otherwise insoluble form (Iyer *et al*., 2012; Matiullah and Muhammad, 2012). The use of phosphate solubilizing microorganisms, for example, *Aspergillus awamori* was found beneficial for phosphate amended compost and vermicompost (Sibi, 2011). Jader *et al*. (2012) reported changes in labile phosphorus forms in matured vermicompost enriched with phosphorus solubilizing and diazotrophic bacteria. According to Saravanan and Aruna, (2013) supplementation with probiotics such as *Lactobacillus sporogenes*, essential microbes and *Saccharomyces cerevisiae*, could enhance the vermicomposting process and improve nutrient content in the compost. Pramanik et *al*. (2009) depicted that microbial inoculation into vermicomposting of different organic substrates increased humic acid content and phosphatase activity, and in turn enhanced the humification and enzyme activity.

(3) Triple superphosphate

Triple super phosphate (TSP) is the most traded water soluble fertilizer made by reacting wet-process phosphoric acid with rock phosphate and contains 46% P_2O_5. TSP is produced in granular and non-granular form and is used both in fertilizer blends (with potassium and nitrogen fertilizers). Rashtbari and Alikhani (2014) reported that vermicompost prepared with triple superphosphate had more polymerization index (PI), humification index (HI) and humification ratio (HR) than urea, sulphur and the control treatments during enrichment treatments on chemical properties of vermicompost. They also reported significant decreases in microbial biomass carbon as well as C: N ratio of TSP enriched compost.

(4) Phosphoric Acid

Phosphoric acid (also known as orthophosphoric acid or phosphoric(V) acid) is a mineral (inorganic) acid having a chemical formula H_3PO_4 and contain 62% P_2O_5 orthophosphoric acid molecules can combine with themselves to form a variety of compounds which are also referred to as phosphoric acids, but in a more general way. Compost enrichment with phosphoric acid has not been documented and, therefore, it will be a novel discovery to explore.

2.12 Parameters for monitoring compost maturity

Various parameters that have been used to evaluate compost maturity include changes in nitrogen species, pH, optical density, temperature, specific gravity, plant assays, respiration, changes in microbial population (Epstein, 1997), specific oxygen uptake rate (SOUR) Scaglia *et al*. (2007), Solvita test (Cabanas-Vargas *et al*. 2005) and activity of cellulolytic microorganisms (Jeanine *et al*. 2006). Other methods often used to measure compost maturity include C/N ratio, electrical conductivity (EC) and pH.

Spectroscopic analysis of compost maturity is carried out by using SEM, FTIR, TGA and ^{13}C-NMR. Thermo-gravimetric analysis (TGA) is used to detect the OM stability in compost as reflected by its sustained presence in the soil. Physical techniques like TGA have been identified as compost maturity indicators (Nada *et al*. 2012). Abouelwafe *et al*. (2008) reported a series of absorption band ratios while using FTIR spectra to determine the extracted fulvic acid from degraded sludge produced from the effluent of a vegetable oil processing plant mixed with household waste from a landfill.

The preceding literature review has revealed that optimizing earthworm stocking density is a novel way to create an ideal optimum level suitable for rapid decomposition of various targeted organic waste. There is yet lack of information on the possibility of optimizing earthworm stocking density for cow- dung- waste paper mixtures. Vermicomposts from cow dung are poor sources of nutrients particularly P and research somewhere else has shown that RP can be used to enrich vermicompost. However, no work has been done on optimizing the RP rate for the cow-dung-waste paper mixtures with a view to improving P availability and shortening the time required to efficient degradation of the waste during vermicomposting. RP is the most widely used additive to enrich composts or vermicomposts. However, it contains high percentage of phosphorus and calcium. There is no reported work on whether it is the phosphorus or calcium in RP that is responsible for improved biodegradation during the vermicomposting of organic wastes and/or cow-dung-waste paper mixtures. This study was carried out to address these gaps in knowledge.

CHAPTER THREE

3 OPTIMIZATION OF *Eisenia fetida* STOCKING DENSITY FOR THE BIOCONVERSION OF PHOSPHATE ROCK ENRICHED COW DUNG -WASTE PAPER MIXTURES

3.1 ABSTRACT

Vermitechnology is gaining recognition as an environmental friendly waste management strategy. Its successful implementation requires that the key operational parameters such as earthworm stocking density be established for each target waste/waste mixture. One target waste mixture in South Africa is waste paper mixed with cow dung and rock phosphate (RP) for P enrichment. This study sought to establish optimal *E.fetida* stocking density for maximum P release and rapid bioconversion of RP enriched cow dung- paper waste mixtures. *E. fetida* stocking densities of 0, 7.5, 12.5, 17.5 and 22.5 g- worms kg^{-1} (which translated to be (0, 0.02, 0.05, 0.07 and 0.09 kg-worms m^{-2} kg^{-1} feedstock)) dry weight of cow dung- waste paper mixtures were evaluated. The stocking density of 12.5 g-worms kg^{-1} feed stock (0.05 kg-worms m^{-2} kg^{-1} feedstock) resulted in the highest earthworm growth rate and humification of the RP enriched waste mixture as reflected by a C: N ratio of < 12 and a humic acid/fulvic acid ratio of > 1.9 in final vermicomposts. A germination test revealed that the resultant vermicompost had no inhibitory effect on the germination of tomato, carrot, and radish. Extractable P increased with stocking density up to 22.5 g-worm kg^{-1} feedstock (22.5 kg-worms m^{-2} kg^{-1} feedstock), suggesting that for maximum P release from RP enriched wastes a high stocking density should be considered.

Keywords: Vermicomposting, Stocking density, *Eisenia fetida*, humification indices, P enrichment.

3.2 Introduction

Paper mill industries continue to flourish in many countries due to the increasing demand for paper. In South Africa, for example, the production and utilization of waste paper has increased from 2.1 million tonnes in 2007 to 2.7 million tonnes in 2012 (PRASA, 2013). On the other hand, intensive livestock farming such as dairy farms generate huge amounts of animal excrement. James *et al*. (2004) estimated that dairy cows in free stall barns produce approximately 1986 kg of manure per animal unit and per year on a dry weight basis (1 Animal unit = 370 kg).

Animal manures are directly used in agriculture to amend the soil as a method of disposal or as nutrient source (Tillman and Surapaneni, 2002; Mupondi *et al*., 2010). Some studies have, however, reported cattle manure to contain pathogenic faecal bacteria and have established a link between application of raw manure and water contamination by faecal coliforms such as *Escherichia coli* (*E. coli*) 0157, which causes intestinal diseases and deaths. The outbreaks of *E. coli* 0157: H7 infections associated with the consumption of crop produce have implicated animal manures as sources of pathogens in fruits and vegetables (Tillman and Surapaneni, 2002; Mupondi *et al*., 2010). Animal manure, therefore, needs to be stabilized before it is applied to soil as a fertilizer or used as a growing medium in the plant nursery industry. Stabilization involves the decomposition of a waste substance which is normally reflected by decreases in microbial activity and concentrations of toxic compounds (Benito *et al*., 2003). Mupondi *et al.* (2010) showed that waste paper, most of which is ordinarily disposed by incineration in most municipalities in South Africa, could be used as a bulking agent for dairy manure and the resulting mixture was effectively stabilized through vermicomposting. Mupondi (2010) further observed that the resulting rock phosphate (RP) enriched vermicompost had a significantly higher total P concentration (2.4%) compared to dairy manure-paper mixtures (0.5% P) where no RP was added. The corresponding values for resin extractable P were 1313 and 431 mg P kg^{-1}, respectively. In addition, Mupondi *et al*. (2010) further justified that, mixture of dairy manure with waste paper with a C: N ratio of 30 was more suitable for producing stabilized vermicompost with more matured, humified materials with high ash content, more total N and P contents than waste with a C: N ratio of 45. Furthermore, a pre-composting period of one week was found suitable for a combined system to stabilizing dairy manure- waste paper mixtures.

Earthworm population density is known to affect earthworm growth and reproduction which in turn could impact the rate of vermicomposting. Considerable work has been done on earthworm stocking densities for vermicomposting of different wastes. Neuhauser *et al.,* (1980) reported a potential *E. fetida* stocking density of 0.8 kg-worms m^{-2} for horse manure and 2.9 kg-worms m^{-2} for activated sludge. They showed using regression analysis that whereas earthworm production increased with population density, growth declined. Yadav *et al*. (2011) reported an optimum *E. fetida* stocking density of 3 kg worms m^{-2} with human faeces. Singh and Kaur (2013) reported an optimum stocking density of 12.5g worms kg^{-1} feed of *E. fetida* for chemical sludge and spent carbon obtained from industrial soft drinks. Ndegwa *et al*. (2000) recommended a stocking density of 1.6 kg worms m^{-2} for bio-solids. In a short duration study of 72 h, Aira *et al*. (2008) working with pig manure as substrate observed a strong and linear density – dependent response of C and N mineralization to *E. fetida* stocking density. Therefore, the rate of decomposition during vermicomposting process depends on the earthworm stocking density and this effect tends to vary with the type of material being vermicomposted, nevertheless, it could also vary with the type of earthworm species used and/or the vermicomposting time (Gajalakshmi, and Abbas, 2004).

Furthermore, most of the work on stocking density effects on vermicomposting has thus far mainly focused on how it affected the growth and development of the earthworms (Dominguez and Edwards 1997; Garg *et al.* 2006) and not on the vermicomposting process. Mupondi *et al*. (2010) in their study on bioconversion of dairy manure-waste paper mixtures enriched with RP used a fixed stocking density of 1.6 kg -worms m^{-2} recommended for bio-solids (Ndegwa *et al.*, 2000). However, given the demonstrated variability of stocking density with substrate used, it is possible that the stocking density used by Mupondi *et al.* (2010) may not be ideal either for dairy manure and paper waste mixture or cow-dung-waste paper mixtures. Mupondi (2010) did not, however, evaluate the possible effects of *E. fetida* stocking density on the release of extractable P during vermicomposting of cow dung-waste paper mixtures spiked with RP. In view of the foregoing, this study, sought to investigate (a) the optimum *E. fetida* stocking density necessary for complete degradation of cow dung - waste paper mixtures enriched with RP by monitoring the effects of *E. fetida* stocking density on cow dung-waste paper vermicompost maturity parameters (C: N ratio, humic acid carbon: fulvic acid carbon ratio (C_{HA}/C_{FA}) or polymerization index (PI), humification index (HI), humification ratio (HR) and seed germination index), and (b) the effect of *E. fetida* stocking density on the release of P during vermicomposting of RP enriched cow-dung-waste paper mixtures.

3.3 MATERIALS AND METHODS

3.3.1 Site description, wastes and earthworms utilized

The study was carried out in a shaded yard at the Research Farm of the University of Fort Hare located at 32°46´ S and 26°50´E in the Eastern Cape Province of South Africa. Cow dung used in the study was obtained from the Keiskammahoek Dairy Project located about 60 km North East of the University of Fort Hare, while shredded waste papers were obtained from the Duplicating Centre of the University. Cow-dung and waste paper were analyzed for selected chemical properties. The RP used was obtained from Phalaborwa in Limpopo Province, South Africa, and was granitic in nature (Van der Line, 2004). The factory identity of the RP sample used is PALFOS 88P and had the following chemical properties: P_2O_5-40.3%; CaO-54.6%, MgO-0.26%; cadmium-2.2 mg kg^{-1}, chromium -18.05 mg kg^{-1}, copper -5.85 mg kg^{-1}, lead -6.05mg kg^{-1} and zinc -13.22 mg kg^{-1} (Forskor, Phalaborwa, South Africa). Epigeic earthworm species *Eisenia fetida* used was obtained from a wormery in East London, South Africa. This epigeic earthworm species was chosen because it is prolific, has a wide temperature tolerance, and can grow and reproduce well in many types of organic wastes (Edward, 1988). Representative samples of the dairy manure and shredded paper were air-dried, ground to pass through a 2 mm sieve and then analyzed for physico-chemical properties.

3.3.2 Experimental Design

The experiment was conducted at the University of Fort Hare Research Farm, Alice, South Africa in worm boxes measuring 0.50 m x 0.40 m x 0.30 m with an open top surface area of 0.2 m^2. The boxes were placed in a well ventilated farm building at an average ambient day temperature of about 25^{o}C. Feedstock (5 kg on dry weight basis) that was prepared by mixing 2.16 kg shredded waste paper and 2.84 kg cow dung to achieve a C: N ratio of 30 was placed in the vermicomposting boxes and thoroughly mixed with 2% P in the form of RP. A C: N ratio of 30 was recommended by Inbar *et al*. (1993) and Reddy *et al*. (2005) as ideal for composting while a 2% RP incorporation rate was found to result in enhanced biodegradation of cow dung-waste paper mixtures by Mupondi, (2010). Juvenile *E. fetida* earthworms having similar body weight were introduced into the worm boxes to achieve different stocking densities of 0, 7.5, 12.5, 17.5 and 22.5 g- worms kg^{-1} dry weight of cow dung- waste paper mixtures. Treatments were replicated three times and arranged in a completely randomized design (CRD). Moisture content in each box was brought to 80% by sprinkling water (Singh *et al*. 2004) and maintained at this level by regular sprinkling throughout the six weeks vermicomposting period. Samples were collected at 0, 14, 28, and 42 days for physico-chemical analysis as described below. Sampling was done by collecting four subsamples from each box at each sampling time. The sampling was done at three depth levels (approximately 6 cm apart) in each box after which the different subsamples were mixed to give a composite sample of about 150 g per treatment. At each sampling period, 10 earthworms were also randomly taken from each box and weighed to determine treatment effects on average worm biomass.

3.3.3 Physico-chemical analyses

Samples were analyzed for pH, electrical conductivity (EC), volatile solids (VS), ash content, total carbon (C), total nitrogen (N), total and extractable phosphorus P, and humic substances. The samples were air-dried until constant weight and then ground (< 2mm) to provide a homogeneous sample for analysis. Electrical conductivity and pH were determined from a vermicompost-water suspension (1: 10) using a pH/Conductivity meter as described by (Ndegwa *et al*., 2000). This suspension was shaken on a mechanical shaker at 230 rpm for 30 min and allowed to stand for an hour prior to pH and EC measurements. Volatile solids (VS) were determined as sample weight loss (previously oven-dried at 105^0C) upon ashing at 550^0C for 4h in a muffle furnace (Ndegwa *et al.*, 2000). Total nitrogen (N) and carbon (C) were determined using a LECO truspec C/N auto analyzerr (LECO Corporation, 2003, St Joseph, Michigan, USA).

Bray 1 P was determined by shaking 5 g of air-dried compost in a 50 ml plastic shaking bottle containing 35 ml of prepared Bray 1 extracting solution. The samples were constantly shaken on a horizontal shaker for 1 minute, and the resulting suspension was immediately filtered through a Whatman No. 5 filter paper. The filtrates were analyzed to determine the phosphorus concentration in the solution by means of the reduced phospho-molybdenum blue method on a Skalar continuous flow analyser (San 2++ Skalar Continuous Flow Analyser, Skalar Analytical B.V. The Netherlands).

Total phosphorus was determined by digesting 0.5 g of air-dried compost samples in a MARS 5 microwave digester (CEM Corporation, Matthews, North Carolina) using aqua regia followed by the determination of phosphorus concentration in the digests by means of the reduced phosphor-molybdenum blue method on a continuous flow analyser (San 2++ Skalar Continuous Flow Analyser, Skalar Analytical B.V. The Netherlands). Humic substances were determined following extraction as described by (Del Carmen Vargas-García *et al.*, 2006). Briefly, compost samples were treated with 0.1-*M* NaOH (1:20 w/v ratio), constantly shaken on a horizontal shaker for 4 h and the resulting solution was centrifuged at 8,000 x g for 15 min. Then the supernatants were divided equally into two fractions, one was analysed for total extractable carbon fraction (C_{EX}) by the Walkley and Black rapid titration method as described by Anderson and Ingram (1996) and the other fraction was adjusted to pH 2 with concentrated H_2SO_4 and allowed to coagulate for 24 h at 4°C. The precipitates that were formed constituted the humic acid–like carbon (C_{HA}) while fraction that remained in solution constituted the fulvic acid-like carbon (C_{FA}). The C_{HA} was calculated by subtracting the C_{FA} from the C_{EX}. The humification ratio (HR) was calculated as HR= (C_{EX}/C) X 100 and humification index (HI) was calculated as HI= (CHA/C) X 100 (Mupondi *et al.*, 2010).

3.3.4 Phytotoxicity study

Phytoxicity was assessed through a seed germination test. Aqueous extracts were prepared from the different vermicomposts with distilled water (1:10 w/v) (Ravindran *et al.*, 2013, Ravindran and Sekenan, 2010). The seed germination bioassay for tomato (*Lycopersicon esculentum*), radish (*Raphanus sativus*) and carrot (*Daucus carota*) was evaluated according to Tam and Tiquia (1994) in which two pieces of Whatman® filter paper were placed inside a sterilized Petri- dish and wetted with the vermicompost extracts. Ten seeds of each crop species were placed on top of the filter paper and incubated for five days in the dark. A control was included for each crop species in which the filter papers were wetted with distilled water. Seed germination, germination index (GI), relative seed germination (RSG) and relative root elongation (RRE) were calculated as follows:

$$\text{RSG (\%)} = \frac{\text{Number of seeds germinated in the sample extract}}{\text{Number of seeds germinated in the control}} \times 100$$

$$\text{RRE (\%)} = \frac{\text{Mean root elongation in the sample extract}}{\text{Mean root elongation in the control}} \times 100$$

$$\text{GI (\%)} = \frac{\text{(\% Seed germination)} \times \text{(\% Root elongation)}}{100}$$

3.3.5 Morphological assessment of vermicomposts

Scanning Electron Microscopy (SEM) images were taken using JOEL (JSM-6390LV, Japan). Briefly, the samples were oven-dried and ground to pass through a 2 mm sieve. Small representative portions of the samples were coated with gold and mounted on SEM. Samples were imaged by scanning them with a high energy beam of electrons in a raster scan pattern.

3.4 Statistical analysis

The data reported herein are the means of three replicates (n=3). Because the sampling was not destructive, statistical analysis of the data obtained was done by repeated measures analysis of variance (ANOVAR). Mean separation was done using Fisher's protected least significant difference (*LSD*) test at $p < 0.05$. Linear regression analysis was done to evaluate relationship between *E. fetida* stocking density and the release of Bray 1 extractable P during the vermidegradation of RP enriched cow-dung-waste paper mixtures. All statistical analyses were done using JMP® Release 10.0 Statistical Package (SAS Institute, Inc., Cary, North Carolina, USA, 2010)

3.5 RESULTS

3.5.1 Chemical characteristics of the waste

Selected chemical properties of the cow dung and waste paper used were significantly different among both wastes as shown in Table 3.1. The cow dung had lower carbon content and higher nitrogen content than the waste paper. The same trend was observed for total P and as a result, cow dung had narrower C: N and C: P ratios than waste paper. The electrical conductivity (EC) and ash content in cow dung was higher than in waste paper whereas the pH of both cow dung and waste paper was alkaline.

Table 3.1: Selected chemical Properties of wastes used in the Study

	Raw Material	
Chemical Property	Cow Dung	Waste Paper
pH	7.6 ± 0.3^{ns}	8.1 ± 0.1^{ns}
EC ($mScm^{-1}$)	5.5 ± 0.2^{a}	0.18 ± 0.1^{b}
Total N (%)	1.46 ± 0.2^{a}	0.04 ± 0.002^{b}
Total Carbon (%)	15.66 ± 1.2^{b}	38.3 ± 2.4^{a}
C : N	10.7 ± 2.6^{b}	957.5 ± 0.1^{a}
Total P (g/kg)	2.8 ± 0.5^{a}	0.4 ± 0.2^{b}
Ash (g/kg)	380 ± 7.5^{a}	175 ± 3.5^{b}
C : P	5.8 ± 0.3^{b}	95.7 ± 0.1^{a}

*Values reported as ± are standard deviations of estimates (n=3); Numbers followed by different letters in each row are significantly different according to the LSD test at $p < 0.05$; n.s = (not significant).

Table 3.2: Repeated measures ANOVA for *E. fetida* biomass, C: N ratio, CHA/CFA, HI, HR and Bray 1 extractable P.

	Effect					
	E. fetida density		Time		*E. fetida* x time	
	$F_{(4,55)}$	P	$F_{(3,55)}$	P	$F_{(12,55)}$	P
E.fetida biomass (mg Kg^{-1})	14.25	<0.0001	5.75	<0.0001	1.44	<0.0001
C: N ratio	35.6	<0.0001	17.45	<0.0001	56.4	<0.001
PI	11.53	<0.0001	14.6	<0.00012	20.6	<0.001
HI (mg Kg^{-1})	120.57	<0.0001	143.15	<0.0001	176.75	<0.0001
HR (mg Kg^{-1})	134.66	<0.001	290.61	<0.0001	401.37	<0.0001
Bray 1P (mg Kg^{-1})	30.50	<0.0001	117.55	<0.0001	132 .31	<0.0001

F values and probabilities shown for each effect at $p < 0.05$ is significant. C: N = carbon to nitrogen ratio, PI = polymerization index, HI = Humification index, HR = Humification ratio.

3.5.2 Effects of earthworm stocking density on earthworm biomass and C: N ratio

E. fetida stocking density had a highly significant effect on worm biomass but this effect was dependent on time as reflected by a significant E. *fetida* x time interaction (Table 3. 2). Worm biomass increased linearly with time up to 28 days but decreased thereafter up to day 42 for all *E. fetida* stocking densities except the 7.5 g-worm kg^{-1} feedstock density in which the biomass continued to increase linearly (Fig. 3.1). The highest worm biomass was observed at a stocking density of 12.5 g-worm kg^{-1} feedstock up to day 28 while higher stocking rates resulted in lower biomass though still greater than what was observed at the lowest stocking density of 7.5 g-worm kg^{-1} feed as shown in (Fig. 3.1).

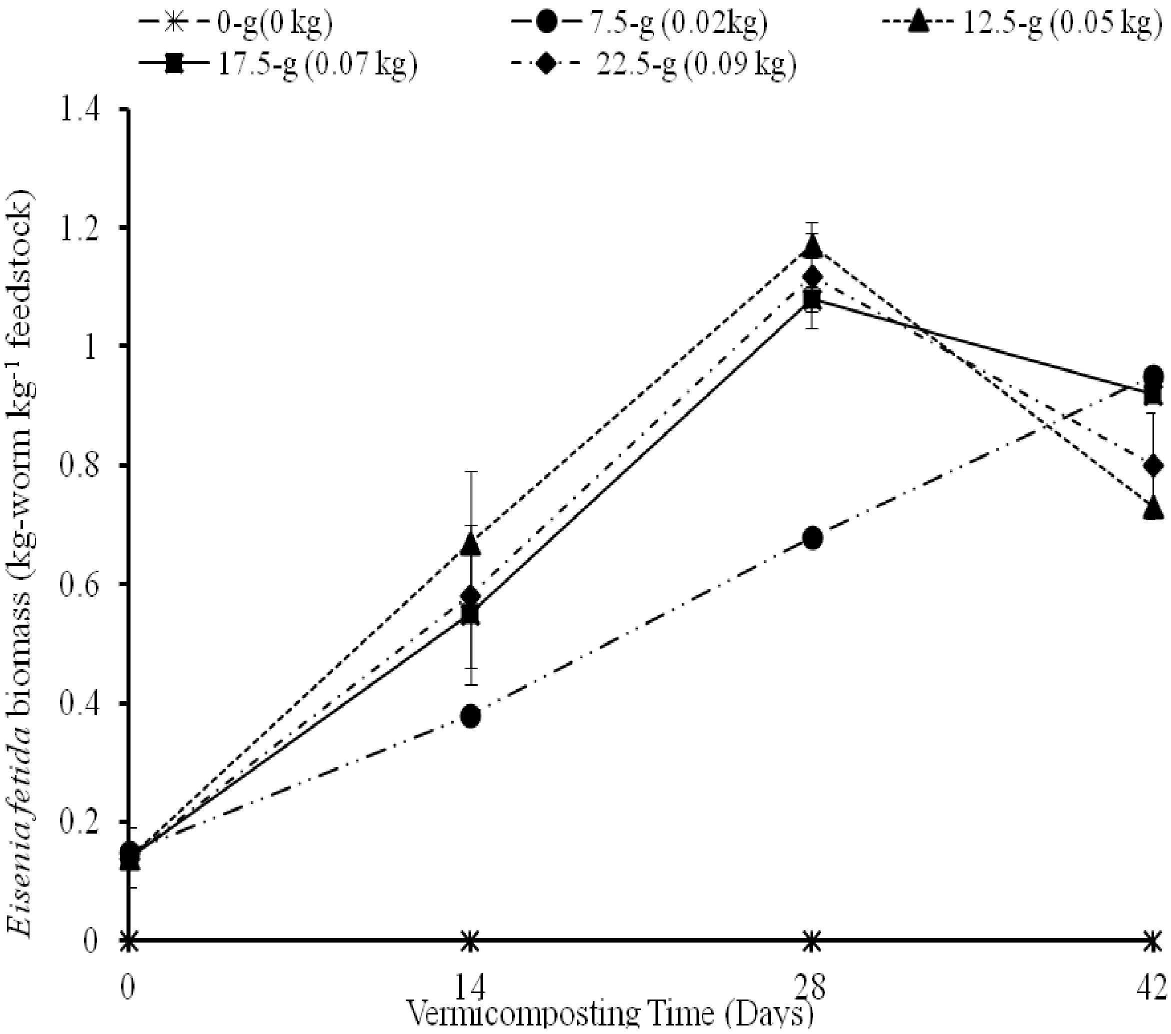


Figure 3.1 : Effect of stocking density on earthworm biomass

The C: N ratio was used for monitoring the effect of *E fetida* stocking density on the vermidegradation of PR enriched cow dung-waste paper mixtures. It reflects changes in carbon and nitrogen concentration of the substrate materials in the vermicompost. *E. fetida* density had a highly significant effect on the C: N ratio of the cow dung-waste paper mixtures but this varied with time as indicated by a significant *E. fetida* x time interaction (Table 3. 2). The C: N ratio declined from 30 in the control to 10, 12, 13, 13, and 25 after 42 days for *E. fetida* stocking densities of 12.5, 22.5, 17.5, 7.5, and 0 g-worms kg^{-1} feedstock, respectively (Fig. 3.2). Wide differences on C: N ratios among the different stocking densities occurred on day 28 but these differences narrowed down by day 42 for all stocking densities except where no worms were included (Fig. 3.2). The *E. fetida* density of 12.5 g-worms kg^{-1} feedstock maintained the lowest C: N ratio throughout the vermicomposting period while the opposite was the case in the control.

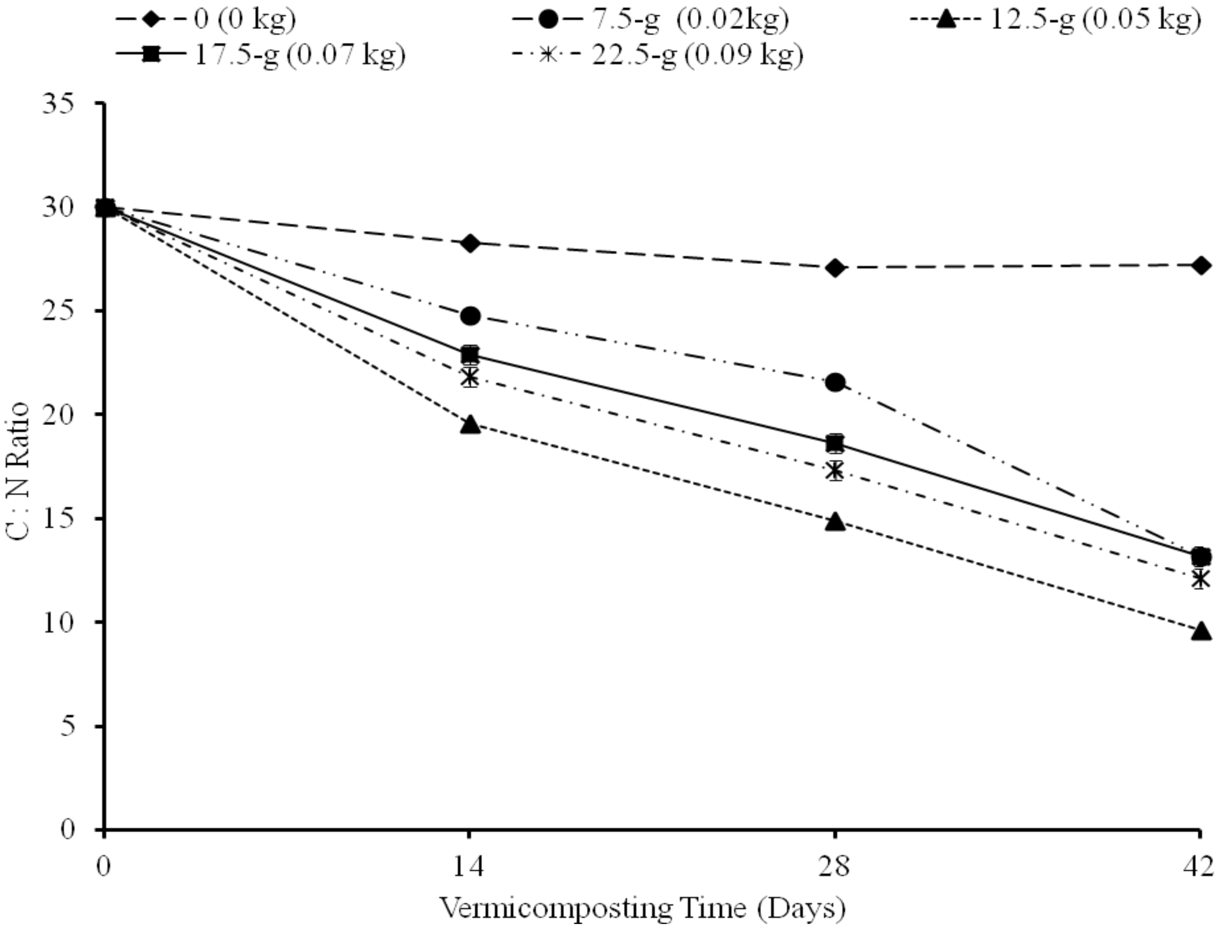


Figure 3.2: Effect of earthworm stocking density on C: N ratio

3.5.3 Effects of earthworm stocking density on humification parameters

The effect of *E. fetida* stocking density on the biodegradation and humification of RP-enriched cow dung-waste paper mixtures was evaluated by monitoring the HI, HR and C_{HA}:C_{FA} ratio also known as polymerization index (PI). All three humification parameters were significantly affected by *E. fetida* stocking density and time as revealed by ANOVAR (Table 3.2). Furthermore, the three parameters showed strong and linear dependency on *E. fetida* stocking density whereby humification increased linearly up to the stocking density of 12.5 g- worms kg^{-1} feedstock but declined at higher stocking densities (Fig. 3.3 to 3.5). The effect of *E. fetida* stocking density on humification parameters varied with time as reflected by a significant *E. fetida* x time interaction (Table 3. 2). Humification increased linearly with time for stocking densities up to 12.5 g -worms kg^{-1} feedstock (Fig. 3.3 to 3.5). For stocking densities greater than the 12.5 g -worms kg^{-1} feedstock, humification increased linearly with time up to 14 days but it gradually declined thereafter.

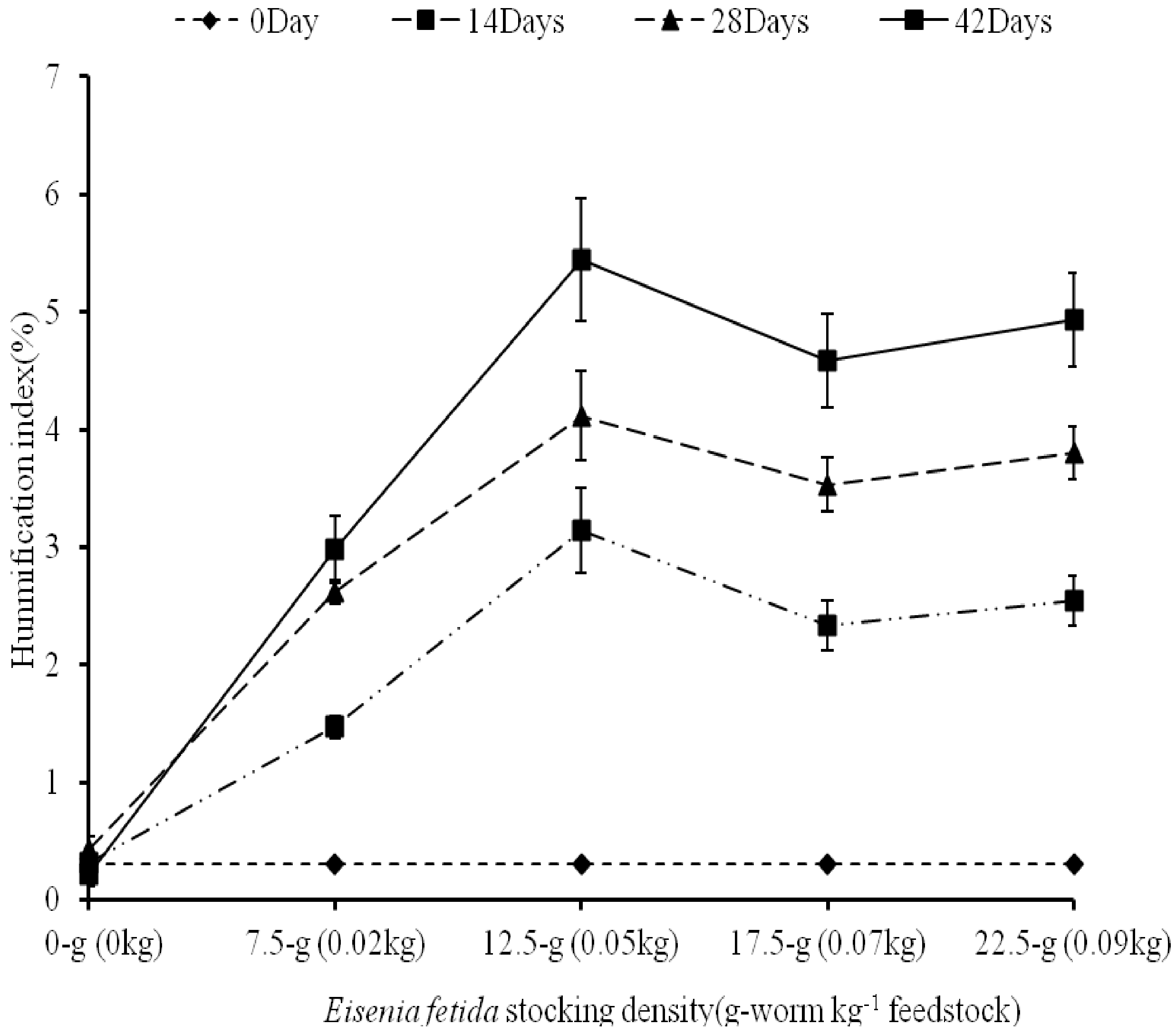


Figure 3.3: Effect of earthworm stocking density on humification index

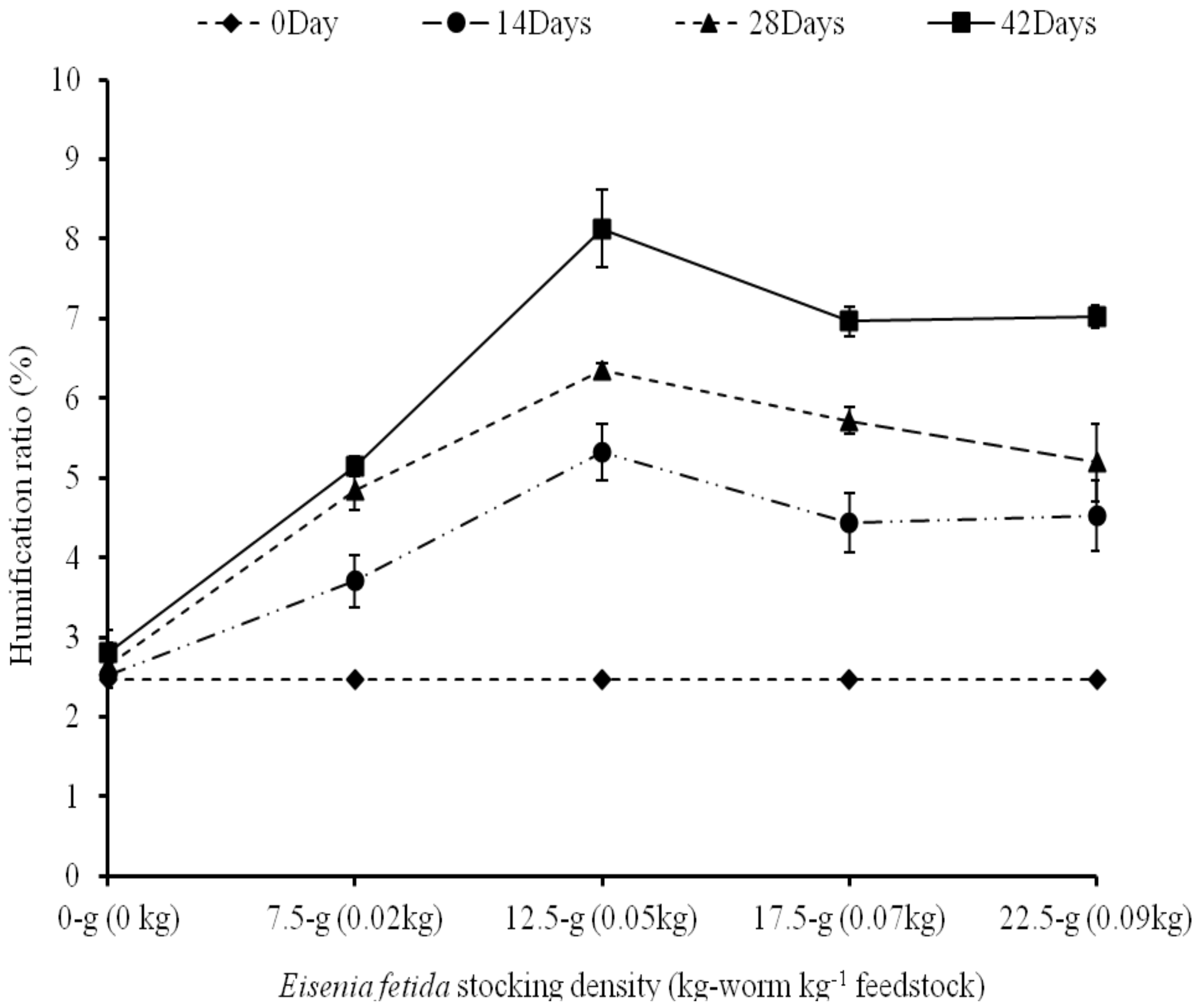


Figure 3.4: Effect of earthworm stocking density on the humification ratio

The humic acid/fulvic acid ratio or PI at different stocking densities was 0.15% at day 0 but it increased progressively from 14 days to 42 days at all earthworm stocking densities relative to the control (Fig. 3.5). The trends of increase at 14, 28 and 42 days were 0.15, 0.66, 2.98, 1.11 and 1.3; 0.17, 1.18, 3.51, 1.58 and 2.78; 0.23, 1.39, 4.63, 1.96 and 2.36 for stocking densities of 0, 7.5, 12.5, 17.5 and 22.5 g worms kg^{-1} substrate, respectively (Fig. 3.5).

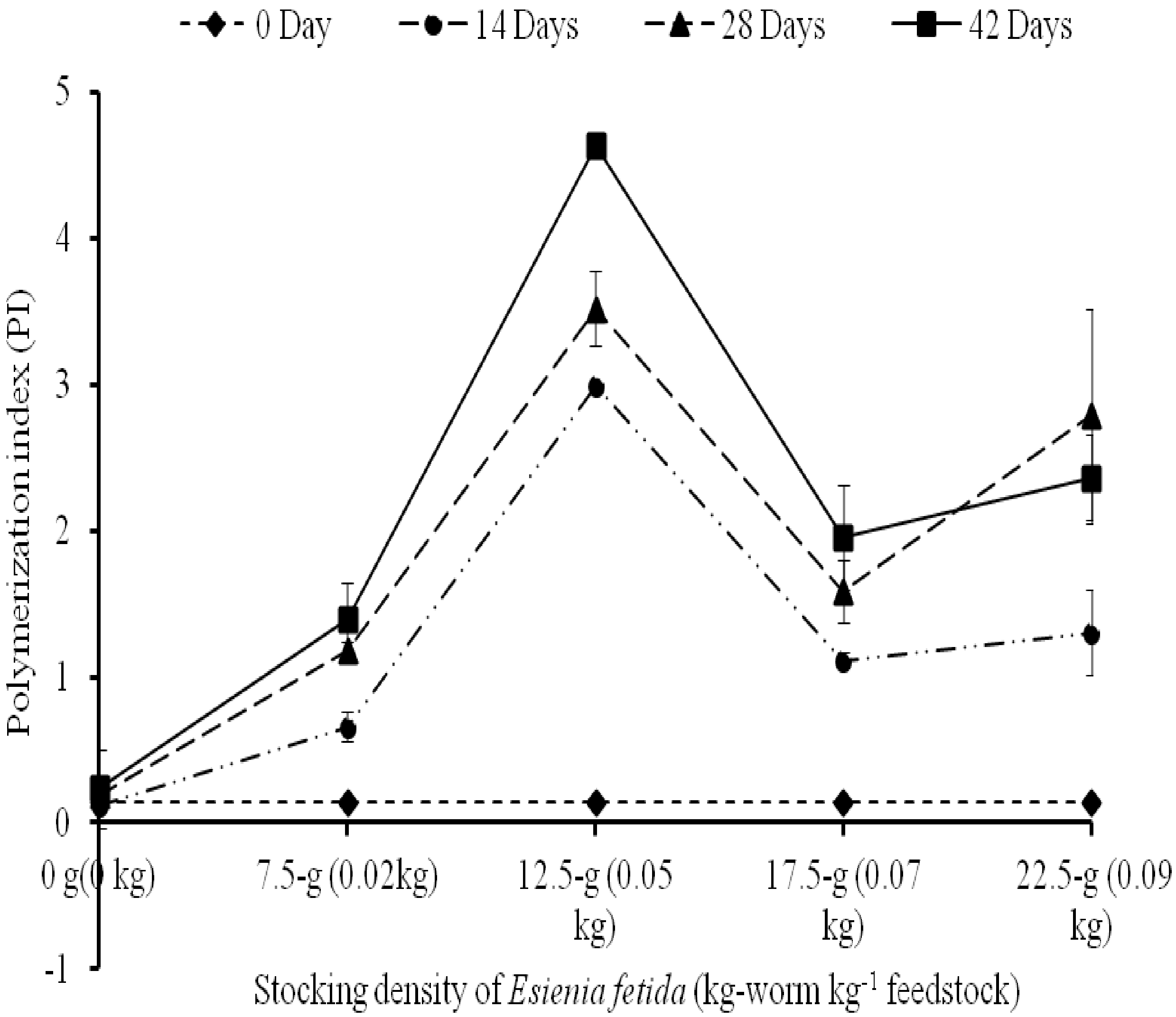
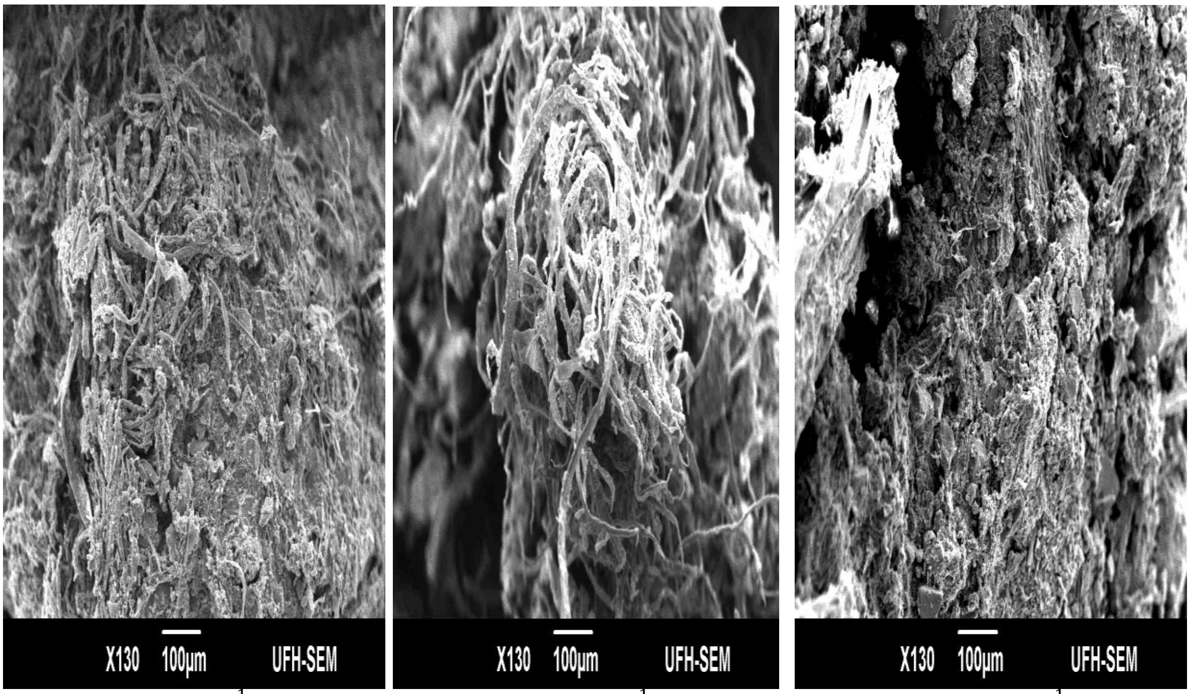
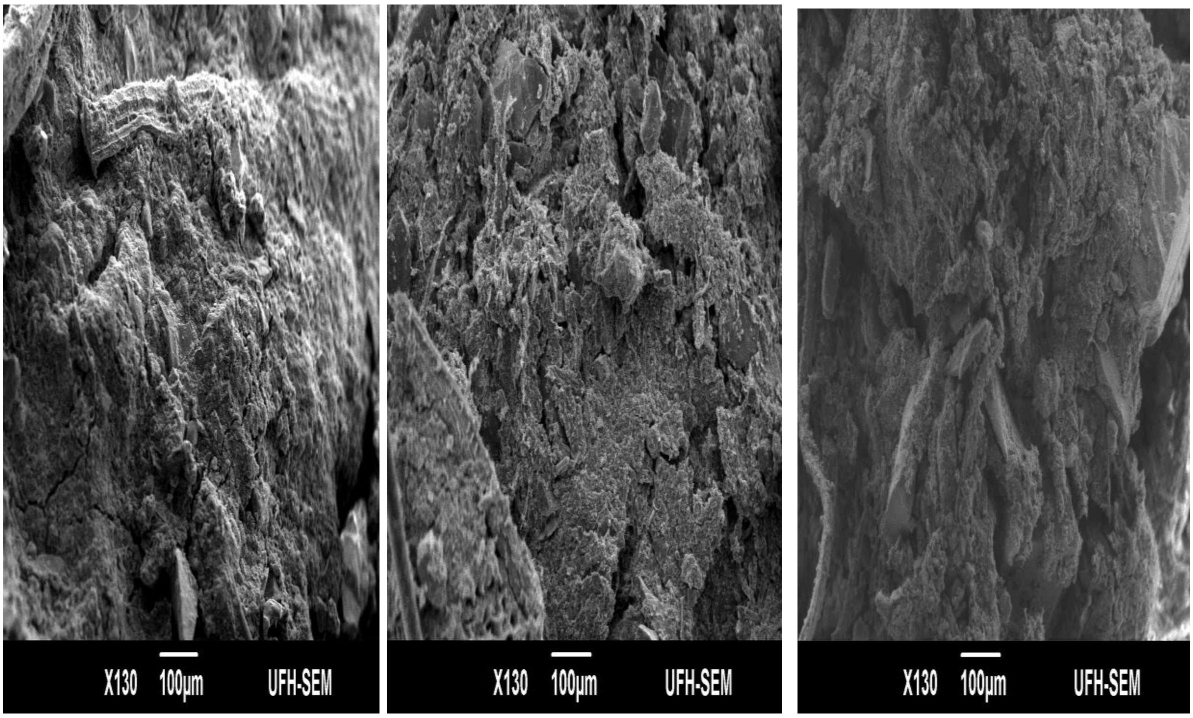


Figure 3.5: Effect of earthworm stocking density on humic acid carbon to fulvic acid carbon or polymerization index (PI)

Scanning electron microscopy (SEM) images of the cow-dung-waste paper mixtures illustrating structural changes during the vermicomposting period are shown in Fig.3.6a-f. SEM images confirmed the extent of humification in the ongoing physical (substrate aeration, mixing, as well as actual grinding in the earthworms' gut and intestine) and biological changes accomplished by enzymatic activities by microbes on the substrate being vermicomposted. Figure 3.6a shows the image of the compacted aggregates of the cellulose and protein fibres in the waste mixtures before vermicomposting which remained more or less the same in the control after 42 days of vermicomposting (Figure 3.6b). However, progressive physical changes characterised by highly degraded fine grain texture vermicompost occurred in all the substrates where earthworms were added (Fig.3.6c-f). Earthworm stocking densities of 12.5 g-worms kg^{-1} -feedstock and above produced well humified products consistent with the observed chemical humification parameters (Fig.3.6d-f).



(a) 0 g worms kg^{-1} (0 day) (b) 0 g worms kg^{-1} (day 42) (c) 7.5 g worms kg^{-1} (day 42)



(d) 12.5 g worms kg^{-1} (day 42) (e) 17.5 g worms kg^{-1} (day 42) (f) 22.5 g worms kg^{-1}(day 42)

Figure 3.6 : SEM images of structural morphology of cow dung waste paper mixtures for day 0 and day 42 at different earthworm stocking density.

3.5.4 Effects of earthworm stocking density on extractable phosphorus during vermicomposting of RP enriched cow dung-waste paper mixtures

Extractable P was monitored to determine the effect of stocking density on the extent of P release from the RP that was added to the cow dung-waste paper mixtures. Both *E. fetida* stocking density and time had a significant effect on Bray 1 extractable P as revealed by ANOVAR (Table 3.2). It increased significantly with time at each stocking density but the extent of release was greater in the highest earthworm density, getting up to 2.1 times over the control (no earthworm) which is consistent with the observed significant *E. fetida* density x time interaction (Table 3.3). The least extractable P (0.17 mg P $kg^{-1}day^{-1}$) occurred in the control while the greatest release occurred at the highest stocking density of 22.5 g- worms kg^{-1} cow dung-waste paper. The rate of P release computed from best fit equations (Fig. 3.7) ranged from 0.17 to 2.11 mg P kg^{-1} day^{-1} (Table 3.3). The greatest rate of P release occurred at a stocking density of 22.5 g worms kg^{-1} substrate.

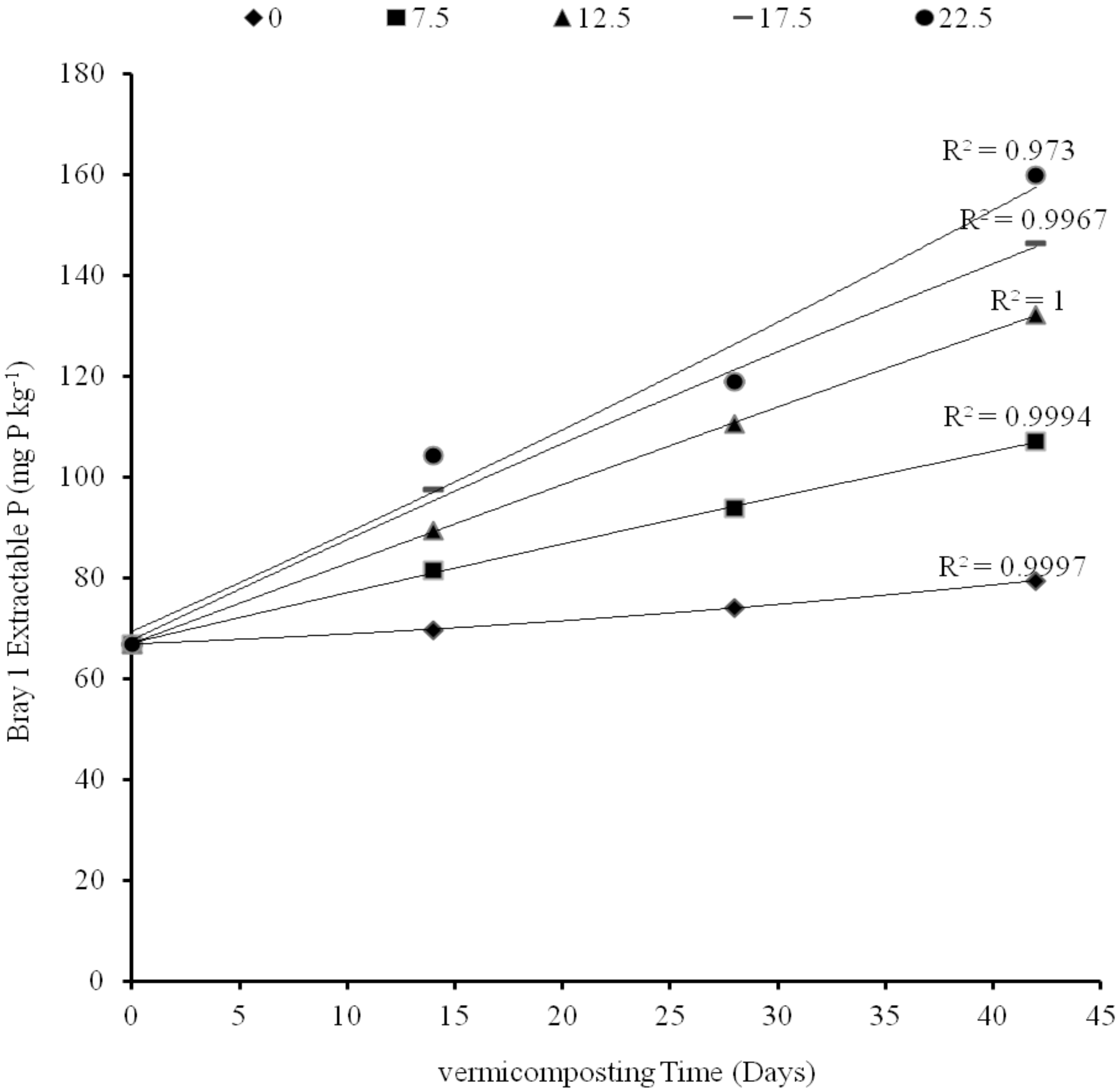


Figure 3.7: Effect of Earthworm stocking density on the Bray 1 extractable P

Table 3.3: Relationship between *E. fetida* stocking density and Bray 1 extractable P during vermicomposting of RP enriched cow dung-waste paper mixtures

*E.fetida*stocking density(gworm Kg^{-1} substrate)	Regression equation	R^2	Rate of Bray 1P release (mg $Pkg^{-1}day^{-1}$)	Predicted Bray1P at 42days (mg P kg^{-1})	Observed Bray 1 P at 42days (mg P kg^{-1})	Net Bray 1 P increase from day 1(mg P kg^{-1})
0	$y = 0.032x^2 + 0.1654x + 66.865$	0.9997	0.17	79.4	79.4	12.5
7.5	$y = -0.0015x^2 + 1.0114x + 67.06$	0.9994	0.99	106.9	107	40.1
12.5	$y = 0.0011x^2 + 1.5975x + 66.965$	1.00	1.57	132.1	132.1	65.2
17.5	$y = 0.0041x^2 + 2.0286x + 67.65$	0.9967	1.89	145.6	146.4	79.5
22.5	$y = 0.0045x^2 + 1.9104x + 69.345$	0.973	2.11	157.5	159.9	93.0
CV			14	14	14	14
P>F			< 0.001	< 0.001	< 0.001	< 0.001

3.5.5 Effect of earthworm stocking density on the phytotoxicity level of RP-enriched cow dung-waste paper vermicomposts

The vermicompost extracts from the final vermicomposts of all treatments had no inhibitory effect on the seed germination of test crops (Table 3.4). In tomato, the relative seed germination increased linearly with *E. fetida* stocking density. The highest seed germination percentage was observed with *E. fetida* stocking densities of 12.5g-worms kg^{-1} feedstock. The same trend was observed for radish and carrot ($P < 0.05$). With respect to relative root elongation, the highest significant value of 163% was recorded in tomato followed by radish 149 % and carrot 108% at an *E. fetida* stocking density of 12.5g-worms kg^{-1} feed. The germination indexes of all crops were above 50% except for the control treatment of carrot seed. *E. fetida* stocking density had a significant effect ($P < 0.05$) on root elongation and germination index in all test crops. The final vermicomposts had GI values that ranged from 98% to 162% (for tomato), 48% to 81% (for carrot) and 51% to 128% (for radish).

Table 3.4 : Effect of earthworm stocking density on the phytotoxicity of cow dung-waste paper vermicompost

Earthworm stocking density(g-wormKg^{-1} feed)	Tomato			Carrot			Radish		
	RSG	RRE	GI	RSG	RRE	GI	RSG	RRE	GI
				(%)					
0	92^{b}	106^{d}	98^{d}	67^{b}	72^{d}	48^{d}	92^{b}	54^{e}	51^{e}
7.5	91^{b}	129^{c}	117^{c}	67^{b}	78^{c}	52^{d}	92^{b}	93^{d}	86^{d}
12.5	100^{a}	163^{a}	163^{a}	83^{a}	108^{a}	90^{a}	100^{a}	149^{a}	149^{a}
17.5	100^{a}	160^{b}	160^{b}	83^{a}	96^{b}	81^{b}	100^{a}	120^{c}	120^{c}
22.5	100^{a}	162^{b}	162^{a}	83^{a}	94^{b}	81^{b}	100^{a}	128^{b}	128^{b}

Numbers followed by different letters in each column are significantly different according to the LSD test $p < 0.05$.

3.6 Discussion

The effect of *E. fetida* stocking density on the biodegradation of RP enriched cow dung-waste paper mixtures was evaluated by monitoring the maturation of the vermicomposts as reflected by selected maturation parameters such as carbon to nitrogen ratio (C: N), humification index (HI), humification ratio (HR) and the ratio of humic acid carbon fraction to fulvic acid carbon fraction (C_{HA}: C_{FA} ratio) or (PI). Decrease in C: N ratio as well as earthworm biomass observed in this study confirmed earlier reports on biodegradation of solid waste management during vermicomposting which reflect the changes in carbon and nitrogen content of substrate materials and the depletion of easily biodegradable components of the feedstock (Iglesias and Garcia, 1992a; Bernal *et al.*, 2009). This decline in worm biomass beyond 28 days could be due to depletion of easily biodegradable feed components in the vermicomposting mixture facilitated by earlier sexual maturity of earthworms in the higher stocking density (Datar *et al.*, 1997; Dominguez and Edwards, 1997). *E. fetida* biomass at the lower stocking density of 7.5 g worm kg^{-1} feedstock, by contrast, increased linearly with time possibly due to limited competition for feed at this low stocking density (Datar *et al.*, 1997).

The general decline in C: N ratio with time could be attributed to the decomposition of organic matter as a result of microbial action (Bernal *et al.*, 2009). A decline in C: N ratio to less than 12 has been advocated to indicate an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity (Chen *et al*, 1996; Cedric *et al.*,2005). Thus, in terms of C: N ratio, all stocking densities resulted in well matured vermicomposts after 42 days though the 12.5 g worms kg^{-1} feed stocking density produced the most matured vermicompost with a C: N ratio of 10. The greater and faster decline in C: N ratio in the presence of *E. fetida* than the control confirmed the effective role that earthworms play in vermidegradation (Datar *et al.*, 1997; Dominguez and Edwards, 1997). Changes in the relative proportions of the fulvic acid (FA) and humic acid (HA) fractions with time is considered to be a good indicator of the maturation status of compost/vermicompost as it indicates the extent of humification of the substrate materials (Chefetz *et al*., 1998; Goyal *et al*., 2005). The increase in the humic acid/fulvic acid ratio or (PI) at all stocking densities with time (Fig. 2c) indicated the transformation of the easily degradable substances that make up the fulvic acid fraction to the more recalcitrant substances of higher molecular weight which make up the humic acid fraction.

The increase in C_{HA} with vermicomposting time resulted in increases in HR (Fig. 3.4) and HI (Fig. 3.3) values which indicated increasing humification of organic matter in the vermicomposts. All *E. fetida* stocking densities greater than 7.5 g-worm kg^{-1} substrate produced vermicomposts with C_{HA}: C_{FA} ratio or PI that exceeded 1.9 which Iglesias and Garcia, (1992b) proposed as the C_{HA}: C_{FA} ratio or PI above which city-refuse and sewage sludge compost could be considered mature. Therefore, *E. fetida* stocking densities greater than 7.5 g worm kg^{-1} cow dung-waste paper produced well humified mature vermicomposts after 42 days but the stocking density of 12.5 g- worms kg^{-1} cow dung-waste paper with a C_{HA}: C_{FA} ratio or PI of 4.63 (Fig 3.5) produced the most humified vermicompost. Singh and Kaur (2013) also reported most humified vermicompost product at a stocking density of 12.5 g-worms kg^{-1} feedstock when they used chemical sludge and spent carbon generated from soft drink industries as feedstock.

Aerobic decomposition of substrates during vermicomposting illustrated by SEM images also confirmed the degree of degradation of the cellulose and protein fibres in the substrate as reflected by the fine grain morphological structure of the resultant vermicompost. The evidence from the microscopy images shows that the biodegradation of cow-dung-waste paper mixtures was characterised by mechanical splitting and fibrillation of the structure. It was also evident that the decomposition of the different components of cow-dung-waste paper mixtures differed, indicating that the highly cellulose fibre components were slowly degraded. At the conclusion of the composting experiment, the cow-dung-waste paper mixture had undergone major breakdown leaving the fibre fragments which were more resistant to bio-degradation (Fig.3.6 c-f). The obvious structural changes demonstrate consumption of cellulose by the actual grinding activity in earthworm intestine and indigenous micro-organisms during vermicomposting.

Increases in the amount of available P during vermicomposting was probably due to mineralization and mobilization of phosphorus from RP facilitated by enhanced phosphatase activity in the guts of the earthworms (Edwards and Lofty, 1972; Khwairakpam and Bhargava, 2009). Improvement in the amount of easily extractable P during vermicomposting was previously reported by Ghosh *et al.* (1999) but the present study has further demonstrated that this effect is strongly influenced by earthworm stocking density. Thus, although vermicompost maturity can be achieved at lower stocking density, a higher stocking density may be preferred if maximum P release from RP incorporated in the waste mixture is a desired goal.

According to Zucconi *et al., (*1981) and Tam *et al..*, (1994, 1995, 1996 and 1998) a germination index of ≥ 80% on compost indicates the disappearance of phytotoxins in composts. Bustamante *et al*., (2001) also corroborated their findings when composting recycled digestates from biogas production. Thus, none of the compost extracts inhibited tomato germination while the germination of carrot and radish was inhibited in mixtures vermicomposted at stocking densities of less than 7.5 and 12.5g- worms kg^{-1} substrate, respectively.

3.7 Conclusions

This study has demonstrated that; (i) C: N ratio, humification index, humification ratio, and the humic acid: fulvic acid ratio are equally effective for monitoring the vermidegradation of cow dung-waste paper mixtures, (ii) a clear dependency of vermidegradation on *E. fetida* stocking density. An *E. fetida* stocking density of 12.5 g- worms kg^{-1} feedstock was found to be ideal for the biodegradation of phosphate-rock enriched cow-dung-waste paper mixtures, (iii) Phosphate release from PR enriched wastes is strongly and linearly dependent on *E. fetida* stocking density and that higher stocking densities favour greater P release. Therefore, although vermicompost maturity can be achieved at lower stocking densities, a higher stocking density may be preferred in situations where maximum P release from RP incorporated in the waste mixture is the desired goal. Future work will seek to optimise the rate of RP incorporation in the vermidegradation of cow-dung-waste paper mixtures as well as unravel the mechanisms by which RP enhances the bioconversion of these waste mixtures.

CHAPTER 4

4 DETERMINATION OF OPTIMUM ROCK PHOSPHATE INCORPORATION RATE FOR EFFICIENT VERMIDEGRADATION OF COW-DUNG-WASTE PAPER MIXTURES

4.1 ABSTRACT

Previous studies have shown that addition of rock phosphate (RP) at rates of application above 2% P accelerated the bioconversion and humification of cow-dung-waste paper mixtures but the optimum rate of RP incorporation was not established. Therefore, the present study evaluated a range of RP incorporation rates (0, 0.5, 1.0, 1.5, 2, and 4% P as RP) with a view to determine the minimum rate of RP incorporation necessary for the efficient vermidegradation of cow-dung-waste paper mixtures. The bioconversion of the waste mixtures was monitored by measuring C: N ratios, polymerization index (PI), and humification index (HI). Scanning electron microscopy (SEM) was used to evaluate the morphological properties of the vermicomposts affected by rates of RP. A germination test was used to determine the phyto-toxicity of the final composts. Phosphorus transformation during vermicomposting was monitored through sequential fractionation of different P fractions while potentially available P was extracted using the Bray 1 method. The C: N ratio and other maturity parameters indicated that incorporation of RP improved the bioconversion of cow-dung-waste paper mixtures with fastest maturation at a rate of 1% P with C: N ratio of 7, PI of 14.4, and HI of 27,1%, respectively. However, higher rates of RP application resulted in final vermicomposts with higher total P contents. A P-fractionation study revealed that the extractability of P increased linearly with the rate of RP application with the water soluble P fraction making the largest (1002.4 mg P kg^{-1}) contribution to the mineralized P.

The enhanced P mineralization was attributed to enhanced microbial activity possibly stimulated by the optimized cow-dung-waste paper mixtures.

Keywords: Vermicompost, carbon to Nitrogen ratio, Humification indices, Germination index, Phosphorus fractions, Rock phosphate.

4.2 INTRODUCTION

Vermicomposting has proved to be a suitable technique of processing biodegradable organic wastes and converting them to organic fertilizers because of its low cost and the large quantity of wastes that can be processed. However, the nutritional values of most compost products are highly variable and determined principally by the types of the substrates used and the degree of composting (Yan *et al*., 2012; Mupondi, 2010, Matiullah and Muhammed, 2012). Biswas and Narayanasamy (2006) reported low P content of (0.37%) in the final compost from straw due to the low P content of the straw material used. (Mupondi, 2010) observed very low P content of (0.6%) in dairy manure and paper waste mixtures vermicompost and also ascribed it to the low P content in the paper and cow dung mixture used as substrate. Yan *et al*. (2012) reported that the rice straw mixed with cow dung vermicompost had very low P content of (0.392%) and attributed it to the low P content of the substrate. Similarly, Matiullah and Muhammad (2012) reported very low phosphorus content in poultry litter (0.3%) during composting and also credited it to the low P content of the poultry litters used as substrate.

In order to increase the acceptability of vermicompost products as sources of nutrients or as potting media, it is essential to increase their P contents. Biswas and Narayanasamy, (2006) were able to increase the total P content of straw compost from 0.37% in control to 2.20% by applying 4% P as RP, while Yan *et al*. (2012) were able also to increase the total P of rice straw vermicompost from 0.392% to 0.82% by applying 2% P as RP. Matiullah and Muhammad (2012) also increased the total P content in poultry litter from 0.3% to 1.02% by applying 4% P as RP. Singh and Amberger (1990) reported that organic solid waste compost to which RP was applied did not only increase its P content but also enhanced the humification of the resultant vermicomposts. This prompted Mupondi (2010) to investigate the effect of RP incorporation on the vermicomposting of dairy manure and paper waste mixtures. The researcher investigated rates of RP incorporation ranging from 2 to 8% P as RP. The results showed that the vermicomposts were highly humified and had high total P and N contents as well as available P and N contents compared with the control. All rates of RP incorporation > 2% P improved humification to more or less the same extent, suggesting that lower rates of RP incorporation could possibly be effective in improving the vermidegradation of dairy manure and paper waste mixtures. The present study, therefore sought to investigate the effectiveness of using low RP application rates of less than 2% P in improving the vermidegradation of cow-dung-waste paper mixtures. Edwards *et al*. (2010) postulated that a range of P forms could be produced during the co-composting of RP with organic wastes which could impact the subsequent bioavailability of P. There is little or no information on the P transformations that take place during the vermicomposting of RP-enriched wastes.

The objectives of this study were, therefore to: (i) determine the minimum amount of rock phosphate required for efficient vermidegradation of cow-dung-waste paper mixtures, and

(ii) determine the effects of rate of RP application on total P, Bray 1 extractable P, and different P fractions in the final cow-dung-waste paper vermicomposts.

4.3 MATERIALS AND METHODS

4.3.1 Site description, wastes and earthworms utilized

The vermicomposting experiment was performed in a closed shaded yard at the University of Fort Hare Teaching and Research farm located (32^{o}46'S and 26^{o}50'E) in the Eastern Cape Province of South Africa under an ambient mean temperature of 25^{o}C. Waste papers used for the study were collected from the University printing press (Xerox) and Faculty offices, while RP was obtained from Phalaborwa in Limpopo Province, South Africa, which is granitic in nature. Earthworms (*E. fetida*) used in the study were collected from our local wormery at the University of Fort Hare Teaching and Research farm. Cow dung was obtained from Keiskammahoek Dairy Project located about 60 km North East of the University of Fort Hare.

4.3.2 Experimental procedure

4.3.2.1 Phosphorus enrichment of vermicomposts with RP

The P enrichment treatments consisted of 6 rates of 0, 0.5, 1, 1.5, 2 and 4 % (elemental P basis) as ground RP. Each was thoroughly mixed with enough feedstock (5 kg on dry weight basis) prepared together by mixing 2.16 kg shredded paper and 2.84 kg cow dung to achieve a C: N ratio of 30. The resultant mixtures were placed in worm boxes measuring 0.50 m x 0.40 m x 0.30 m (length x width x depth) with an exposed top surface area of 0.2 m^2 and placed in a well-ventilated farm building at an average mean ambient temperature of about 25^{0}C. Unuofin and Mnkeni (2014) established a stocking density of 12.5 g worms kg^{-1} feedstock as the optimum density for the bioconversion of cow dung–waste paper mixtures. Hence, for this present study matured, worms were introduced at this predetermined stocking density of 12.5g worms kg^{-1} feed. Treatments were arranged in a completely randomized design (CRD) with three replications. The moisture content was determined on a weekly basis according to (Reinecke and Venter 1985) and it was maintained at 80% moisture content level by sprinkling water when necessary throughout 56 days of the vermicomposting period.

Sample collection was carried out at day 0, 14, 28, 42 and 56 of vermicomposting and analyzed for volatile solids, ash content, total C, total N and inorganic N (nitrate- N and ammonium – N), organic and inorganic phosphorus and microbial biomass carbon. The waste mixture samples collected at day zero and day 56 were subjected to sequential P fractionation as described below.

4.3.2.2 P Fractionation of vermicomposts

Vermicompost samples that were collected at the beginning (0 day) and at termination (56 days) of vermicomposting were sequentially fractionated to determine different P fractions using a modified version of the Hedley *et al.* (1982) procedure as described by Dou *et al.* (2000). Briefly, moist vermicompost samples were successively extracted with de-ionized water, 0.5 M $NaHCO_3$, 0.1 M NaOH, and 1 M HCl. In the first extraction with de-ionized water, 0.3g (oven dry basis) of vermicompost samples was weighed into a 50 ml centrifuge tube along with 30 ml of de-ionized water and shaken for 1h at room temperature on an end-to-end shaker at 150 excursions per minute. The samples were centrifuged at 10,000 g for 15 min and filtered using Whatman No. 42 filter paper.

The compost residues that remained in the centrifuge tubes were subsequently successively extracted using 30 ml each of 0.5 M $NaHCO_3$ (pH 8.5), 0.1 M NaOH and 1.0 M HCl. Following shaking with each extractant for 16 h, centrifugation of the suspensions was done and afterwards filtered. A part of the $NaHCO_3$ and NaOH extracts were acidified to precipitate out the extracted organic matter and the resultant supernatant was then analysed for inorganic P (Pi).

The remaining bit of $NaHCO_3$ and NaOH extracts were digested with acidified potassium persulphate and analyzed for total P (Pt). The organic P (Po) in $NaHCO_3$ and NaOH extracts was determined as the difference between the Pt and Pi contents. The P concentration in all extracts and digests was determined by the molybdenum blue colorimetric method of Murphy and Riley (1962) on a San 2++ Skalar Continuous Flow Analyser (CFA) (Skalar Analytical B.V. the Netherlands).

4.3.2.3 Microbial biomass carbon

The initial microbial biomass carbon (MBC) content and that of the resulting vermicompost at day 56 was determined by means of the chloroform fumigation and extraction method (Vance *et al*., 1987). The process involved using 2 g (oven-dry basis) moist compost which was afterwards fumigated for 24 h at 25 ºC with ethanol free chloroform. After the removal of fumigant, the sample was then extracted with 60 ml of 0.5 *M* potassium sulphate solution. The measurement of the organic C content in the extracts was done by dichromate oxidation (Kalembasa & Jenkinson, 1973) and the microbial biomass carbon (MBC) was calculated by means of the equation:

$$MBC = E_C / K_{EC}$$

Where E_C represent the organic carbon extracted from fumigated soil minus organic carbon that was extracted from unfumigated soil while K_{EC} (The proportion of the microbial C that is extracted from the compost) = 0.38 (Vance *et al*., 1987).

4.3.2.4 Physico-chemical analyses

The resultant vermicompost samples were analyzed for volatile solids (VS), ash content, total carbon and total nitrogen, extractable phosphorus P and humic substances. These vermicomposts were first air dried until constant weight was achieved and subsequently pulverized (< 2 mm) to offer a uniform sample for analysis. The (VS) were determined as sample weight loss (previously oven-dried at 105^{o}C) upon ashing at 550^{o}C for 4 h in a muffle furnace (Ndegwa *et al*. 2000) while total nitrogen (N) and carbon (C) were determined using a Truspec CN Carbon/ Nitrogen analyser (LECO Corporation, 2003). Bray 1 P was determined as described in chapter 3.

Total phosphorus was determined by digesting 0.5 g of air-dried composts samples in a MARS 5 microwave digester (CEM Corporation, Matthews, North Carolina) using aqua regia followed by the determination of phosphorus concentration in the digests by means of the reduced phosphomolybdenum blue method on a continuous flow analyser (San 2++ Skalar Continuous Flow Analyser, Skalar Analytical B.V. the Netherlands). Humic substances were determined following extraction as described by Del Carmen Vargas-García *et al*. (2006).

To the composted samples 0.1 *M* NaOH (1:20 w/v ratio) was added and continuously shaken on a horizontal shaker for 4 h, followed by centrifugation of the resultant solution at 8,000 x g for 15 min. After that, the supernatants were separated into two portions, one portion was analysed for total extractable carbon fraction (C_{EX}) through the Walkley and Black rapid titration method as described by Anderson and Ingram (1996) and the remaining portion was adjusted to pH 2 using concentrated H_2SO_4 and set aside to coagulate for 24 h at 4°C. The resulting precipitates that were formed comprised the humic acid–like carbon (C_{HA}) while fraction that remained in solution constituted the fulvic acid-like carbon (C_{FA}). The C_{HA} was calculated by subtracting the C_{FA} from the C_{EX}. The humification indices were calculated using the following equations (Ciavatta *et al*., 1988; Mupondi *et al*., 2010):

Humification ratio (HR) $= \left(\frac{CEX}{C}\right) x\ 100$

Polymerization index (PI) = (C_{HA}/C_{FA}) x 100

Humification index (HI) $= \left(\frac{CHA}{C}\right) x\ 100$.

4.3.2.5 Morphological assessment of the resultant vermicomposts

Scanning Electron Microscopy (SEM) images of the samples were taken using a scanning electron microscope model JOEL (JSM-6390LV, Japan). Briefly, the samples were oven dried and ground to pass through a 2mm sieve. A small representative portion of the samples were coated with gold and mounted on SEM. Samples were imaged by scanning it with a high energy beam of electrons in a raster scan pattern.

4.3.2.6 Phytotoxicity study

Phytoxicity was determined as described in Unuofin and Mnkeni (2014). Briefly, two pieces of Whatman® filter paper were placed inside a sterilized petri dish and wetted with the vermicompost extracts. Ten seeds of each crop species were placed on top of the filter paper and incubated for five days in the dark. A control was included for each crop species in which the filter papers were wetted with distilled water. Seed germination index (GI), relative seed germination (RSG) and relative root elongation (RRE) were calculated as follows:

$$\text{RSG (\%)} = \frac{\text{Number of seeds germinated in the sample extract}}{\text{Number of seeds germinated in the control}} \text{ x } 100$$

$$\text{RRE (\%)} = \frac{\text{Mean root elongation in the sample extract}}{\text{Mean root elongation in the control}} \text{ x } 100$$

$$\text{GI (\%)} = \frac{\text{(\% Seed germination) x (\% Root elongation)}}{100}$$

4.4 Statistical analysis

Data reported herein are the means of three replicates (n=3). Statistical analysis was done using repeated measures analysis of variance (ANOVAR) since destructive sampling was not carried out. Fisher's protected least significant difference (*LSD*) test at $p<0.05$ was used for means separation. All statistical analyses were done using JMP® Release 10.0 statistical package (SAS Institute, Inc., Cary, North Carolina, USA, 2010).

4.5 RESULTS

4.5.1 Effects of RP rate on ash and volatile solids contents

Both percent ash and volatile solids contents of cow-dung-waste paper vermicompost were significantly affected by added RP (Table 4.1).

Table 4.1: Repeated measures ANOVAR for C: N ratio, C_{HA}: C_{FA}, HI, HR, Total P, Total N, Bray1 extractable P, MBC, VS, Ash content, NH_4-N and NO_3-N

	Effect					
	Added RP rates		Time		Added RP rates X Time	
	$F_{(5, 60)}$	P	$F_{(4, 60)}$	P	$F_{(20, 60)}$	P
C: N ratio	1023.3	<0.0001	22341.2	<0.0001	460.3	<0.0001
Polymerization index (PI)	42.8	<0.0001	376.8	<0.0001	30.1	<0.0001
HI (%)	425.1	<0.0001	2357.6	<0.0001	101.7	<0.0001
Total P (g Kg^{-1})	2332.1	<0.0001	1064.1	<0.0001	894.4	<0.0001
Total N (%)	201	<0.0001	540	<0.0001	25	<0.0001
Bray 1P (mg Kg^{-1})	1895.5	<0.0001	1637.5	<0.0001	1034	<0.0001
MBC (mg Kg^{-1})	100.5	<0.0001	10224.7	<0.0001	58.7	<0.0001
VS (%)	314.2	<0.0001	903.4	<0.0001	17.9	<0.0001
Ash content (%)	396.3	<0.0001	784.3	<0.0001	16.2	<0.0001
NH_4^+-N (mg Kg^{-1})	20.5	<0.0001	701.7	<0.0001	8.2	<0.0001
NO_3^-- N (mg Kg^{-1})	6.1	<0.0001	1003.7	<0.0001	23.3	<0.0001
NH_4^+ : NO_3^-	3.36	<0.0001	230.2	<0.0001	5.6	<0.0001

F values and probabilities are shown for each effect. P < 0.05 in italics is significant. n=3, C: N=carbon to Nitrogen ratio, PI = Polymerization index, HI= Humification index, HR= Humification ratio, VS=Volatile solid, MBC= Microbial biomass carbon NH_4^+-N= Ammonium- Nitrate, NO_3^-- N = Nitrate- Nitrite, NH_4^+: NO_3^- = Ammonium: nitrate ratio.

The percent ash increased significantly with time at each added RP rate with the 1 % P rate of RP application resulting in consistently the highest ash content and the absolute control where no RP was added the lowest ash levels (Fig. 4.1a). The other rates of RP application resulted in intermediate ash contents which followed the order 4%P ≈ 2%P ≈ 1.5%P > 0.5%P (Fig.4.1a). Final percent ash content ranged from 42% to 49% in vermicompost where RP was added, while in the control where no RP was added the ash content was 40% (Fig.4.1a). The ash content was lowest for each RP treatment at day 0 but increased as time progressed with wide differences between treatments occurring on days 28 and 42.

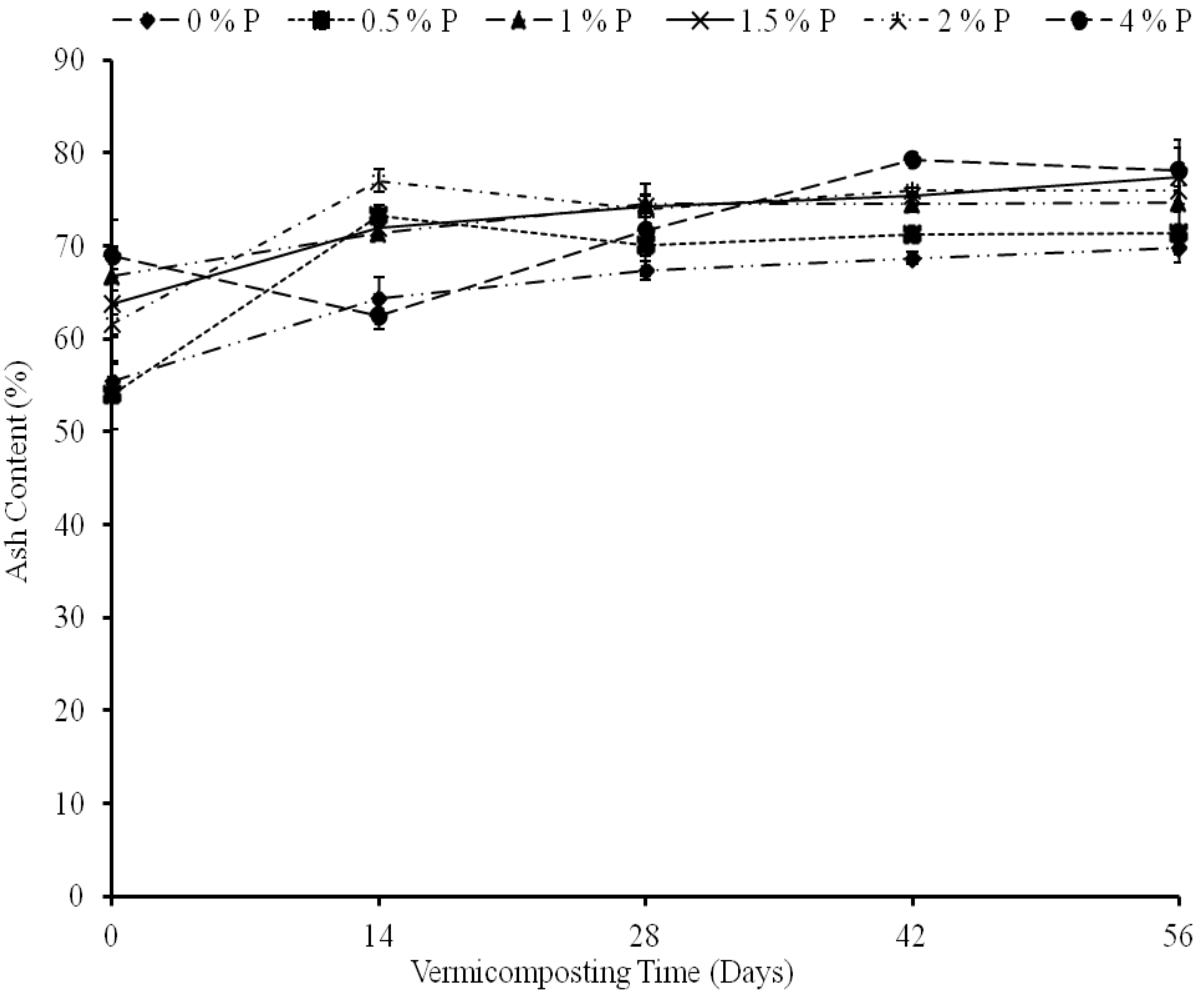


Figure 4.1 (a): Effect of added RP rates and vermicomposting time on ash contents during vermicomposting of cow dung-waste paper mixtures

The (VS) in cow-dung-waste paper vermicompost followed the same but opposite trend to ash content whereby the VS decreased significantly with time at each added RP rate (Fig.4.1b).

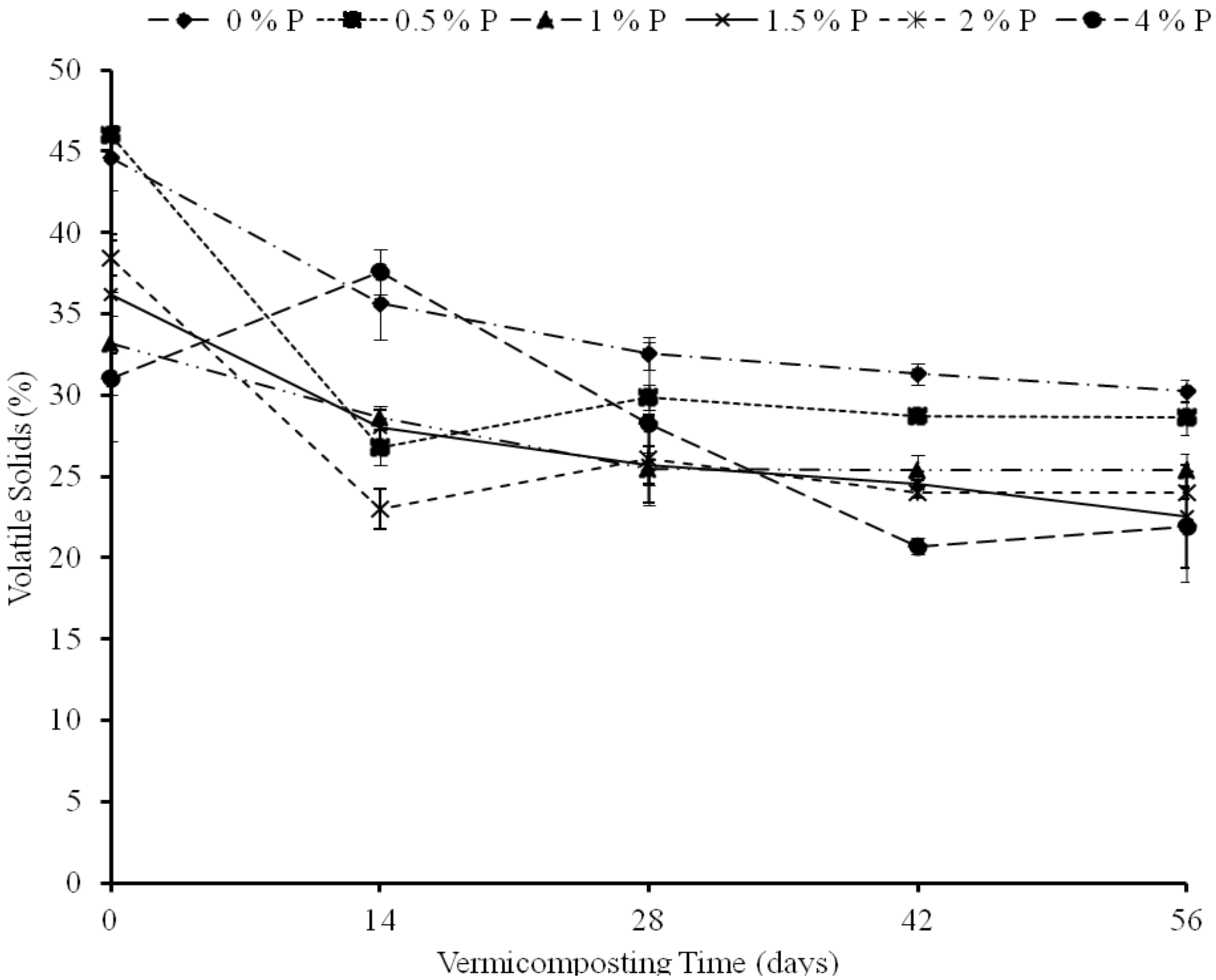


Figure 4.1 (b): Effect of added RP rates and vermicomposting time on volatile solids contents during vermicomposting of cow dung-waste paper mixtures

4.5.2 Effect of RP rate on the carbon to nitrogen ratio

Both added RP rates and time significantly affected C: N ratio of cow-dung-waste paper vermicompost (Table 4.1). The C: N ratio decreased significantly with time at each added RP rate but pronounced differences between rates of RP application were only observed up until day 28 beyond which differences were minimal (Fig.4.2). The greatest decrease in C: N ratio at day 14 occurred where RP was added at a rate of 1% while the least decline in C: N ratio was observed where no RP was added.

Further decline in C: N ratio was observed beyond day 28 till the termination of the experiment but the effects of added RP rates were not significantly different except for 0% and 0.5% P at days 42 and 56, respectively. Final C: N ratios ranged from 6.9 to 10.6 in vermicompost where RP was added, while in the control where no RP was added the C: N ratio was 12 (Fig. 4.2). Thus, the incorporation of RP hastened the vermidegradation of cow dung-waste paper mixtures during the early stages of vermicomposting up to 28 days but beyond this the RP effect was minimal. Time required to vermicompost maturity was 56 days where no RP was added but it was 42 days or less where RP was added (Table 4.2). The shortest time to vermicompost maturity was 33 days and was realized with the application of 1% P as RP which shortened the time to vermicompost maturity by 28% (Table 4.2).

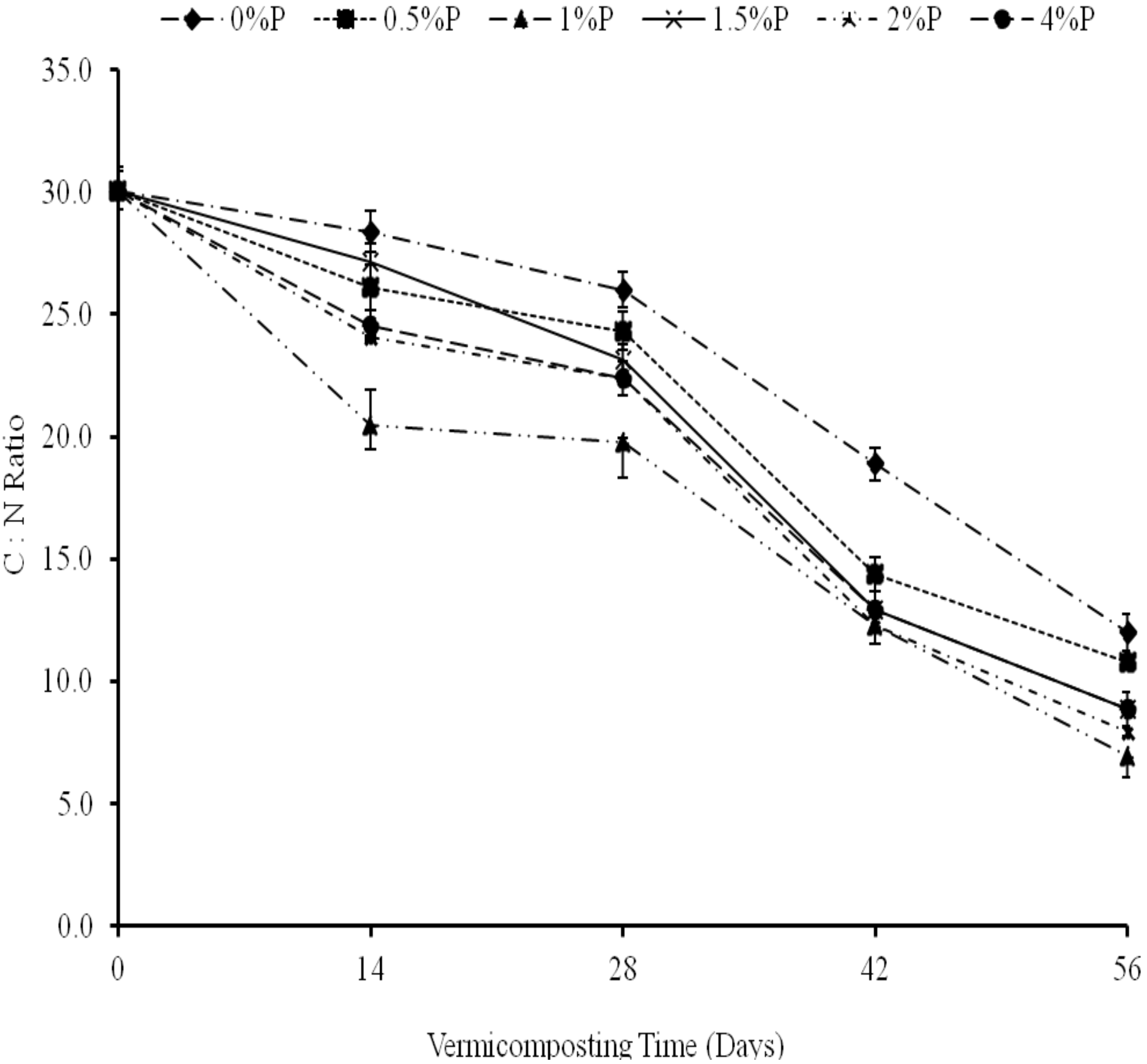


Figure 4.2: Effect of added P as rock phosphate (RP) and vermicomposting time on the C: N ratio of cow dung- waste paper mixtures during vermicomposting with *Eisenia fetida*.

Table 4.2 : Effect of rate of RP application on the vermidegradation time of cow-dung-waste paper mixtures as reflected by C: N ratio

Treat. No.	Treatment % P as RP	Regression Equations (C:N ratio VS Incubation time in days)	Days required to maturity(i.e.at C/N ratio=12)	% improvement in reducing time to vermicompost maturity
1	0	$y = -0.0026x^2 - 0.2072x + 30.8$	46	
2	0.5	$y = 0.002x^2 - 0.1234x + 31.0$	42	9*
3	1.0	$y = 0.0066x^2 - 0.7714x + 29.229$	33	28*
4	1.5	$y = 0.005x^2 - 0.6458x + 31.6$	41	11*
5	2.0	$y = 0.0059x^2 - 0.7038x + 30.4$	41	11*
6	4.0	$y = 0.007x^2 - 0.7646x + 29$	40	13*
		P>F	<0.0001	

*Relative to treatment 1 (where cow-dung-waste paper mixtures were only inoculated with *Eisenia fetida* and no RP addition).

4.5.3 Effect of RP rate on humification parameters

4.5.3.1 Polymerization index (PI)

Addition of RP into cow-dung-waste paper mixtures during vermicomposting had significant effects on the polymerization index (PI) of the vermicompost with significant interaction between added RP and time (Table 4. 1). The (PI) increased significantly with time at each added RP rate ($P< 0.0001$). Whereas all RP rate treatments had the same (PI) at time 0, differences started to emerge as time progressed. These differences between rates of RP application were not pronounced up to 28 days, but wider significant differences were observed on days 42 and 56 (Fig. 4.3a). The highest increase in PI at both day 42 and 56 occurred where RP was added at a rate of 1% P while the smallest increase occurred where no RP was added. The addition of RP at 1.5, 2 and 4% P had more or less the same effect on (PI) on these two days. Thus the trend of the (PI) of the waste mixtures on days 42 and 56 can be summarized as 1 % P > 1.5%P ≈ 2%P ≈4%P >> 0.5%P >0%P (Fig 4.2a).

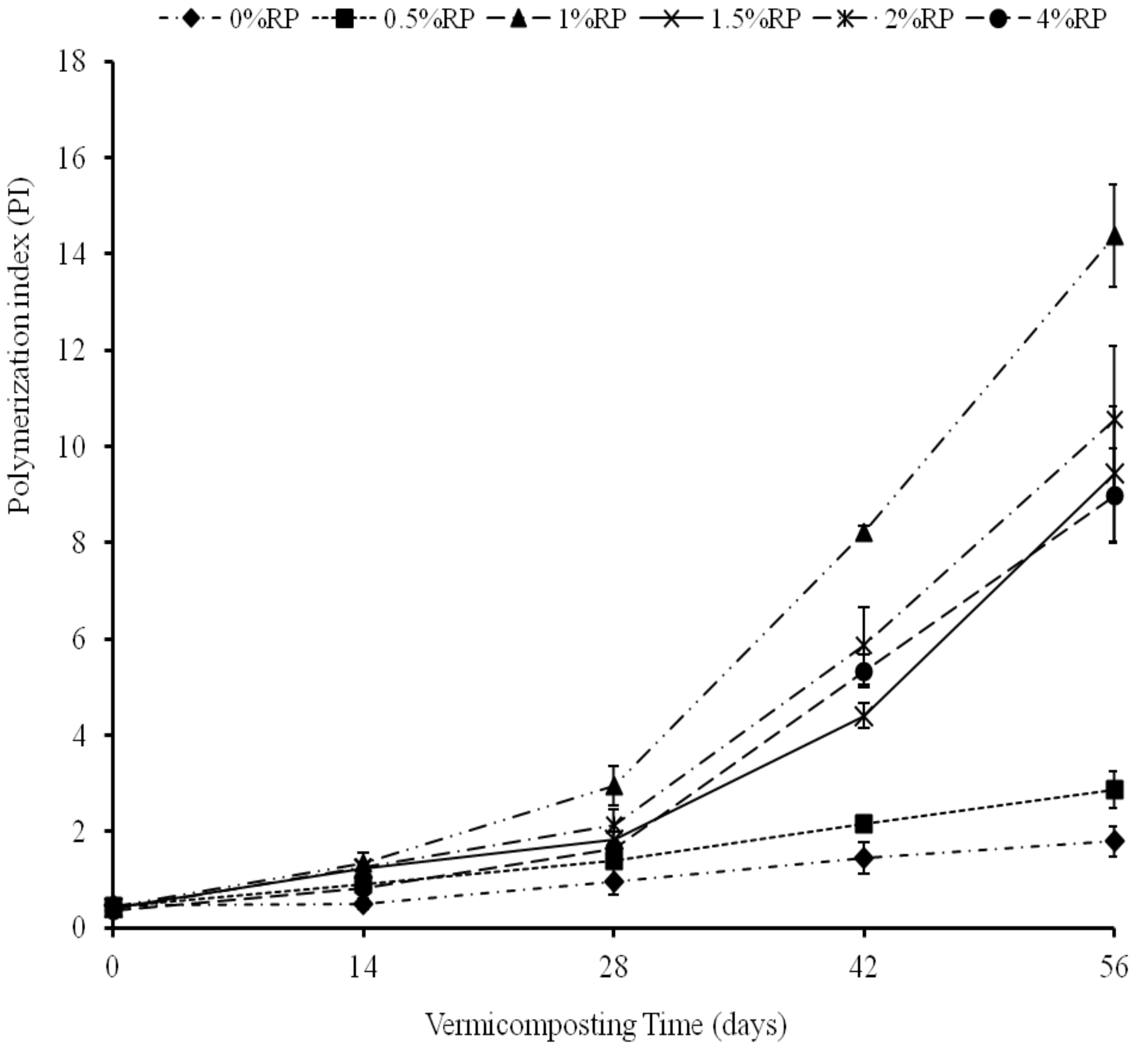


Fig. 4.3a: Effects of added RP rate and vermicomposting time on Polymerization index. Bars indicate standard deviations

4.5.3.2 Humification index

The HI of the resultant vermicompost (Table 4.1, Fig 4.3b) followed the same pattern as the (PI). The HI increased significantly with time at each added RP rate ($P< 0.0001$) with no striking differences between rates of RP application up to 28 days (Fig 4.3b). However, wide differences on HI were observed on day 42 and 56 (Fig 4.3b) consistent with the observed significant RP x time interaction ($P< 0.0001$) (Table 4.1). The differences on HI followed the order of 1%P=4%P >2%P >1.5%P >0.5%P >0%P at day 42, whereas for day 56, the order was 1%P.>2%P=4%P=1.5%P> 0.5%P >0%P, respectively (Fig.4.3b).

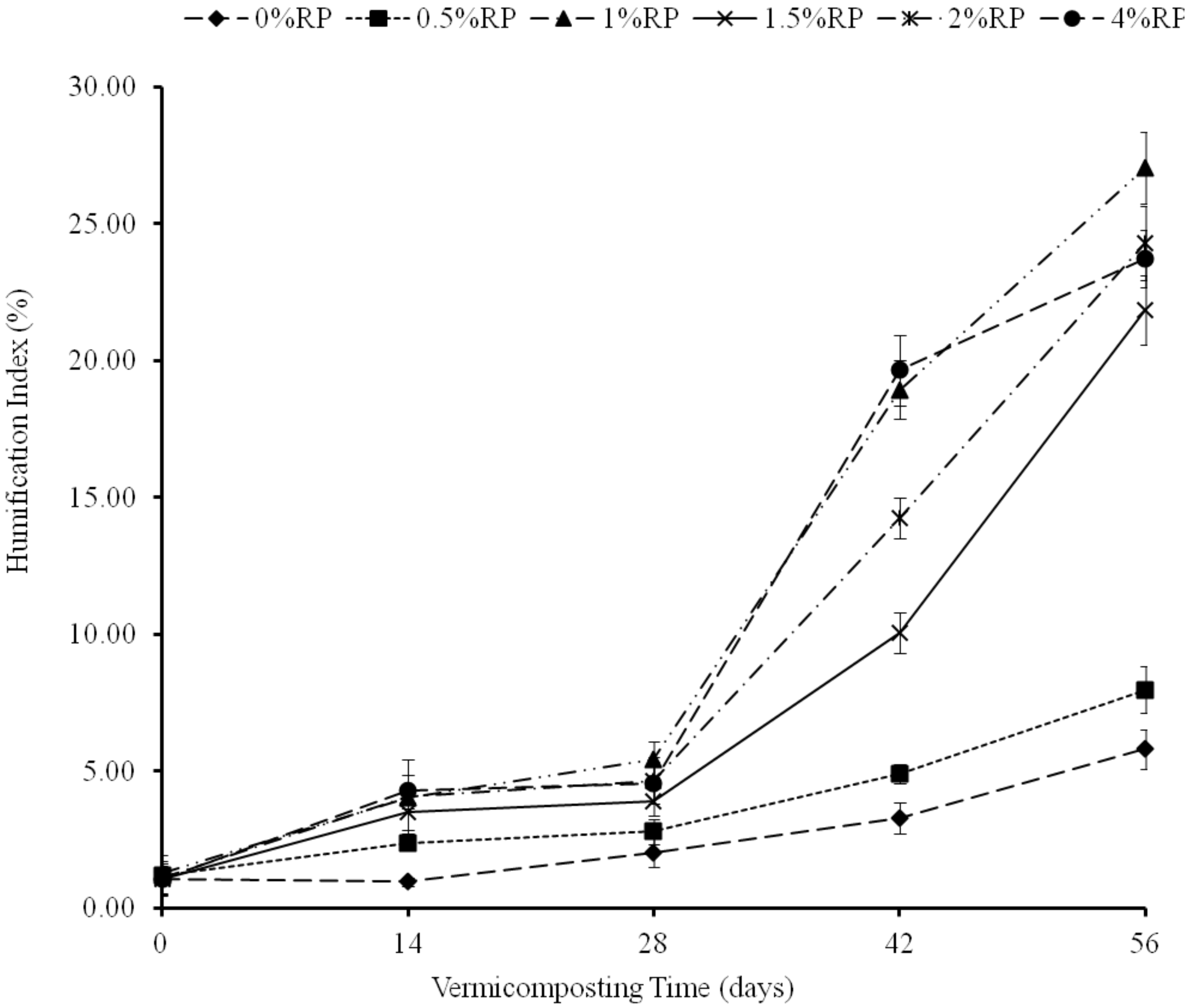
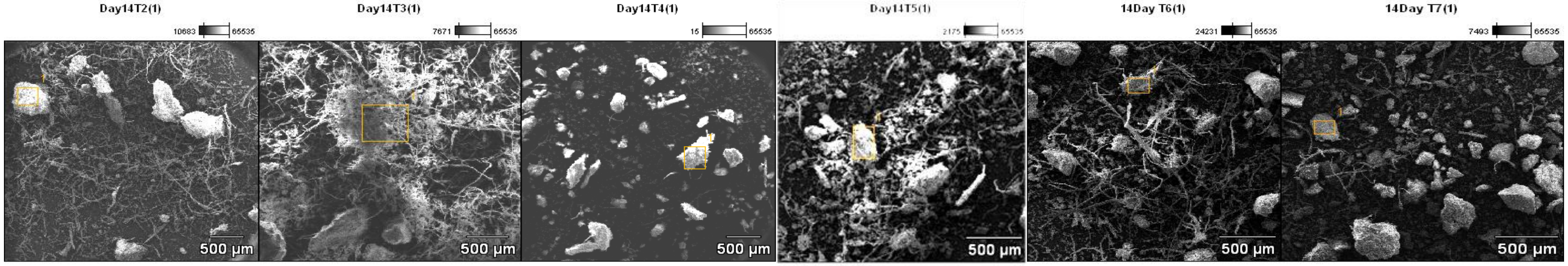


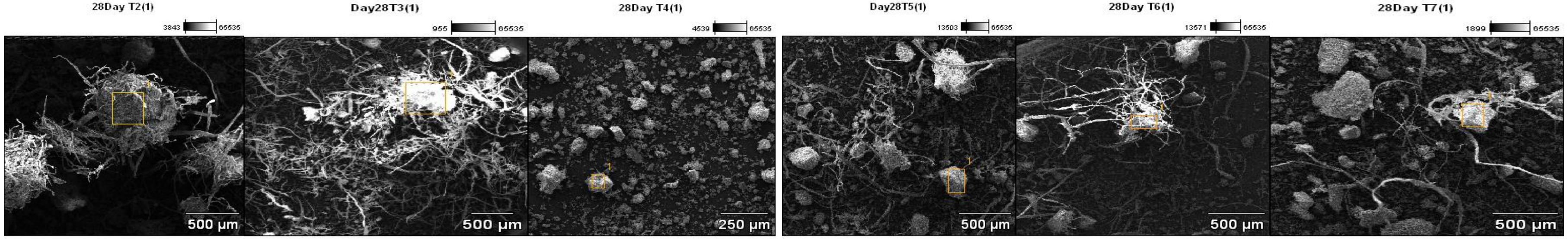
Fig. 4.3b: Effects of added RP rate and vermicomposting time on Humification index of cow dung waste paper vermicompost. Bars indicate standard deviations

4.5.4 Changes in the morphological structure of cow-dung-waste paper mixture during vermicomposting

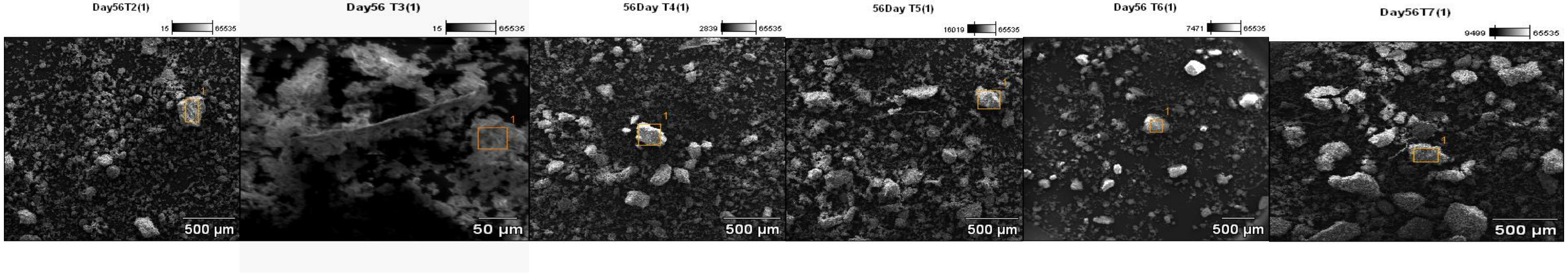
Figures 4.4a-c show the effects of rate of RP application on the morphological structure of cow-dung-waste paper mixtures during vermicomposting recorded using scanning electron microscopy (SEM). The SEM images of the cow-dung-waste paper mixtures at the beginning of the experiment (not shown) showed compacted aggregates of cellulose and protein fibres. However, inoculation of earthworm and addition of RP facilitated the breakdown of the recalcitrant fibre in the paper and the cow dung to produce degraded vermicompost to varying degrees depending on the rate of added RP and vermicomposting time (Fig. 4.4a-c). The SEM images show that degradation of the cow-dung-waste paper mixtures intensified with time at each rate of RP application. Wide differences in extent of degradation at different rates of RP application are seen on days 14 and 28 but by day 56 the vermicomposting mixtures appear to be equally degraded visually except where RP was added at a rate of 1% P where greater degradation was observed (Fig 4.4 a-c). On day 28 there were pronounced differences in the segregation of the wastes mixture aggregate particles which appeared to follow the order 1%P>4%P>2%P>1.5%P>0%P>0.5%P, respectively. Generally, the incorporation of RP at the rate of 1% P resulted in consistently greater vermidegradation of the cow-dung-waste mixtures at each sampling date than with the other RP treatments. This was reflected in higher segregation of the waste mixtures aggregate particles.



(a)0%RP (day14) 0.5%RP (day14) 1%RP (day14) 1.5%RP (day14) 2%RP (day14) 4%RP (day14)



(b)0%RP (day28) 0.5% RP (day28) 1%RP (day28) 1.5%RP (day14) 2%RP (day14) 4%RP (day14)



(c) 0%RP (day56) 0.5% RP (day56) 1%RP (day56) 1.5%RP (day56) 2%RP (day56) 4%RP (day56)

Figure 4.4(a-c): Scanning electron microscope images showing effects of RP application rate and time on vermicompost morphological properties

4.5.5 Effects of rate of RP on MBC

Added RP had significant effect on the MBC of cow-dung-waste paper vermicompost but this effect varied with time as revealed by the significant interaction between RP rates and time (Table 4.1 and Fig. 4.5). The MBC increased sharply up to day 14 at each added RP rate but declined sharply thereafter up to day 28 where after the decline in MBC was gradual up to day 56 (Fig.4.5). The level of MBC at each rate of RP application followed the order 4%P > 2%P >1.5%P ≈ 1.0 %P >0.5%P > 0% P (Fig.4.5). The significant RP x Time interaction on MBC was largely due to the fact that initially all RP treatments had the same level of MBC but this changed as time progressed, peaking at day 14 and declining thereafter.

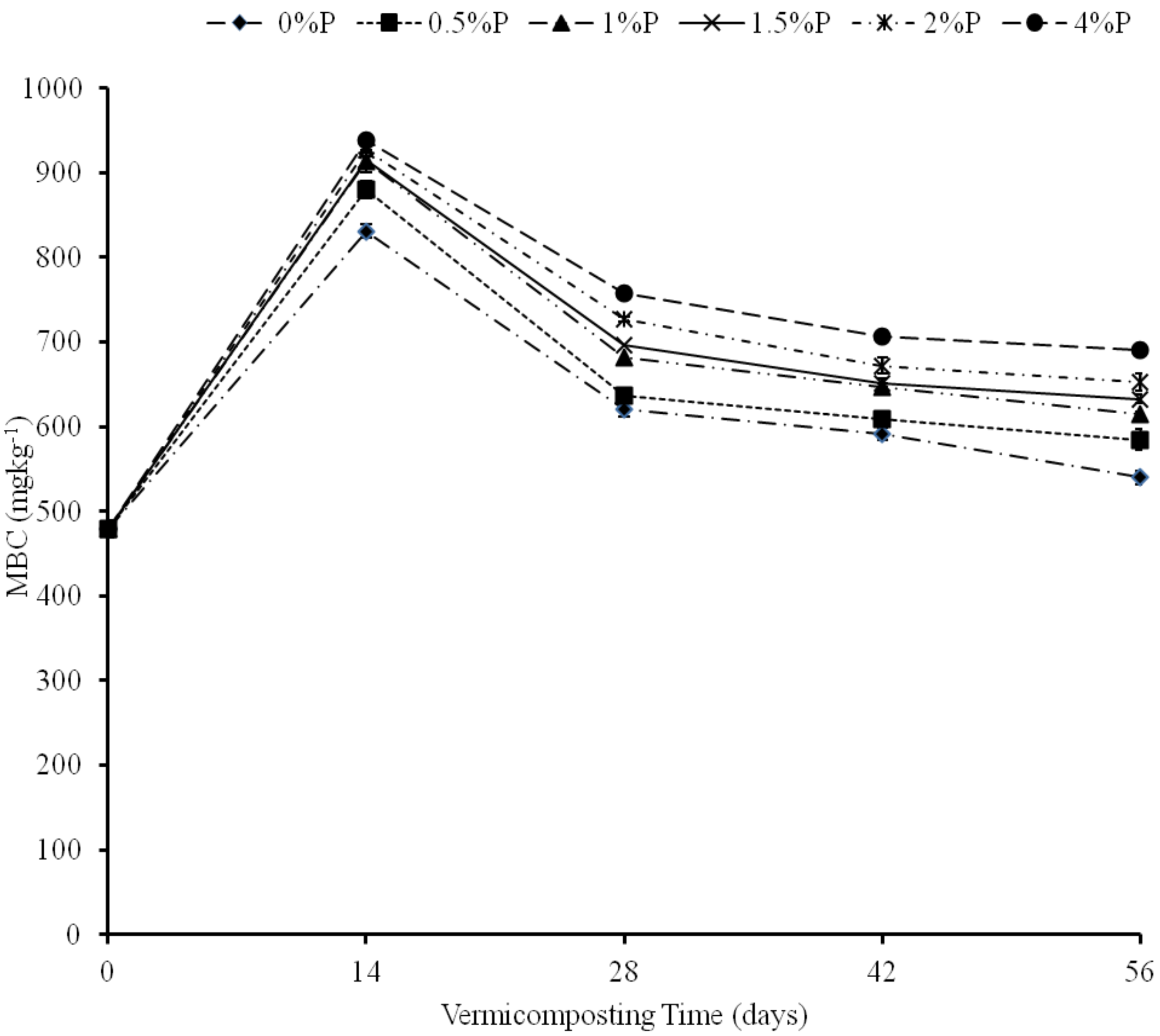


Figure 4.5: Effects of added RP on microbial biomass carbon of cow-dung-waste paper mixtures during vermicomposting. Bars indicate standard deviations

4.5.6 Effects of RP rate on, Nitrite-N NH_4^+-N and NH_4^+: NO_3^- ratio

Nitrite-N, Ammonium-N, and the NH_4^+:NO_3^- ratio of cow-dung-waste paper vermicompost were significantly affected by added RP but these effects varied with time as reflected by a significant interaction between RP rates and time (Table 4.1). The Nitrite-N content of the waste mixtures increased significantly with time at each added RP rate with the 1% P rate of RP application resulting in consistently the highest Nitrite-N and the absolute control where no P was added had the lowest Nitrite-N (Fig.4.6a). The other rates of RP application resulted in intermediate Nitrite-N contents which followed the order 4%P ≈ 2%P ≈ 1.5%P > 0.5% P (Fig.4.6a). The final Nitrite-N contents ranged from 35 mg kg^{-1} to 19.1 mg kg^{-1}in vermicomposts where RP was added, while in the control where no RP was added the Nitrite-N was 18 mg kg^{-1} (Fig.4.6a).The observed significant RP x Time interaction on Nitrite-N content was largely as a result of the fact that the Nitrite-Ncontent was the same for each RP treatment at day 0 but differences set in as time progressed. These differences between treatments were wide on days 28 and 42 but much narrower on day 56 except for the 1%P rate of RP incorporation which resulted in substantially higher values than the rest of the treatments (Fig. 4.6a).

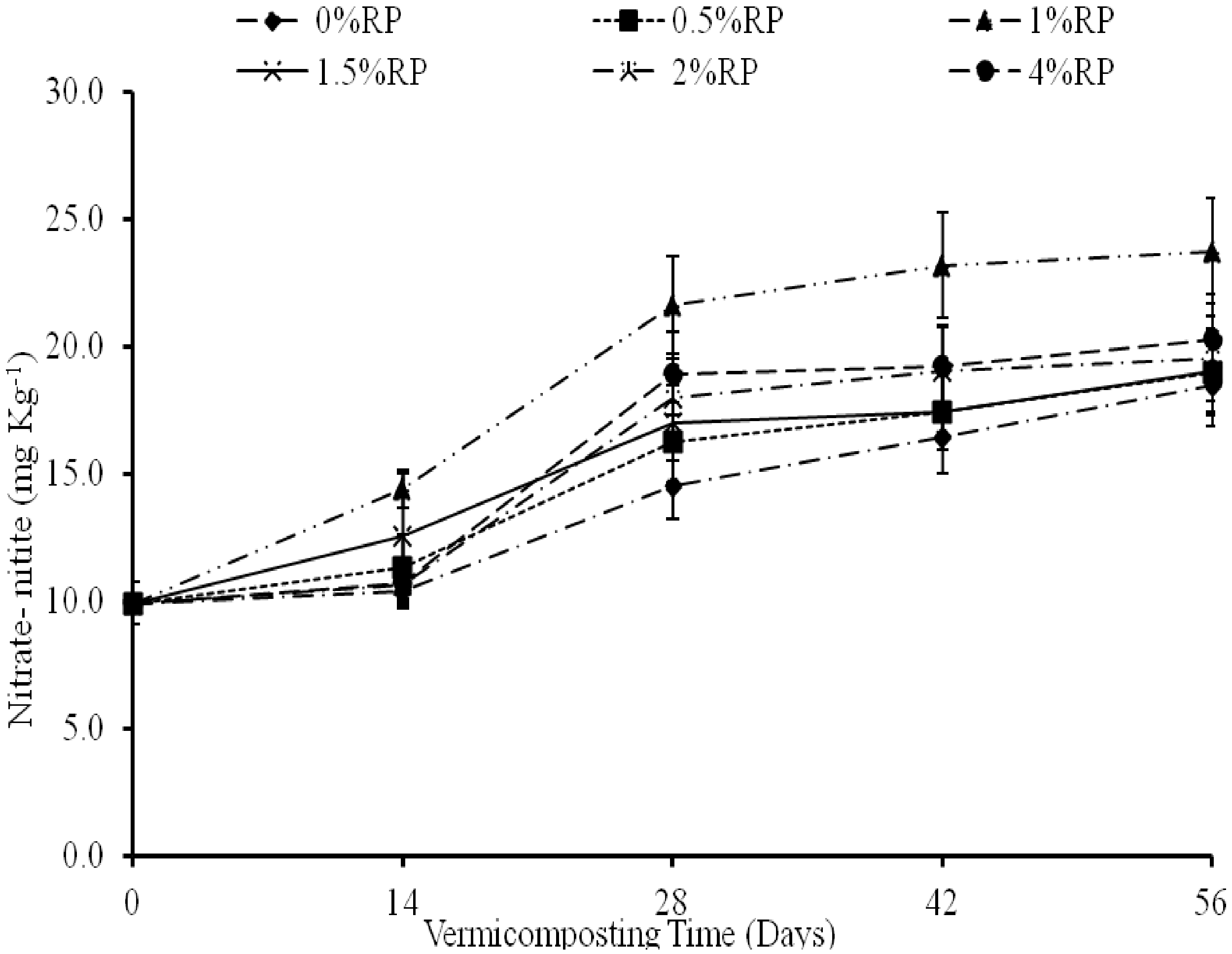


Figure 4.6a: Effect of added RP rates and vermicomposting time on Nitrite-N during vermicomposting of cow-dung-waste paper mixtures

The ammonium-N (NH_4^+-N) and the ratio of NH_4^+:NO_3^- in cow-dung-waste paper vermicompost followed the same trend whereby they both increased up to day 14 with no striking differences in added RP, thereafter decreased linearly to day 28. However, beyond day 28, sharper significant decreases were observed with time at each added RP rate (Fig.4.6 b and c). The NH_4^+-N and the NH_4^+:NO_3^- ratio of the waste mixtures both increased significantly with time at each rate of added RP. The control where no P was added had the highest NH_4^+-N and the NH_4^+:NO_3^- ratio, while 1% P rate of RP application resulted in consistently the lowest contents (Fig.4.6b and c). The other rates of RP application followed the order 4%P ≈ 2%P ≈ 1.5%P > 0.5% P (Fig.4.6b and c). Final NH_4^+-N contents and the NH_4^+:NO_3^- ratio values ranged from 12 mg kg^{-1} to 4 mg kg^{-1} and 0.8 to 0.1 where RP was added, respectively (Fig. 4b and c). In the control where no RP was added, the NH_4^+-N content and the NH_4^+: NO_3 ratio were 16.4 mg kg^{-1} and 0.9, respectively (F.g.4.6b and c).

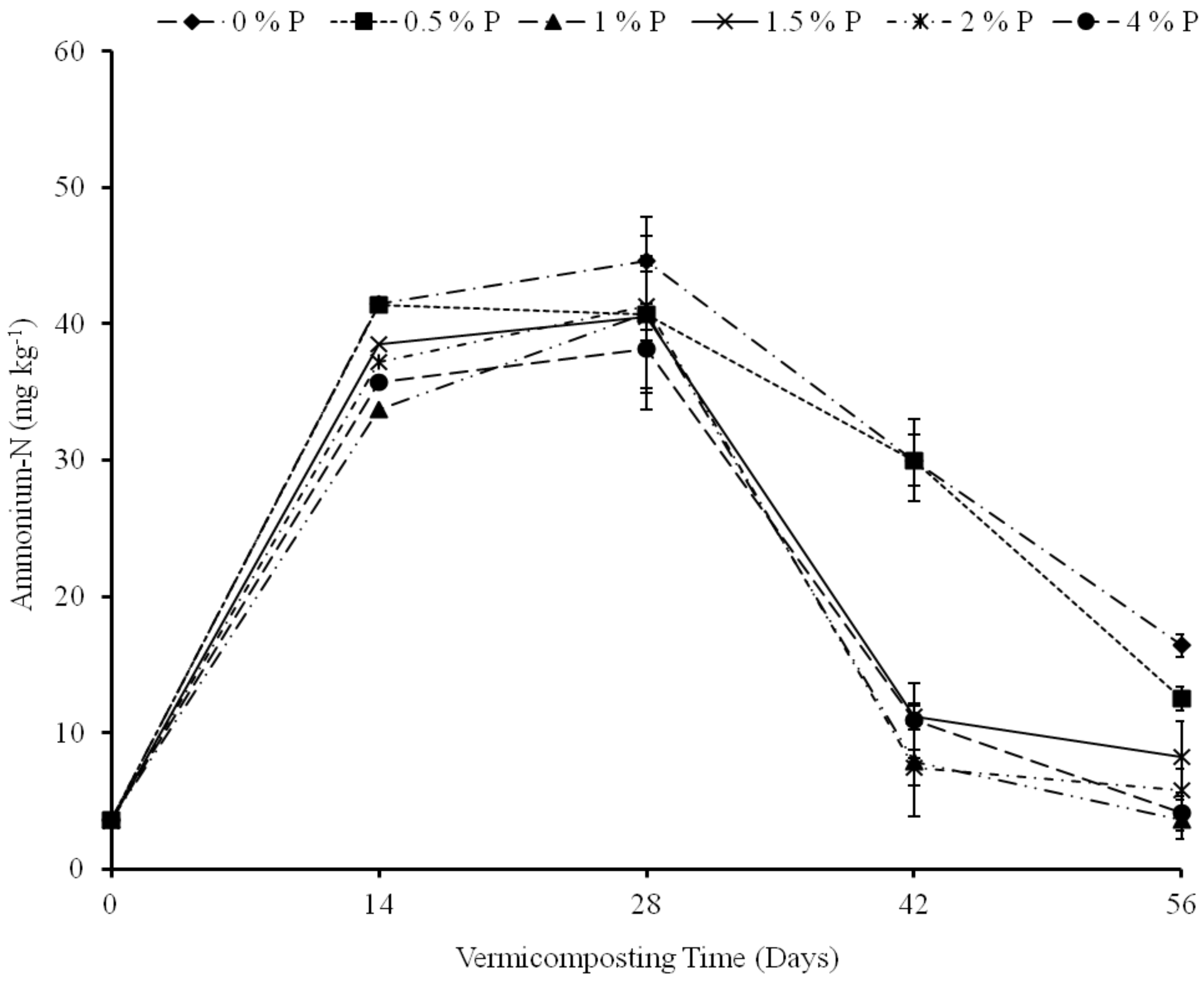


Figure 4.6 (b): Effect of added RP rates and vermicomposting time on NH_4^+-N during vermicomposting of cow-dung-waste paper mixture

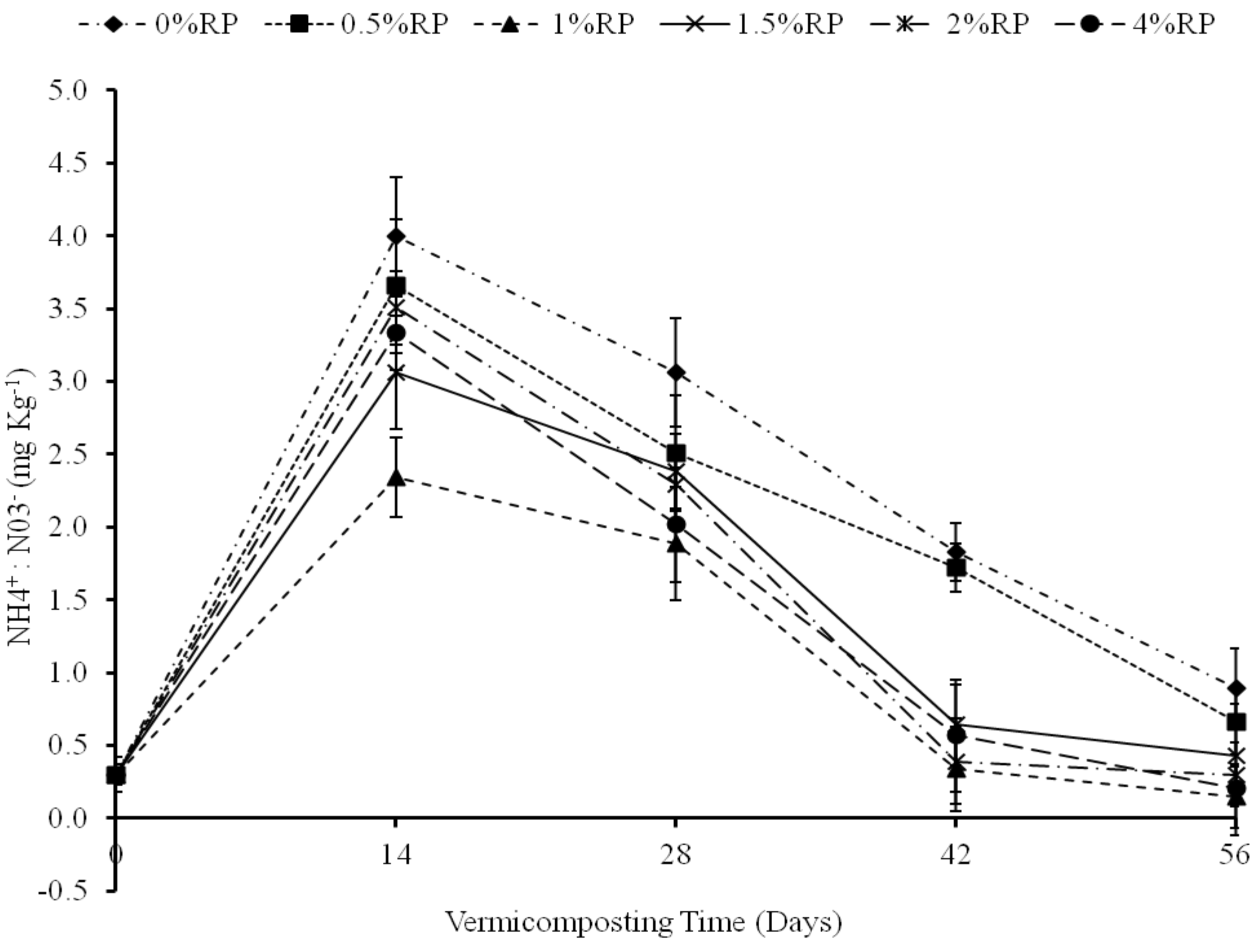


Figure 4.6 (c): Effect of added RP rates and vermicomposting time on NH_4^+: NO_3^- ratio during vermicomposting of cow-dung-waste paper mixture

4.5.7 Effect of rate of RP on the phytotoxicity level

Three parameters (relative root elongation (RRE), relative seed germination (RSG), and germination index (GI)) were used to evaluate the phytotoxicity of the final vermicomposts using tomato, carrot and radish as test crops. All three parameters were significantly ($p < 0.05$) influenced by added RP (Table 4.3). For each test crop, the three indices for phytotoxity increased with rate of RP application, peaking at the 1% P rate of RP application and declining thereafter with each increment in RP application (Table 4.3). The GI ranged from 90 - 138, 93 – 151and 92 – 152 for tomato, carrot and radish, respectively. The highest GI was realized at the 1% P rate of RP application and was significantly different from the other RP treatments (Table 4.3)

Table 4.3: Effect of added RP on the phytotoxicity of cow dung-waste paper vermicomposts

	Tomato			Carrot			Radish		
Treatment	RRE	RSG	GI	RRE %	RSG	GI	RRE	RSG	GI
0%P	110d	84d	90e	96c	93e	93f	86d	108e	92e
0.5%P	116c	92c	108d	102b	100d	102e	91c	117d	107d
1%P	124a	110a	138a	109a	135a	151a	101a	150a	152a
1.5%P	120b	100b	120c	107a	110c	116d	95b	125c	119c
2%P	121b	102b	125bc	104a	115c	127c	99a	125c	122c
4%P	126a	102b	129b	108a	125b	140b	99a	134b	132b
CV (%)	5	9	10	13	7	5	4	2	4
P-Value	<. 028	<. 028	<. 028	<. 028	<. 028	<. 028	<. 028	<. 028	<. 028

Numbers followed by different letters in each column are significantly different according to the LSD test at $p < 0.05$. RRE=Relative root elongation, RSG= Relative seed germination; GI=Germination index.

4.5.8 Effect of RP rate on total P, Bray 1 extractable P and different P fractions

Addition of RP to cow-dung-waste paper mixtures resulted in increases in both total P and Bray 1 extractable P contents with each increment of added RP (Table 4.4). The total P content of the final vermicomposts ranged from 0.18% where no RP was added to 2.31% P when RP was added at a rate of 4 % P. Corresponding values for Bray 1 extractable P were 80 mg P kg^{-1} to 207 mg P kg^{-1} (Table 4.4). The Bray-1 P content of the waste mixtures increased significantly with time at each rate of added RP rate with the 4%P rate of RP application resulting consistently in the highest Bray-1 P and the absolute control where no P was added had the lowest Bray-1P (Fig.4.7). It increased sharply up to day 14 at each added RP rate but declined sharply thereafter up to day 28. Beyond day 28 there was a sharp increase in Bray 1 P up to day 42 followed by a gradual decline up to day 56 (Fig.4.7).

Table 4.4: Effects of added RP on Total P and Bray 1 extractable contents of the final cow dung waste paper vermicomposts

Added RP	Total P (%)	Bray 1 P (mgKg^{-1})	Increase in Extractability of P (%)
0% P	0.18	80	
0.5% P	0.55	101	26
1% P	0.97	141	76
1.5% P	1.32	152	90
2% P	1.76	170	113
4% P	2.31	207	159
CV%	5	3	
P-Value	<0.0001	<0.0001	

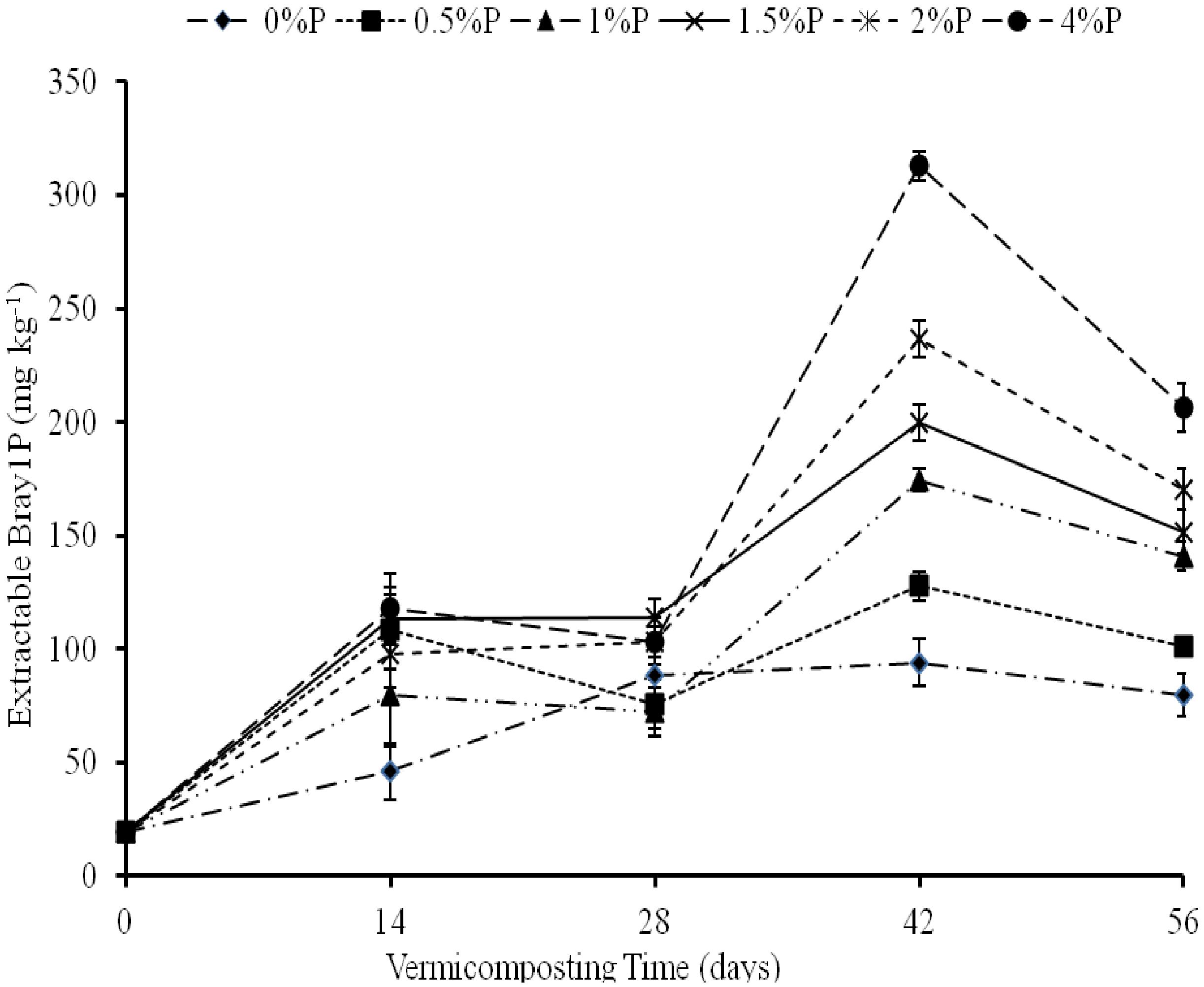


Figure 4.7 Effects of added RP on Bray 1 extractable P during vermicomposting of cow-dung-waste paper mixtures. Bars represent standard deviation.

The sequential fractionation results revealed that total inorganic P (P_i) and organic P (P_o) were not affected by rate of RP application at the beginning (time 0) but after 56 days of vermicomposting the rate of RP application had contrasting effects on the two P fractions (Figure 4.8). The total inorganic P (P_i) fraction increased with rate of RP application whilst the total organic (Po) fraction declined (Figure 4.8).

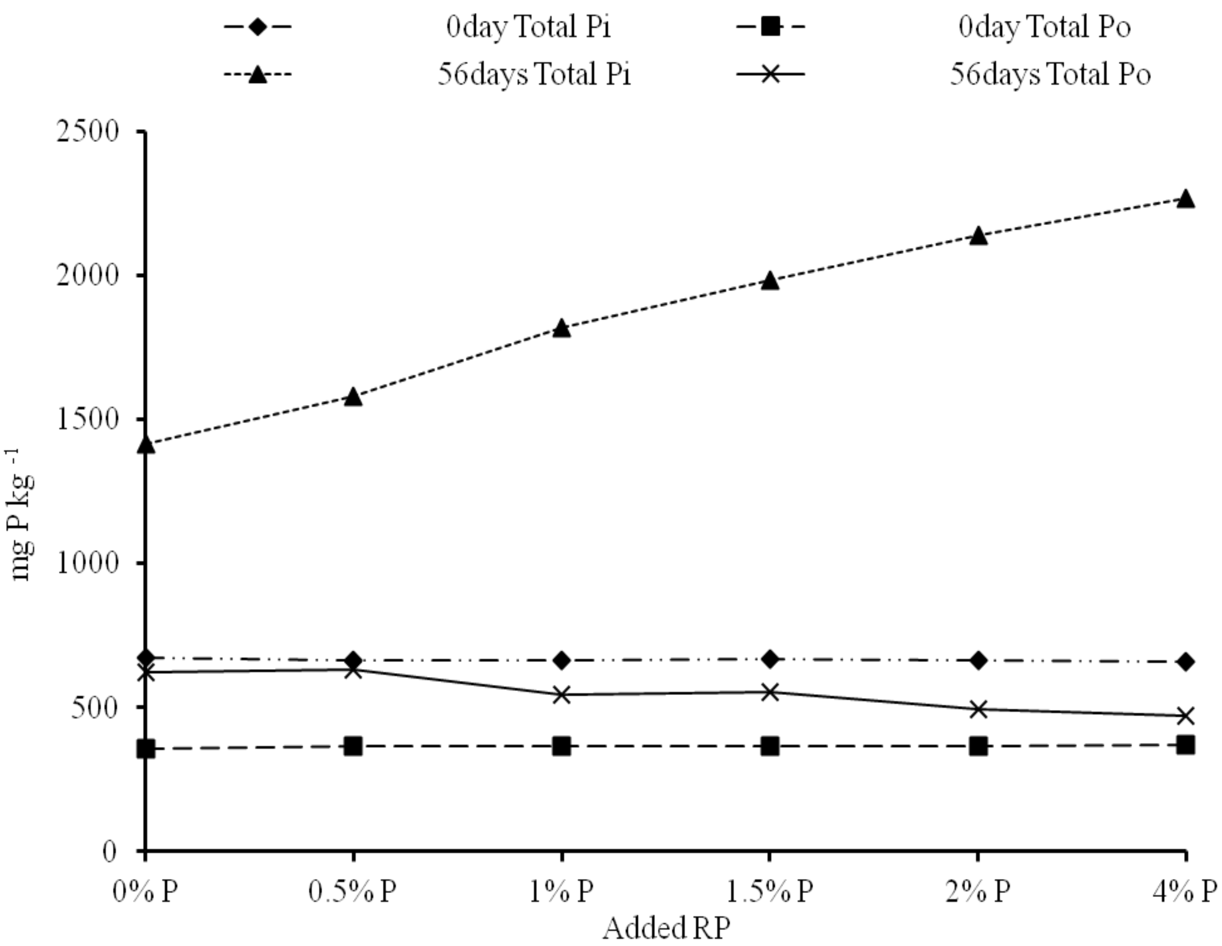


Figure 4.8: Effects of added RP on total Pi and Po fractions of initial and final vermicompost. (Bars indicate standard deviation).

Examination of the sequential P fractionation results of individual fractions showed that the H_2O-P_i fraction made the largest contribution to total Pi while $NaHCO_3$- P_i made the least contribution (Fig.4.9a). The other Pi fractions also increased with rate of RP applications but only marginally (Fig.4.9a).The Pi content of the four vermicompost fractions at each added RP rate followed the order H_2O-P_i >> NaOH-P_i > HCl-P_i > $NaHCO_3$- P_i (Fig. 4.9a).

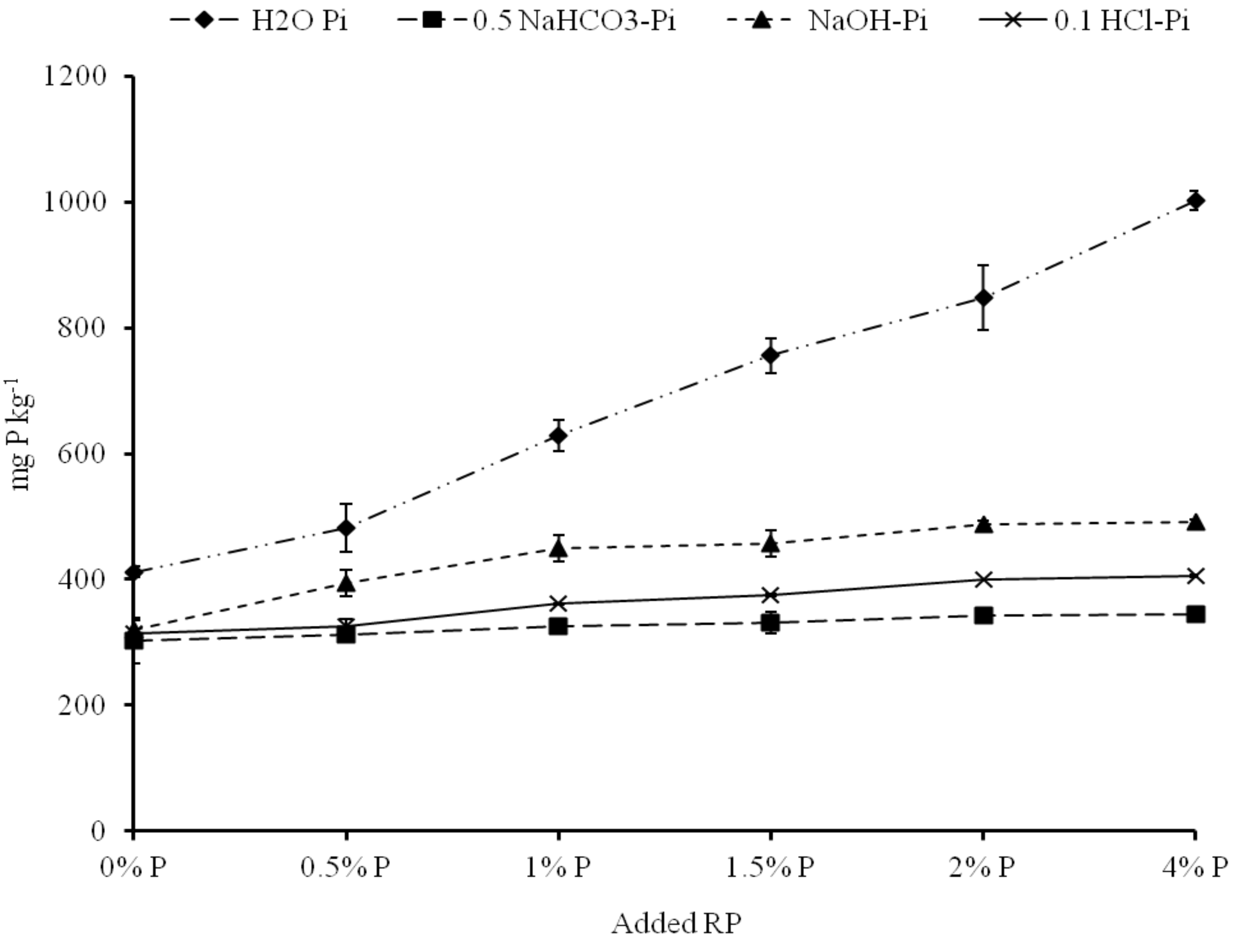


Figure 4.9a: Effects of added RP on individual Pi fractions of final vermicompost. Bars indicate standard deviation.

With the exception of the H_2O-Po fraction, the other organic P fractions (NaOH, HCl, and $NaHCO_3$ declined with rate of RP application (Fig. 4.9b), suggesting that these fractions were responsible for the observed decline in total (Po) (Fig. 4.8).

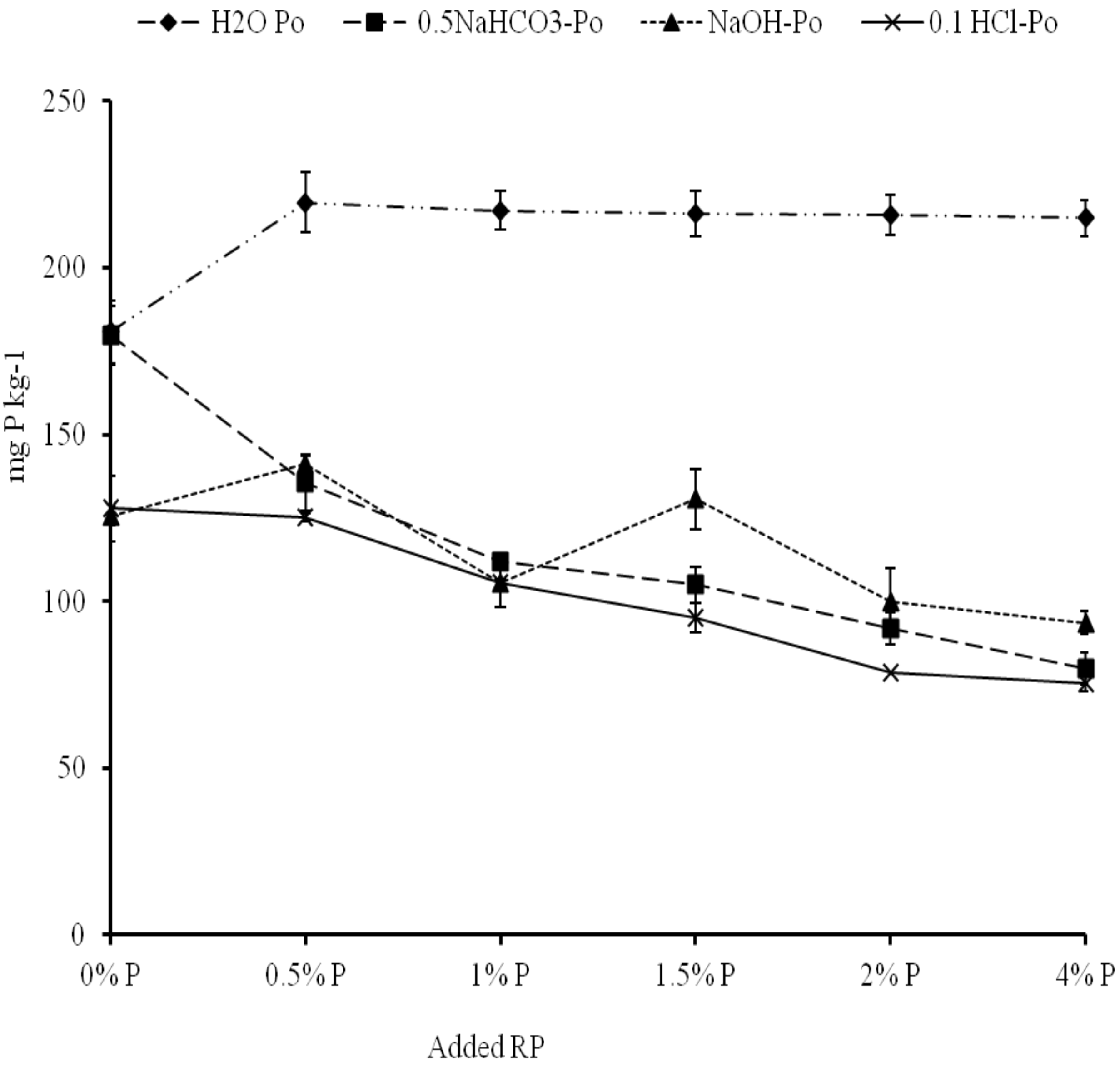


Figure 4.9b: Effects of added RP on individual Po fractions of final vermicompost. (Bars indicate standard deviation)

4.6 DISCUSSION

4.6.1 Effects of RP rate on selected maturity

One of the main aims of this study was to explore the possibility of enhancing the vermidegradation of cow-dung-waste paper mixtures with lower rates of RP incorporation. The observed significant increase in ash and decrease in volatile solids contents with time at each rate of added RP (Fig.4.1a and b) indicated degradation of organic matter (OM) in the vermicomposting mixtures as also reported by Mupondi *et al*. (2010). This trend was confirmed by the maturation parameters used to monitor the stabilization of the vermicomposts, namely C: N ratio (Fig.4.2), polymerization index (PI) and humification index (HI) (Fig.4.3a and b), and SEM imagery (Fig 4.4a-c).

The C: N ratio results (Fig.4.2) confirmed the findings of Mupondi (2010) that incorporation of RP accelerated the vermidegradation of the cow-dung-waste paper mixtures in that by day 42 the C: N ratio of the waste mixtures had declined from 30 when the experiment was initiated (Day 0) to less than 14 where RP was incorporated compared with 18 where it was not. The results further revealed that the improved degradation was fastest when RP was added at a rate of 1% P which allowed compost maturity to be achieved within 33 days. This indicated that a rate lower than the lowest rate of 2% P as RP used by Mupondi (2010) could be used to improve the rate of biodegradation of cow-dung-waste paper mixtures using *E. fetida*. The possibility of using lower rates of RP incorporation to enhance the vermidegradation of cow-dung-waste paper mixtures and possibly other waste will mean less transportation costs to places far from the Limpopo Province of South Africa where RP is mined.

The rate of C: N ratio decline was fastest between day 14 and 28 and slowest after day 42 explaining the observed interaction between significant RP rate and time (Table 4.1). Bernal *et al*., (2009) reported that the decline in C: N ratio could be attributed to the decomposition of organic matter as a result of microbial action. This is supported by the MBC data (Fig. 4.5) which show that microbial activity was highest on day 14 followed by day 28, a period coinciding with the sharpest decline in C: N ratio at each rate of added RP. This is further supported by the nitrate and ammonium data (Fig 4.6 a and b) which show that ammonification and nitrification, both microbially-mediated processes, were most intense during this period. The MBC increased with rate of RP application, suggesting that RP had a stimulatory effect on microbial activity. Mupondi (2010) reported a similar trend of highest microbial activity on day 14 followed by day 28 during the vermicomposting of dairy manure-paper waste mixtures as well as enhanced increase in available P. The author ascribed this increase in available P to the presence of high microbial populations as shown by large amount of microbial biomass C and P at the early vermicomposting stage. This increase, the author further noted, could be as a result of an increase in phosphate-solubilising microorganisms. Similarly, Bhattacharya and chattopadhyay (2002) reported an increase in phosphate-solubilising microorganisms while vermicomposting flyash-amended cattle manure. Although these microorganisms were not specified in this study, but they have been reported capable of solubilising RP by acidifying the media (Raju and Reddy, 1999).

The increase in the HI with time indicated that the vermidegradation increased humification of the cow-dung-waste paper mixtures. Corresponding increases in the PI with time (Fig.4.3a) indicated that the lower molecular weight fulvic acid fraction was progressively converted to the more recalcitrant molecules of higher molecular weight which make up the humic acid fraction. This transformation was influenced by the rate of RP application and interestingly it was highest at the 1 % P rate of RP application. This pattern of response was further confirmed by scanning electron microscopy (Fig.4.3a-c) which also showed that RP application intensified the degradation of cow-dung-waste paper mixtures and that the 1% P rate of RP application resulted in consistently greater vermidegradation of the waste mixtures at each sampling date than with the other RP treatments. This was reflected in higher segregation of the waste mixtures aggregate particles. The SEM images show that degradation of the cow dung-waste-paper mixtures intensified with time at each rate of RP application. The wide differences in extent of degradation at different rates of RP application observed on days 14 and 28 which coincided with the period of maximum microbial activity (Fig.4.5) is further proof that the observed improved vermidegradation of waste mixtures mixed with RP could be related to its stimulatory effect on soil microorganisms. The RP constituent responsible for this stimulation remains to be established.

The seed germination index is a more direct indicator of both vermicompost and compost maturity as it directly tests whether the finished vermicompost can inhibit plant growth or not when used as a growth media. The over 80% GI observed for all test crops in this study indicated that addition of P as RP to cow-dung-waste paper mixtures in the presence *E. fetida* resulted in vermicompost that was free of phytotoxins according to (Zucconi *et al.*, 1981; and Tam *et al.*, 1998). These results are also in agreement with those of Bustamante *et al*. (2001) which showed that a germination index of ≥ 80% indicated the disappearance of phytotoxins in composts.

4.6.2 Effects of RP rate on the P enrichment

The total P content of 2.31 % P in the final cow-dung-waste paper vermicompost where RP was added at a rate of 4% P is consistent with results of Biswas and Narayanasamy (2006) who reported increases in the total P content of straw compost from 0.37% in control to 2.20% by applying 4% P as RP. Yan *et al.* (2012) also reported an increase in the total P of rice straw compost from 0.392% to 0.82% by applying 2% P as RP. More significantly, however, in the present study Bray 1 extractable P increased from a low 80 mg P kg^{-1} where no RP was added to 207 mg P kg^{-1} where RP was added at a rate of 4% P (Table 4.4). This has contributed to the increase in the extractable P and thus implies significant increases in the effectiveness of vermicompost in providing available P.

The improvement in the extractability of P with rate of RP application (Table 4.4) mirrored the increase in microbial biomass with rate of RP application (Fig. 4.5), suggesting that among the microorganisms stimulated by added RP were phosphate bacteria which facilitated the mineralization and, therefore, extractability of P from the added RP. The P release is mediated by phosphate enzymes produced by microorganisms in the earthworm guts and those in the earthworm casts (Yan et al., 2012). The observed increase in humic acids reflected by the humification index (Fig.4.3b) could also account for the enhanced mineralization and dissolution of P. According to Singh and Amberger (1990), they pointed out that humic acids can absorb significant quantities of calcium ions and release H^{+} ions which further facilitate the dissolution of RP. In addition, the functional groups in humic acids such as carboxylic and phenolic groups can also chelate Ca^{++} ions, providing a driving force for the mineralization and dissolution of P from RP.

The increase in Bray 1 P up to day 42 and its decline thereafter (Fig. 4.7) suggested that after 42 days the mineralized P underwent precipitation reactions hence the decreased extractability. Sequential extraction showed that the H_2O-Pi fraction made the largest contribution to the total inorganic P extracted (Fig. 4.8), suggesting that most of the mineralized P during the vermicomposting of the cow-dung-waste paper mixtures enriched with RP would be available for plant uptake. These results indicate that vermicomposting cow-dung-waste paper mixtures enriched with phosphate rock improves the solubilisation of the Phalaborwa RP used in this study and thus improved its fertilizer value. According to Edwards *et al*. (2010) RP is an acceptable source of P for organic agriculture but its use is limited by its slow rate of P release. The high total P and extractable P contents observed in the cow dung waste paper vermicomposts enriched with RP point to their potential as organic P fertilizers. Future studies should explore this potential.

4.6.3 CONCLUSIONS

The results of this study have confirmed that incorporation of rock phosphate improves the biodegradation of cow-dung-waste paper mixtures and further revealed that optimal vermidegradation can be achieved with the application of 1% P as RP. At this rate of RP incorporation, the vermicomposting mixtures required only 33 days to reach maturity. The improvement in vermicomposting occurred mostly between days 14 and 28. The observed effect of RP in improving the vermidegradation of cow-dung-waste paper mixtures indicated a need to establish the active agent responsible for this effect. The quantity of P recovered in the different fractions and their availability was closely related to the added RP rates with greatest concentration resulting from water fraction. Although a 1% P rate of RP application is all that was needed for fast maturation of the vermicompost, higher rates of RP application are necessary for enhanced P fertilizer value of the resultant vermicompost. Therefore, higher rates of RP incorporation may be necessary where final composts with higher P contents and thus better P fertilizer value are desired. Future studies will need to examine the agronomic value of these composts. Nevertheless, the results of the present study have shown that cow-dung-waste paper vermicomposts enriched with phosphate rock have potential use as organic fertilizers which would be acceptable in organic farming.

CHAPTER FIVE

5 PHOSPHORUS ENHANCES THE VERMIDEGRADATION OF COW-DUNG-WASTE PAPER MIXTURES ENRICHED WITH PHOSPHATE ROCK

5.1 ABSTRACT

Earlier studies have demonstrated that incorporation of rock phosphate (RP) enhanced the biodegradation of cow-dung-waste paper mixtures and improved the N and P contents of resultant vermicomposts. However, the constituent element in RP responsible for the observed enhanced biodegradation of the cow-dung-waste paper mixtures remained to be established. The main objective of this study was to determine which of the two dominant elements in RP, phosphorus or calcium could be most responsible for the improved bioconversion of the waste mixtures. This was investigated by comparing the vermidegradation of cow-dung-waste paper mixtures enriched with phosphorus (1 % P) in the form of RP, triple superphosphate (TSP), and phosphoric acid (PHA) with Ca in the form of $CaCl_2$. The vermidegradation of the waste mixtures was monitored by measuring percent volatile solids (VS), percent ash content (AS), C: N ratios, and humification indices. Scanning electron microscopy (SEM) was used to evaluate the morphological properties of the resultant vermicomposts while a germination test was used to determine the phyto-toxicity levels of the vermicomposts. Results indicated that TSP, a water soluble P source resulted in greater and faster degradation of the waste mixtures than RP while the Ca source had the least effect. With TSP incorporation, the compost maturity C: N ratio of 12 was reached within 28 days while RP, PHA and $CaCl_2$ needed 42, 56 and more than 56 days, respectively. The results indicated that P was largely responsible for the enhanced bioconversion of the waste mixtures. This coincided with the effect of P to stimulate microbial growth as reflected by higher microbial biomass carbon levels where water soluble

P sources were applied. The C: N ratios of the final vermicomposts at day 56 were 10, 11.5, 13, 14, and 23 with TSP, RP, PHA, Control (no P added) and $CaCl_2$ treatments, respectively. The results indicated that TSP was superior but in practice RP may still be the preferred additive in the vermicomposting of cow-dung-waste paper mixtures as it is cheaper and produces mature compost in a reasonably short time.

Keyword: vermicomposting, triple superphosphate, phosphoric acid, microbial biomass carbon, humification indices, rock phosphate, pH, electrical conductivity.

5.2 INTRODUCTION

Incorporation of Rock phosphate and P-solubilising microorganisms into various wastes for nutrient enrichment during vermicomposting have been widely studied (Mupondi *et al.*, 2010, Biswas and Narayanasamy, 2006, Edwards *et al.*, 2004, Masciandaro *et al.*, 1997; Senesi *et al.*, 1992; Kaushik *et al.*, 2008; Kumar and Singh, 2001). This is because most waste materials used for compost or vermicompost production, for example, agricultural wastes, dairy wastes, paper waste, industrial wastes and household wastes, in their original form have low nutrient contents particularly phosphorus and nitrogen among the essential primary macro- nutrient required by plants for maximum growth (Mupondi *et al.*, 2010, Biswas and Narayanasamy, 2006, Edwards *et al.*, 2004).

Mupondi (2010) in a vermicomposting study reported that incorporation of rock phosphate (RP) in dairy manure-paper waste mixtures at rates greater than 2% P effectively improved the bioconversion and humification of the waste mixture as well as enhanced P availability through increases in the labile pools and less labile pools of P. They attributed this to the combined effects of degradation of organic matter and the dissolution of the applied RP. Mupondi (2010) further observed that added RP improved the total N content of the vermicomposting mixture with time. This was attributed to a concentration effect caused by weight reduction of the composting mixtures as a result of organic matter degradation and loss of C as carbon dioxide, coupled with enhanced nitrogen mineralization in the substrate (Atiyeh *et al*., 2000).

The study reported in Chapter 4 of this work was a follow – up on the work of Mupondi *et al.* (2010) which explored the effectiveness of RP incorporation in cow-dung-waste paper mixtures at rates of application less than 2 % P. It was observed that improved biodegradation and humification of cow-dung-waste paper mixtures could be realized even at 0.5 % P rate of RP application though faster degradation and maturation was observed at 1 % P. Although a 1% P rate of RP application is all that was needed for fast maturation of the vermicompost, higher rates of RP application were necessary for enhanced P fertilizer value of the resultant vermicompost.

The observed effect of RP in improving the vermidegradation of dairy manure-paper waste mixtures reported by Mupondi (2010) and confirmed in the study reported in Chapter 4 indicated a need to establish the active agent in RP responsible for this effect. The RP used by Mupondi *et al* (2010) and in the study as reported in Chapter 4 was obtained from Phalaborwa in Limpopo Province, South Africa and had the following chemical properties: P_2O_5- 40.3%; CaO-54.6%, MgO-0.26%; cadmium-2.2 mg kg^{-1}, chromium -18.05 mg kg^{-1}, copper -5.85 mg kg^{-1}, lead -6.05 mg kg^{-1} and zinc -13.22 mg kg^{-1} (Unuofin and Mnkeni, 2014). Based on this composition, calcium and phosphorus were the dominant elements in the RP used as it is the case for most rock phosphates. It was, therefore, hypothesized that since Ca and P were the dominant nutrient elements in RP, they could be responsible for the observed improved bioconversion of cow-dung waste-paper mixtures. This question was investigated by comparing the vermidegradation of cow-dung-waste paper mixtures enriched with phosphorus (1 % P) but supplied in the forms of RP, triple superphosphate (TSP), and phosphoric acid (PHA) with Ca in the form of $CaCl_2$. Therefore, the main objective of this study was to determine if P or Ca in the rock phosphate could be predominantly responsible for the observed enhanced bioconversion of cow-dung-waste paper mixtures during vermicomposting. A second objective was to determine the extractability of P in vermicomposts treated with the different P sources.

5.3 MATERIALS AND METHODS

5.3.1 Site description, wastes and earthworms utilized

The vermicomposting experiment was carried out in a closed shaded yard at the University of Fort Hare Teaching and Research Farm located (32^{o}46’S and 26^{o}50’E) in the Eastern Cape Province of South Africa under an ambient mean temperature of 25^{o}C. Waste papers used for the study were collected from the University Printing Press (Xerox) and Faculties, while phosphate rock was obtained from Phalaborwa in Limpopo Province, South Africa, which is granitic in nature and with a low P content. The *Eisenia fetida* earthworms used for the study were collected from the local wormery at the University Teaching and Research Farm. Cow dung was obtained from Keiskammahoek Dairy Project located about 60 km North East of the University of Fort Hare, whereas, soluble P- fertilizer (Triple super phosphate) was purchased from a fertilizer distribution company in East London, phosphoric acid and calcium chloride where supplied by Merck, a chemicals distribution company in Pretoria, South Africa.

5.3.2 Experimental procedure

Worm boxes measuring 0.50 m x 0.40 m x 0.30 m (length x width x depth) were used. This provided 0.2 m^{2} of exposed top surface. Matured worms were introduced at a stocking rate of 12.5 g worms kg^{-1} feed (Unuofin and Mnkeni, 2014). Enough feedstock (5 kg on dry weight basis) prepared by thoroughly mixing 2.16 kg shredded paper with 2.84 kg cow dung to achieve a C: N ratio of 30 was placed in the vermicomposting boxes to meet the needs of the earthworms for the entire 56 days. Treatments were arranged in completely randomized design (CRD) with three replications. Moisture content in each box was kept at 80% (Reinecke and Venter 1985) by sprinkling water when necessary throughout the vermicomposting period.

5.3.3 Experimental treatments

The treatments were rock phosphate (RP), Triple superphosphate (TSP), Phosphoric acid (PHA) and calcium chloride. The P treatments were applied to cow-dung-waste paper mixtures at a rate of 1% elemental P since this was found to be the optimum rate of RP application that resulted in fastest vermidegradation of cow-dung-waste paper mixtures reported in Chapter 4. Calcium chloride was also applied to cow-dung-waste paper mixtures at the level of Ca supplied by RP when applied at a rate of 1 % P. A control (with earthworms but no P source) was also included. RP, TSP and PHA were applied each at a rate of 1 % P but $CaCl_2$ was applied at the level of Ca supplied by RP when applied at a rate of 1 % P to each 5 kg (dry matter basis) initially prepared by mixing thoroughly 2.16 kg shredded paper and 2.84 kg cow dung together to give a C: N ratio of 30. All the treated cow-dung-waste paper mixtures were vermicomposted in the worm boxes with 0.2 m^2 exposed top surfaces. Cow-dung-waste paper mixtures were inoculated separately with the different treatments, and inoculated with matured worms at a stocking density of 12.5 g worms kg^{-1} feedstock. Volatile solids (VS), ash content, total C, total N and inorganic N (nitrate- N and ammonium – N), microbial biomass carbon were determined at the beginning of the experiment. Samples were collected at day 0, 14, 28, 42 and 56 during vermicomposting and were analysed as described below.

5.3.4 Microbial biomass carbon

Determinations of microbial biomass carbon (MBC) contents in the resultant vermicomposts were carried out following the chloroform fumigation and extraction method of (Vance *et al*., 1987). Two g (oven-dry basis) moist compost was fumigated for 24 h at 25 ºC using ethanol-free chloroform. Samples were then immediately extracted with 60 ml of 0.5 *M* potassium sulphate solution. Organic C in the extracts was measured through dichromate oxidation

(Kalembasa and Jenkinson, 1973) and used as a basis for calculating microbial biomass carbon (MBC) using the equation:

$MBC= E_C/K_{EC}$

E_C is the organic carbon extracted from fumigated soil minus organic carbon extracted from unfumigated soil while K_{EC} (The proportion of the microbial C that is extracted from the compost) = 0.38 (Vance *et al*., 1987).

5.3.5 Physico-chemical analyses

Analysis of samples to determine pH, electrical conductivity (EC), VS, ash content, total carbon (C), total nitrogen (N), extractable phosphorus P, and humic substances was done as follows: First, the samples were air dried to a constant weight and then ground and sieved (< 2mm) for analysis. Electrical conductivity and pH were determined in a vermicompost-water suspension (1: 10) with a pH/Conductivity meter as described by Ndegwa *et al*. (2000).The suspensions were allowed to stand for one hour after constant shaking using a mechanical shaker at 230 rpm for 30 minutes prior to pH and EC measurements. Volatile solids were determined as sample weight loss (previously oven-dried at 105^oC) upon ashing at 550^oC for 4h in a muffle furnace (Ndegwa *et al*., 2000). The total nitrogen (N) and carbon (C) were measured using a Truspec CN Carbon/ Nitrogen analyser (LECO Corporation, 2003). The extractable P of organic wastes was estimated using Bray 1 extractant. The orthophosphate in the extracts was determined using a continuous flow analyser (San 2++ Skalar CFA, Skalar Analytical B.V. The Netherlands) employing the ammonium molybdate-antimony potassium tartrate- ascorbic acid method.

5.3.6 Determination of Humic Substances

Humic substances were determined following extraction as described by Del Carmen Vargas-García *et al*. (2006). Vermicompost samples were treated with 0.1 *M* NaOH (1:20 w/v ratio), and were shaken on a horizontal shaker for 4 h. The resultant suspension was centrifuged at 8,000 x g for 15min. After that, the clear solution was separated into two portions, one portion was analysed for total extractable carbon fraction (C_{EX}) following the Walkley and Black rapid titration method as described by Anderson and Ingram (1996) and the other portion was adjusted to pH 2 by adding concentrated H_2SO_4 and then left to coagulate for 24 h at 4°C. The resulting precipitates constituted the humic acid–like carbon (C_{HA}) while portion that remained in solution constituted the fulvic acid-like carbon (C_{FA}). The C_{HA} was calculated by subtracting the C_{FA} from the C_{EX}. The humification ratio (HR) was calculated as $HR = (CEX/C)\ X\ 100$ and humification index (HI) was calculated as $HI = (CHA/C)\ X\ 100.$ PI was calculated as the ratio of C_{HA} to C_{FA} (Mupondi 2010).

5.3.7 Phytotoxicity study

Phytoxicity was assessed through a seed germination test. Aqueous extracts were prepared from the different vermicomposts with distilled water (1:10 w/v) (Ravindran *et al*., 2013). The seed germination bioassay for tomato (*Lycopersicon esculentum*), radish (*Raphanus sativus*) and carrot (*Daucus carota*) was evaluated according to Tam and Tiquia (1994) in which two pieces of Whatman® filter paper were placed inside a sterilized petri dish and wetted with the vermicompost extracts. Ten seeds of each crop species were placed on the filter paper and incubated for five days in the dark. A control was included for each crop species in which the filter papers were wetted with distilled water. Seed germination, germination index (GI), relative seed germination (RSG) and relative root elongation (RRE) were calculated as follows:

$$\text{RSG (\%)} = \frac{\text{Number of seeds germinated in the sample extract}}{\text{Number of seeds germinated in the control}} \times 100$$

$$\text{RRE (\%)} = \frac{\text{Mean root elongation in the sample extract}}{\text{Mean root elongation in the control}} \times 100$$

$$\text{GI (\%)} = \frac{\text{(\%germination)} \times \text{(\% Root elongation)}}{100}$$

5.3.8 Morphological assessment of vermicomposts

Scanning Electron Microscopy (SEM) images were taken using JOEL (JSM-6390LV, Japan) as described in Unuofin and Mnkeni (2014).

5.4 Statistical analysis

The data reported herein are the means of three replicates. Results were subjected to repeated measure analysis of variance (ANOVAR). Means separation were done using Fisher's protected least significant difference (*LSD*) test at $p \leq 0.05$. All statistical analyses were done using JMP® Release 10.0 statistical package (SAS Institute, Inc., Cary, North Carolina)

5.5 RESULTS

5.5.1 Treatment effects on selected compost stabilization and maturity parameters

The interaction between treatments and time during bioconversion of cow-dung-waste paper mixtures are summarized in Table 5.1. Results showed that there was high significant interaction between treatments and time, except for pH and pH and time interaction.

Table 5.1: Repeated measures ANOVA for C: N ratio, PI, HI, HR, pH, EC, Bray1 extractable P, MBC, VS, Ash content, NH_4-N and NO_3-N

	Effect					
	Treatments		Time		Treatments x time	
	$F_{(4,50)}$	P	$F_{(4,50)}$	P	$F_{(16, 50)}$	P
C: N ratio (%)	476.431	<.0001	2107.199	<.0001	89.579	<.0001
(PI)	507.648	<.0001	1881.033	<.0001	399.833	<.0001
HI (%)	700.819	<.0001	2578.356	<.0001	673.436	<.0001
HR (%)	1064.597	<.0001	3399.654	<.0001	560.465	<.0001
pH	1.825	< .201	1.081	<.379	0.993	<.483
EC (m S cm^{-1})	135.9	<.0001	7.195	<.0001	2.582	<.008
Bray 1P (mg kg^{-1})	8763.3	<.0001	17986.31	<.0001	2285.007	<.0001
MBC (mg kg^{-1})	823.205	<.0001	3689.370	<.0001	117.714	<.0001
Volatile solids (%)	24.954	<.0001	1105.3	<.0001	5.603	<.0001
Ash content (%)	41.623	<.0001	853.453	<.0001	8.336	<.0001
NH_4^+-N (mg kg^{-1})	1105.975	<.0001	12530.3	<.0001	397.681	<.0001
NO_3^--N (mg kg^{-1})	1297.168	<.0001	2142.724	<.0001	364.07	<.0001

F values and probabilities are shown for each effect. $p < 0.05$ are significant n = 3, C: N = carbon to Nitrogen ratio, PI = Polymerization index, HI = Humification index, HR = Humification ratio, MBC = microbial biomass carbon, EC = electrical conductivity.

5.5.2 Effect of P and Ca bearing sources on the percentage Ash and VS

Incorporating P and Ca sources into cow-dung-waste paper mixtures had a significant effect on the reduction of volatile solids and corresponding increase in the percent ash of the vermicompost, but this effect was time dependent as shown by a significant treatment x time interactions (Table 5.1). Percent ash increased with time in all treatments but there were variations in the magnitude among the different treatments (Fig 5.1a). The lowest values were observed in the absolute control while the highest values were observed where phosphoric acid (PHA) was added. Generally, the water-soluble P sources resulted in the higher percent ash values than the RP, $CaCl_2$ and control treatments.

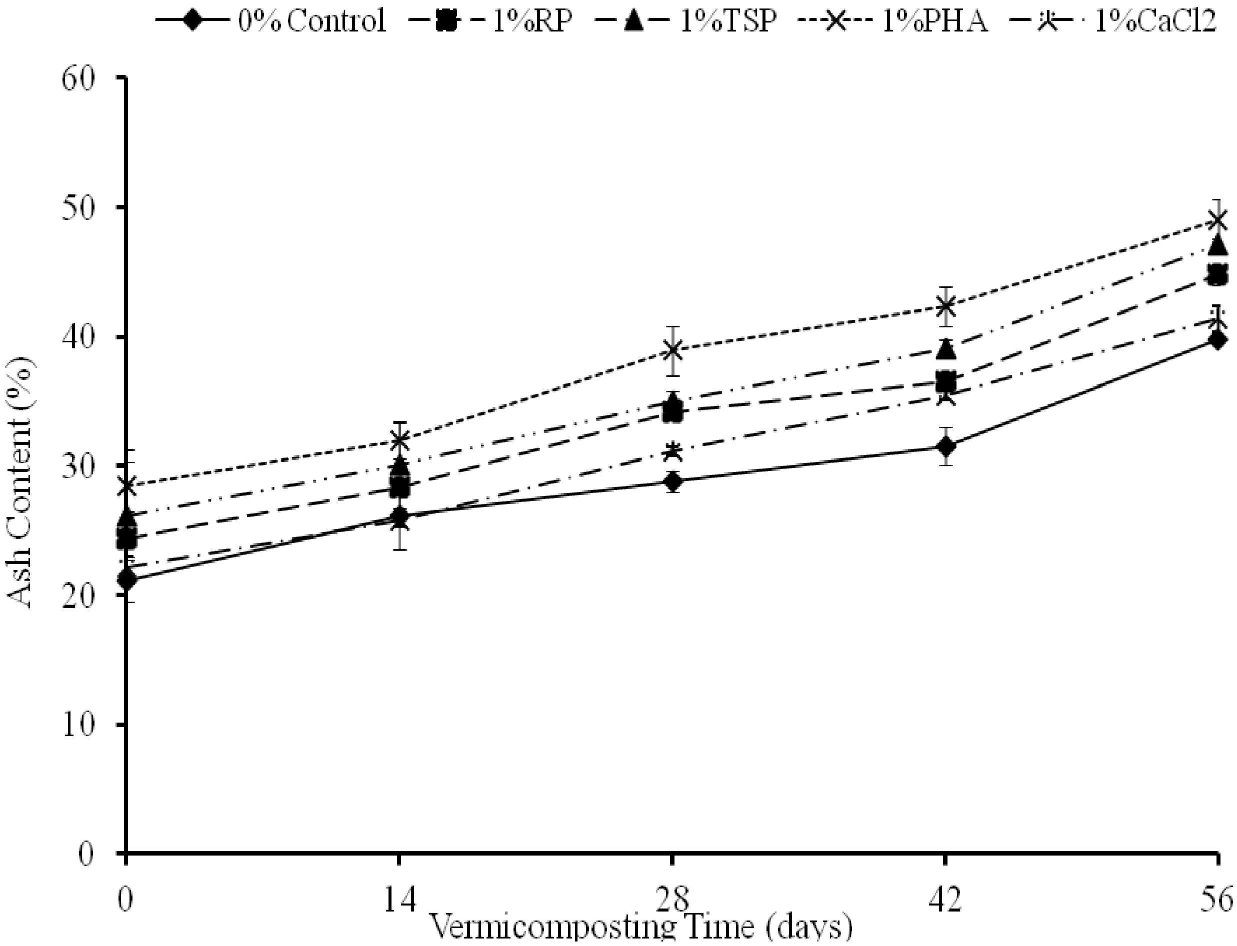


Figure 5.1a: Effect of different P and Ca sources on percent ash of cow-dung-waste paper mixtures during vermicomposting.

Volatile solids which varied between 71 and 78% in the cow-dung-waste paper mixtures, decreased in all the treatments between first and last sampling dates (Fig.5.1b). At day 14 and 28, except for PHA-treated waste mixtures, decrease in VS values of $CaCl_2$-treated vermicompost was not noticeably different from the control treatment, while TSP and RP - treated vermicompost followed similar pattern with a sudden drop between day 42 and 56, respectively. The greatest decrease of 50% in VS at day 56 corresponded to PHA treated vermicompost while the lowest increase in percent ash content (35%) occurred in the control.

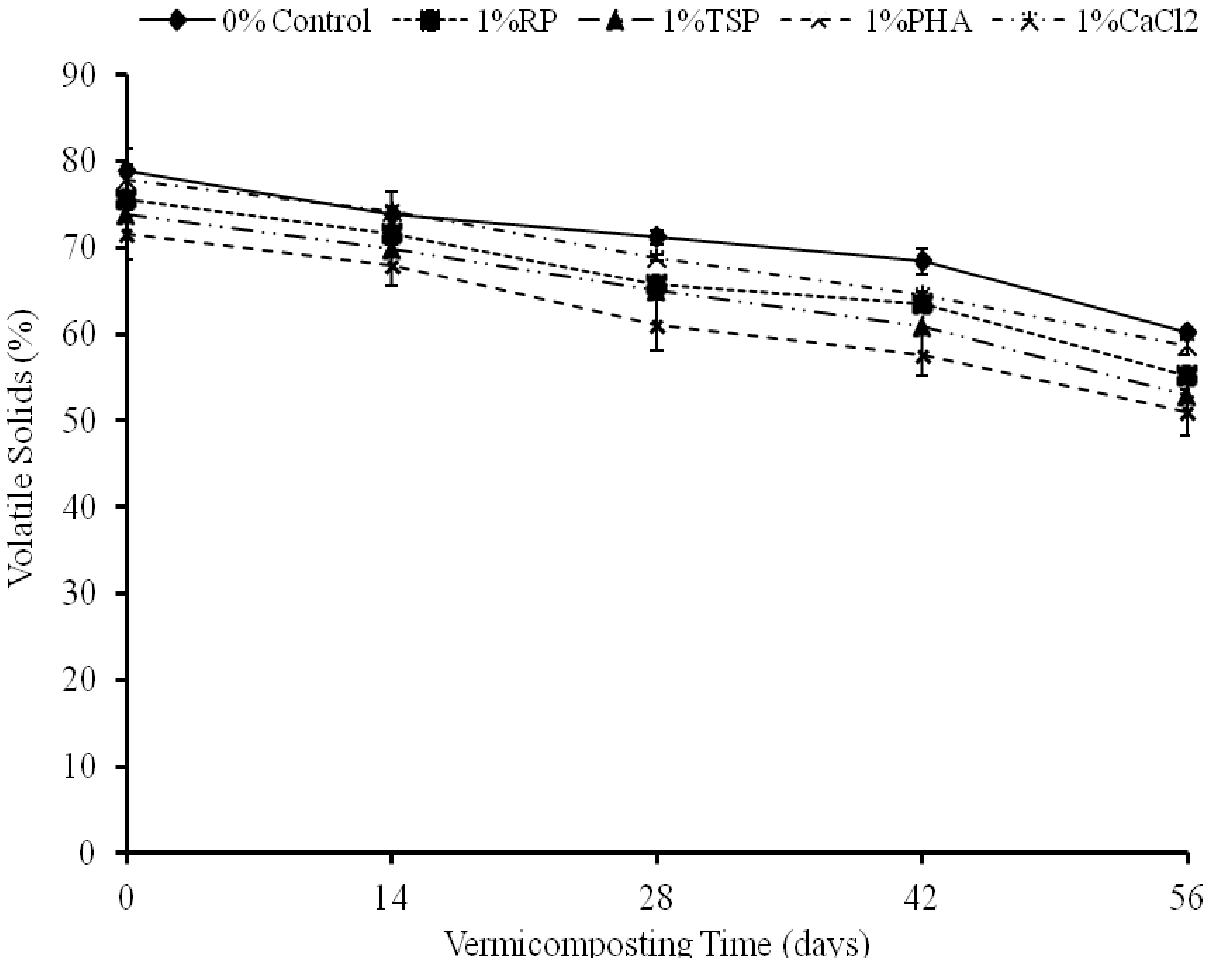


Figure 5.1b: Effect of different P and Ca sources on VS of cow-dung-waste paper mixtures during vermicomposting.

5.5.3 Effect of P and Ca sources on pH and EC

At the beginning of the experiment, pH of vermicomposts ranged between 5.3 and 10.2 (Fig.5.2a). The lowest pH was recorded where PHA was applied and the greatest where $CaCl_2$ was added. While most of the treatments showed a pH decrease (RP, PHA, and $CaCl_2$), others (control and TSP) showed no differences over time. This showed that P and Ca probably did not affect the pH of the vermicompost.

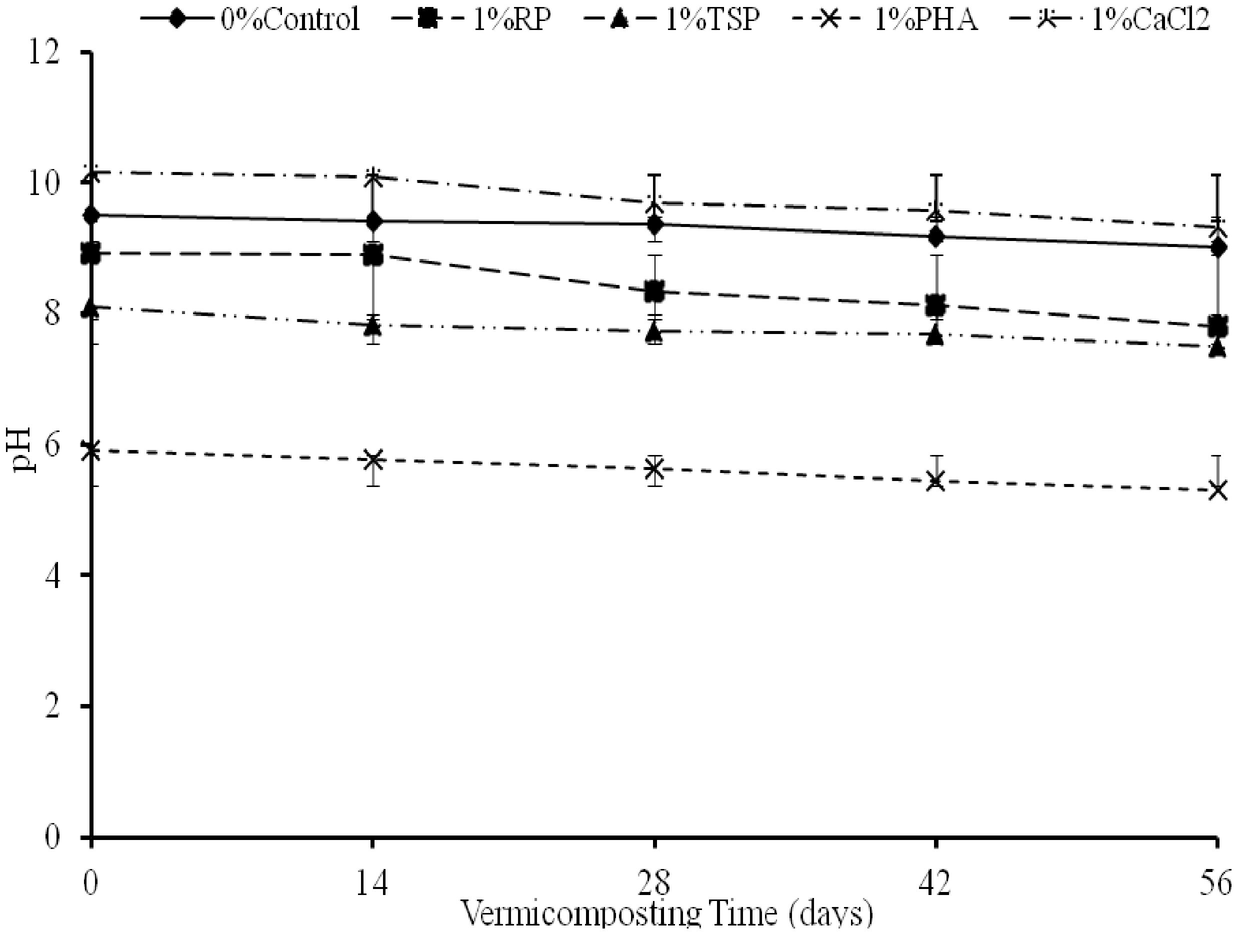


Figure 5.2a: Effect of different P and Ca sources on pH of cow-dung-waste paper mixtures during vermicomposting.

Electrical conductivity, which varied between 3 and 9 m S cm^{-1}, decreased in all treatments between first and last sampling dates with the highest EC values recorded where $CaCl_2$ was applied (Fig. 5.2b). Two treatments, RP and control followed very similar patterns, with a sudden drop between days 42 and 56 (Fig. 5.2b). However, PHA-treated vermicompost followed a different trend, with a lowest EC of 2.8 m S cm^{-1} at day 14 but gradually increased beyond others except $CaCl_2$-treated vermicompost at day 28 and thereafter followed similar pattern with RP and control, while TSP treated vermicompost had the lowest EC values of 2.6 m S cm^{-1} after day 14 and remained steadily low up till day 56 (Fig.5.2b) revealing that Ca probably had no critical role in the vermicomposting process.

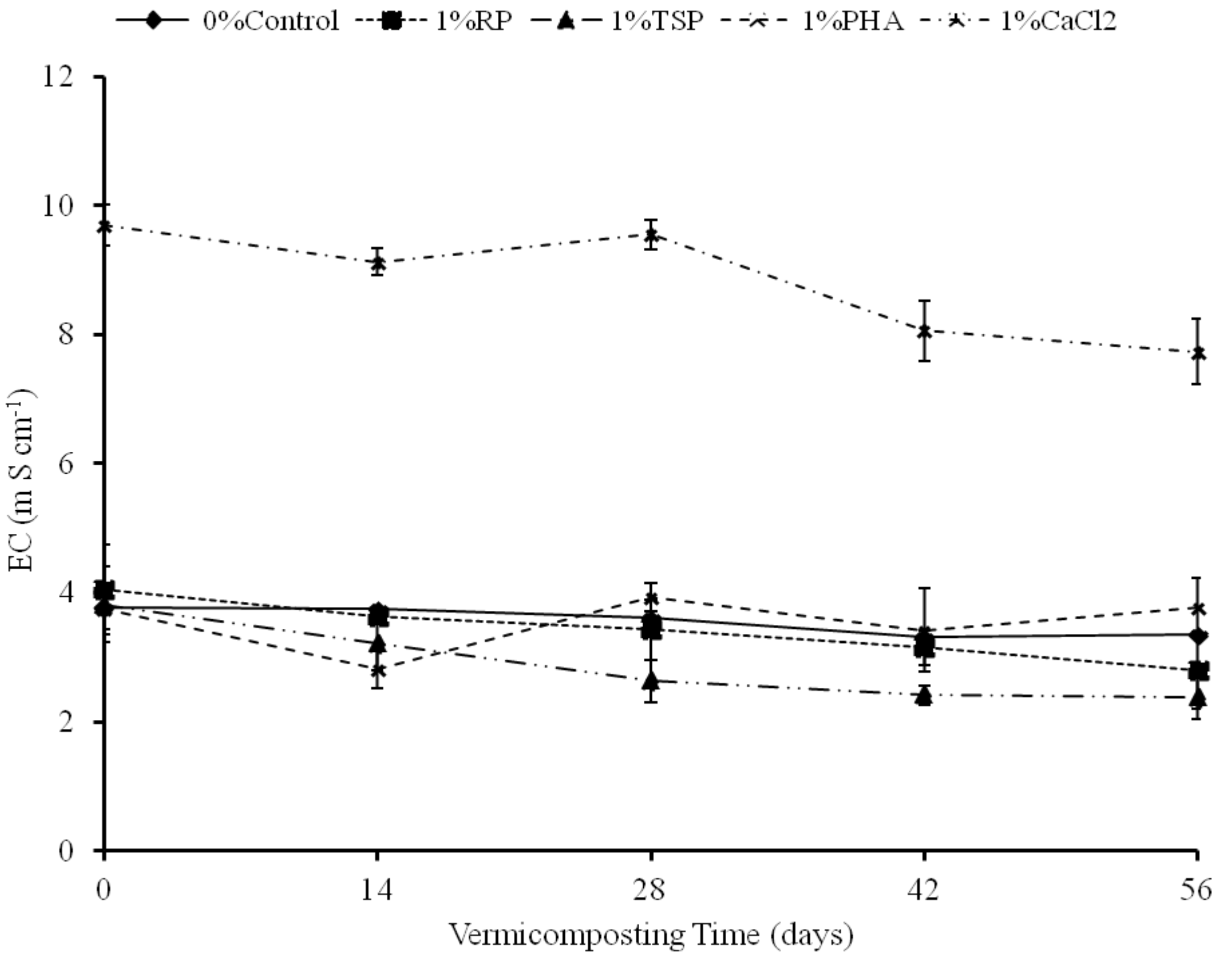


Figure 5.2b: Effect of different P and Ca sources on EC of cow dung waste paper mixtures during vermicomposting.

5.5.4 Effect of P and Ca sources on MBC

There was an increase in MBC during the first 14 days of vermicomposting which was followed by a sharp decline on the 28th day and a steady decline to the end of the 56th day of vermicomposting (Figures 5.3). However, there was a significant interaction between treatments and time on MBC (Table 5.1). At the beginning of vermicomposting there was no difference in the MBC contents of the cow-dung-waste paper mixtures but from day 14 onwards application of different P-source fertilizers and Ca sources affected the MBC content of vermicomposts. At the end of the vermicomposting period the MBC of vermicomposts was higher than their initial content meaning that P source fertilizers played critical role in the bioconversion cow-dung-waste paper.

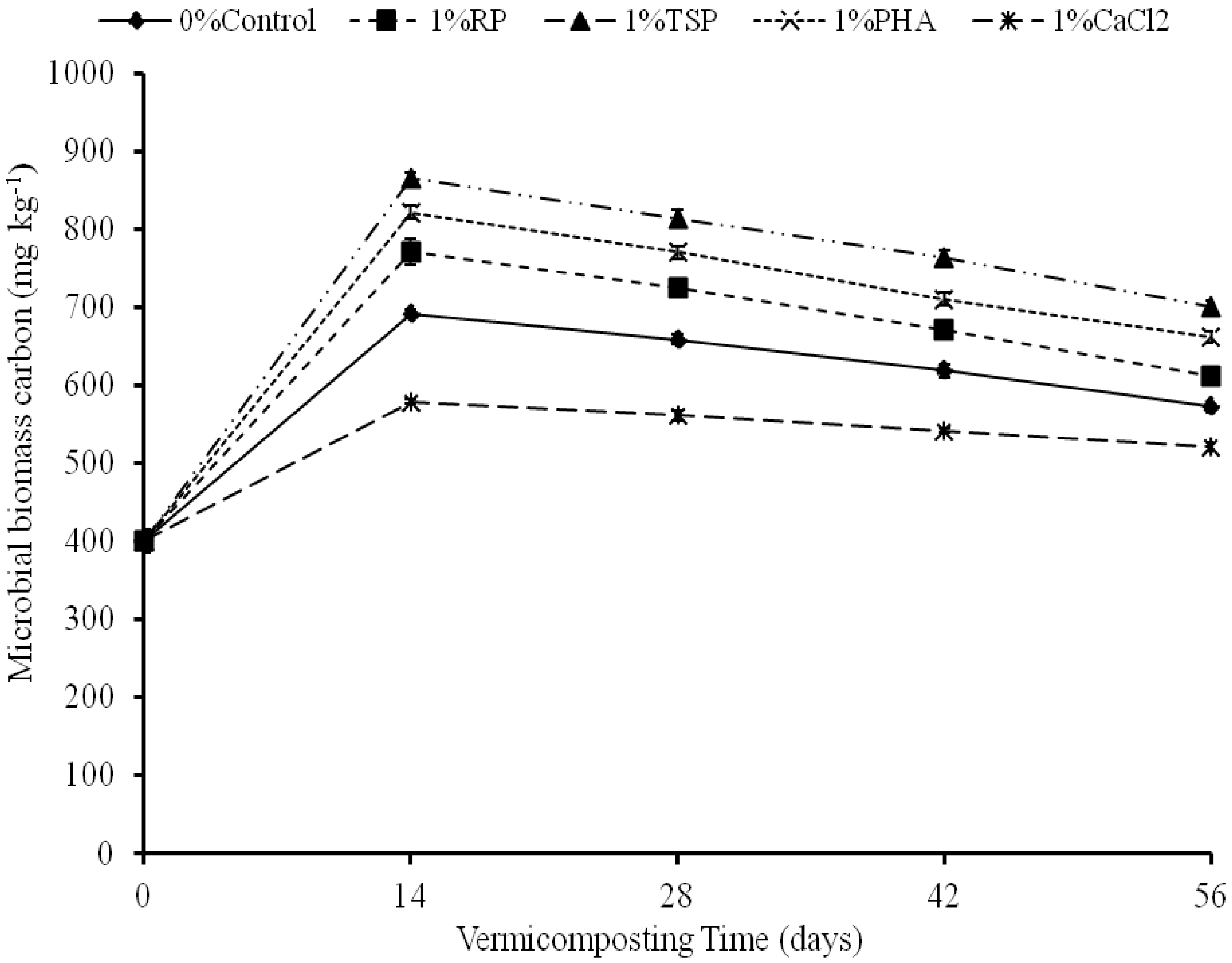


Figure 5.3: Effect of P and Ca sources on MBC during vermicomposting of cow dung-waste paper mixtures (Error bars represent standard deviation).

5.5.5 Effects of P and Ca sources on inorganic nitrogen dynamics

There was a significant interaction between treatments and time on ammonium N (Table 5.1). A linear increase in ammonium was observed from day zero to 28 days and sharply declined thereafter till termination of the experiment at day 56 (Fig.5.4a). The highest increase of about 179 mg kg^{-1} ammonium-N was observed in cow-dung-waste paper mixtures mixed with TSP, whilst the control samples had the least value of NH_4^+-N (93 mg kg^{-1}). Other treatments increased and followed the order of RP> Control > PHA > $CaCl_2$. At day 56, $CaCl_2$-treated vermicompost had the greatest NH_4^+-N content, suggesting that vermicomposting process was down showing that Ca had no critical role in the bioconversion of cow-dung-waste paper mixtures.

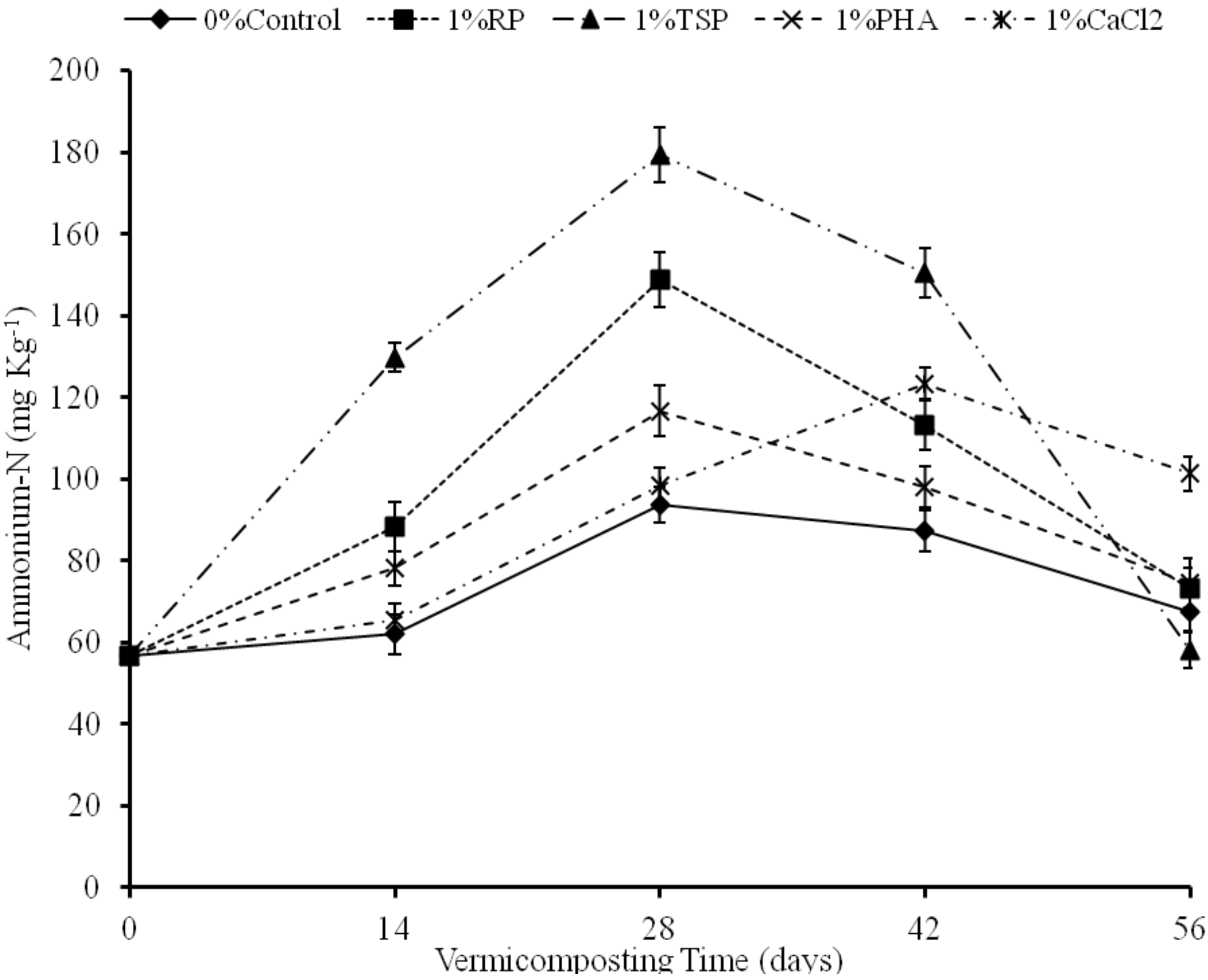


Figure 5.4a: Effect of P and Ca sources application on NH_4^+-N during vermicomposting of cow dung-waste paper mixtures (Error bars represent standard deviation).

Nitrate-nitrite in the vermicompost followed the same linearly increasing trend up till day 14 with highest increase observed where TSP was added, whilst the lowest nitrate–N contents were observed in cow-dung-waste paper mixtures mixed with $CaCl_2$ till 28 days (Fig.5.4b). A sharp increase was observed from day 28 up till day 42 in all the treatments except for waste mixtures mixed with $CaCl_2$ showing that Ca probably had no critical role in the bioconversion of cow-dung-waste paper mixtures. At day 56 TSP, RP, Control and PHA treatments increased linearly with TSP having the highest nitrate concentration of (49 mg kg^{-1}), RP (30 mg kg^{-1}), Control (20 mg kg^{-1}) and PHA (13 mg kg^{-1}), respectively.

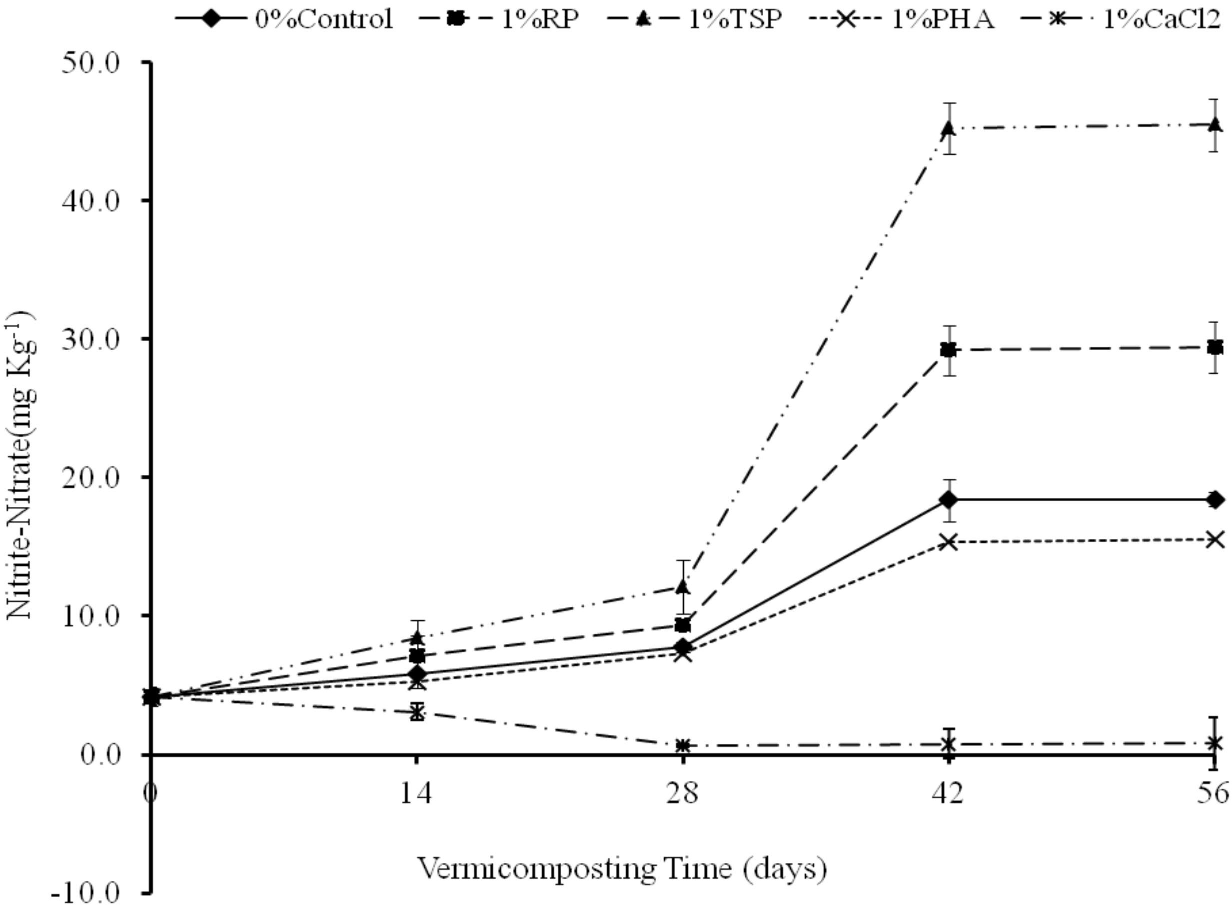


Figure 5.4b: Effect of P and Ca sources application on inorganic nitrogen during vermicomposting of cow dung-waste paper mixtures (Error bars represent standard deviation).

5.5.6 Effect of P and Ca sources on the phytotoxicity

None of the extracts from the final vermicomposted products treated with P or Ca sources had any inhibitory effect on the seed germination of Tomato, Carrot and Radish (Table 5.2). In tomato, each treatment increased the RSG except where PHA and $CaCl_2$ were added. The highest seed germination percentage was observed with vermicompost mixed with TSP. The same statistically significant ($P< 0.0001$) trend was observed for radish and carrot except where $CaCl_2$ was added. With respect to relative root elongation, tomato had the highest significant value of 125.7% followed by radish 109 % and carrot the least at 102% upon addition of TSP (Table 5.2). All tested crops had GI of more than 80% on vermicomposts treated with P but less than 80% where $CaCl_2$ was applied.

Table 5.2: Effect of different P and Ca sources on the phytotoxicity of cow dung-waste paper vermicompost

	Tomato			Carrot			Radish		
Treatment	RRE	RSG	GI	RRE %	RSG	GI	RRE	RSG	GI
Control	80.0c	113.4c	89.5d	90.8ab	108.3c	97.4c	95.6b	91.7c	87.4c
RP	77.1d	133.4b	108.8c	99.3ab	108.3c	120.6b	91.2b	91.7c	95.6b
TSP	125.7a	143.4a	155.6a	102.3a	133.4a	129.9a	109.1a	108.4a	103.2a
PHA	111.6b	106.7d	115.7b	93.4ab	125.0b	115.2b	85.7c	104.2b	91.2c
$CaCl_2$	75.8d	90.0e	69.6e	84.8b	91.7d	75.8d	77.7d	75.0d	60.1d
CV (%)	8	6	7	6	5	4	7	7	7
P> F	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Numbers followed by different letters in each column are significantly different according to the LSD test at $p \leq 0.05$

5.5.7 Effect of P and Ca source on the C: N ratio

Added P-source and Ca source had significant effects in reducing the C: N ratio of cow-dung-waste paper vermicompost, but this effect differed with time as shown by the significant relationship between treatment x time (Table 5.1). The highest decrease in C: N ratio at day 14 corresponded to TSP > RP > PHA > Control, respectively. Further decline in C: N ratio was observed beyond day 28 till the termination of the experiment but the effects of added P-nutrient sources were not significantly different except for $CaCl_2$ treated vermicompost. Final C: N ratios were in the range of 10 to 12 in vermicompost treated with P-nutrient source fertilizer, whereas control and $CaCl_2$ -treated vermicompost had C: N ratios of 14 and 22, respectively (Fig.5.5). Thus, the addition of P-nutrient bearing source speeded up the process and in turn enhanced cow-dung-waste paper mixtures vermidegradation at the early stages of vermicomposting up to day 28, beyond which treatment effect was minimal. However, vermicompost treated with $CaCl_2$ declined minimally but not lower than 22 at day 56 showing that Ca probably played no critical role in the bioconversion process (Fig.5.5).

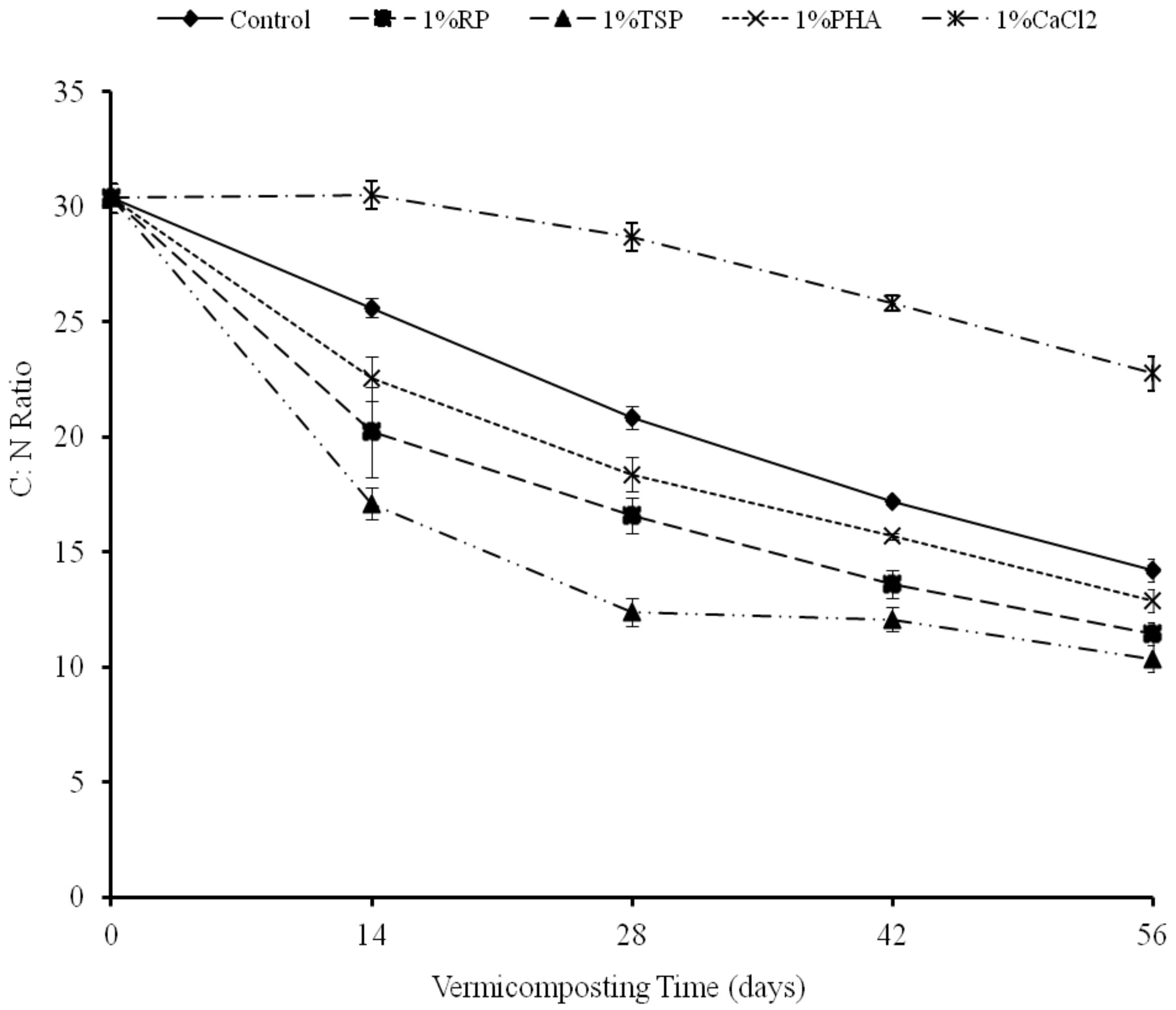


Figure 5.5: Effect of P and Ca sources on the C: N ratio after 56 days of vermicomposting cow dung – waste paper mixture. (Bars represent standard deviations)

5.5.8 Effect of P and Ca source on the humification parameter

Humification parameters (polymerization index (PI), HI, and HR) of cow-dung-waste paper mixtures were influenced by all the treatments during vermicomposting period. These effects were similar to the trend observed in C: N ratio as shown in (Table 5.1). At the beginning of vermicomposting up till 14 days, all treated waste materials had the same PI of 0.2 mg kg^{-1} (Fig. 5.6a). However, as vermicomposting progressed noticeable increases in the polymerization index were evident and followed the order TSP > RP > PHA > Contol > $CaCl_2$. The least PI of 0.3 was observed where $CaCl_2$ was added suggesting that Ca played no signinificant role in the bioconversion process of cow-dung-waste paper mixtures. Substantial changes in PI were observed at day 56 with cow-dung-waste paper mixtures where TSP was added had the greatest PI value of (4.5) followed by RP (2.5), control and PHA had equal PI value of (1.2), while $CaCl_2$ had (0.1) respectively.

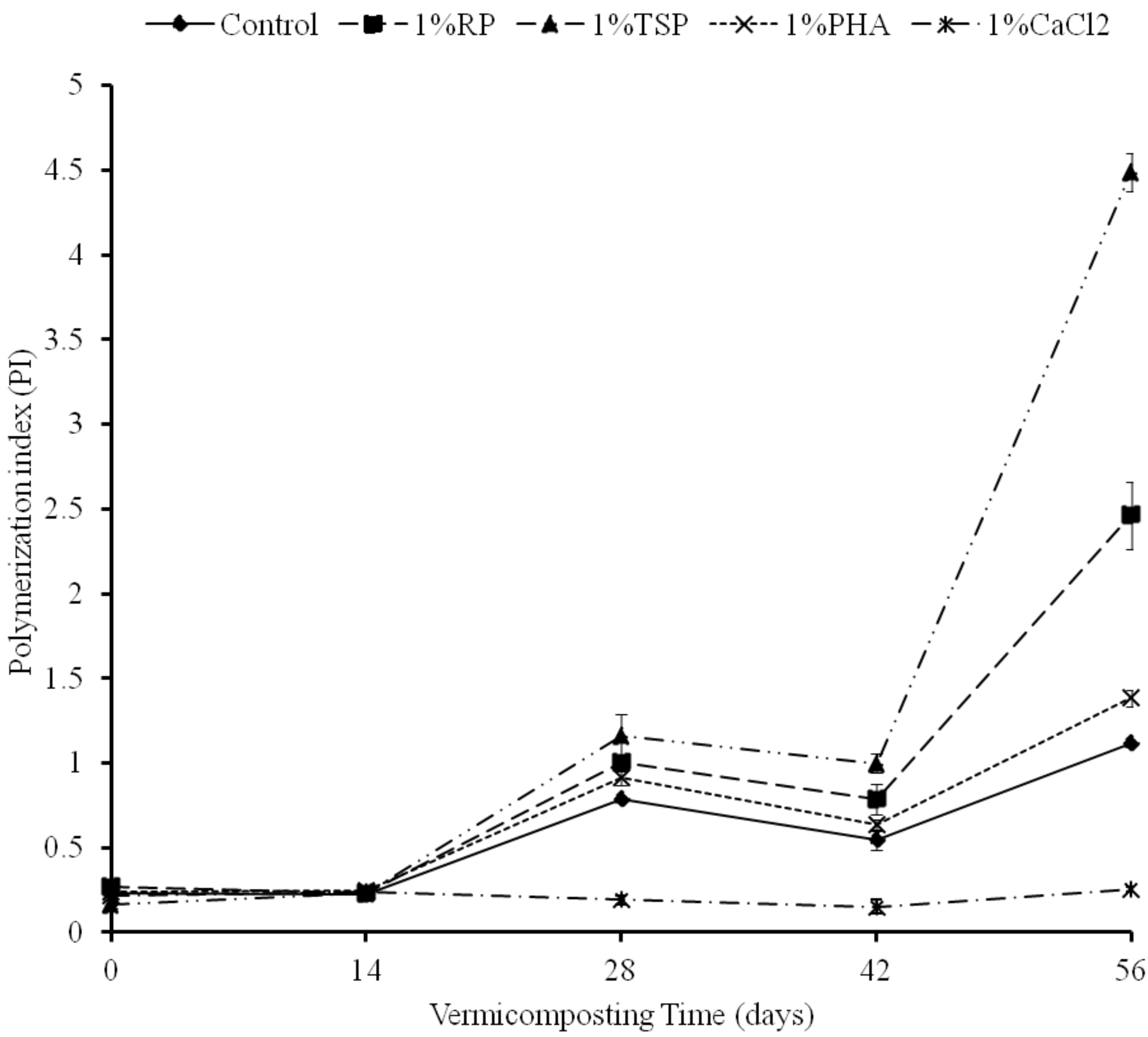


Figure 5.6a: Effect of P and Ca source addition on humification parameters (PI) during vermicomposting of cow dung-waste paper mixtures (Error bars represent standard deviation).

Similarly, humification indexes (HI) from day zero to 14 days of vermicomposting were not different when compared with the control (Fig 5.6 b). However, from 28 days to 56days, waste mixed with TSP had the highest HI values of (4.5 and 22 mg kg^{-1}, respectively), whilst the least HI was recorded in cow-dung-waste paper mixed with $CaCl_2$ in contrast to control. The added P-nutrient sources followed the same trend for humification ratio (HR) in vermicompost from the beginning to the end of the study period showing the importance of P rather than Ca ($CaCl_2$) (Fig. 5.6 c).

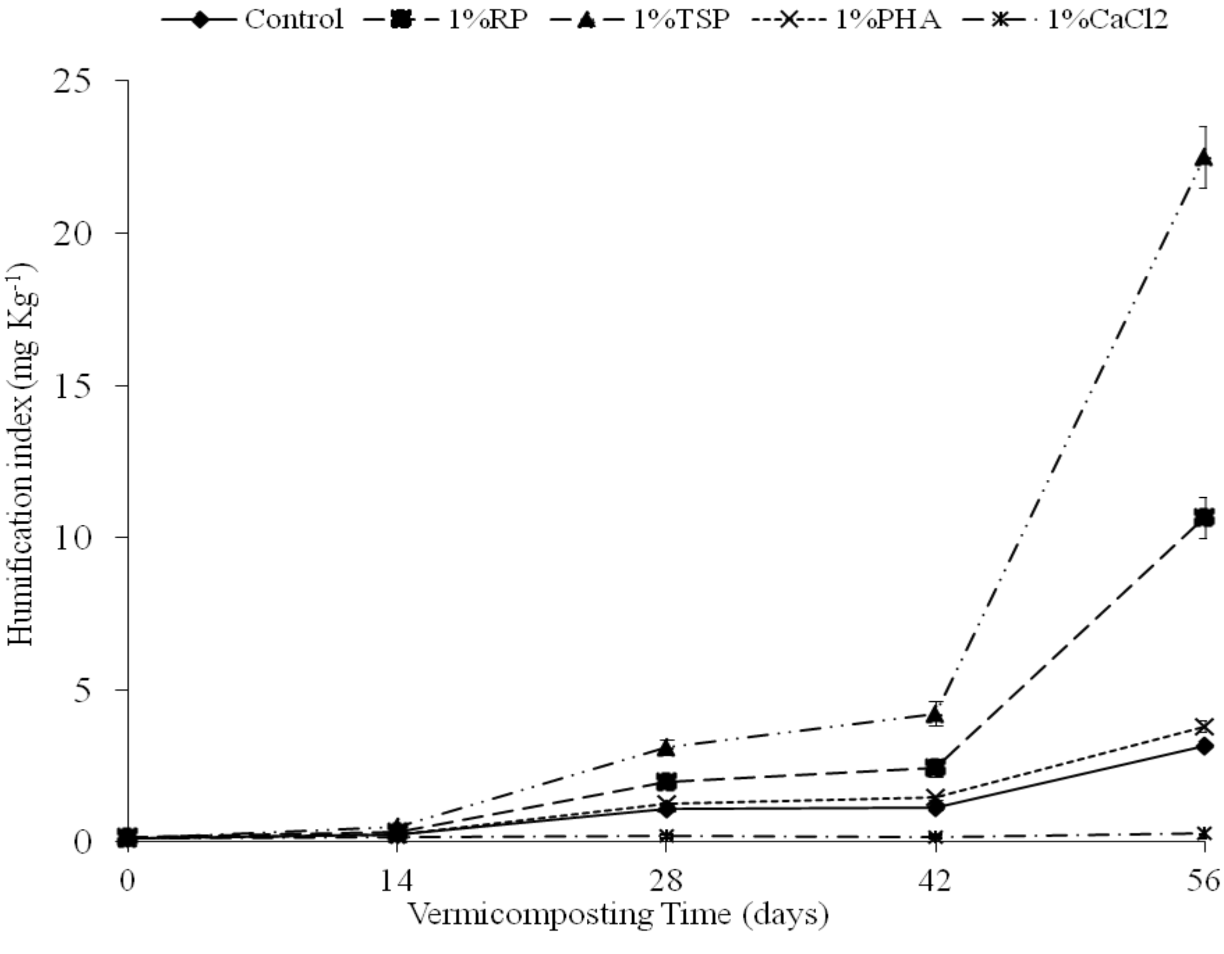


Figure 5.6(b): Effect of P and Ca source addition on humification parameters during vermicomposting of cow dung-waste paper mixtures (Error bars represent standard deviation).

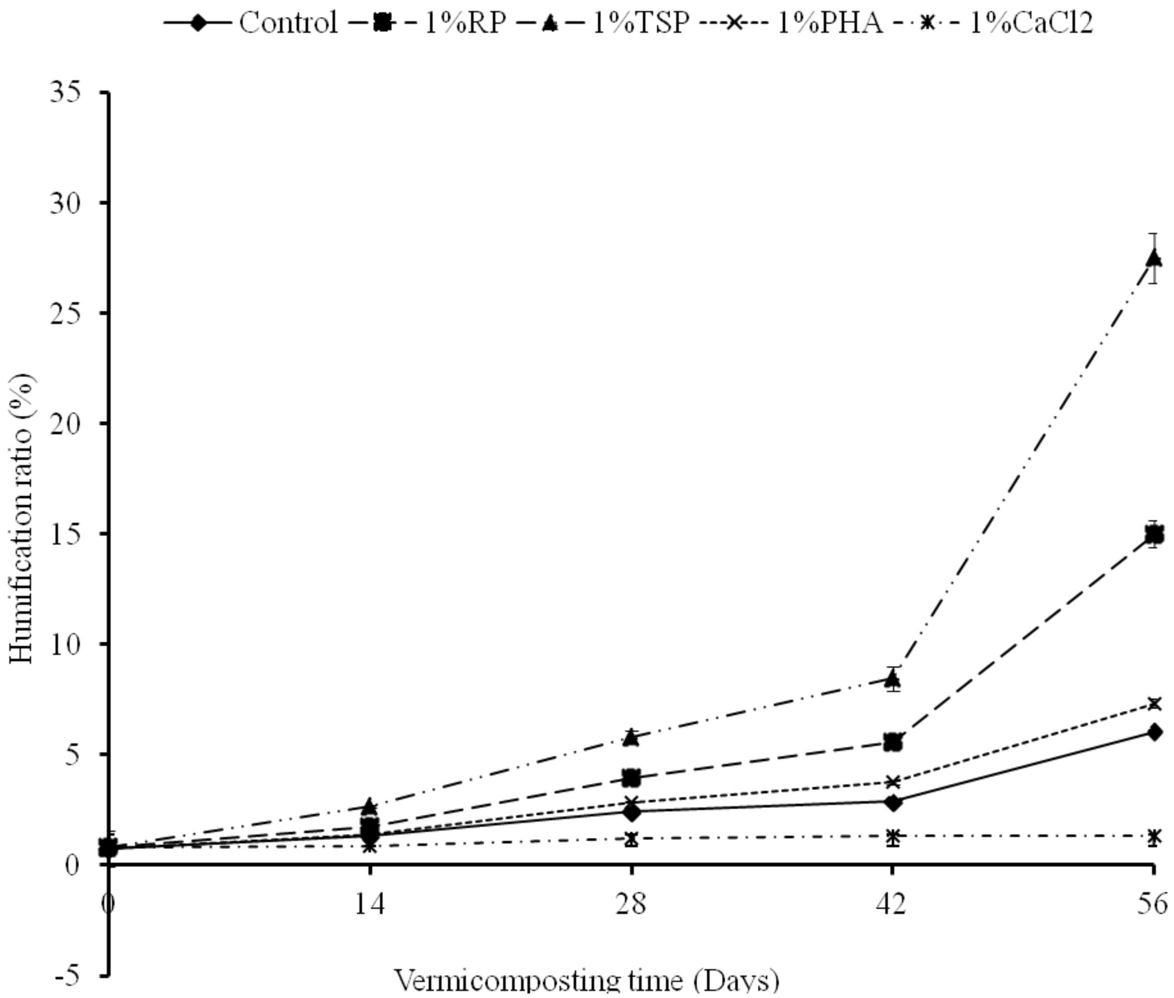
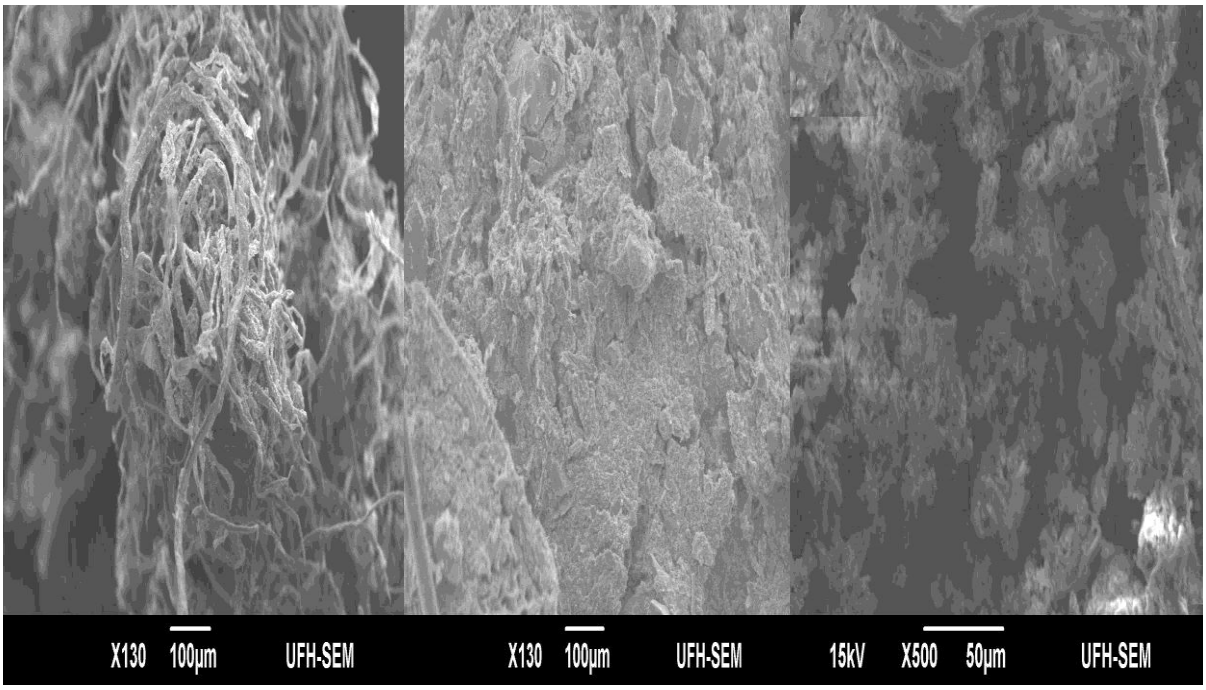


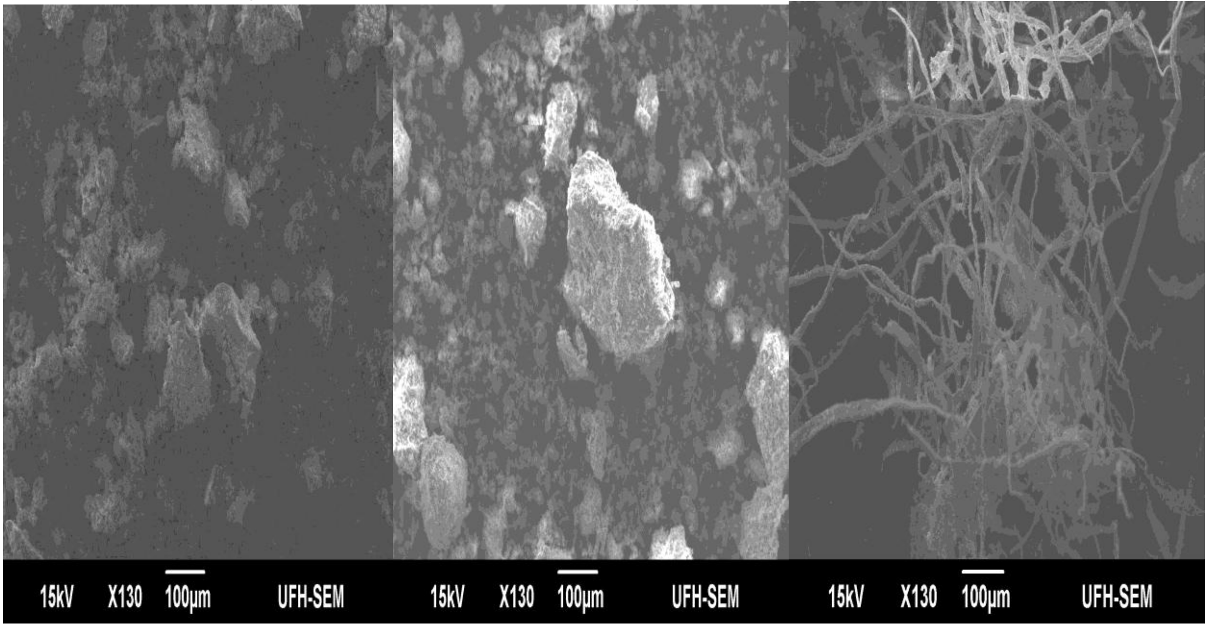
Figure 5.6(c): Effect of P and Ca source addition on humification parameters during vermicomposting of cow dung-waste paper mixtures (Error bars represent standard deviation).

5.5.9 Morphological structure of the vermicompost using scanning electron microscopy

Scanning electron microscopy (SEM) was used to confirm the extent of humification of the vermicomposts (Fig. 5.7 a-f). The morphological structure of the cow-dung-waste paper mixtures at the beginning of the experiment (Fig. 5.7a) showed squashed aggregates of cellulose and protein fibres which were disintegrated by the activities of microbes in enzyme’s gut after 56 days (Fig. 5.7b). However, where earthworms and TSP, RP, PHA were added the cow-dung -waste paper mixtures were highly degraded by earthworm grinding activity as reflected by the fine grain textures (Fig. 5.7c-e) except for $CaCl_2$-treated vermicompost (Fig. 5.7f). The level of grinding activities of the waste materials by earthworms became more intensified with treatments. Vermicompost produced from TSP (readily available P) enrichment had well humified and high aggregate particles which is consistent with the observed chemical humification parameters, showing the importance of P rather than Ca ($CaCl_2$) in the bioconversion of cow-dung-waste paper mixture



(a) control at (0 Day) (b) Control at (56days) (c) RP treated vermicompost (56days)



(d)TSP vermicompost (56 days)(e)PHA vermicompost(56 days)(f)CaCl$_2$ vermicompos (56 days)

Figure 5.7 (a - f): Scanning electron microscope pictures showing P and Ca source effects on vermicompost morphological properties.

5.6.0 Effects of P and Ca source on extractable phosphorus

Addition of treatments into cow-dung-waste paper mixture vermicompost had significant effect on the extent of P release from waste mixtures. However, this effect varied significantly with time as indicated by the significant relationship between treatments and time (ANOVAR) during the study period (Table 5.1). The highest initial Bray 1 extractable P (713 mg kg^{-1}) corresponded to the TSP-treated waste mixture and the lowest to the $CaCl_2$ -treated waste mixtures (Fig.5.8). In all treatments, the release of P by each applied treatments (TSP, RP, PHA and control) either increased or decreased with time and at some points remained linear. At day 14, PHA treated samples had the highest increase (464 mg kg^{-1}) in extractable Bray P compared with TSP treated waste (328 mg kg^{-1}). However, two products (RP and Control) followed very similar pattern from the beginning to the 56th day, while $CaCl_2$ treated vermicompost remained the lowest up to termination of the experiment (Fig.5.8).

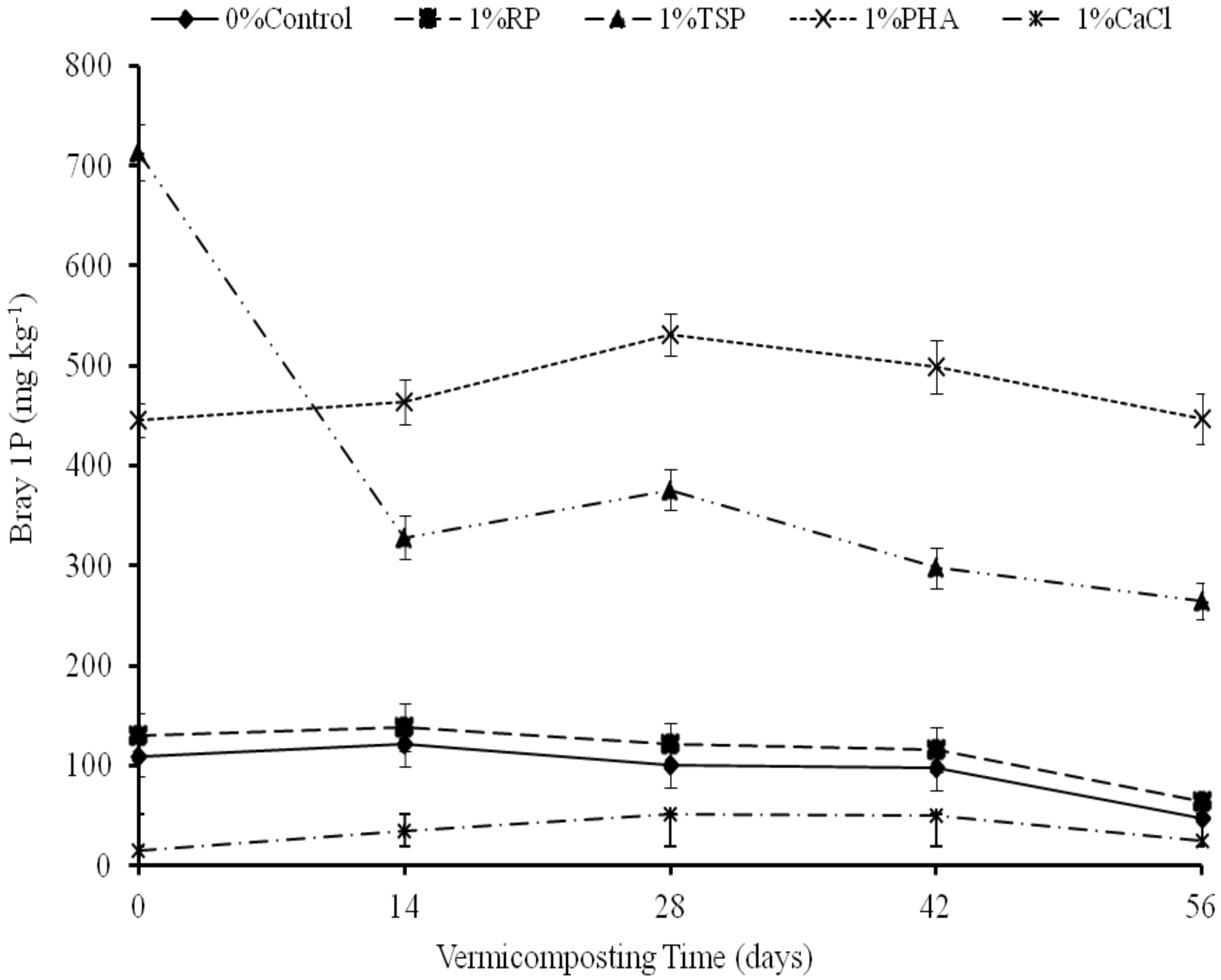


Figure 5.8**:** Effect of P and Ca source on bray 1 P during vermicomposting of cow dung-waste paper mixtures (Error bars represent standard deviation)

5.7 DISCUSSION

5.7.1 Effects of added P and Ca source on pH, EC and selected maturity parameter

The major aim of this study was to find out the constituent elements in RP responsible for the observed enhanced vermidegradation of cow-dung-waste paper mixture enriched with rock phosphate. The significant increase in ash and decrease in volatile solids contents, pH and EC observed with time at each added P-nutrient bearing source and Ca source (Figs. 5.1a and b; 5.2 a and b) indicated the degradation of organic matter (OM) in the vermicomposting mixtures as also depicted by (Mupondi *et al*. 2010; Levannon and Pluda, 2002).

The decreasing trend in pH in all applied P- source results (Fig. 5.2a and b) showed the importance of P rather than Ca ($CaCl_2$) in the degradation of cow-dung-waste paper mixtures. The decrease in pH may be as a result of the accumulation of organic acids from microbial metabolism and/or from the production of fulvic acids and humic acids during decomposition (Campitelli *et al*., 2012). Ebrahim *et al*. (2014) also reported decreased pH in sheep dung and leaf compost with more pronouced effect in the presence of RP and attributed it to the accumulatiom of organic acids from microbial metabolism and RP dissolution. This is also reported by Mupondi, 2010, who co-composted dairy manure paper waste with RP. Atiyeh *et al*. (2008) reported the same trend when they co-composted organic waste with RP. In another study, Rapheal and Velmourougane, (2011), reported decreased pH during the vermicomposting of coffee pulp.

Vermicomposting caused a decrease in the EC of cow-dung-waste paper vermicompost at all P- nutrient bearing sources and its effect seems to be independent of the presence or type of P-nutrient bearing source. The EC of P-nutrient sources varied from 4.1 m S $cm^{-1}$to 3.8 m S cm^{-1} at the beginning and decreased from 3.5 m S $cm^{-1}$to 2.4 m S cm^{-1} at the end of the experiment (Fig. 5.2 b). Decrease in the EC with vermicomposting time was probably due to loss by leaching of salts released during decomposition. These results are consistent with the findings of Ebrahim *et al*. (2014) who reported similar results using RP-enriched leaf and sheep dung vermicompost.

The rate of C: N ratio decline was fastest between day 14 and 28 and slowest after day 42 explaining the observed significant interaction between treatments and time (Table 5.1). The observed decline in C: N ratio could be attributed to the decomposition of organic matter as a result of microbial action. These results are consistent with the findings of Swati and Vikram, (2010); Khwairakpam and Bhargava, (2009), who both reported reported declined C: N ratio of organic waste composts. The increase in the HI with time indicated that the vermidegradation increased humification of the cow dung waste paper mixtures. Corresponding increases in the PI with time (Fig. 5.6 a) indicated that the lower molecular weight of fulvic acid fraction was progressively converted to the more recalcitrant molecules of higher molecular weight which make up the humic acid fraction.

The treatment effects on the C: N ratio followed the trend TSP < RP < PHA < Control << $CaCl_2$ (Fig 5.5). A similar response pattern was observed on NO_3^- and NH_4^+ data (Fig 5.4 a and b) and humification parameters (Fig 5.6 a-c). This trend suggests that phosphorus rather than calcium had the strongest influence on the vermidegradation of cow-dung-waste paper mixtures with the water soluble TSP enhancing vermidegradation the most followed by RP and PHA. This pattern of response was further confirmed by scanning electron microscopy (Fig 5. 7 b-e) which also revealed that incorporation of P-nutrient bearing sources intensified the degradation of cow- dung-waste paper mixtures the most and that TSP applications resulted in consistently greater vermidegradation of the waste mixtures at each sampling date compared with the other sources. This was reflected in higher segregation of the waste mixtures aggregate particles.

The MBC data (Fig. 5.3) followed the trend TSP > PHA> RP > Control > $CaCl_2$ which more or less mirrored that which was followed by the maturation parameters highlighted above. The decline of MBC overtime indicated reduced microbial demand after the rapid increase in MBC values peaking at day 14. This result suggested that over time, the easily available compounds in the mixtures became depleted resulting in the turnover of the microbial biomass. The increase in the MBC appeared linked to the release of P as it coincided with an increase in available P during this time. It is suggested that the enhanced microbial activity by the P bearing sources was largely responsible for the observed enhanced vermidegradation of cow dung waste paper mixtures. The greater effectiveness of TSP relative to PHA which is also water soluble P source could be related to the fact that PHA had a greater acidifying effect (Fig 5.2a) which may have had an unfavourable effect on microbial activity. Calcium chloride did not have a positive effect on vermidegradation possibly due to its failure to stimulate microbial activity (Fig 5.3) in the absence of phosphorus. A possible toxic effect of the chloride ions on microbes in the decomposing mixture could not be ruled out, though it seems unlikely. Nevertheless, it is an aspect that could be investigated in future studies.

The C: N results (Fig 5.5) show that at 28 days, the C: N ratios of the different cow-dung-waste paper mixtures were 12, 16, 18, 21, and 29 for waste mixtures treated with TSP, RP, PHA, no P (control), and $CaCl_2$, respectively. The corresponding values for day 56 were 10, 11.5, 13, 14 and 23, respectively. According to Iglesias and Garcia. (1992), Chen *et al.* (1996), and Cedric *et al* .(2005), a decline in C: N ratio to less than 12 indicates an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity. Thus, based on the results of the present study only, the incorporation of TSP resulted in a mature cow-dung-waste paper vermicompost within 28 days. However, except for the $CaCl_2$ treatment, by day 56, all composts including the control where no P was applied had reached maturity. Therefore, as observed with RP treatments in Chapter 4, phosphorus seems to accelerate the vermidegradation of cow-dung -waste paper mixtures during the early stages of vermicomposting but given enough time for cow-dung-waste paper mixtures mixed with the different P carriers reach more or less the same level of maturity. The results of this study support the hypothesis that phosphorus accelerates the bioconversion and maturation of cow-dung-waste paper mixtures during vermicomposting and explains the observed effects of RP and other P-bearing minerals in enhancing the biodegradation of waste materials. However, the use of expensive water-soluble P sources in vermicomposting cannot be justified as enhanced vermidegradation can be achieved with less expensive impure sources of P such as rock phosphate.

The seed germination index is a more direct indicator of both vermicompost and compost maturity as it directly shows whether the finished vermicompost can inhibit plant growth or not when used as a growth media. The over 80% GI observed for all test crops in this study indicated that addition of P as TSP, PHA and RP to cow-dung-waste paper mixtures in the presence of *E. fetida* resulted in vermicompost that was free of phytotoxins according to Zucconi *et al.* (1981) and Tam *et al.*(1998). Bustamante *et al*. (2001) also linked a germination index of $\geq$ 80% with the disappearance of phytotoxins in composts. Of the three P sources tested, water soluble P vermicomposts had the highest germination indices for all crops tested indicating the superiority of these vermicomposts.

5.7.2 Effects of added P and Ca sources on P enrichment of compost

Adding P-nutrient sources into cow-dung-waste paper mixture vermicompost had significant effect on the Bray 1 extractable P. Extractable P increased from a low 108 mg P kg^{-1} where no P- nutrient source was added to 447 and 264 mg P kg^{-1} where PHA and TSP were added (Fig.5.8). The increase in the available P with P-nutrient sources especially in PHA and TSP application (Fig.5.8)) was accompanied by an increase in microbial biomass with P-nutrient source application (Fig. 5.3), suggesting that among the microorganisms stimulated by added P-Nutrient source were phosphate bacteria which facilitated the dissolution and the availability of P from the added P-nutrient sources. According to Bhattacharya and Chattopadhyay (2002), phosphate bacteria facilitate the dissolution of P from P bearing minerals through the phosphatase enzymes they produce. The P release is mediated by phosphate enzymes produced by microorganisms in the earthworm guts and those in the earthworm casts (Yan et al., 2012). The observed increase in humic acids reflected by a high humification index (Fig. 5.6 b) could also account for the enhanced dissolution of P. The high extractable P contents observed in the cow-dung-waste paper vermicomposts enriched with P-nutrient source point to their potential as organic P fertilizers.

5.8 CONCLUSIONS

The results of this study have demonstrated that phosphorus and not calcium is responsible for the enhanced biodegradation of waste mixtures mixed with P bearing minerals such as rock phosphate during vermicomposting. However, the use of water soluble P sources to enhance vermicomposting may not be justifiable where impure and less expensive P sources such as rock phosphates are available as their use can result in equally mature and P enriched vermicomposts in 6 to 8 weeks. The effectiveness of added P in enhancing vermicomposting appears linked to its ability to stimulate microbial growth, reduction in the C: N ratio, increase in the PI, HI and HR. However, it could also be partially linked to meeting the P nutritional requirements of the earthworms. This latter effect will need to be explored in future studies.

CHAPTER SIX

6 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS FOR FOLLOW UP STUDIES

6.1 GENERAL DISCUSSION

There is a worldwide crave for organic farming by adding recycled organic waste of various kinds with the aim of improving the fertility of the soil to boost crop productivity by incorporating their vermicompost product into the soil, and thus, sustaining constant agriculture as well as for creating pollution free environment (Kumar and Singh, 2000). For the enhancement of a sustainable farming, waste enrichment is very important. Involvement of earthworms, especially the epigeic species e.g. *Eisenia fetida* for the bioconversion of organic waste into bio-fertilizer is of utmost importance (Dominguez, 2004, Suthar 2007; Lazcano *et al*. 2008) as it helps to minimize carbon loss from agricultural waste and animal manure when the vermicompost is the source of organic matter. There is the possibility of optimizing the vermidegradation of these organic wastes (Aira and Dominguez, 2007; Manyuchi and Phiri, 2013) through addition of appropriate rate of rock phosphate and earthworm number during vermicomposting which in turn increase microbial activities that help to solubilise phosphorus and increase phosphorus availability to plant. In addition, for the vermicomposting process to progress there is the need for the earthworms, enrichment additives, organic waste and operating conditions to be optimized.

These studies, which were aimed at the optimization of the vermidegradation of cow-dung-waste paper mixtures encompassed the evaluation of the effect of *Eisenia fetida* stocking density on the bioconversion of cow-dung-waste paper mixtures enriched with rock phosphate (RP) and identified an ideal stocking density; determined the rate of Phalaborwa RP (low grade South African Rock Phosphate) incorporation and time required for efficient vermicomposting of cow-dung-waste paper mixtures and determined if phosphorus or calcium which were predominant elements in RP was responsible for improved biodegradation during the vermicomposting of cow dung-waste paper mixtures. This chapter discusses the findings from these studies and explores research gaps and aspects that need further research.

As described in Chapter 3, the aim of optimizing *Eisenia fetida* stocking density was to establish an ideal stocking density effective for the bioconversion of cow-dung-waste paper mixtures enriched with rock phosphate during vermicomposting. This study revealed that, a stocking density of 22.5 g-worms kg^{-1} feedstock resulted in maximum P released from RP enriched vermicompost, while earthworm stocking density of 12.5 g-worms kg^{-1}feedstock resulted in the highest earthworm growth rate and humification of RP enriched waste mixture. This was reflected by a C: N ratio of < 12 and a polymerization index that exceeded 1.9 in final vermicomposts of P enriched mixtures whilst in the control all maturity parameters evaluated did not attain the critical maturity levels.

The changes in C: N ratio demonstrated the extent of organic waste mineralization and stabilization during vermicomposting. A C: N ratio of less than 12 is suggestive of an advanced level of stabilization and acceptable maturity, while a ratio of 20 or less is agronomically preferable as plants cannot assimilate nitrogen unless the ratio is in the order 20 or less (Morais and Queda, 2003; Subrata and Vinod, 2011). Hence, in this study, the high degree of organic matter humification obtained at an earthworm stocking density of 12.5 g-worms kg^{-1} feedstock was well below 12 and definitely indicative of agronomic potential of vermicompost. The lowering of C: N ratio during vermicomposting attributed to loss of organic carbon as CO_2 due to microbial respiration and increased levels of nitrogen introduced by earthworm casts (Subrata and Vinod, 2011). Singh and Kaur (2013) also reported an optimum stocking density of 12.5 g-worms

kg^{-1} feedstock with chemical sludge and spent carbon obtained from industrial soft drinks. There are other studies that have evaluated earthworm stocking density of different waste but their results are reported items of surface area rather than the mass of waste to be biodegraded. These include Neuhauser *et al*. (1980) who reported an optimum stocking density of 0.8 kg-worms m^{-2} for horse manure and 2.9 kg-worms m^{-2} for activated sludge. Ndegwa *et al*. (2000) recommended a stocking density of 1.6 kg worms m^{-2} for bio-solids while Yadav *et al*. (2011) reported an optimum stocking density of 3 kgworms m^{-2} for human faeces. These studies underscored the need for establishing optimum stocking densities for different waste materials. However, reporting the stocking density on surface area basis is practically less feasible than when reported on mass basis as done in the present study. This present study has established an optimum stocking density of 12.5 g-worm kg^{-1} cow dung-waste paper mixtures for the bioconversion of cow-dung-waste paper mixtures but a higher stocking density of 22.5 g-worm kg^{-1} cow-dung-waste paper mixtures is recommended when maximum P release is the desired goal. The latter recommendation is informed by the fact that the greatest amount of P release was observed at the highest stocking density of 22.5 g-worm kg^{-1} substrate (Chapter 3).

While stocking density is vital in vermicomposting, the maturity parameters are equally essential.The significance of humic materials in soil ecosystem, fertility and structure and their valuable effects on plant growth make humification a key factor in quality of composts (Inbar *et al*., 1990). Equally important is the nutrient content of the resultant vermicomposts. Chapter 4 explored the possibility of enhancing the vermidegradation of cow-dung-waste paper mixtures by applying low RP rates (≤ 2%) as against higher rates used by Mupondi (2010). Addition of RP to cow-dung-waste paper mixtures even at low rates of application, resulted in increased C_{EX}, C_{HA} and a corresponding decrease in C_{FA} which resulted in increases in HR and HI over time. This was attributed to the possible improvement in P content of the waste mixtures which perhaps influenced more microbial activity and caused degradation of the RP-enriched mixtures. The results further revealed that the improved degradation was fastest when RP was added at a rate of 1% P. At this rate of application vermicompost maturity was achieved within 33 days. This result indicated that rates lower than 2% P could be used to improve the rate of biodegradation and humification of cow-dung-waste paper mixtures. However, the total P content of the resultant vermicomposts increased with rate of RP application reaching as high as 2.31% P in the final cow-dung-waste paper vermicompost where RP was added at the rate of 4% P. RP rate beyond 4% P could be necessary if higher P contents and better fertilizer value were the desired goal. This was consistent with result of Biswas and Narayanasamy (2006) who reported increases in the total P content of straw compost from 0.37% in control to 2.20% by applying 4% P as RP. Yan *et al.* (2012) also reported an increase in the total P content of rice straw compost from 0.392% to 0.82% by applying 2% P as RP. Of greater nutritional significance is that in the present study Bray 1 extractable P increased from a low 80 mg P kg^{-1} where no RP was added to 207 mg P kg^{-1} where RP was added at a rate of 4% P.

The increase in the extractability of P with RP application is thought to be the result of greater stimulation of phosphate bacteria which facilitated the dissolution and extractability of P from the added RP. Bhattacharya and Chattopadhyay (2002) reported that phosphate bacteria facilitate the dissolution of P from P bearing minerals through the phosphatase enzymes they produce. The observed increase in humic acids reported in Chapter 4 could also account for the enhanced mineralization and dissolution of P. According to Singh and Amberger (1990) humic acids can adsorb significant quantities of calcium ions and release H^{+} ions which further facilitate the dissolution of RP. In addition, the functional groups in humic acids such as carboxylic and phenolic groups can also chelate Ca^{++} ions, providing a driving force for the dissolution and dissolution of P from RP. Most of the inorganic P extracted was water extractable (Chapter 4) which implied that most of the P that was released during the vermicomposting of RP enriched cow-dung- waste paper mixtures is available for plant uptake. Edwards *et al*. (2010) reported that RP is an acceptable source of P for organic agriculture but its use is limited by its slow rate of P release. The high total P and extractable P contents observed in the cow-dung-waste paper vermicomposts enriched with RP point to their potential as valuable organic P fertilizers. Future studies should explore this potential. It can, therefore, be concluded that although lower rates of RP application is all that is needed to achieve fast maturation of the vermicompost, higher rates of RP application from 4% to 8% are necessary for increasing P content of the resultant vermicompost for better P fertilizer value.

The study reported in Chapter 5 sought to establish which of the two major constituents of RP, phosphorus or calcium, was responsible for the bioconversion enhancement properties of RP. The study compared RP with two water soluble P sources and $CaCl_2$ in terms of their effectiveness in enhancing vermidegradation. All vermicompost maturation parameters (C: N ratio, polymerization index (PI), and humification index (HI)) showed that by 56 days, all treatments resulted in well matured vermicomposts except $CaCl_2$. For example, the C: N ratios were 9, 11,12,13 and 25 for triple superphosphate (TSP), rock phosphate (RP), control (C), phosphoric acid (PHA) and calcium chloride ($CaCl_2$), respectively (Chapter 5). These results indicated that phosphorus and not calcium is largely responsible for the enhanced biodegradation of waste mixtures mixed with rock phosphate during vermicomposting. However, the use of water-soluble P sources to enhance vermicomposting may not be justifiable where impure and less expensive P sources such as rock phosphates are available as their use can result in equally mature and P enriched vermicomposts as shown by compost maturity parameters that were monitored.

6.2 GENERAL CONCLUSIONS

The main objectives of this study were to find ways of optimizing the vermi-degradation of cow dung waste paper mixtures through establishing the optimum earthworm stocking density and optimum RP application rate; and to determine whether phosphorus and calcium played key roles in enhancing the bioconversion of cow dung waste paper mixtures during vermicomposting. Based on the findings from the study it can be concluded that:

1. An *E. fetida* stocking density of 12.5g worms kg^{-1} feedstock is an ideal stocking density for the bioconversion of RP-enriched cow-dung-waste paper mixtures during vermicomposting. This stocking density resulted in the most matured and humified vermicompost. However, where higher levels of extractable P are desired in the final vermicompost, higher stocking densities should be considered. In the present study, a stocking density of 22.5 g-worms kg^{-1} cow dung-waste paper mixture resulted in the highest extractable P levels in the final vermicompost.

2. Application of phosphate rock does enhance the bioconversion of cow-dung-waste paper mixtures and that the critical application rate for optimum bioconversion is $\leq$ 1% P as RP. This rate of RP application resulted in a mature final vermicompost with a C: N ratio of less than 12 which was also highly humified as reflected by high levels of the polymerization index, humification index, and humification ratio. However, higher rates of RP application may be necessary to achieve final vermicomposts with high total and extractable P contents for better P fertilizer value.

3. Phosphorus seems to be the main constituent of RP that is responsible for the observed enhanced biodegradation of waste mixtures enriched with rock phosphate during

vermicomposting. This was reflected by the better performace of TSP, a relatively more water –soluble P source, in enhancing vermidegradation when compared with RP. Triple superphosphate may, therefore, be used to enhance the vermidegradation of cow-dung-waste paper mixtures where RP is not available. However, RP should be the preferred P source where it is readily available as it is an inexpensive P source.

6.3 GENERAL RECOMMENDATION FOR FOLLOW-UP STUDIES

The following follow–up studies will add value to the findings of the present study:

1. The present study has demonstrated the importance of optimizing key parameters for the vermidegradation of cow-dung-waste paper mixtures. The types of optimizing studies reported herein need to be extended to other target waste mixtures for vermicomposting.

2. Other P-bearing minerals such as coal flyash and bone meal need to be investigated for their ability to serve as sources of P using vermidegradtion.

3. There is the need to study the agronomic effectiveness of the vermicompost enriched with lower RP rates as well as their suitability for use in organic farming.

4. Effective microorganism (EM) need to be investigated for their effectiveness in enhancing the vermidegradation of cow dung waste paper mixtures.

5. Many kinds of waste papers are produced with different textures used for various purposes both in different institutions and media houses. There is need to validate the utility of the

approaches used in this study on other types of waste papers as possible feed stocks for cow dung based vermicomposts.

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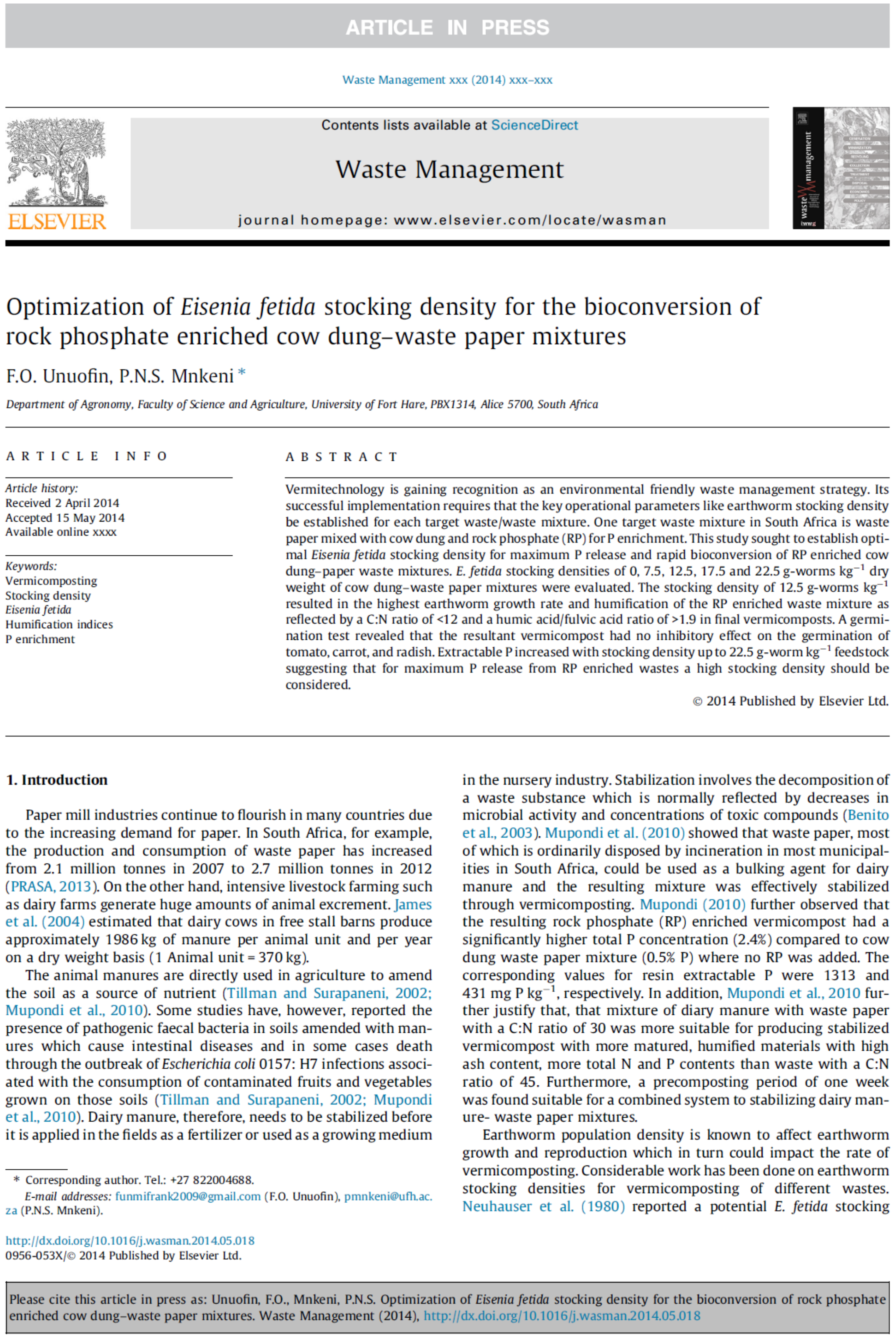
APPENDIX 1



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Optimization of *Eisenia fetida* stocking density for the bioconversion of rock phosphate enriched cow dung–waste paper mixtures

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ABSTRACT

Vermitechnology is gaining recognition as an environmental friendly waste management strategy. Its successful implementation requires that the key operational parameters like earthworm stocking density be established for each target waste/waste mixture. One target waste mixture in South Africa is waste paper mixed with cow dung and rock phosphate (RP) for P enrichment. This study sought to establish optimal *Eisenia fetida* stocking density for maximum P release and rapid bioconversion of RP enriched cow dung–paper waste mixtures. *E. fetida* stocking densities of 0, 7.5, 12.5, 17.5 and 22.5 g-worms kg^{-1} dry weight of cow dung–waste paper mixtures were evaluated. The stocking density of 12.5 g-worms kg^{-1} resulted in the highest earthworm growth rate and humification of the RP enriched waste mixture as reflected by a C:N ratio of <12 and a humic acid/fulvic acid ratio of >1.9 in final vermicomposts. A germination test revealed that the resultant vermicompost had no inhibitory effect on the germination of tomato, carrot, and radish. Extractable P increased with stocking density up to 22.5 g-worm kg^{-1} feedstock suggesting that for maximum P release from RP enriched wastes a high stocking density should be considered.

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1. Introduction

Paper mill industries continue to flourish in many countries due to the increasing demand for paper. In South Africa, for example, the production and consumption of waste paper has increased from 2.1 million tonnes in 2007 to 2.7 million tonnes in 2012 (PRASA, 2013). On the other hand, intensive livestock farming such as dairy farms generate huge amounts of animal excrement. James et al. (2004) estimated that dairy cows in free stall barns produce approximately 1986 kg of manure per animal unit and per year on a dry weight basis (1 Animal unit = 370 kg).

The animal manures are directly used in agriculture to amend the soil as a source of nutrient (Tillman and Surapaneni, 2002; Mupondi et al., 2010). Some studies have, however, reported the presence of pathogenic faecal bacteria in soils amended with manures which cause intestinal diseases and in some cases death through the outbreak of *Escherichia coli* 0157: H7 infections associated with the consumption of contaminated fruits and vegetables grown on those soils (Tillman and Surapaneni, 2002; Mupondi et al., 2010). Dairy manure, therefore, needs to be stabilized before it is applied in the fields as a fertilizer or used as a growing medium in the nursery industry. Stabilization involves the decomposition of a waste substance which is normally reflected by decreases in microbial activity and concentrations of toxic compounds (Benito et al., 2003). Mupondi et al. (2010) showed that waste paper, most of which is ordinarily disposed by incineration in most municipalities in South Africa, could be used as a bulking agent for dairy manure and the resulting mixture was effectively stabilized through vermicomposting. Mupondi (2010) further observed that the resulting rock phosphate (RP) enriched vermicompost had a significantly higher total P concentration (2.4%) compared to cow dung waste paper mixture (0.5% P) where no RP was added. The corresponding values for resin extractable P were 1313 and 431 mg P kg^{-1}, respectively. In addition, Mupondi et al., 2010 further justify that, that mixture of diary manure with waste paper with a C:N ratio of 30 was more suitable for producing stabilized vermicompost with more matured, humified materials with high ash content, more total N and P contents than waste with a C:N ratio of 45. Furthermore, a precomposting period of one week was found suitable for a combined system to stabilizing dairy manure- waste paper mixtures.

Earthworm population density is known to affect earthworm growth and reproduction which in turn could impact the rate of vermicomposting. Considerable work has been done on earthworm stocking densities for vermicomposting of different wastes. Neuhauser et al. (1980) reported a potential *E. fetida* stocking

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by means of the reduced phosphomolybdenum blue method on a continuous flow analyser (San 2++ Skalar Continuous Flow Analyser, Skalar Analytical B.V. The Netherlands). Humic substances were determined following extraction as described by Del Carmen Vargas-García et al. (2006). Briefly, compost samples were treated with 0.1 M NaOH (1:20 w/v ratio), constantly shaken on a horizontal shaker for 4 h and the resulting solution was centrifuged at 8000*g* for 15 mins. Then the supernatants were divided equally into two fractions, one was analyzed for total extractable carbon fraction (C_{EX}) by the Walkley and Black rapid titration method as described by Anderson and Ingram (1996) and the other fraction was adjusted to pH 2 with concentrated H_2SO_4 and allowed to coagulate for 24 h at 4 °C. The precipitates that were formed constituted the humic acid-like carbon (C_{HA}) while fraction that remained in solution constituted the fulvic acid-like carbon (C_{FA}). The C_{HA} was calculated by subtracting the C_{FA} from the C_{EX}. The humification ratio (HR) was calculated as $HR = (C_{EX}/C) \times 100$ and humification index (HI) was calculated as $HI = (CHA/C) \times 100$ (Mupondi et al., 2010).

2.3. Phytotoxicity study

Phytoxicity was assessed through a seed germination test. Aqueous extracts were prepared from the different vermicomposts with distilled water (1:10 w/v) (Ravindran et al., 2013). The seed germination bioassay for tomato (*Lycopersicon esculentum*), radish (*Raphanus sativus*) and carrot (*Daucus carota*) was evaluated according to Tam and Tiquia (1994) in which two pieces of Whatman® filter paper was placed inside a sterilized petri dish and wetted with the vermicompost extracts. Ten seeds of each crop species were placed on top of the filter paper and incubated for five days in the dark. A control was included for each crop species in which the filter papers were wetted with distilled water. Seed germination, germination index (GI), relative seed germination (RSG) and relative root elongation (RRE) were calculated as follows:

$$\mathrm{RSG}\ (\%) = \frac{\text{Number of seeds germinated in the sample extract}}{\text{Number of seeds germinated in the control}} \times 100$$

$$\mathrm{RRE}\ (\%) = \frac{\text{Mean root elongation in the sample extract}}{\text{Mean root elongation in the control}} \times 100$$

$$\mathrm{GI}\ (\%) = \frac{(\%\ \text{Seed germination}) \times (\%\ \text{Root elongation})}{100}$$

2.4. Morphological assessment of vermicomposts

Scanning electron microscopy (SEM) images were taken using JOEL (JSM-6390LV, Japan). Briefly, the samples were oven dried and ground to pass through a 2 mm sieve. Small representative portions of the samples were coated with gold and mounted on SEM. Samples were imaged by scanning it with a high energy beam of electrons in a raster scan pattern.

2.5. Statistical analysis

The data reported herein are the means of three replicates ($n = 3$). Because the sampling was not destructive, statistical analysis of the data obtained was done by repeated measures analysis of variance (ANOVAR). Means separation was done using Fisher's protected least significant difference (*LSD*) test at $P < 0.05$. Linear regression analysis was done to evaluate relationship between *E. fetida* stocking density and the release of Bray 1 extractable P during the vermidegradation of RP enriched cow dung waste paper mixtures. All statistical analyses were done using JMP® Release 10.0 statistical package (SAS Institute, Inc., Cary, North Carolina, USA, 2010).

3. Results and discussion

3.1. Chemical characteristics of the waste

Selected chemical properties of the cow dung and waste paper used were significantly different among both wastes as shown in Table 1. The cow dung had lower carbon content and higher nitrogen content than the waste paper. The same trend was observed for total P as a result cow dung had narrower C:N and C:P ratios than waste paper. The electrical conductivity (EC) and ash content in cow dung was higher than in waste paper whereas, both cow dung and waste paper pH was alkaline but waste paper had a higher pH. Previous research has showed that dairy manure had higher nitrogen and lower carbon content than waste paper that resulted in a lower C:N, C:P ratios (Mupondi et al., 2010).

3.2. Effects of earthworm stocking density on earthworm biomass and C:N ratio

E. fetida stocking density had a highly significant effect on worm biomass but this effect was dependent on time as reflected by the significant interaction between earthworm density and time (Table 2). Worm biomass increased linearly with time up to 28 days but decreased thereafter up to day 42 for all *E. fetida* stocking densities except the 7.5 g-worm kg^{-1} feedstock density in which the biomass continued to increase linearly (Fig. 1a). The highest worm biomass was observed at a stocking density of 12.5 g-worm kg^{-1} feedstock up to day 28 while higher stocking rates resulted in lower biomass though still greater than what was observed at the lowest stocking density of 7.5 g-worm kg^{-1} feed as shown in (Fig. 1a). The decline in worm biomass beyond 28 days could be due to depletion of easily biodegradable feed components in the vermicomposting mixture facilitated by earlier sexual maturity of earthworms in the higher stocking density (Datar et al., 1997; Dominguez and Edwards, 1997). *E. fetida* biomass at the lower stocking density of 7.5 g-worm kg^{-1} feedstock, by contrast, increased linearly with time possibly due to limited competition for feed at this low stocking density (Datar et al., 1997).

The C:N ratio was used for monitoring the effect of *E. fetida* stocking density on the vermidegradation of PR enriched cow dung–waste paper mixtures. It reflects changes in carbon and nitrogen concentration of the substrate materials in the vermicompost. *E. fetida* density had a highly significant effect on the C:N ratio of the cow dung–waste paper mixtures but this varied with time as indicated by the significant interaction between earthworm density and time (Table 2). The C:N ratio declined from 30 in the control to 10, 12, 13, 13, and 25 after 42 days for *E. fetida* stocking densities of 12.5, 22.5, 17.5, 7.5, and 0 g-worms kg^{-1} feedstock, respectively (Fig. 1b). Wide differences on C:N ratios among the different stocking densities occurred on day 28 but these differences narrowed down by day 42 for all stocking densities except where no worms were included (Fig. 1b). The *E. fetida* density of 12.5 g-worms kg^{-1} feedstock maintained the lowest C:N ratio throughout the vermicomposting period while opposite was the case where no worms were included.

The general decline in C:N ratio with time could be attributed to the decomposition of organic matter as a result of microbial action (Bernal et al., 2009). A decline in C:N ratio to less than 12 has been advocated to indicate an advanced degree of organic matter stabilization and reflects a satisfactory degree of maturity

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Table 2
Repeated measures ANOVA for *E. fetida* biomass, C:N ratio, C_{HA}/C_{FA}, HI, HR and Bray 1 extractable P.

	Effect					
	E. fetida density		Time		*E. fetida* × time	
	$F_{(4,55)}$	*P*	$F_{(3,55)}$	*P*	$F_{(12,55)}$	*P*
E. fetida biomass (mg/kg)	14.25	<.0001	5.75	<.0001	1.44	<.0001
C:N ratio	35.6	<.0001	17.45	<.0001	56.4	<.001
C_{HA}/C_{FA} (mg kg^{-1})	11.53	<.0001	14.6	<.00012	20.6	<.001
HI (mg kg^{-1})	120.57	<.0001	143.15	<.0001	176.75	<.0001
HR (mg kg^{-1})	134.66	<.001	290.61	<.0001	401.37	<.0001
Bray 1P (mg kg^{-1})	30.50	<.0001	117.55	<.0001	132.31	<.0001

F values and probabilities are shown for each effect. $P < 0.05$ significant, C:N = carbon to Nitrogen ratio, C_{HA}/C_{FA} = Humic acid carbon and fulvic acid carbon, HI = Humification index, HR = Humification ratio.

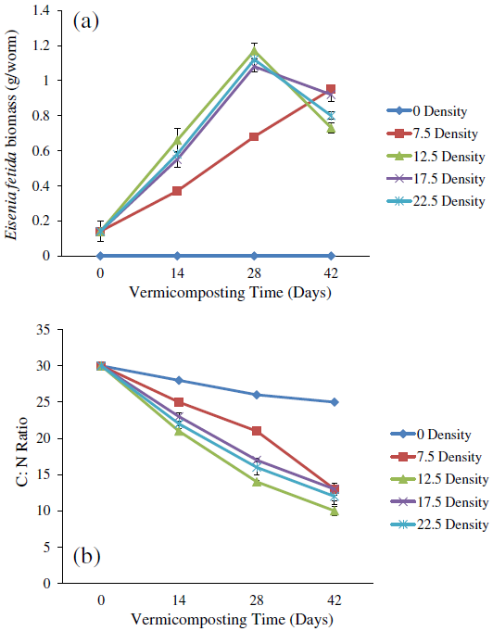

Fig. 1. *Eisenia fetida* stocking density effects on (a) worm biomass and (b) C:N ratio of cow dung–waste paper mixtures mixed with rock phosphate during vermicomposting. [Error bars are standard deviations].

(Iglesias-Jimenez and Perez- Garcia, 1992a; Chen et al., 1996; Cedric et al.,2005). Thus, in terms of C:N ratio all stockings densities resulted in well matured vermicomposts after 42 days though the 12.5 g-worms kg^{-1} feed stocking density produced the most matured vermicompost with a C:N ratio of 10. The greater and faster decline in C:N ratio in the presence of *E. fetida*, relative to the control, further confirmed the effective role that earthworms play in vermidegradation.

3.3. Effects of earthworm stocking density on humification parameters

The effect of *E. fetida* stocking density on the biodegradation and humification of RP enriched cow dung–waste paper mixtures was evaluated by monitoring the C_{HA}/C_{FA} ratio, HR, and HI. All three humification parameters were significantly affected by *E. fetida* stocking density and time as revealed by ANOVAR (Table 2).

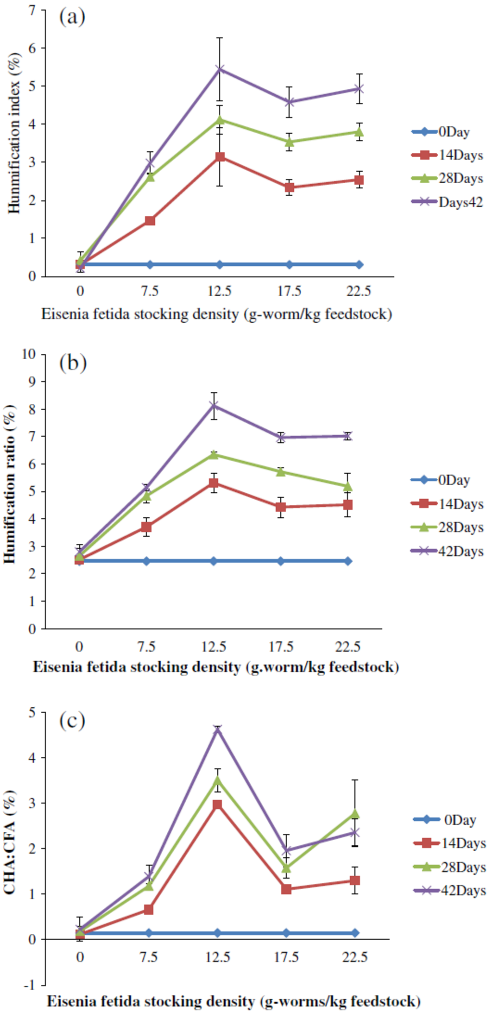

Fig. 2. *Eisenia fetida* stocking density effect on the: (a) humic acid/fulvic acid (C_{HA}/C_{FA}) ratio, (b) humification ratio (HR) of cow dung waste paper- mixtures during vermicomposting, and (c) Humification Index (HI) [Error bars are standard deviations].

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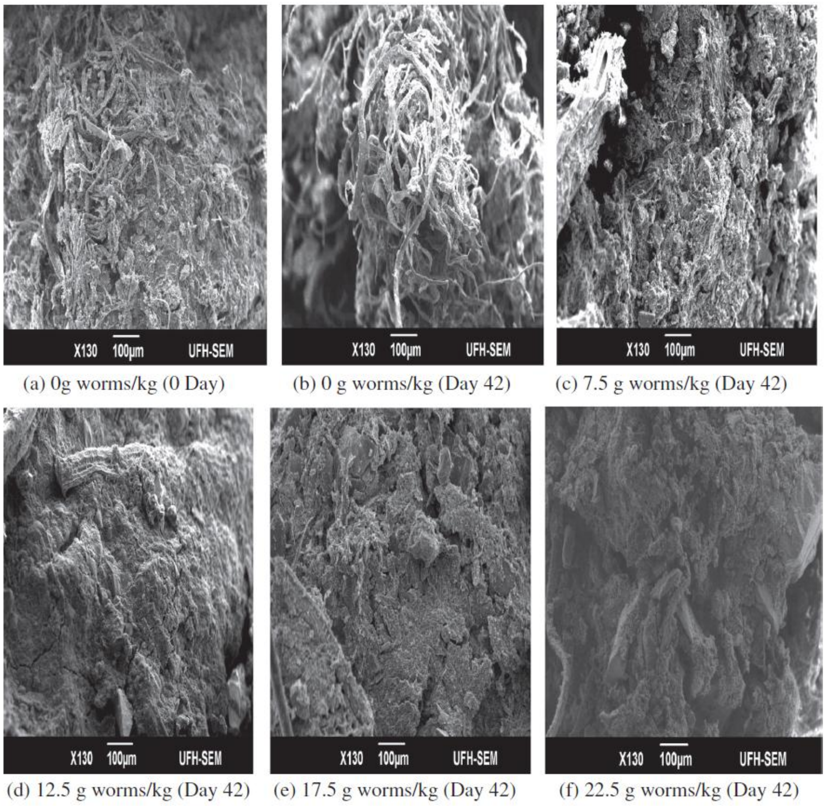

(a) 0g worms/kg (0 Day) (b) 0 g worms/kg (Day 42) (c) 7.5 g worms/kg (Day 42)

(d) 12.5 g worms/kg (Day 42) (e) 17.5 g worms/kg (Day 42) (f) 22.5 g worms/kg (Day 42)

Fig. 3. Scanning electron microscope pictures showing *Eisenia fetida* stocking density effects on vermicompost morphological properties.

The three parameters showed a strong and linear dependency on *E. fetida* stocking density whereby humification increased linearly up to the stocking density of 12.5 g-worms kg^{-1} feedstock but declined at higher stocking densities (Fig. 2a–c). The effect of *E. fetida* stocking density on humification parameters varied with time as reflected by the significant interaction between earthworm density and time (Table 2). Humification increased linearly with time for stocking densities up to 12.5 g-worms kg^{-1} feedstock (Fig. 2a–c). For stocking densities greater than the latter, humification increased linearly with time up to 14 days but it gradually declined thereafter.

Changes in the relative proportions of the fulvic acid (FA) and humic acid (HA) fractions with time is considered to be a good indicator of the maturation status of compost/vermicompost as it indicates the extent of humification of the substrate materials (Chefetz et al., 1998; Goyal et al., 2005). The increase in the humic acid/fulvic acid ratio at all stocking densities with time (Fig. 2c) indicated the transformation of the easily degradable molecules that make up the fulvic acid fraction to the more recalcitrant molecules of higher molecular weight which make up the humic acid fraction. The increase in C_{HA} with vermicomposting time resulted in increases in HR (Fig. 2b) and HI (Fig. 2a) values which indicated increasing humification of organic matter in the vermicomposts. The humic acid/fulvic acid ratio at different stocking densities was 0.15% at 0 day. There was a corresponding increase from 14 days to 42 days in the entire earthworm stocking densities relative to the control (Fig. 2c). The trends of increase at 14, 28 and 42 days were 0.15, 0.66, 2.98, 1.11 and 1.3; 0.17, 1.18, 3.51, 1.58 and 2.78; 0.23, 1.39, 4.63, 1.96 and 2.36 for 0, 7.5, 12.5, 17.5 and 22.5 g worms kg^{-1} substrate, respectively (Fig. 2c). All *E. fetida* stocking densities greater than 7.5 g-worm kg^{-1} substrate produced vermicomposts with C_{HA}/C_{FA} ratios that exceeded 1.9 which (Iglesias-Jimenlez and Perez- Garcia, 1992b) proposed as the C_{HA}/C_{FA} ratio above which city-refuse and sewage sludge compost could be considered mature. Therefore, *E. fetida* stocking densities greater than 7.5 g-worm kg^{-1} cow dung–waste paper produced well humified mature vermicomposts after 42 days but the stocking density of 12.5 g-worms kg^{-1} cow dung–waste paper with a C_{HA}/C_{FA} ratio of 4.63 (Fig 2c) produced the most humified vermicompost. Singh and Kaur (2013) also reported most humified vermicompost product at a stocking density of 12.5 g-worms kg^{-1} feed when they used chemical sludge and spent carbon generated from soft drink industries as feedstock.

Scanning electron microscopy (SEM) confirmed the extent of humification of the vermicomposts (Fig. 3a–f). The morphological structure of the initial cow dung–waste paper mixtures at the beginning of the experiment (Fig. 3a) showed compacted aggregates of cellulose and protein fibres which remained more or less the same after 42 days where no earthworms were included (Fig. 3b). However, where earthworms were added the cow dung

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Table 3
Relationship between *E. fetida* stocking density and Bray 1 extractable P during vermicomposting of RP enriched cow dung–waste paper mixtures.

E. fetida stocking density (g worms kg^{-1} substrate)	Regression equation	R^2	Rate of Bray 1 P release (mg P/kg^{-1} day^{-1})	Predicted Bray 1 P at 42 days (mg P kg^{-1})	Observed Bray 1 P at 42 days (mg P kg^{-1})	Net Bray 1 P increase from day 1 (mg P kg^{-1})
0	$y = 0.032x^2 + 0.1654x + 66.865$	0.9997	0.17	79.4	79.4	12.5
7.5	$y = -0.0015x^2 + 1.0114x + 67.06$	0.9994	0.99	106.9	107	40.1
12.5	$y = -0.0011x^2 + 1.5975x + 66.965$	1.00	1.57	132.1	132.1	65.2
17.5	$y = -0.0041x^2 + 2.0286x + 67.65$	0.9967	1.89	145.6	146.4	79.5
22.5	$y = 0.0045x^2 + 1.9104x + 69.345$	0.973	2.11	157.5	159.9	93.0
CV			14	14	14	14
$P > F$			<0.001	<0.001	<0.001	<0.001

waste paper mixtures were highly degraded by earthworm grinding activity as reflected by the fine grain textures (Fig. 3c–f). Earthworm stocking densities of 12.5 g-worms kg^{-1} feedstock and above produced well humified products consistent with the observed chemical humification parameters.

3.4. Effects of earthworm stocking density on extractable phosphorus during vermicomposting of RP enriched cow dung–waste paper mixtures

Extractable P was monitored to determine the effect of stocking density on the extent of P release from the RP that was added to the cow dung–waste paper mixtures. Both *E. fetida* stocking density and time had a significant effect on Bray 1 extractable P as revealed by ANOVAR (Table 2). It increased significantly with time at each stocking density but the extent of release was greater in the highest earthworm density, getting up to 2.1 times over the control consistent with the observed significant *E. fetida* density × time interaction (Table 3). The least extractable P occurred where no earthworms were added while the greatest release occurred at the highest stocking density of 22.5 g-worms kg^{-1} cow dung–waste paper. The rate of P release computed from best fit equations (Fig. 4) ranged from 0.17 to 2.11 mg P kg^{-1} day^{-1} (Table 3). The greatest rate of P release occurred at a stocking density of 22.5 g-worms kg^{-1} substrate. Increases in the amount of available P during vermicomposting was probably due to mineralization and mobilization of phosphorus from RP facilitated by enhanced phosphatase activity in the guts of the earthworms (Edwards and Lofty, 1972; Khwairakpam and Bhargava, 2009). Improvement in the amount of easily extractable P during vermicomposting was previously reported by Ghosh et al. (1999) but the present study has further demonstrated that this effect is strongly influenced by earthworm stocking density. Thus, although vermicompost maturity can be achieved at lower stocking density, a higher stocking density may be preferred if maximum P release from RP incorporated in the waste mixture is a desired goal.

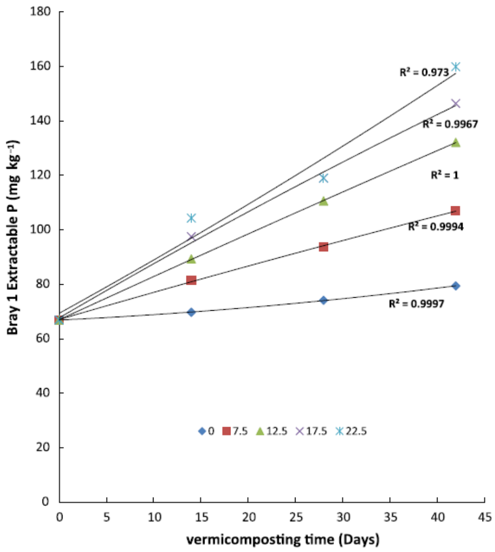

Fig. 4. Effect of *Eisenia fetida* stocking density on Bray1 extractable P during vermicomposting of cow dung–waste paper mixtures spiked with 2% rock phosphate.

3.5. Effect of earthworm stocking density on the phytotoxicity level of RP enriched cow dung–waste paper vermicomposts

The vermicompost extracts from the final vermicomposts of all treatments had no inhibitory effect on the seed germination of test crops (Table 4). In tomato, the relative seed germination increased linearly with *E. fetida* stocking density. The highest seed germination percentage was observed with *E. fetida* stocking densities of 12.5 g-worms kg^{-1} feedstock. The same trend was observed for radish and carrot ($P < 0.05$). With respect to relative root elongation, the highest significant value of 163% was recorded in tomato followed by radish 149% and carrot 108% at an *E. fetida* stocking density of 12.5 g-worms kg^{-1} feed. The germination indexes of all crops were above 50% except control treatment of carrot seed. *E. fetida* stocking density had a significant effect ($P < 0.05$) on root elongation and germination index in all test crops. The final vermicomposts had GI values that ranged from 98% to 162% (for tomato), 48–81% (for carrot) and 51–128% (for radish). According to (Zucconi et al., 1981; Tam and Tiquia, 1994; Tam and Wong, 1995; Tam et al., 1996; Tam and Tiquia, 1998) a germination index of ⩾80% indicate the disappearance of phytotoxins in composts. (Bustamante et al., 2001) also corroborated their findings when composting recycled digestates from biogas production. Thus none of the compost extracts inhibited tomato germination while the germination of carrot and radish was inhibited in mixtures

Table 4
Effect of earthworm stocking density on the phytotoxicity of cow dung–waste paper vermicompost.

Earthworm stocking density (g-worms kg^{-1} feed)	Tomato			Carrot			Raddish		
	RSG (%)	RRE (%)	GI (%)	RSG (%)	RRE (%)	GI (%)	RSG (%)	RRE (%)	GI (%)
0	92^b	106^d	98^d	67^b	72^d	48^d	92^b	54^e	51^e
7.5	91^b	129^c	117^c	67^b	78^c	52^d	92^b	93^d	86^d
12.5	100^a	163^a	163^a	83^a	108^a	90^a	100^a	149^a	149^a
17.5	100^a	160^b	160^b	83^a	96^b	81^b	100^a	120^c	120^c
22.5	100^a	162^b	162^a	83^a	94^b	81^b	100^a	128^b	128^b

Numbers followed by different letters in each column are significantly different according to the LSD test at $P < 0.05$.

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vermicomposted at stocking densities of less than 7.5 and 12.55 g-worms kg^{-1} substrate, respectively.

4. Conclusions

This study has demonstrated: (i) that C:N ratio, humification index, humification ratio, and the humic acid/fulvic acid ratio are equally effective for monitoring the vermidegradation of cow dung–waste paper mixtures, (ii) a clear dependency of vermidegradation on *E. fetida* stocking density but this effect tends to vary with time. Nevertheless, an *E. fetida* stocking density of 12.5 g-worms kg^{-1} feedstock was found to be ideal for the biodegradation of phosphate rock enriched cow dung waste paper mixtures, and (iii) Phosphate release from PR enriched wastes is strongly and linearly dependent on *E. fetida* stocking density and that higher stocking densities favour greater P release. Therefore, although vermicompost maturity can be achieved at lower stocking densities, a higher stocking density may be preferred in situations where maximum P release from RP incorporated in the waste mixture is a desired goal. Future work will seek to optimise the rate of RP incorporation in the vermidegradation of cow dung waste paper mixtures as well as unravel the mechanisms by which RP enhances the bioconversion of these waste mixtures.

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